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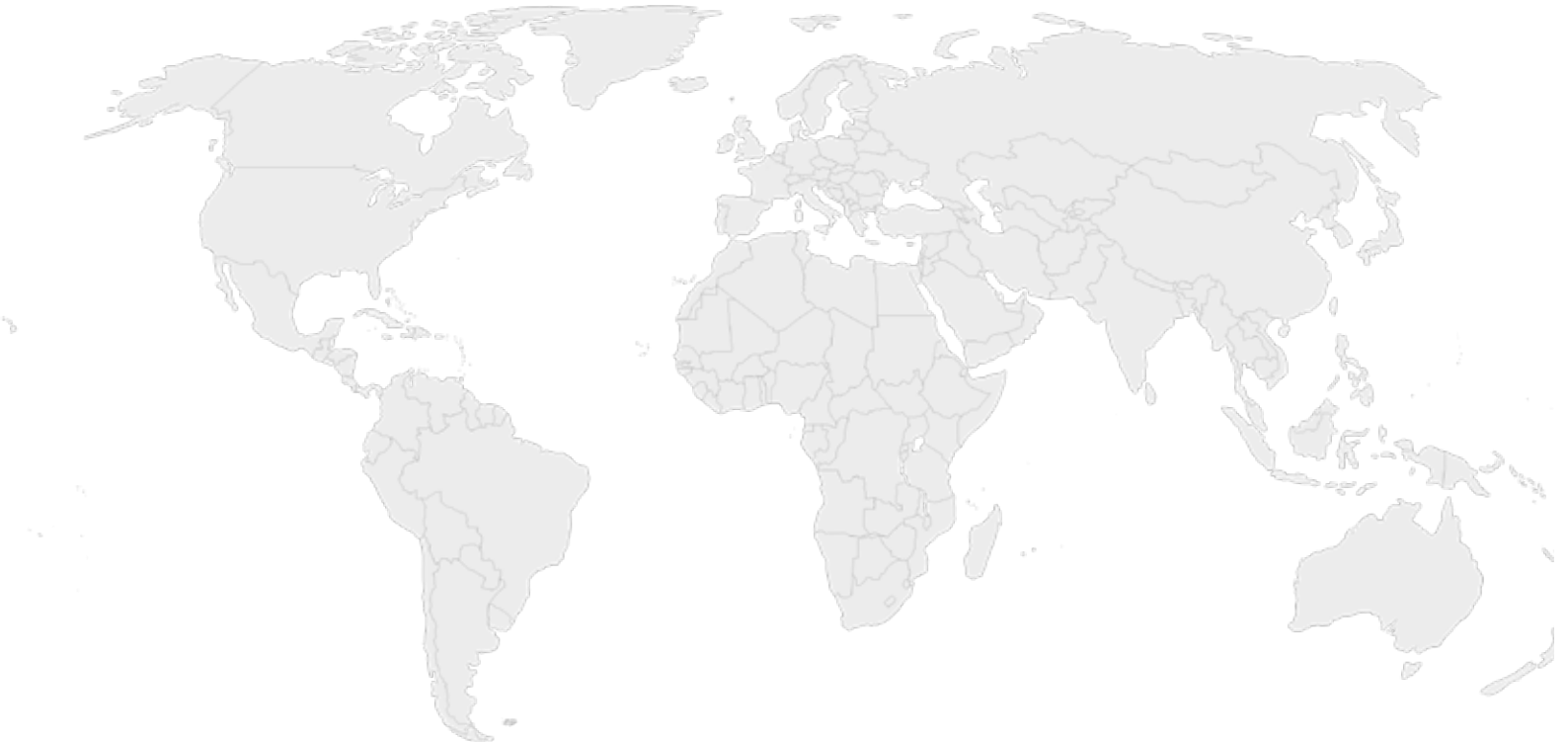

In this Issue

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Letter to the Editor

Magnesium - The Silent Partner or the Next Vitamin D? Shifting Paradigm in Mineral Metabolism in Health and Disease

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Magnesium is a critical yet greatly overlooked micronutrient, acting as a cofactor in over 300 enzymatic reactions [1-4]. Its role is integral in nucleic acid synthesis, energy metabolism, and antioxidant defenses, serving as a cofactor for key enzymes including superoxide dismutase and glutathione peroxidase [1, 2, 5]. It also regulates extracellular calcium, acts as a messenger in intracellular signaling and modulates circadian clock genes [6]. Despite its significance, standard serum magnesium testing is unreliable; patients may have normal serum levels of 0.75-0.95 mmol/L yet present with evidence of chronic, underlying deficiency [1,4,5,7]. Evidence suggests that up to 42% of young adults exhibit undetected insufficiency and nearly half of the US population fails to meet the RDA of 300-400 mg/day [1, 4, 6]. This can be met through magnesium rich foods like leafy green vegetables, legumes, whole grains, nuts and fortified products [8]. Inclusion of such foods in routine diet will meet overall magnesium requirements safely and effectively, with supplementation used only when dietary intake is inadequate and under medical supervision.

There are a multitude of causes for deficiency ranging from insufficient nutritional intake, increased excretion, malabsorption disorders, renal elimination, common medications along with aging and stress [1, 5, 6].

Studies have shown that daily doses reduce depression scores and anxiety [2, 3, 9]. This effect may stem from magnesium's role in converting tryptophan to serotonin [2, 4]. Its benefits extend into sleep medicine, 500 mg/day of magnesium improves insomnia severity index, sleep efficiency, shortens latency and restores cortisol, and

melatonin [4,10]. Beyond neuropsychiatric benefits, Type 1 diabetics supplemented with 300 mg/day showed reduced neuropathy progression relative to the control. Furthermore, Magnesium has reduced the frequency, intensity, and duration of migraines by 41% versus 15.8 % with placebo [1, 4, 6].

In relation to cardiovascular health, it improves blood pressure, decreases carotid intima thickness, with cohort data highlighting 77% reduction in sudden cardiac death in individuals with the highest percentile of magnesium intake [1, 4, 7, 11]. Supplementation has also been beneficial in inhibiting thrombus formation in patients with coronary artery disease [12].

Current evidence is limited with studies employing small sample sizes or short supplementation periods making it difficult to draw conclusions. Moreover, positive outcomes weren't consistent across the study population as some studies showed that Magnesium has no effect on anxiety of premenstrual or postpartum women while benefiting others. Additionally, while Magnesium can be beneficial, excessive intake can cause toxicity, emphasising the need for supplementation under medical supervision.

Table 1 summarizes evidence from PubMed and Google Scholar, keywords used included "magnesium supplementation" "magnesium deficiency" "neurological function" "sleep quality", prioritizing RCTs, systematic reviews, and large-scale studies, while incorporating smaller studies where relevant. Studies from 2018 onwards were included. Human studies were emphasized to ensure clinical applicability.

The rise of Vitamin D from being an overlooked micronutrient to becoming a part of routine evaluation illustrates how easily key nutrients can be overlooked. Magnesium seems to be following a similar pattern, with research steadily emphasising its role in neurological and cardiovascular health. Its benefits are recognised but underutilised, highlighting the need for well-designed studies to guide its application in patient care.

Table 1: Summary of selected studies on Magnesium and related health outcomes.

Author	Study type	Population	Sample size	Intervention	Outcome
Mohammed S. Razzaque1, 2018	Commentary	N/A	N/A	N/A	Magnesium deficiency is linked to clinical complications like muscle spasms, fibromyalgia, arrhythmias, and migraines.
Violeta Cepeda2, 2025	Systematic Review	General population, rats and mice with or without pathology	Systematic Reviews- 28 Meta-Analysis-6	Oral Mg intake	Magnesium supplementation, alone or with zinc, Vitamin C, Vitamin E, or selenium, reduces blood pressure in diabetes and cardiovascular disease, and, when combined with zinc, alleviates anxiety and depression symptoms.
Mahdi Moabedi3, 2023	Systematic Review	Adults with depression (20-60 years old)	7 RCTs (n=325)	Oral Magnesium supplementation	Depression scores have shown marked reduction when treated with Magnesium relative to placebo.
Gerry K. Schwalfenberg4, 2017	Review	Various studies on Mg supplementation	N/A	N/A	It reduces migraine frequency and severity, slows peripheral neuropathy progression, improves depression and anxiety, prevents arteriosclerosis changes, and relieves symptoms in mitral valve prolapse.
Veer Patel5, 2024	Review	Various studies on Magnesium supplementation	N/A	N/A	Evidence suggests association between magnesium intake and cognitive function in healthy individuals.

Ligia J. Dominguez6 ,2025	Review	Acute and Chronic Migraines	12 RCT for Acute Migraines and 8 RCT for Chronic Migraines	IV Magnesium supplementation in Acute and Oral in chronic migraines patients.	Evidence supports the benefits of both intravenous and oral magnesium administration acute migraine episodes as well as migraine prophylaxis.
Remi Fritzen7, 2023	Review	Various studies on Magnesium deficiency.	N/A	N/A	Magnesium deficiency increases risk of cardiovascular. Evidence, including NHANES data, shows magnesium is protective, with supplementation linked to lower risk of hypertension, atrial fibrillation, and heart failure, and improved blood pressure.
Ligia J. Dominguez 8, 2020	Review	Hypertensives	18 Studies	Magnesium supplementation	Evidence shows a close relationship between magnesium deficit and high blood pressure.
Alexander Rawji 9, 2024	Systematic Review	Adults with anxiety and sleep disorders.	15 studies	Oral Magnesium supplementation	A majority of included trials demonstrated at least modest positive results in regards to sleep quality and anxiety, with higher doses of magnesium being more effective.
Micheal J. Breus 10, 2024	Research Article	Adults with poor sleep quality	N = 31 adults, M age = 46.01	Participants were randomized to Magnesium Condition or Placebo Condition.	Magnesium supplementation had significant improvements compared to the placebo for sleep quality, mood, and activity outcomes
Lianbin Xu 11,2023	Systematic Review	1,325 T2D Individuals	24 RCTs	Oral Magnesium supplementation	Magnesium supplementation lowers glycated hemoglobin, systolic and diastolic blood pressure.
James J DiNicolantonio12, 2018	Review	General population	N/A	N/A	Subclinical magnesium deficiency linked to cardiovascular disease.

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Review Article

Revisiting the Platelet–β-Cell Axis: Insights into How Platelet-Derived Mediators, Lipid Signaling, and DOC2B Pathways Converge to Drive β-Cell Dysfunction in Type 2 Diabetes

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Abstract

Type 2 diabetes mellitus (T2DM) is a multifactorial disorder where platelet-derived mediators, lipid metabolic pathways, and exocytotic proteins intersect to drive β-cell dysfunction. Activated platelets release serotonin, platelet factor 4 (PF4), sphingosine-1-phosphate (S1P), and microvesicles that trigger oxidative and endoplasmic reticulum (ER) stress in pancreatic islets. CD36-mediated lipid uptake and sphingolipid imbalance intensify ceramide-driven mitochondrial damage. These insults converge on exocytotic failure through disruption of DOC2B, a Ca^{2+}-sensitive mediator of insulin vesicle fusion. Revisiting this axis clarifies how thromboinflammation and lipotoxicity orchestrate β-cell failure and highlights emerging therapeutic targets for T2DM. This review introduces a novel integrative perspective linking platelet-derived mediators, lipid dysregulation, and DOC2B-mediated exocytotic failure as a unified model of β-cell dysfunction in T2DM.

Background and Rationale

Type 2 diabetes mellitus (T2DM) is not solely a metabolic disease but also a vascular-inflammatory disorder [1–4]. Platelets in T2DM exhibit hyperactivity and release mediators capable of influencing β-cell function and viability [9–17]. This concept has evolved into the platelet–β-cell axis, a bidirectional pathway linking thromboinflammation to insulin secretion defects. The diversity of platelet mediators-PF4, serotonin, S1P, and platelet-derived microvesicles-illustrates how hemostatic cells can influence pancreatic islets beyond coagulation. Although previous studies have recognized platelet–β-cell crosstalk in the context of inflammation, oxidative stress, and lipid overload, none have comprehensively integrated the DOC2B-mediated exocytotic machinery with platelet-derived lipid signaling and CD36-driven lipotoxic stress as a unified mechanism of β-cell failure. This review introduces a novel tri-axis model-encompassing platelet mediators, lipid dysregulation, and DOC2B dysfunction-that provides a systems-level explanation of how thromboinflammatory and lipotoxic pathways converge to precipitate β-cell demise in T2DM.

Table 1: Summary of Platelet-Derived Mediators Involved in β-Cell Dysfunction.

Platelet Mediator	Main β-Cell Target	Molecular Effect	Key Mechanism	References
Platelet Factor 4 (PF4)	Heparan sulfate receptor on β-cell membrane	Increases intracellular Ca^{2+} and ROS via NADPH oxidase activation	Induces oxidative stress and impairs insulin gene transcription	[11, 14, 15, 17]
Serotonin (5-HT)	5-HT_2B receptor on β-cells	Stimulates acute insulin exocytosis; chronic exposure causes desensitization	Protein serotonylation of SNARE components	[11, 37]
Sphingosine-1-Phosphate (S1P)	S1PR2/S1PR3 receptors	Regulates Ca^{2+} signaling and mitochondrial activity; protective at physiological levels, toxic when excessive	Ceramide–S1P rheostat in β-cell survival	[6, 7, 31, 38]
Platelet Microvesicles (PMVs)	β-cell cytoplasm and islet endothelium	Transfer lipids and miRNAs; induce ER stress and inflammation	Vesicular crosstalk between platelet and islet cells	[18, 33]
Platelet-Derived ROS	β-cell mitochondria	Oxidizes DOC2B and impairs vesicular fusion	NOX2/NOX4-mediated chronic oxidative stress	[34, 36, 40]

Of note, the clinical relevance of platelet hyperactivity in T2DM extends beyond hemostasis. Several population-based analyses demonstrate that abnormal platelet indices-such as mean platelet volume and platelet distribution width-correlate strongly with metabolic control and the development of microvascular complications [9,10,11,13,15]. Such platelet activation is closely related to systemic inflammation and oxidative imbalance, both of which accelerate β-cell exhaustion and insulin secretory decline [14,16,17]. These findings support the notion that platelet dysfunction may precede overt hyperglycemia and serve as an early biomarker of metabolic deterioration [11,13,17]. Collectively, these insights strengthen the hypothesis that the platelet–β-cell axis represents a pivotal link between vascular inflammation and endocrine dysregulation in T2DM pathogenesis [9–17].

Platelet Activation and Thromboinflammatory Mediators

Activated platelets in T2DM release a complex repertoire of cytokines, lipid messengers, and extracellular vesicles. PF4 and serotonin stimulate intracellular Ca^{2+} signaling and oxidative stress, while platelet-derived microvesicles (PMVs) deliver ceramides and miRNAs that impair insulin gene expression [11,14,16–18,33]. PMV-induced activation of NADPH oxidase (NO X 2/4) creates persistent ROS that propagates ER stress and apoptosis.

Lipid Signaling: CD36 and Sphingolipid Dysregulation

CD36 acts as a lipid gatekeeper for long-chain fatty acids and oxidized LDL. Its overactivation in β-cells promotes ceramide accumulation and mitochondrial ROS production [5,30,32]. Simultaneously, sphingolipid imbalance-especially elevated ceramide and diminished S1P-drives ER stress and apoptosis [6–8,31,38].

CD36 serves as a metabolic gatekeeper that facilitates the

uptake of long-chain fatty acids and oxidized lipoproteins into β-cells. While transient activation of CD36 supports membrane remodeling and energy supply for insulin secretion, chronic overexpression under hyperglycemic and lipotoxic conditions drives excessive fatty acid influx, ceramide accumulation, and reactive oxygen species (ROS) generation [5, 30, 32]. This oxidative and ER stress environment disrupts mitochondrial respiration and promotes apoptotic cascades, contributing directly to β-cell dysfunction [31, 38].

In parallel, sphingolipid metabolism-particularly through sphingosine-1-phosphate (S1P) and ceramide-plays a dual role in maintaining β-cell integrity. Physiological S1P signaling via S1PR2 and S1PR3 supports insulin secretion and cellular resilience; however, imbalance in the ceramide/S1P ratio shifts the intracellular milieu toward apoptosis and inflammation [6, 7, 31, 38]. Activation of neutral sphingomyelinase-2 (nSMase2) under hyperglycemic conditions further amplifies ceramide production, establishing a self-perpetuating cycle of lipotoxic stress.

Taken together, aberrant CD36 signaling and sphingolipid dysregulation form a lipid-centric framework of β-cell injury that intertwines metabolic overload with oxidative stress. Importantly, these lipid pathways also sensitize β-cells to platelet-derived mediators-such as S1P and microvesicle lipids-linking lipid metabolism to thromboinflammatory crosstalk within the platelet–β-cell axis [6, 7, 18, 30–33, 38]

This integrative lipidomic dysfunction couples metabolic overload with platelet activation, reinforcing the feed-forward cycle of inflammation and β-cell failure.

Table 2: Relationship between CD36 and Sphingolipid Pathways in β-Cell Dysfunction.

Lipid Pathway	Core Components	Effect on β-Cells	Mechanistic Evidence	References
CD36-mediated lipid uptake	CD36, oxidized LDL, long-chain fatty acids	Ceramide accumulation, ROS generation, apoptosis	CD36 overexpression triggers mitochondrial and ER stress	[5, 30, 32]
Ceramide synthesis pathway	Neutral sphingomyelinase-2 (nSMase2), serine palmitoyl transferase	Activates caspase-3, CHOP, and suppresses GSIS	Ceramide inhibits SNARE complex assembly	[6, 7, 31, 38]
S1P signaling	S1PR2/S1PR3	Maintains cell survival at moderate levels, pro-apoptotic when excessive	Regulates balance between β-cell proliferation and apoptosis	[6, 31, 38]
Lipid vesicle crosstalk	Platelet-derived microvesicles	Transfers ceramide and inflammatory lipids to β-cells	Mediates intercellular lipotoxicity via vesicle transport	[18, 28, 33]

DOC2B and Exocytotic Failure

DOC2B (Double C2-like Domain β) is a calcium-sensitive protein critical for insulin granule fusion. Under oxidative and nitrosative stress, DOC2B undergoes conformational changes and post-translational modifications that impair its SNARE interaction [29,35,40]. Ceramide accumulation and ROS from platelet activity synergistically reduce DOC2B stability, leading to defective glucose-stimulated insulin secretion (GSIS). DOC2B (Double C2-like Domain β) serves as a critical Ca^{2+} sensor that regulates insulin granule fusion with the plasma membrane. Functionally, it bridges glucose-induced Ca^{2+} influx to the SNARE complex-comprising syntaxin-1A, SNAP-25, and VAMP2-to ensure the timing and efficiency of insulin release [29, 35]. In healthy β-cells, rapid DOC2B phosphorylation at tyrosine residues enables synchronous exocytosis during the first-phase glucose-stimulated insulin secretion (GSIS). However, diabetic stressors-such as ROS accumulation and S-nitrosylation-induce structural alterations that weaken DOC2B–SNARE interactions, resulting in delayed or incomplete vesicle fusion [34, 36, 40].

Beyond its exocytotic function, DOC2B also modulates cytoskeletal rearrangement and vesicle trafficking, processes that depend on balanced redox signaling. Ceramide-mediated proteasomal degradation and endoplasmic-reticulum stress markedly decrease DOC2B expression, linking lipid overload to secretory insufficiency [31, 38]. Interestingly, recent evidence shows that β-cells under metabolic stress can release DOC2B within extracellular vesicles, suggesting a potential adaptive or compensatory mechanism and a measurable biomarker of β-cell distress [29].

Taken together, these findings position DOC2B as a convergence point where oxidative, nitrosative, and lipotoxic stress intersect to impair insulin exocytosis. Targeting post-translational regulation of DOC2B-by preventing S-nitrosylation, enhancing phosphorylation, or stabilizing its protein structure-may represent a promising strategy to restore β-cell secretory competence [29, 35, 36, 40].

Table 3: Molecular Links between Oxidative Stress and Impaired Insulin Exocytosis via DOC2B.

Mediator / Pathway	Effect on DOC2B	Impact on Insulin Secretion	References
ROS (NOX2/NOX4)	Oxidation of cysteine residues in DOC2B	Weakens SNARE complex binding	[36]
S-nitrosylation	Modification of tyrosine residues in DOC2B	Disrupts vesicle docking and exocytosis	[40]
Ceramide	Promotes proteasomal degradation of DOC2B	Reduces GSIS and granule stability	[31, 38]
Loss of tyrosine phosphorylation	Inactivates DOC2B	Impairs insulin granule fusion and release	[35]
Extracellular vesicle export	Induces DOC2B secretion into plasma vesicles	Serves as a marker of β-cell stress	[29]

Interestingly, DOC2B acts as a calcium sensor that interacts dynamically with syntaxin-1A, Munc18-1, and SNAP25 to fine-tune the timing and amplitude of insulin vesicle release [35]. Under physiologic conditions, Ca^{2+} influx triggers conformational changes within its tandem C2 domains, ensuring synchronized glucose-stimulated insulin secretion (GSIS) [29,35]. However, oxidative stress and nitrosative modifications (such as S-nitrosylation of tyrosine residues) disrupt DOC2B conformation and its binding to SNARE proteins, resulting in asynchronous vesicle docking and diminished first-phase insulin secretion [34,36,40]. Lipid-induced ceramide accumulation and proteasomal degradation further exacerbate DOC2B loss, forming a mechanistic bridge between lipotoxicity and exocytotic failure [31,38]. These molecular events not only explain the blunted GSIS observed in T2DM but also highlight DOC2B as a promising therapeutic checkpoint to restore β-cell competency [29,35,40].

Integrative Mechanism

The Platelet–β-Cell Axis Revisited

This section introduces the novel integrative model proposed in this review, which unifies platelet activation, lipid dysregulation, and DOC2B exocytotic failure into a single mechanistic framework. This conceptual triad has not been previously described in existing platelet or β-cell literature.

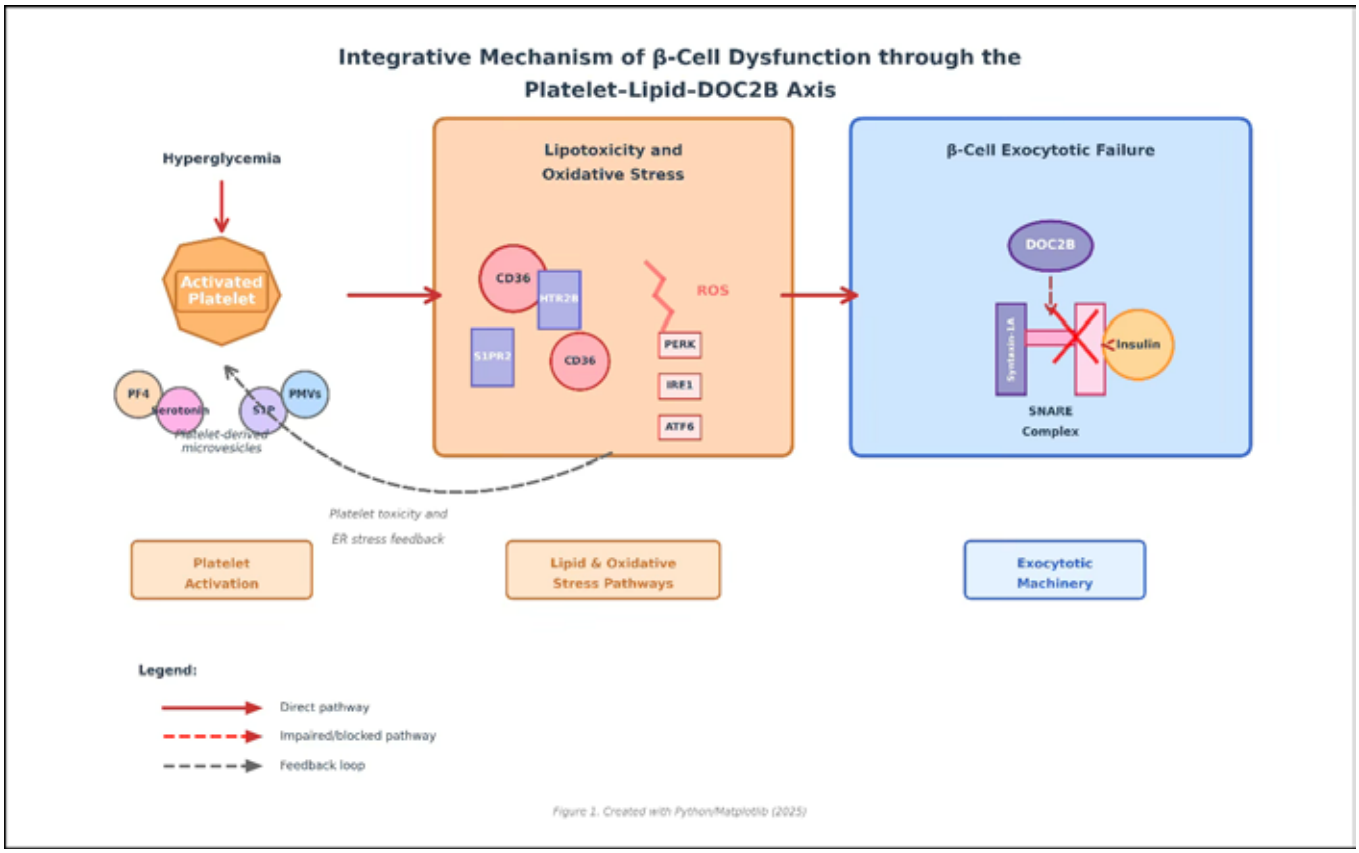
The platelet–β-cell axis can be visualized as a unified network: platelet activation → release of mediators (PF4, S1P, PMVs) → CD36/nSMase-2-driven ceramide accumulation → ROS/ER-stress-mediated DOC2B dysfunction → insulin exocytosis failure → hyperglycemia → further platelet activation [5–8,11–18,28–36,38–40].

The illustration should display three interconnected modules:

1. Activated Platelets (releasing PF4, S1P, serotonin, PMVs)
2. Lipotoxic Pathway (CD36, ceramide, NOX2/4, ER stress)
3. Exocytotic Machinery (DOC2B, SNARE, Insulin Vesicle)

Arrows between modules represent the self-reinforcing loop from metabolic overload to β-cell demise.

Figure 1: Integrative Mechanism of β-Cell Dysfunction through the Platelet–Lipid–DOC^{2}BAxis Created with BioRender.com (accessed 2025).



Clinical and Translational Implications

Therapeutically, targeting the platelet–β-cell axis could complement glucose-centric care. Antiplatelet agents, CD36 inhibitors, sphingolipid modulators, and DOC2B-stabilizing compounds hold potential to restore β-cell integrity [5,11–17,30–32,35,38–40]. Circulating biomarkers such as platelet microvesicles, sphingolipid ratios, and soluble DOC2B warrant further evaluation as indicators of β-cell stress and treatment response.

Beyond pharmacologic modulation, integrating platelet and lipid biomarkers into metabolic screening may refine early detection of β-cell deterioration. Combined assessment of circulating platelet-derived vesicles, ceramide/S1P ratios, and soluble DOC2B levels could identify subclinical β-cell stress before overt hyperglycemia manifests [18,28,31,33]. Furthermore, experimental models demonstrate that simultaneous targeting of platelet activation, CD36-mediated lipid uptake, and sphingolipid imbalance can synergistically preserve insulin secretion and metabolic stability [5,6,30–32]. In particular, S1P receptor modulators, ceramide synthesis inhibitors, and CD36 antagonists are emerging as novel adjuncts to glucose-lowering therapy [6,7,31,38]. Translating these mechanistic insights into clinical practice could open new therapeutic avenues by attenuating platelet-driven oxidative stress and maintaining DOC2B functionality [11,15,29,35,40].

Conclusion and Future Perspectives

Revisiting the platelet–β-cell axis highlights an underappreciated dimension of metabolic crosstalk in type 2 diabetes mellitus, where thromboinflammatory signaling, lipid imbalance, and vesicular exocytotic failure act in concert to drive β-cell demise. This integrative model not only bridges vascular and endocrine pathology but also reframes diabetes as a disorder of intercellular communication rather than isolated metabolic dysfunction. Future investigations should employ longitudinal platelet transcriptomic and lipidomic profiling alongside molecular indices of β-cell exocytosis-such as DOC2B, SNARE integrity, and ceramide/S1P ratios-to delineate temporal disease trajectories and reveal precision-targetable pathways [1, 5–7, 11–12, 20–22, 28–31, 34–36, 38–40].

Ultimately, translating these mechanistic insights into clinical practice could yield novel biomarkers for early detection and therapeutic strategies aimed at restoring β-cell resilience through modulation of platelet and lipid signaling networks. In conclusion, our review contributes a new conceptual understanding by framing T2DM as a disorder of intercellular communication, in which platelet-derived mediators, lipid metabolic stress, and DOC2B dysfunction act synergistically. This integrative approach not only connects vascular and endocrine pathology but also establishes a mechanistic bridge

that may guide the development of platelet-targeted and DOC2B-modulating therapies.

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Conflict Of Interest Statement
The author declares that there are no commercial or finansial relationship that could be construed as a potential conflict of interest. The content of this article was developed solely based on scientific evidence and without any influence from external parties.

Ethics Statement
This article is narrative review based entirely on previously published research and publicly available data. No. new studies involving human participants or animals were conducted by the authors. Therefore,ethical approval and informed consent were not required. All original sources were appropriately cited to maintain academic integrity and to acknowledge prior work. The review was performed in accordance with the principles and guidelines of the Committee on Publication Ethics(COPE).

Artificial Intelligence (AI) Assistance Declaration
ChatGPT (OpenAI, GPT-5) was used to support linguistic refinement, structural formatting, and reference alignment in this manuscript. All conceptual ideas, data interpretation, and final conclusions are entirely the authors' own responsibility.

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Research Article

Opinion Paper: Smartwatches in Healthcare: Revolutionizing Health or Creating Data Confusion?

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Abstract

Smartwatches have gained significant attention for their role in advancing digital health interventions and enhancing wellbeing. These modern technologies have the potential to revolutionize patient care by making healthcare more accessible and personalized. By promoting preventive care, shifting services from hospitals to communities, and transitioning from analog to digital healthcare systems, smartwatches can help individuals stay healthier and reduce hospital visits.

A key aspect of this transformation is the integration of smartwatch data into a unified patient record, ensuring comprehensive access to health information. However, with over 20 smartwatch manufacturers, offering multiple models with diverse health-monitoring capabilities, critical questions arise: What data is collected? How is it stored? Should any of it be integrated into medical records? Beyond the risks of data misuse for financial purposes, persistent challenges include ensuring accuracy, reliability, and standardization. A recent IFCC best practice publication on incorporating patient-generated health data into Electronic Health Records (EHRs) [1] highlights essential questions that must be addressed before smartwatch data can contribute meaningfully to healthcare. This paper explores these issues, weighing the potential benefits of smartwatches against the complexities of data integration and management.

Introduction

Globally, more than 20 smartwatch manufacturers produce at least 29 models with health tracking capabilities. Basic models measure heart rate, oxygen levels, sleep patterns, step count, distance, and calories expenditure. More advanced versions can provide ECG (Electrocardiogram) readings, mental and physical well-being scores, menstrual health tracking, skin temperature variation, breathing rate, body composition metrics, blood pressure monitoring, and blood glucose tracking. Despite these innovations, significant variations exist even within the same manufacturer's line-up.

For instance, Apple Watches rely on photoplethysmography (PPG) for heart rate variability (HRV) tracking, whereas Samsung Watches also employ ECG sensors. Such disparities highlight the broader issue of limited standardization, particularly for PPG-based devices, which dominates the market but often face accuracy challenges during physical activity. However, automated biosensor detection of unwitnessed cardiac arrest, followed by immediate dispatch of medical assistance, could greatly improve survival rates, underscoring the importance of reliable, standardized measurements. Although some devices achieve continuous monitoring during sleep, consistency remains a challenge [2], these challenges among others are explored in detail by Li et al [3].

User-entered demographic data-used to calibrate step distance and calorie counts can introduce inaccuracies, as can the step-tracking feature which relies on a mix of location data and watch movement. In some cases, users can inflate their step counts simply by shaking the watch without actually walking. By moving your watch arm up and down around 6 inches and going from left to right (or vice versa) across the width of your body the Fitbit sense will count steps for as long as the movement keeps going. Despite these limitations, smartwatches provide tangible personal benefits: daily activity goals, heart rate monitoring, and sleep analysis can all foster healthier habits and an increased sense of well-being. A recent review including 35 studies and other publications pointed out that smartwatches can serve as valuable tools for monitoring health by increased acceptance, but they should not be solely relied upon for diagnosing illnesses in most instances. According to the editors, one problem is that smartwatches focus on aggregating biomedical data and do not take a holistic view of the patient, and the over-reliance on smartwatches and the fewer face-to-face doctor visits lead to a violation of the principle of non-maleficence [4].

Device choice is frequently tied to user smartphone ecosystems. Apple Watches work only with iPhones, while Fitbit and Garmin devices support both iOS and Android. More budget-friendly options are often tied to a single phone brand or Android-based systems.

Although some standardization exists through licensing agreements among manufacturers. The overall variability in hardware and software poses a hurdle for integrating smartwatch data into clinical or population-wide health studies. Nevertheless, as data accuracy and interoperability continue to improve, smartwatches hold significant potential for supporting evidence-based health interventions and personalized wellness strategies.

Methods

Below is an assessment of the listed data and trends regarding smartwatch health functionalities (Table 1-4). Since these figures come from a specific (and somewhat future-dated) source (July 2024 internet search), it's not possible to verify them against publicly available references at this time. The initial data searches were carried out to create a spreadsheet of smartwatch capabilities within the healthcare arena.

The search engines used were google.com and bing.com. Both search engines were used to ensure that the maximum number of smart watches were discovered. The initial search terms used were 'what smart watches are available globally' from this a list of watches was collated. For each watch manufacturer the question was then asked, "Huawei smart watch for health monitoring" (Huawei used as an example. These produced smartwatch versions that were advertised for their health monitoring features. Then for each watch manufacturer up to 3 of the latest versions were chosen to get detailed information and the search term "Huawei smart watch 4 technical specifications" was entered and the spreadsheet updated from the manufacturers technical specifications.

Given the rapid expansion of smartwatch manufacturers and the frequent release of new models with diverse features, any list of currently available devices is likely to become outdated by the time the article reaches publication. Nonetheless, the underlying rationale and insights presented in this paper remain relevant and enduring.

However, from a purely logical and industry-knowledge standpoint, the numbers and features mentioned appear internally consistent and align reasonably well with known smartwatch capabilities. Here are some points to consider:29 of 30 models included heart rate sensors, while only 13 offered ECG monitoring.

- **Heart Rate Sensors (29 of 30 models):** It is very common for modern smartwatches to include optical heart rate sensors. Having 97% coverage is highly plausible
- **Blood Oxygen Monitoring (20 of 30 models):** SpO□ sensors have become more prevalent, especially after 2020, but only 2 included blood pressure monitoring
- **Blood Glucose Logging (6 of 30 models):** Actual non-invasive glucose measurement is still in development, so most likely these devices support manual logging or integration with third-party sensors.
- **ECG Monitoring (13 of 30 models):** ECG functionality requires more advanced hardware and regulatory approvals, so fewer models offer it

- **Sleep Monitors (25 models) and Activity Tracking (26 models):** These features are fairly standard.
- **Distinct Health-related metrics (28 in total):** It's very plausible that across 30 models, manufacturers advertise nearly 30 distinct metrics (e.g., steps, calories, VO_2 max, HRV, stress scores, etc.). The statement that no single metric is universally available across all models underscores the lack of industry-wide standardization.
- **Some models included unique features**
  - **Garmin Venu 3:** Known to have advanced health reports and nap detection, (provides a comprehensive overview of sleep quality, including sleep stages, stress levels, heart rate variability (HRV), and recovery status, while its "nap detection" feature automatically tracks and logs naps, allowing the user to see how they impact overall sleep quality and providing insights on ideal nap duration and timing) which aligns with Garmin's emphasis on detailed fitness and wellness data.
  - **ASUS HC A05:** While less well-known, the mention of pulse transit time measurement (this is a measure of blood flow speed, which can indicate emotional stress and overall health) is consistent with ongoing industry experiments to refine blood pressure or stress estimates.

This overview of the smartwatch market, based on direct manufacturer specifications, provides a snapshot of market trends and illustrates the wide range of features and health indicators offered by different brands, reflecting the state of smartwatch technology projected through July 2024. The diversity of metrics and features presents a well-known challenge for integrating smartwatch data into clinical or population health research. The variability of measurement methods and the lack of standardized health metrics further complicate harmonization efforts.

Table 1: Health Functions of 10 Available Smart Watches (1).

Functionality	Apple watch v9.0	Apple watch ultra	Apple watch SE	Fitbit sense 2	Fitbit Charge 4	Fitbit Versa 4	Fitbit inspire 3	Google pixel watch	Google Pixel watch 2	Samsung galaxy watch 4
Blood oxygen measurement, the percentage of blood saturated with oxygen	Blood Oxygen App	Blood Oxygen App		SpO2 (blood oxygen) tracking + breathing rate	SpO2 (blood oxygen) tracking + breathing rate	SpO2 (blood oxygen) tracking + breathing rate	SpO2 (blood oxygen) tracking + breathing rate	SpO2 (blood oxygen) tracking + breathing rate	SpO2 (blood oxygen) tracking + breathing rate	Bioactive sensor measures SP02 in real time
ECG (Electrocardiogram) measures the electrical activity of the heart	ECG App	ECG App		ECG App	ECG App	ECG App		ECG App	ECG App	ECG in real time
Heart rate notifications alert the user if the heart rate deviates from a predetermined level	High and low heart rate notifications	High and low heart rate notifications	High and low heart rate notifications	High and low heart rate notifications	High and low heart rate notifications	High and low heart rate notifications	High and low heart rate notifications	High and low heart rate notifications	High and low heart rate notifications	Electrical and optical heart rate sensor
Estimated blood pressure by monitoring blood flow changes										
Sleep monitor measures sleep duration, quality and sleep stages.	Sleep stages	Sleep stages	Sleep stages	Sleep stages, sleep profile, sleep score + smart wake alarm	Sleep stages, sleep profile, sleep score + smart wake alarm	Sleep stages, sleep profile, sleep score + smart wake alarm	Sleep stages, sleep profile, sleep score + smart wake alarm	Sleep stages, sleep profile, sleep score	Sleep stages, sleep profile, sleep score	Sleep monitor, holistic analysis, sleep coaching
Health Mental well being provides insights into individual health and mental state	Mental well being	Mental well being	Mental well being	Wellness report	Wellness report	Wellness report	Wellness report	Wellness report	Wellness report	
Menstrual cycle tracking logs dates and symptoms to predict future periods.	Cycle tracking	Cycle tracking	Cycle tracking	Menstrual health tracking	Menstrual health tracking	Menstrual health tracking	Menstrual health tracking	Menstrual health tracking	Menstrual health tracking	
Skin temperature is used for insights like sleep monitoring.	Temperature sensor	Temperature sensor		Skin temperature variation	Skin temperature variation	Skin temperature variation	Skin temperature variation		Skin temperature variation	
Stress App, Reflections, Daily Readiness and Medical Information				EDA scan app for stress management & reflections & daily readiness score	EDA scan app for stress management & reflections & daily readiness score	EDA scan app for stress management & reflections & daily readiness score	EDA scan app for stress management & reflections & daily readiness score	EDA scan app for stress management & reflections & daily readiness score	EDA scan app for stress management & reflections & daily readiness score	

Activity tracking monitors and records various phsical activity throughout the day	Activity tracking	Activity tracking	Activity tracking	Active zone minutes	Active zone minutes	Active zone minutes	Active zone minutes	Active zone minutes	Active zone minutes	Activity tracking
Steps, distance and calories				Steps, distance and calories + floor climbed	Steps, distance and calories	Steps, distance and calories + floor climbed	Steps, distance and calories	Steps, distance and calories + floor climbed	Steps, distance and calories + floor climbed	
Exercise modes monitor specific types of physical activity e.g. cycling				Exercise modes with smart track auto recognition & workouts	Exercise modes with smart track auto recognition & workouts	Exercise modes with smart track auto recognition & workouts	Exercise modes with smart track auto recognition & workouts	Exercise modes with smart track auto recognition & workouts & pace training	Exercise modes with smart track auto recognition & workouts & pace training	
Blood glucose tracking is currently made by entering glucose levels in an App				Glucose tracking (App)	Glucose tracking (App)	Glucose tracking (App)	Glucose tracking (App)	Glucose tracking (App)	Glucose tracking (App)	

Table 2: Health Functions of 10 Available Smart Watches (2).

Functionality	Samsung Galaxy 5	Samsung Galaxy 6	One Plus watch 2	CMF watch Pro	Garmin Venu 3	Huawei watch ultimate	Huawei watch 4	ASUS smart watch HC A05	Acer Leap ware	HTC Grip
Blood oxygen measurement, the percentage of blood saturated with oxygen	SpO2 (blood oxygen) tracking +VO2 Max	SpO2 (blood oxygen) tracking +VO2 Max	blood oxygen monitoring single point or all day	blood oxygen monitor + breathing training	Pulse oxygen sensor + VO2 Max + breathing rate			Pulse oxygen sensor		
ECG (Electrocardiogram) measures the electrical activity of the heart	ECG App	ECG App					ECG sensor	ECG sensor		
Heart rate notifications alert the user if the heart rate deviates from a predetermined level	Electrical and optical heart rate sensor	Electrical and optical heart rate sensor	High/low heart rate warning.	Heart rate alert	Heart rate variability notifications	Optical heart rate sensor	Optical heart rate sensor	Aerobic heart rate sensor	Heart rate sensor	
Estimated blood pressure by monitoring blood flow changes	Blood pressure monitoring	Blood pressure monitoring								
Sleep monitor measures sleep duration, quality and sleep stages.	Sleep stages	Sleep stages	Sleep monitor - Sleep stages	Sleep monitor	Sleep monitoring + nap detection+ jet lag adviser			Sleep monitoring	Sleep monitoring	Sleep monitoring
Health Mental well being provides insights into individual health and mental state										
Menstrual cycle tracking logs dates and symptoms to predict future periods.					Menstrual health tracking					
Skin temperature is used for insights like sleep monitoring.	Infrared Temperature Sensor	Infrared Temperature Sensor			Skin temperature variation	Temperature sensor	Temperature sensor			
Stress App, Reflections, Daily Readiness and Medical Information	Stress level management, Body fat, skeletal musce & body water %,	Stress level management, Body fat, skeletal musce & body water %,	Stress monitoring	Stress monitoring	Stress tracking + Meditation & Health Report			Stress tracking		
Activity tracking monitors and records various phsical activity throughout the day	Activity tracking	Activity tracking	Activity tracking	Activity tracking	Activity tracking			Activity tracking	Activity tracking	Activity tracking

Steps, distance and calories			Steps, distance and calories		Steps, distance and calories + floors climbed			Steps, distance and calories + floors climbed	Steps and pace	Distance and calories
Exercise modes monitor specific types of physical activity e.g. cycling			100+ sports modes with 6 types of auto recognition	100+ sports modes					Multi sport mode	
Blood glucose tracking is currently made by entering glucose levels in an App										

Table 3: Health Functions of 10 Available Smart Watches (3).

Functionality	Honor watch GS3	LG Smartwatch W110	HD Smart Fitness Watch	Motorola Moto 100 smart watch	NOKIA Steel HR	Oppo Smart watch	Real Me Watch S	Withings scan watch 2	Xiaomi Mi Watch S2	ZTE Watch
Blood oxygen measurement, the percentage of blood saturated with oxygen	Blood Oxygen Monitor 24/7 tracks blood oxygen.			SpO2 monitor			SpO2 sensor	SpO2 sensor + respiratory rate	SpO2 sensor	SpO2 sensor
ECG (Electrocardiogram) measures the electrical activity of the heart								ECG		
Heart rate notifications alert the user if the heart rate deviates from a predetermined level	8 Channel PPG Heart Rate Sensor.	PPG (Heart rate monitor)	Heart rate sensor	HRM	Heart rate infrared PPG sensor	Heart rate sensor	Heart rate sensor	Heart rate sensor	Heart rate sensor	Heart rate sensor
Estimated blood pressure by monitoring blood flow changes										
Sleep monitor measures sleep duration, quality and sleep stages.	Sleep tracking		Sleep monitor		Time it takes to fall asleep, sleep cycles	Sleep monitor	Sleep monitor	sleep monitor		Sleep monitor
Health Mental well being provides insights into individual health and mental state										
Menstrual cycle tracking logs dates and symptoms to predict future periods.								Menstrual cycle tracking		
Skin temperature is used for insights like sleep monitoring.								Body temperature sensor	Body temperature sensor	
Stress App, Reflections, Daily Readiness and Medical Information							Meditation		BMI composition	
Activity tracking monitors and records various phsical activity throughout the day	Activity tracking		Activity tracking	Activity tracking	Activity tracking	Activity tracking	Activity tracking	Activity tracking		Activity tracking
Steps, distance and calories					Steps, distance and calories		Steps, distance and calories	Steps distance		Steps, distance and calories
Exercise modes monitor specific types of physical activity e.g. cycling	>100 sport modes			26 sports modes	Running and swimming	Runs, swims, bike rides, walks +	16 sport modes	>10 modes		12 sports modes
Blood glucose tracking is currently made by entering glucose levels in an App										

Results and Discussion

Smartwatches track movement, exercise bands assess users' sleep habits, and flexible bandages monitor body temperature, heart rate, and fluid levels. These devices provide real-time data from integrated sensors and analyze the information gathered. Consumers can use their smartphones or other mobile apps to track their health using data from wearable devices [5].

To achieve clinical utility, smartwatches must measure validated, accurate, and actionable health parameters. Although most consumer-grade devices do not meet medical-grade standards, certain sensors have demonstrated promise in clinical monitoring, early disease detection, and chronic disease management. Standardizing key health metrics across devices could improve their integration into clinical workflows and enhance evidence-based decision-making. The following section outlines what health care data is collected; it is normally synchronized with the users smartphone on an application that is proprietary to the Smartwatch manufacturer which may also be the same for the phone e.g. Apple. The integration into medical records would need to follow the considerations outlined in 3.2.

Key Health-Tracking Features Requiring Standardization

1. **Heart Rate Monitoring (HR)**: Detects trends in resting heart rate, stress, infections, and arrhythmias. While optical PPG sensors are reliable for resting HR, they are less accurate during intense activity.
2. **Electrocardiogram (ECG/EKG)**: Identifies atrial fibrillation and other arrhythmias. FDA-cleared models (e.g., Apple Watch, Samsung Galaxy Watch) provide single-lead ECGs but cannot replace full 12-lead ECGs.
3. **Blood Oxygen Saturation (SpO2)**: Useful for sleep apnoea screening and respiratory conditions. While less accurate than medical pulse oximeters, they can aid trend analysis.
4. **Heart Rate Variability (HRV):** Reflects autonomic nervous system balance. Low HRV correlates with cardiovascular risk, stress, and chronic fatigue.
5. **Blood Pressure Monitoring:** Emerging technology, currently requiring external calibration. Most wearables lack FDA clearance.
6. **Sleep Tracking:** Detects sleep duration, disruptions, and stages, aiding in identifying insomnia and apnoea.
7. **Skin Temperature Trends:** Tracks menstrual cycles and early signs of infection. Relative changes, rather than absolute values, are more informative.
8. **Activity and Step Tracking:** Supports exercise adherence and rehabilitation.
9. **Continuous Glucose Monitoring (CGM) Integration:** Emerging partnerships (e.g., Apple Watch with the Dexcom G7 system able to display glucose data directly on compatible Apple Watch model. s. At present, no smartwatch on the market is capable of measuring blood glucose independently. The partnership between Apple and Dexcom-is cited to illustrate the level of regulatory rigor and technological performance that a consumer-grade smartwatch would need to meet before being acceptable for healthcare use.
10. **Fall Detection and Emergency Alerts:** Crucial for elderly users or those with mobility issues, automatically alerting emergency contacts when needed.

Clinical Integration Considerations

• Regulatory approval (e.g., US Food and Drug Administration (FDA) clearance) and validation through peer-reviewed studies, the paper by Li et al describes the basis for designing a study to evaluate the heart rate variability measurements across smart watches [3]. There are multiple regulatory agencies globally and the term FDA is used in this paper to represent regulatory oversight. Dependent on the country where the healthcare data is collected and used would determine which regulatory body would give approval. This adds extra time and costs for commercial smart watch manufacturers if they want to sell their watches for healthcare purposes in multiple regulatory jurisdictions. The following challenges need to be addressed for manufacturers to achieve FDA or other regulatory approval, ensuring that smartwatch health features are both safe and effective for consumer use:

- **Accuracy and Reliability**: Smartwatches must provide precise and consistent health measurements. For instance, the FDA has cautioned against using smartwatches or smart rings for measuring blood glucose levels due to concerns about accuracy, emphasizing the potential risks of relying on unauthorized devices for critical health decisions [6].
- **Regulatory Compliance**: Manufacturers must adhere to FDA guidelines, which can be complex and time-consuming. The FDA has initiated pilot programs to streamline the approval process for digital health technologies, aiming to balance innovation with patient safety [7].
- **Post-Market Surveillance**: Continuous monitoring of device performance after-market release is essential. The FDA conducts post-market evaluations to assess how wearable devices influence medical care and patient outcomes, ensuring ongoing compliance and effectiveness [8].
- **Data Privacy and Security:** Protecting user data is crucial. Wearable devices collect sensitive health information, necessitating robust security measures to prevent unauthorized access and ensure compliance with privacy regulations [9].
- **Data standardization for interoperability with EHRs:**

By focusing on the following areas, the healthcare industry can move towards seamless integration of smartwatch-generated health data into EHRs, enhancing patient care and facilitating comprehensive health monitoring:

- **Adopt Established Interoperability Standards:** Implementing guidelines like the ITU interoperability guidelines for FHIR ensures that personal health devices and systems can communicate effectively. These guidelines

promote interoperability, security, and ease of use by building on existing industry standards such as Bluetooth and ISO/IEEE 11073 [10].

- **Implement Standardized Data Formats:** Utilizing uniform data formats and terminologies, such as those recommended by the American Nurses Association (ANA), facilitates consistent data capture and representation across different systems. This standardization supports accurate documentation and reporting of health information [11].
- **Ensure Secure Data Sharing Protocols:** Developing secure methods for data transmission and storage is crucial. Ensuring that health data is shared in compliance with privacy regulations protects patient information and maintains trust in digital health technologies [12].
- **Enhance EHR System Interoperability:** Improving the interoperability of EHR systems themselves is essential. Addressing issues related to poor system interoperability can lead to better quality of care and patient safety [13].
- **Educate Healthcare Providers:** Training healthcare professionals on the effective use of smartwatch data ensures that they can interpret and integrate this information into clinical decision-making processes, maximizing the potential benefits of wearable health technology [8].
- Secure, user-controlled data sharing.
- Minimizing false alarms that may cause unnecessary anxiety.
- Ensuring inclusivity in algorithm training to avoid biases.

If the challenges associated with glucose monitoring [6] are taken as an example, the following would need to be met:

1. **Accuracy and Precision**: Non-invasive glucose monitoring methods, such as those potentially used in smartwatches, face difficulties in achieving the accuracy required for medical decision-making. Inaccurate readings could lead to inappropriate treatment adjustments, posing risks to patient health.
2. **Technological Limitations:** Current sensor technologies may struggle to accurately detect glucose levels without invasive methods. Factors like skin thickness, temperature, and hydration can affect sensor readings, leading to variability and potential inaccuracies.

3. **Regulatory Compliance:** Devices intended for medical use must undergo rigorous evaluation to demonstrate safety and effectiveness. The FDA classifies continuous glucose monitoring (CGM) systems as Class II devices, requiring special controls and premarket notification.
4. **Clinical-Grade Specifications Required:**

- **Analytical Accuracy:** Devices should meet specific accuracy thresholds, such as those outlined in the FDA's recognized consensus standards for glucose monitoring systems.
- **Precision**: Consistent performance across multiple measurements is essential to ensure reliability in monitoring and treatment decisions.
- **Detection of Critical Values:** The ability to accurately identify hypoglycaemic and hyperglycaemic events is crucial for timely interventions and patient safety.
- **Trend Analysis:** Providing accurate trends and patterns in glucose levels can aid in long-term diabetes management and therapy adjustments.

Chronic Condition Use Cases

- **Diabetes**: With 38 million Americans diagnosed and 98 million with prediabetes [14], CGM integration and activity tracking are valuable for disease management. In 2022, diabetes-related costs reached $413 billion [15].
- **Hypertension**: Smartwatch blood pressure monitoring could aid management if validated.
- **Heart Disease**: More than 944,800 annual U.S. deaths result from heart disease and stroke [16]. ECG, HR, and HRV tracking could support early detection and intervention. Cardiovascular disease costs are projected to reach $2 trillion by 2050 [17]. These diseases take an economic toll, as well, costing the US health care system $254 billion per year and causing $168 billion in lost productivity on the job [18].

Beyond technological advancements, adequate user education is essential for maximizing the clinical utility of consumer smartwatches and other wearables. Integrating intuitive "poka-yoke" (error-proofing) design directly into apps can promote correct device usage and enhance patient adherence. There is a major question around digital literacy, a 2024 good things foundation report detailed that 1.6 million people in the UK don't have a smartphone, tablet or PC. Of a population of 84 million, 30% are unaware of local access points for device access or internet connection, 77% of those with no basic skills are over 65. This is a major consideration for the use of smart watches in healthcare as a major target for their value is keeping elderly people out of hospital whilst being able to monitor their wellbeing. If the digital literacy is poor in the UK, then the challenges for lower economic countries may provide significant challenges. [19]. Battery life remains a critical factor, as longer-lasting devices - such as the Withings ScanWatch 2 with its 30-day battery life - facilitate continuous data collection, reducing data gaps in long-term monitoring. Anecdotally most smart watches have a battery life after a full charge of between 18 hours and 7 days with the time being aligned with the number of associated apps and features that are used; most watches can be connected to your phone to read text messages and take calls, watches can also have wallet features turned on and dependent on frequency of usage of these features then battery life can be affected. If watches can become a clinical tool, then the need for continuous collection of data or the ability to record when charging is taking place becomes important.

Despite their promise, consumer wearables are not yet

diagnostic tools and should serve as adjuncts to, rather than replacements for, clinical assessments. Refining algorithms to minimize false positives is crucial to avoid unnecessary anxiety and clinical burden. Determining what constitutes a false positive or false alarm in wearable health monitoring raises important ethical considerations. An algorithm must be robust enough to flag clinically significant events without being so permissive that it generates frequent false alarms - or so restrictive that it misses true positives. While the scientific community sometimes employs "grey zones" to account for uncertainty near decision thresholds, such ambiguity can be difficult for users to interpret clearly.

Moreover, responsibility for interpreting results must be clearly defined. If the user is responsible, the device must convey clear guidance on what actions to take. If interpretation rests with the healthcare provider, systems must allow for seamless digital monitoring and alerting-ideally integrated with clinical data and workflows - for providers to respond appropriately. [20,21], By enabling early detection of health anomalies, smartwatches could enhance preventive care, potentially reducing disease burden and healthcare costs.

The integration of AI-driven avatars and cloud-based data collection further expands the impact of wearable technology. Virtual health assistants powered by AI can provide real-time feedback, behavioral coaching, and personalized health recommendations. Meanwhile, cloud platforms facilitate seamless data aggregation, remote monitoring, and AI-powered analytics, enabling healthcare providers to make more informed clinical decisions [22]. While unlimited access to digital health information can inspire healthier behaviors in some individuals, excessive data monitoring may inadvertently lead to pathological symptom tracking and impaired functioning in others. For instance, continuous cardiac monitoring via commercially available smartwatches has been linked to new-onset health anxiety in certain patients. This highlights the need to balance the benefits of digital health technologies with potential psychological harm, especially in vulnerable populations [23]. Additionally, manufacturers must prioritize the inclusion of diverse populations in training datasets to ensure equitable performance across different demographics, ultimately improving patient outcomes. Understanding how different populations, such as young adults, older adults, and individuals with chronic conditions, interact with this technology is essential to optimize its impact. Researchers have developed and studied frameworks based upon smartwatches such as ROAMM (Real-time online assessment and mobility monitoring) in which the health applications present in the smartwatches are used to collect and preprocess data and dedicated servers are utilized to store and recover data and perform remote monitoring [24].

A significant barrier to integrating smartwatch data into healthcare systems lies in data ownership [25]. When users synchronize their watch with a mobile app, they digitally consent to the manufacturer's ownership of that data. For healthcare providers to access this information, a formal agreement with the smartwatch manufacturer is required - ensuring that data-sharing adheres to privacy policies, user consent, and any applicable regulations.

Table 4: Potential Health Monitoring Functions of Smart Watches.

	Heart Rate function	Blood Oxygen Monitoring	ECG	Activity monitoring	Sleep monitoring	Glucose Monitoring (App)
Apple watch v9.0	x	x	x	x	x	x
Apple watch ultra	x	x	x	x	x	x
Apple watch SE	x			x	x	
Fitbit sense 2	x	x	x	x	x	x
Fitbit Charge 4	x	x	x	x	x	x
Fitbit Versa 4	x	x	x	x	x	x
Fitbit inspire 3	x	x		x	x	
Google pixel watch	x	x	x	x	x	x
Google Pixel watch 2	x	x	x	x	x	x
Samsung galaxy watch 4	x	x	x	x	x	x
Samsung Galaxy 5	x	x	x	x	x	x
Samsung Galaxy 6	x	x	x	x	x	x
One Plus watch 2	x	x		x	x	
CMF watch Pro	x	x		x	x	
Garmin Venu 3	x	x		x	x	
Huawei watch ultimate	x					
Huawei watch 4	x		x			x

ASUS smart watch HC A05	x	x	x	x	x	x
Acer Leap ware	x			x	x	
HTC Grip				x	x	
Honor watch GS3	x	x		x	x	
LG Smartwatch W110	x					
HD Smart Fitness Watch	x			x	x	
Motorola Moto 100 smart watch	x	x		x	x	
NOKIA Steel HR	x			x	x	
Oppo Smart watch	x			x	x	
Real me watch S	x	x		x	x	
Withings scan watch 2	x	x	x	x	x	x
Xiaomi Mi watch S2	x	x				
ZTE watch	x	x		x	x	

Conclusion

The integration of smartphones and smartwatches into healthcare has transformed the way health data is transmitted, monitored, and acted upon, enabling more effective real-time communication between patients, participants, and healthcare providers. The flexibility of bidirectional, real-time control within healthcare applications offers a dynamic interface that adapts to individuals' evolving needs and environments. Smartwatches, with their discreet and continuous monitoring capabilities, have the potential to shift healthcare delivery from traditional clinical settings to the rhythm of patients' daily lives. This decentralization empowers individuals to take an active role in managing their health, increasing awareness, and encouraging healthier lifestyles. As such, wearable technology plays a pivotal role in enhancing patient engagement and fostering a culture of prevention.

Maximizing these benefits requires active collaboration among diverse stakeholders, including IVD companies, medical device manufacturers, healthcare practitioners, researchers, patients, and caregivers. Understanding how different populations, such as young adults, older adults, and individuals with chronic conditions, interact with this technology is key to tailoring its applications for maximum impact. Notable frameworks, such as ROAMM (Real-time Online Assessment and Mobility Monitoring), illustrate how smartwatch-based applications can collect, preprocess, and securely store health data for remote monitoring.

To fully realize the promise of smartwatches in medicine, critical challenges must be addressed: standardization, validation, data security, and regulatory compliance. Overcoming these barriers will allow smartwatches to progress from consumer fitness tools to indispensable instruments of personalized, preventive, and patient-centered care, integrated seamlessly into the future of modern medicine.

Declaration of Conflict of Interests

No authors work for or have any financial interests with any smart watch vendor, no funding was requested or received by any author.

Ethical Approval

Ethical approval was not required for this research; all data is openly available on the internet.

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Authors Contribution

All authors had opportunity to provide diverse commentary to the published work and were equal contributors.

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Review Article

Endothelial-to-Mesenchymal Transition in Post-Myocardial Infarction Fibrosis: A Maladaptive but Targetable Pathway

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Endothelial-to-Mesenchymal Transition (EndMT), Myocardial Infarction, Cardiac Fibrosis, TGF-β Signaling, Anti-fibrotic Therapy, Biomarkers, Translational Cardiology

Abstract

Myocardial infarction (MI) initiates a healing response in which fibroblasts and other cells deposit extracellular matrix to form a stabilizing scar. This scarring is essential for preventing ventricular rupture, yet when excessive or diffuse, it becomes maladaptive: fibrosis stiffens the ventricle, impairs filling, and drives progression to heart failure. Traditional antifibrotic approaches, such as broad TGF-β blockade or collagen cross-linking inhibition, have largely failed because fibroblast activity is required for early scar integrity, while established fibrosis is difficult to reverse.

This review highlights endothelial-to-mesenchymal transition (EndMT) as a distinct and underappreciated contributor to post-MI fibrosis. Experimental studies indicate that EndMT supplies 10–30% of fibroblast-like cells, and evidence of EndMT is present in human ischemic cardiomyopathy. Unlike fibroblast-driven repair, EndMT is maladaptive in the adult heart: it promotes fibrosis without enhancing scar strength and reduces endothelial cell numbers, leading to microvascular rarefaction and impaired perfusion.

EndMT is regulated by discrete, targetable pathways-including TGF-β/Smad, Notch, Wnt/β-catenin, HIF-1α, and microRNA networks (e.g., miR-21, miR-29)-and exhibits partial reversibility. This opens opportunities for time-limited, pathway-specific interventions during the proliferative phase of healing. Emerging diagnostic tools, such as extracellular volume mapping, fibroblast activation protein PET, collagen peptide assays, and circulating fibrosis-related microRNAs, provide clinical means to detect EndMT activity.

By integrating mechanistic insights with advances in molecular imaging and biomarker profiling, this review proposes EndMT-directed, biomarker-guided therapies as a precision strategy to limit maladaptive fibrosis, preserve vascular networks, and improve outcomes after MI.

Introduction

Myocardial infarction (MI) is among the leading causes of morbidity and mortality worldwide, with millions of patients each year surviving the acute ischemic event but facing long-term complications related to adverse remodeling of the heart. The healing process after MI is marked by a tightly regulated cascade that ultimately replaces necrotic myocardium with a fibrotic scar. This fibrotic tissue is crucial for survival, as it prevents ventricular wall rupture and preserves structural integrity during systole [1,2]. However, fibrosis has both protective and detrimental aspects. When scar formation extends excessively beyond the infarct zone or when interstitial collagen deposition accumulates disproportionately, the result is increased ventricular stiffness, impaired diastolic filling, arrhythmogenic substrate, and progressive heart failure [1,2]. This paradox, fibrosis as both necessary and detrimental, has framed decades of research into myocardial repair.

Much of the literature has focused on the role of cardiac fibroblasts. Fibroblasts are the most abundant non-myocyte cell type in the heart and the principal source of extracellular matrix (ECM) proteins. In response to injury, fibroblasts become activated through signals such as transforming growth factor-β (TGF-β), angiotensin II, and inflammatory cytokines, transitioning into contractile, α-smooth muscle actin (α-SMA)–positive myofibroblasts that deposit collagens I and III [2,7]. These myofibroblasts are necessary for early scar formation; without them, ventricular rupture is inevitable. This central role has naturally led to a fibroblast-centric paradigm in both basic research and therapeutic development. However, this paradigm may be too narrow. The infarcted heart is a highly dynamic microenvironment where multiple cell types contribute to remodeling, and recent advances have revealed that the mechanisms of fibrosis extend beyond resident fibroblasts. Other cellular sources of scar-forming myofibroblasts have been identified, but with variable contributions depending on species, model, and disease stage. These include epithelial-to-mesenchymal transition (EMT) from the epicardium, bone marrow–derived fibrocytes recruited into the injured myocardium, and perivascular cells such as pericytes and vascular smooth muscle cells [2,7]. Historically, these sources were considered minor or secondary, but a growing body of evidence suggests they may influence the quality and persistence of fibrosis in ways not captured by a purely fibroblast-centered view. Among these, EndMT has emerged as particularly significant. EndMT represents a process by which endothelial cells progressively downregulate endothelial markers (e.g., CD^{31}, VE-cadherin) and upregulate mesenchymal markers such as vimentin, fibronectin, and α-SMA, ultimately acquiring a migratory and matrix-secreting phenotype [8–10]. In the context of MI, this transition does more than add scar-forming cells; it simultaneously subtracts from the endothelial cell pool, reducing vascular density and impairing microcirculatory perfusion. This dual maladaptive effect, amplifying collagen deposition while promoting vascular rarefaction, suggests that EndMT may be a crucial but underappreciated driver of adverse remodeling.

Experimental lineage-tracing studies support this view. Zeisberg et al. demonstrated that in murine models, approximately one-quarter to one-third of fibroblast-like cells in the fibrotic myocardium originated from endothelial cells through EndMT [10]. Subsequent work confirmed that TGF-β is sufficient to induce this transition, while bone morphogenetic protein-7 (BMP-7) can oppose it [11]. Evidence of EndMT has since been observed across cardiovascular diseases in both animal models and human tissues [8,9,12], reinforcing its relevance beyond experimental limitations. Yet despite these insights, EndMT remains less visible in mainstream fibrosis research and has not been systematically integrated into post-MI therapeutic frameworks.

The limited progress in translation may be partly explained by the disappointing performance of broad anti-fibrotic therapies. Interventions targeting master regulators such as TGF-β or collagen cross-linking enzymes have failed in clinical trials, largely because they lacked selectivity and were poorly timed [1,7]. Fibroblast activity in the early phase of repair is essential for scar integrity; thus, global suppression during this window risks catastrophic rupture. Conversely, once the scar has matured, fibrosis is largely irreversible, and late interventions provide little functional recovery. These challenges highlight the need for a more careful approach that distinguishes between essential and unnecessary sources of fibrosis. In this regard, EndMT offers a unique therapeutic opportunity. Unlike fibroblasts, EndMT-derived cells are not required for scar stability. Their contribution is maladaptive, adding to fibrotic burden without providing structural benefit and simultaneously compromising vascular supply [8–10]. Moreover, EndMT is regulated by defined and targetable signaling pathways, including TGF-β/Smad, Notch, Wnt/β-catenin, hypoxia-inducible factor-1α, and microRNAs such as miR-21 and miR-29 [8–11]. These nodes provide potential entry points for selective therapies that could modulate EndMT without broadly impairing fibroblast-dependent repair.

Recent advances in imaging and biomarker technologies strengthen the translational potential of such an approach. Cardiac magnetic resonance imaging (MRI) with extracellular volume (ECV) mapping provides quantitative assessment of interstitial expansion and has proven more sensitive than late gadolinium enhancement for diffuse fibrosis [3,6]. Fibroblast activation protein (FAP) positron emission tomography (PET) imaging has emerged as another modality, allowing spatiotemporal visualization of fibroblast activity in vivo and demonstrating dynamic changes in activation after MI [13,14]. At the molecular level, circulating collagen turnover peptides and fibrosis-associated microRNAs, particularly elevated miR-21 and suppressed miR-29, are being developed as non-invasive biomarkers of fibrogenic activity [11]. Together, these tools make it feasible to detect excessive or persistent fibrotic activity and to stratify patients for targeted interventions.

Thus, these developments emphasize an important gap in current knowledge. Contemporary reviews and therapeutic strategies continue to emphasize fibroblast biology and broad anti-fibrotic approaches, which have consistently struggled in the MI setting due to lack of specificity and timing constraints [1,2,7]. By contrast, EndMT represents a non-essential but maladaptive pathway that contributes to fibrosis while simultaneously undermining vascular health. Its regulation by discrete signaling mechanisms, coupled with the availability of emerging imaging and biomarker tools, creates the foundation for a novel therapeutic paradigm. This review therefore seeks to reframe post-MI fibrosis as a process partly driven by endothelial plasticity and to highlight EndMT modulation as a selective, monitoring-guided, and time-limited strategy to reduce pathological fibrosis while preserving the essential role of fibroblasts in scar stability.

Overview of Post-MI Remodeling

Myocardial remodeling after infarction is a dynamic, multi-stage process that determines whether the ventricle recovers or progresses toward dysfunction. Each phase of healing is important for survival but carries risks when unbalanced. Understanding these stages, and the cellular contributors active within them, is critical to identifying therapeutic targets.

The inflammatory phase dominates the first several days following MI (days 0–4). Necrotic cardiomyocytes release danger-associated molecular patterns (DAMPs) such as high-mobility group box 1 and heat shock proteins, which activate complement cascades and Toll-like receptor signaling [15]. This initiates a robust influx of neutrophils, which not only phagocytose cellular debris but also release proteases and reactive oxygen species that can extend tissue injury if unchecked. Monocytes subsequently infiltrate and differentiate into macrophages, with M^1-like pro-inflammatory subtypes promoting clearance and M^2-like reparative subtypes fostering resolution [16]. Failure to transition from inflammation to repair leads to sustained matrix degradation, infarct expansion, and poor outcomes. Thus, while inflammation is necessary to clear necrotic tissue, its resolution is equally important to prevent excessive damage.

The proliferative phase (approximately weeks 1–3) represents the critical window in which infarct stabilization and fibrotic expansion are determined. In this stage, fibroblasts become the dominant effector cells. Activated by TGF-β, angiotensin II, endothelin-1, and inflammatory cytokines, fibroblasts differentiate into myofibroblasts characterized by α-smooth muscle actin (α-SMA) expression, enhanced contractility, and robust production of collagens I and III [17]. These cells form the backbone of the scar, supplying tensile strength that prevents ventricular rupture. However, fibroblast activity alone does not fully account for the heterogeneity of scar composition.

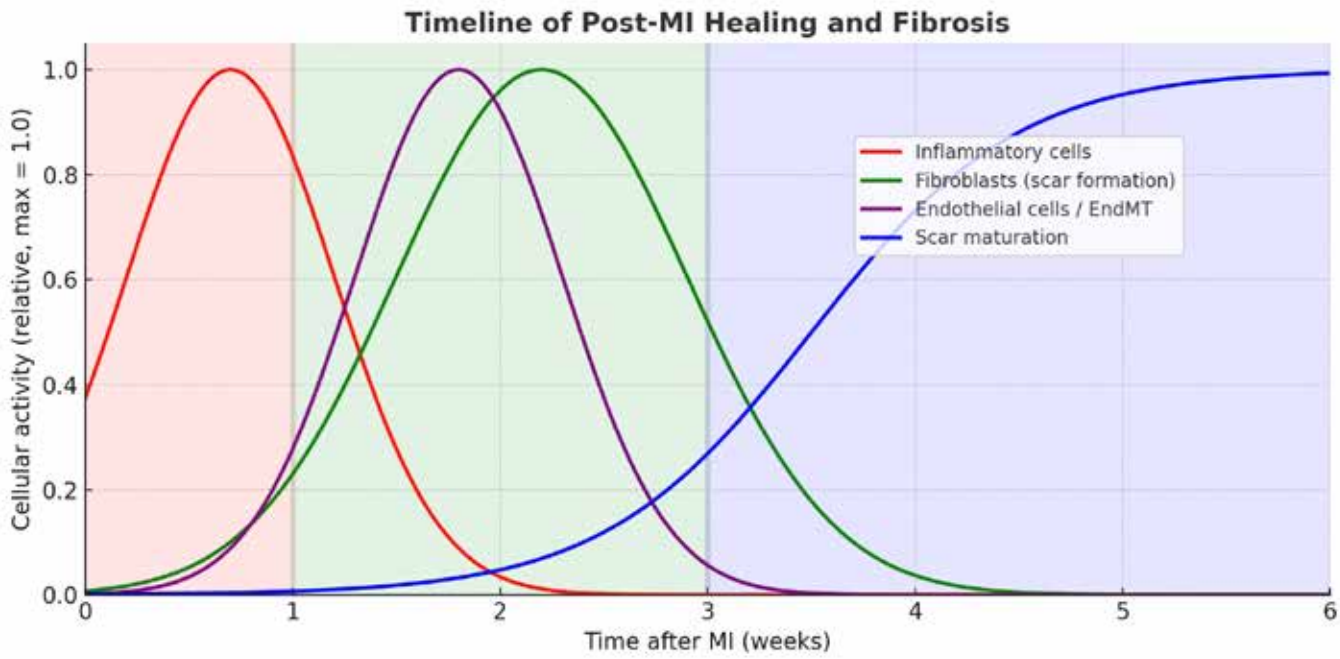
EndMT emerges during this same window, generating fibroblast-like cells of endothelial origin, as confirmed by lineage tracing [18]. EndMT contributes maladaptively by both increasing collagen-producing cells and reducing endothelial populations, impairing neovascularization when oxygen delivery is most needed. Unlike fibroblast-driven repair, which provides structural stability, EndMT-derived cells add fibrosis without proportional benefit-making this pathway an attractive therapeutic target. In parallel, angiogenesis supplies nutrients to the metabolically active scar, guided by VEGF and angiopoietins. Yet when EndMT predominates, endothelial loss restricts vessel growth, promotes hypoxia, and accelerates fibrosis. Thus, the proliferative phase represents a balance between reparative and maladaptive processes.

The maturation phase (from ~4–6 weeks) consolidates earlier changes. Myofibroblasts undergo apoptosis or inactivation, leaving behind an acellular scar. Collagen fibers cross-link and align, increasing tensile strength but reducing compliance [19]. At this point, the scar resists rupture, but stiffness predisposes to diastolic dysfunction. Because structural changes are essentially fixed, therapies after this stage have limited ability to reverse fibrosis.

Underlying all phases is the principle of extracellular matrix (ECM) homeostasis. In healthy myocardium, ECM accounts for ~20–25% of tissue volume, maintained by a balance between matrix metalloproteinases (MMPs), which degrade ECM, and tissue inhibitors (TIMPs), which prevent excessive breakdown [20]. After MI, this balance collapses: early MMP spikes destabilize the infarct wall [21], whereas subsequent TIMP activity and fibroblast-driven deposition overshoot into pathological fibrosis. Excess collagens I and III, fibronectin, and periostin stiffen the ventricle and impair function.

Together, these sequential phases reveal the proliferative stage as the key therapeutic window, where selective modulation of maladaptive processes such as EndMT may improve outcomes without compromising scar stability.

Figure 1: Timeline of Post-MI Healing and Fibrosis.



Schematic of cellular dynamics after myocardial infarction. Inflammation dominates days 0–4, followed by fibroblast activation and peak EndMT during weeks 1–3, driving scar formation and vascular loss. By weeks 4–6, maturation consolidates the scar through myofibroblast inactivation and collagen cross-linking. The overlap of fibroblast and EndMT activity highlights a potential therapeutic window.

Sources of Fibrosis After MI

Fibrosis after MI arises from multiple cellular origins, each contributing to the population of myofibroblasts that deposit extracellular matrix. Although resident cardiac fibroblasts dominate the reparative response, alternative sources, including endothelial-to-mesenchymal transition (EndMT), epicardial epithelial-to-mesenchymal transition (EMT), circulating fibrocytes, and perivascular cells, also participate to varying degrees.

Resident cardiac fibroblasts are the principal and indispensable source of scar-forming cells. In the healthy heart, fibroblasts maintain extracellular matrix turnover and structural integrity. Following MI, they are rapidly activated by TGF-β, angiotensin II, and pro-inflammatory cytokines, differentiating into myofibroblasts characterized by α-smooth muscle actin (α-SMA) expression and high collagen synthetic capacity [22]. These cells accumulate within the infarct, where they produce fibrillar collagens I and III, fibronectin, and periostin, which collectively form the structural scaffold that prevents wall rupture. Without fibroblast activation, repair fails and mortality from rupture is inevitable. Thus, fibroblasts are essential, but if they stay too active, they can cause excessive scarring and ventricular stiffening.

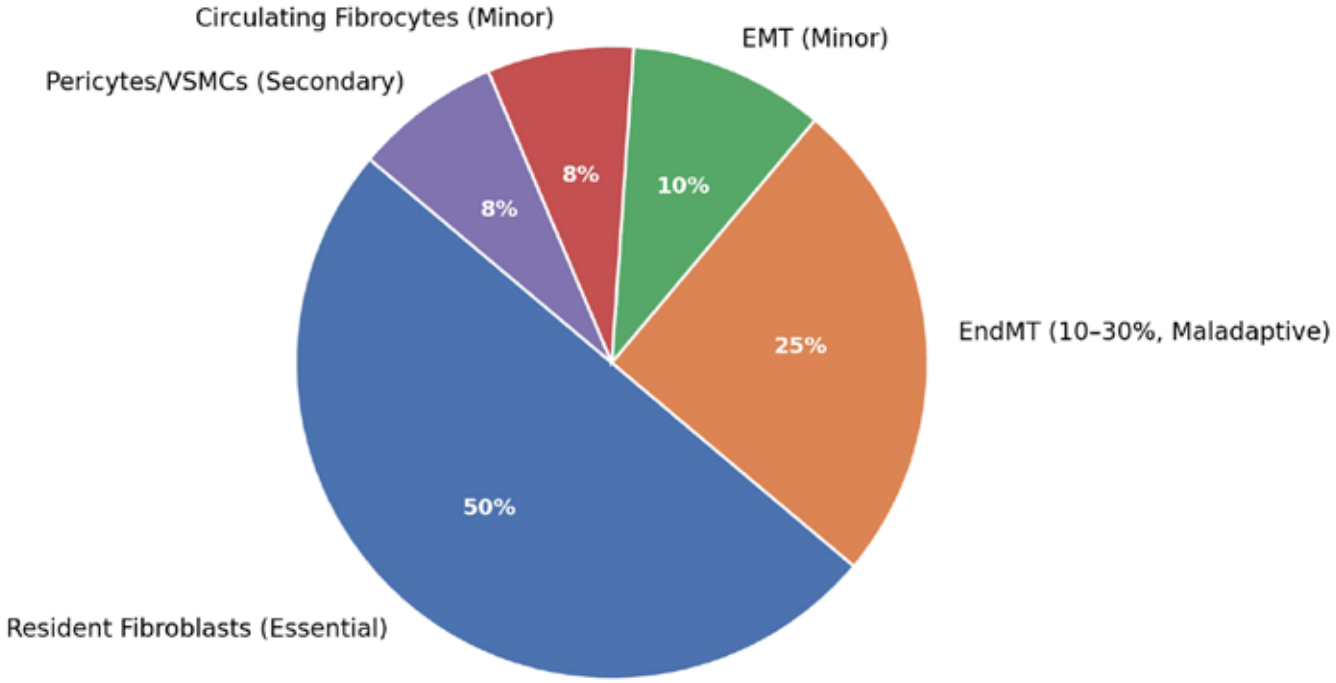
EndMT provides a distinct, maladaptive contribution to the fibrotic response. In EndMT, endothelial cells progressively lose endothelial identity markers such as VE-cadherin and CD^{31} while gaining mesenchymal markers including vimentin, fibronectin, and α-SMA [23]. Lineage-tracing studies in murine models have demonstrated that approximately 10–30% of fibroblast-like cells in the infarcted myocardium arise from EndMT [24]. Unlike fibroblasts, these cells are not required for structural integrity; instead, they amplify scar expansion while simultaneously depleting endothelial cells and impairing neovascularization. Human biopsy studies corroborate the presence of EndMT-derived cells in ischemic heart disease [10,12]. This dual effect, adding scar-forming cells while subtracting endothelial capacity, positions EndMT as a particularly maladaptive and therapeutically attractive target.

EMT represents another source of mesenchymal cells. During embryonic development, EMT is critical for forming the coronary vasculature and interstitial fibroblasts [25,26]. In adult hearts, epicardial cells retain a limited ability to undergo EMT after MI, giving rise to fibroblast-like cells. However, their contribution in adult mammalian models appears minor compared with fibroblasts and EndMT [27]. EMT therefore plays a developmental role but is less significant in the adult fibrotic response.

Circulating fibrocytes are bone marrow–derived cells that enter the circulation and migrate to sites of injury. They express hematopoietic markers such as CD^{45} along with mesenchymal proteins, and they are capable of producing collagen and other ECM components [28]. In the post-MI setting, fibrocytes typically contribute less than 10% of the scar-forming cell population [29]. Their role may be more supportive, interacting with inflammatory cells and fibroblasts rather than serving as major collagen producers.

Pericytes and vascular smooth muscle cells (VSMCs) can also differentiate into matrix-secreting myofibroblast-like cells under stress conditions. Pericytes, normally supportive cells that stabilize capillaries, have been observed to migrate and acquire mesenchymal characteristics in ischemic injury [30]. Similarly, VSMCs from coronary vessels may contribute to the fibrotic response, though their quantitative impact is relatively small [31]. These populations may become more relevant in chronic remodeling or hypertension-associated fibrosis but remain secondary compared to fibroblasts and EndMT.

Figure 2: Sources of fibroblasts in post-myocardial infarction (MI) fibrosis.



Pie chart showing that resident fibroblasts are the main source for scar stability, while EndMT contributes ~10–30% of fibroblast-like cells, driving maladaptive fibrosis and endothelial loss. Smaller contributions arise from EMT, circulating fibrocytes, and pericytes/VSMCs.

Biology of EndMT in the Heart

EndMT is a form of cellular plasticity in which endothelial cells gradually lose their vascular identity and acquire mesenchymal characteristics. Although EndMT is essential during embryonic development and has been described in vascular diseases such as pulmonary hypertension and atherosclerosis, its role in MI is particularly significant and mechanistically distinct [32,33]. The combination of excessive fibrosis and vascular rarefaction makes EndMT uniquely maladaptive in post-infarction remodeling [34].

In the infarcted heart, endothelial cells undergoing EndMT downregulate classical endothelial markers such as VE-cadherin, PECAM-1 (CD^{31}), and von Willebrand factor while gaining mesenchymal proteins including α-smooth muscle actin (α-SMA), vimentin, fibronectin, and collagen type I [32]. Morphologically, this corresponds to a transition from a cobblestone monolayer to an elongated spindle-shaped form with enhanced contractility and migratory capacity [33]. Many cells remain in hybrid states that co-express endothelial and mesenchymal markers, as shown in biopsies from human ischemic cardiomyopathy [35]. These intermediate phenotypes highlight that EndMT is not a discrete process but a continuum. This plasticity has profound therapeutic implications because cells in partial transition may still be reversible if interventions are applied during the right phase of infarct healing.

The timing and distribution of EndMT after MI emphasize its maladaptive role. It occurs predominantly during the proliferative phase, roughly one to three weeks after infarction, when fibroblast activity is peaking and the scar is consolidating [15,36]. Spatially, EndMT is concentrated in the border zone, where viable but stressed myocardium is exposed to hypoxia, inflammatory infiltration, abnormal mechanical strain, and progressive extracellular matrix stiffening. This is the very region where angiogenesis is most critically required to preserve surviving myocardium. By diverting endothelial cells toward a mesenchymal program, EndMT simultaneously adds to the fibrotic burden and undermines vascular repair, thereby shifting the process away from regeneration.

The infarct microenvironment provides a combination of signals that cooperate to induce EndMT. Inflammatory cytokines such as interleukin-1β and tumor necrosis factor-α, secreted by neutrophils and macrophages, together with

damage-associated molecular patterns released from necrotic cardiomyocytes, activate NF-κB signaling in endothelial cells [37]. NF-κB not only promotes inflammatory gene expression but also sensitizes endothelial cells to transforming growth factor-β (TGF-β), the canonical driver of EndMT. TGF-β is produced by activated fibroblasts, myofibroblasts, macrophages, and can also be released from latent stores in the extracellular matrix [37,41]. Notch ligands such as Jagged and Delta-like proteins, expressed on fibroblasts, immune cells, and stressed endothelial cells, activate endothelial Notch receptors, and release the Notch intracellular domain, which synergizes with Smads to reinforce mesenchymal differentiation [42]. Wnt ligands, including $Wnt^{3}a$ and $Wnt^{5}a$, are secreted by myofibroblasts, macrophages, and endothelial cells under hypoxic stress and bind Frizzled/LRP receptors, stabilizing β-catenin and facilitating its nuclear translocation where it partners with Smads to drive profibrotic transcription [43]. Hypoxia in the border zone stabilizes hypoxia-inducible factor-1α (HIF-1α), which directly induces transcription factors such as Snail and Twist [38]. At the same time, disturbed shear stress and cyclic strain are sensed by mechanosensory complexes composed of PECAM-1, VE-cadherin, $VEGFR^{2}$, and $Piezo^{1}$, which activate MAPK, $PI^{3}K$/ERK, and Rho/ROCK cascades [39]. As the scar matures, deposition and cross-linking of collagen stiffen the matrix; integrins and focal adhesions detect this stiffening and activate YAP/TAZ, which further reinforce mesenchymal gene expression [40]. MicroRNAs add another layer of regulation: miR-21, induced by TGF-β and inflammatory cues, suppresses $Smad^{7}$ and thereby amplifies $Smad^{2}$/3 activity; miR-29 normally restrains fibrosis by targeting collagen transcripts but is downregulated after MI; and endothelial-enriched miRNAs such as miR-126 and members of the miR-200 family help maintain vascular identity but are suppressed under hypoxia and inflammation, favoring EndMT [45].

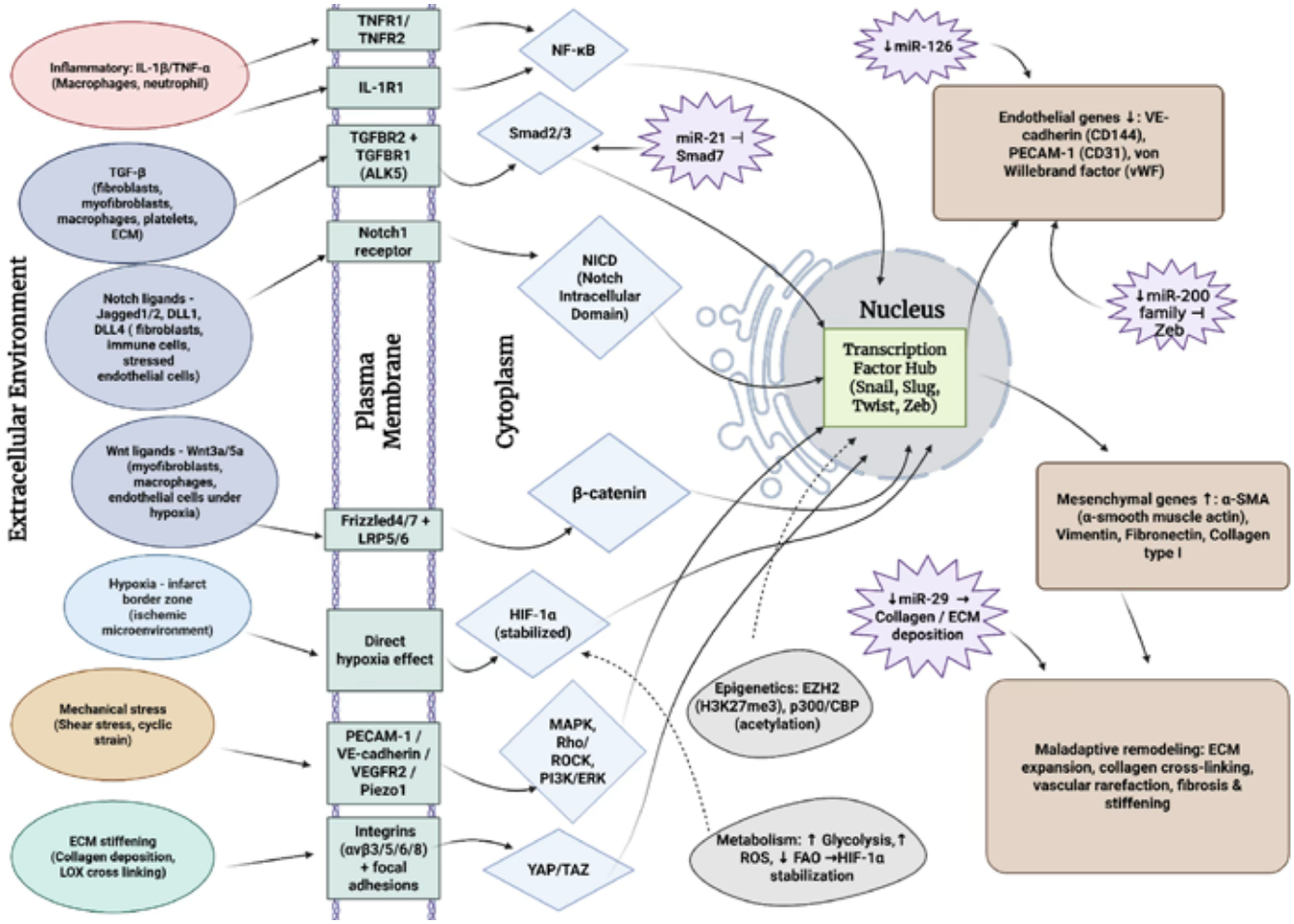
At the molecular level, these diverse inputs converge on a limited set of master transcription factors. The TGF-β/$Smad^{2}$/3 axis induces Snail, Slug, Twist, and Zeb, which directly repress endothelial genes and activate mesenchymal ones [41]. Notch and Wnt signaling cooperate with Smads to reinforce the transcriptional switch [42,43], while NF-κB and HIF-1α amplify inflammatory and hypoxic responses [37,38]. MAPK, $PI^{3}K$/ERK, and Rho/ROCK cascades remodel the actin cytoskeleton and drive motility [44]. Collectively, these signals establish a self-sustaining transcriptional network that decisively shifts endothelial cells toward the mesenchymal state.

The stability of this program is further reinforced by epigenetic and metabolic changes. Endothelial genes are silenced through promoter methylation and repressive histone modifications such as $H^{3}K^{27}$ trimethylation mediated by EZH^{2}, while mesenchymal loci are activated by histone acetylation through p^{300}/CBP [46]. Snail and Twist recruit these chromatin modifiers to enforce silencing of endothelial identity. Metabolically, endothelial cells shift from their typical reliance on glycolysis and fatty acid oxidation to enhanced glycolysis with reduced fatty acid oxidation. This metabolic reprogramming increases reactive oxygen species, which stabilize HIF-1α and further enhance TGF-β and Wnt signaling [47]. Together, these epigenetic and metabolic adaptations create a feed-forward loop that locks cells into a mesenchymal fate even after the initiating inflammatory triggers have subsided.

The tissue-level consequences of EndMT in MI are consistently maladaptive. EndMT-derived cells secrete collagens I and III, fibronectin, periostin, and other extracellular matrix proteins that expand the fibrotic matrix [48]. They promote collagen cross-linking through lysyl oxidase activity, which stiffens the ventricular wall [49], while the loss of endothelial cells reduces capillary density and leads to vascular rarefaction. This impairs oxygen delivery, exacerbates hypoxia, and sustains EndMT, creating a vicious cycle of fibrosis and ischemia. Thus, unlike fibroblast-driven scar formation, which is required to prevent rupture, EndMT-derived fibrosis adds stiffness without providing mechanical benefit and simultaneously undermines tissue perfusion, thereby accelerating progression from infarct healing to chronic heart failure.

A key therapeutic insight is that EndMT appears to be partially reversible. Studies in renal and pulmonary fibrosis show that bone morphogenetic protein-7 and other counter-regulatory signals can reverse TGF-β–induced EndMT and restore endothelial characteristics [52]. This raises the possibility that in the infarcted heart, EndMT-derived or hybrid cells could be rescued if targeted during the proliferative phase. Such interventions might prevent the generation of new mesenchymal cells while rescuing those in partial transition, preserving the vasculature and permitting fibroblast-driven scar stabilization to proceed. Unlike broad antifibrotic therapies that indiscriminately block fibroblast activation and risk destabilizing the scar, approaches directed at EndMT may provide specificity and reversibility. This makes EndMT not only a uniquely maladaptive process but also a uniquely targetable one, opening the door to therapeutic strategies that move beyond traditional antifibrotic approaches.

Figure 3: Molecular pathways driving EndMT after MI.



Signals from inflammation, TGF-β, Notch, Wnt, hypoxia, mechanical stress, and ECM stiffening converge on endothelial cells, activating transcription factors (Snail, Slug, Twist, Zeb) that repress endothelial genes and induce mesenchymal markers. EndMT-derived cells deposit ECM, promote fibrosis, and contribute to ventricular stiffening and heart failure, with regulation by microRNAs, epigenetic, and metabolic changes.

Clinical Consequences of EndMT in Myocardial Infarction

A major clinical consequence of EndMT in MI is diffuse fibrosis, which directly links to the growing burden of heart failure with preserved ejection fraction (HFpEF). EndMT-derived cells secrete collagens, fibronectin, and periostin, expanding the extracellular matrix in border and peri-infarct zones during the proliferative phase [4,7,48]. Unlike fibroblast-mediated fibrosis that stabilizes the scar, this redundant deposition stiffens the ventricle, impairs relaxation, and elevates filling pressures. Clinically, these structural changes manifest as dyspnea, pulmonary congestion, and exercise intolerance despite preserved systolic function-hallmarks of HFpEF. Imaging studies provide quantitative evidence: cardiac magnetic resonance with extracellular volume (ECV) mapping shows that each 1% increase in ECV corresponds to a measurable rise in risk for hospitalization and mortality among HFpEF patients [3,6,42]. Post-MI individuals with high ECV often develop diastolic dysfunction even when ejection fraction is maintained, highlighting how EndMT-driven fibrosis transforms an initially adaptive scar into a substrate for chronic dysfunction and a key driver of HFpEF progression [53].

Equally significant is microvascular rarefaction. By converting endothelial cells into mesenchymal phenotypes, EndMT reduces capillary density and limits oxygen delivery to viable myocardium [35]. The resulting hypoxia stabilizes hypoxia-inducible factor-1α, which further promotes EndMT, establishing a reinforcing cycle [38]. Animal models demonstrate that reduced capillary density correlates with ongoing cardiomyocyte apoptosis and expansion of replacement fibrosis [36]. Clinically, coronary microvascular dysfunction predicts poorer prognosis, impaired exercise capacity, and higher mortality [54]. Thus, EndMT drives both structural scarring and ischemic injury in viable tissue, accelerating the progression toward heart failure.

Beyond reducing vascular supply, EndMT further aggravates adverse remodeling by altering matrix quality. EndMT-derived cells increase collagen cross-linking through lysyl oxidase activity, raising matrix rigidity and compounding ventricular dysfunction [49]. In this way, vascular loss and fibrosis act synergistically, together driving the transition from adaptive

repair to pathological remodeling. Over time, the ventricle becomes progressively stiffened and underperfused, resulting in worsening diastolic dysfunction, reduced stroke volume, and symptomatic heart failure [41]. Imaging studies provide translational evidence of these processes: cardiac magnetic resonance imaging with ECV mapping detects diffuse fibrosis and correlates with outcomes [3,6,42], while fibroblast activation protein (FAP) positron emission tomography (PET) identifies sustained fibroblast activity, often persisting in patients who later develop adverse remodeling [55]. Together, these imaging modalities may indirectly capture ongoing EndMT activity and serve as early warning markers of maladaptive fibrogenesis.

Evidence from experimental and human studies reinforces the prognostic importance of EndMT. Zeisberg et al. demonstrated that up to one-third of fibroblast-like cells after MI arise from endothelial origins [24], and Hashimoto et al. showed that inhibiting TGF-β reduced endothelial-derived fibroblasts and attenuated fibrosis without compromising scar stability [50]. In humans, Evrard et al. identified endothelial–mesenchymal hybrid cells in ischemic cardiomyopathy, with their presence correlating to microvascular loss and poor clinical outcomes [35]. Single-cell RNA sequencing by Farbehi et al. confirmed that endothelial cells in post-MI myocardium occupy transitional states along the mesenchymal continuum, underscoring the clinical relevance of this process [51]. Collectively, these findings position EndMT not as a secondary contributor but as a central driver of pathological remodeling and poor prognosis in MI.

Current Therapeutic Approaches to Fibrosis and Limitations

Therapeutic efforts to reduce post-MI fibrosis have historically focused on broad suppression of profibrotic signaling pathways or on indirect modulation through conventional cardioprotective drugs. While some approaches have shown preclinical efficacy, translation to clinical benefit has been limited by a lack of selectivity, poor timing, and significant safety concerns.

The most direct antifibrotic strategies have focused on TGF-β, a central regulator of fibroblast activation and extracellular matrix deposition. In preclinical models, broad TGF-β blockade consistently reduced collagen accumulation, improved diastolic compliance, and attenuated remodeling [7,41]. However, these promising results have not been translated into human therapies because of what has been termed the “temporal paradox” of TGF-β inhibition. TGF-β is necessary during the early proliferative phase of healing, when fibroblast activation and matrix deposition are required to stabilize the infarct and prevent rupture. Global inhibition at this stage reduces scar tensile strength, predisposing the ventricle to thinning and catastrophic rupture [1,2]. Conversely, when TGF-β inhibition is delayed until the scar has matured, its impact is negligible, since collagen fibers are already cross-linked and resistant to remodeling [36]. Thus, TGF-β–directed therapies have failed because they cannot be administered without either undermining early survival or offering no meaningful late benefit.

Other molecular targets such as connective tissue growth factor (CTGF), lysyl oxidase, and matricellular proteins like periostin have also shown encouraging results in experimental models, where their inhibition reduces extracellular matrix deposition and attenuates fibrosis. Yet these approaches suffer from a lack of selectivity. CTGF, periostin, and lysyl oxidase play physiological roles in normal wound healing and tissue homeostasis; systemic inhibition risks slowing repair of surgical wounds, weakening host defense against infection, or disrupting extracellular matrix dynamics in other organs [41]. Because these proteins are widely expressed outside the heart, their blockade produces organ-specific toxicities and limits clinical feasibility.

Conventional post-MI pharmacotherapies provide indirect antifibrotic benefits but remain nonspecific. Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers reduce fibroblast activation through suppression of angiotensin II signaling, yet their antifibrotic effects are modest and secondary to hemodynamic unloading. β-blockers mitigate adrenergic drive and reduce oxygen demand but do not specifically target fibrogenic pathways. Mineralocorticoid receptor antagonists attenuate aldosterone-driven collagen synthesis but have systemic side effects, including hyperkalemia and renal dysfunction, that constrain dosing. Sodium-glucose cotransporter 2 inhibitors have recently been linked to reduced adverse remodeling, but their antifibrotic effects are indirect and mechanistically unclear [2,7,36]. While all of these drug classes improve survival and reduce heart failure progression, their ability to directly restrain maladaptive fibrosis is limited, diffuse, and nonspecific.

A final limitation that unites all of these strategies is the problem of systemic exposure. Broad antifibrotic drugs circulate throughout the body and cannot distinguish between adaptive fibroblast-mediated scar formation, which is necessary for survival, and maladaptive sources of fibrosis such as EndMT or pericyte activation. This nonspecific action risks interfering with essential repair processes in the heart while simultaneously producing toxicities in other organs including the lungs, kidneys, and skin. The absence of precision targeting explains why, despite decades of research, no antifibrotic drug has been successfully integrated into standard post-MI therapy.

Why Focus on EndMT?

The relative contribution of EndMT to fibrosis, estimated at 10–30% of fibroblast-like cells in lineage-tracing models [24,50], may appear modest compared to fibroblasts, but its clinical impact is disproportionately harmful. By converting endothelial cells into mesenchymal phenotypes, EndMT adds to the fibrotic burden while subtracting from vascular supply, creating a dual pathology: excessive fibrosis and vascular

rarefaction [35,38]. No other source of fibrosis simultaneously increases stiffness and decreases perfusion. Targeting EndMT therefore offers a unique opportunity to reduce both fibrotic load and microvascular loss, two key determinants of adverse post-MI remodeling.

Equally important, EndMT is governed by well-defined and targetable signaling pathways. Standard mediators such as TGF-β/Smad, Notch, Wnt/β-catenin, and HIF-1α converge on transcription factors including Snail, Slug, Twist, and Zeb, which repress endothelial genes and activate mesenchymal programs [41–44]. MicroRNAs such as miR-21 and miR-29 further modulate this process, creating opportunities for RNA-based therapeutics [45]. Because these pathways are distinct from those driving fibroblast activation, it is possible to design interventions that selectively block EndMT without impairing fibroblast-mediated scar formation. This separation of pathways provides the foundation for the safety advantage of EndMT-directed therapy.

Another reason to prioritize EndMT is its partial reversibility. Unlike terminally differentiated fibroblasts, EndMT-derived cells often remain in intermediate or hybrid states, co-expressing endothelial and mesenchymal markers [35,51]. Experimental studies have shown that such transitional cells can be coaxed back toward an endothelial phenotype by signals such as bone morphogenetic protein-7 (BMP-7), Notch modulation, or microRNA manipulation [52]. This plasticity suggests that therapeutic intervention could not only prevent the emergence of new mesenchymal cells but also restore vascular function by reprogramming hybrid cells. Clinically, this would translate into both reduced fibrosis and preserved microvascular density, a combination rarely achievable with current therapies.

Finally, focusing on EndMT aligns with the principle of precision medicine. Because EndMT activity peaks during the proliferative phase of MI healing (weeks 1–3) [36], therapies could be delivered within this defined window, guided by imaging and biomarker surveillance [3,6,55]. Such temporal and mechanistic specificity would overcome the general limitations of broad antifibrotic approaches. Instead of chronically suppressing fibroblasts, clinicians could intervene selectively and transiently against EndMT, achieving therapeutic benefit without jeopardizing scar integrity.

Table 1: Comparison of broad antifibrotic strategies versus EndMT-targeted therapy.

Therapy type	Mechanism	Pros	Cons	Timing / Safety
Pan–TGF-β blockers	Neutralizing antibodies, ligand traps, or receptor kinase inhibitors suppress global TGF-β signaling	Potent reduction of fibrosis in preclinical models	High risk of impaired wound healing, immune dysregulation, and cardiac rupture post-MI	Unsafe in early proliferative phase; systemic toxicity limits clinical translation
Collagen cross-linking inhibitors	Inhibit enzymes such as lysyl oxidase (LOX) to reduce collagen maturation and scar stiffening	Lower myocardial stiffness; potential benefit in chronic remodeling	Ineffective once fibrosis is established; risk of scar fragility	Narrow window, best late after MI; limited clinical benefit shown
ACEi / ARB / SGLT2i (indirect)	Neurohormonal modulation reduces fibroblast activation and interstitial fibrosis	Well-established safety; proven reduction in HF progression and mortality	Indirect, nonspecific antifibrotic effect; incomplete suppression of remodeling	Safe across MI timeline; cornerstone in current guidelines
Anti-EndMT therapies (e.g., miRNA modulation, pathway inhibitors, BMP-7, glycocalyx stabilizers)	Selectively block or reverse endothelial-to-mesenchymal transition by targeting TGF-β/Smad, Notch, Wnt/β-catenin, HIF-1α, or ncRNAs (miR-21, miR-29, MALAT^{1}, H^{19})	Spares essential fibroblast-driven scar formation; preserves endothelial pool and microvascular perfusion; partially reversible process allows rescue	Still experimental; delivery and specificity challenges; long-term safety untested	Best suited for proliferative phase (weeks 1–3 post-MI) when EndMT peaks; time-limited, precision-guided therapy

How to Detect Excessive Fibrosis and EndMT?

Translating EndMT into a clinical target requires reliable methods to detect when it is occurring at maladaptive levels after MI. Unlike fibroblast activation, which can be broadly inferred by fibrosis imaging or serum collagen markers, EndMT is more elusive because it represents a cellular fate transition rather than a single product. Nevertheless, converging advances in cardiac imaging, circulating biomarkers, and functional assessments now provide indirect yet clinically meaningful ways to detect excessive EndMT activity.

Imaging Approaches

Cardiac magnetic resonance imaging (CMR) is currently the standard for assessing fibrosis in vivo. Late gadolinium enhancement (LGE) identifies areas of focal scar but does not capture diffuse interstitial fibrosis, which is a hallmark of maladaptive remodeling. To overcome this, T1 mapping and extracellular volume (ECV) quantification are employed. These techniques measure the proportion of myocardium occupied by extracellular space, which expands as collagen accumulates. In patients after MI, persistently elevated ECV has been shown to correlate with collagen fraction on biopsy and to predict hospitalization, arrhythmias, and death [3,6,42,57,58]. In the context of EndMT, the key signal is when ECV rises out of proportion to infarct size, indicating that new collagen-producing cells are contributing to diffuse fibrosis outside the scar core. Repeated CMR over time adds an additional layer of insight: physiological scarring stabilizes after a few weeks, whereas continued expansion of ECV on serial scans suggests ongoing EndMT activity [59].

FAP-PET adds a functional dimension by detecting fibroblast activation. Fibroblast activation protein (FAP) is expressed by activated fibroblasts in healing myocardium, and PET tracers against FAP can visualize this activity in vivo. Normally, FAP uptake peaks during weeks 1–3 of scar formation and declines as fibroblasts enter a quiescent state [55]. However, in patients who later develop adverse remodeling, FAP uptake remains abnormally elevated well beyond the expected healing window, implying persistent fibroblast activation [60]. Because EndMT generates new fibroblast-like cells that join the activated pool, prolonged FAP signal is an indirect but powerful indicator of excessive EndMT. Even though FAP-PET cannot differentiate fibroblast origin, its persistence points toward a non-physiological source of ongoing activation, of which EndMT is a prime candidate. Combining FAP-PET with CMR in PET-MR protocols strengthens detection by linking fibrotic burden (ECV) with fibroblast activity (FAP uptake), providing a two-dimensional view of abnormal remodeling. Moreover, some novel tracers like experimental live-cell EndMT reporter systems have recently been developed to visualize endothelial–mesenchymal hybrid states. For example, a CNN1-Rep fluorescent construct allows real-time tracking of endothelial cells as they downregulate endothelial identity and acquire mesenchymal traits in 2D, 3D, and organ-on-chip platforms. While still experimental, such tracers hold promise for enabling molecularly specific detection of EndMT in physiologically relevant settings [61,62].

Circulating Biomarkers

Collagen turnover peptides (PICP, PINP, and PIIINP) are among the oldest serum markers of fibrosis. They represent fragments released during collagen synthesis and degradation. Elevated peptide levels after MI indicate that the myocardium is actively remodeling [36]. In cases where infarct size is stable but collagen peptides remain persistently high, this suggests a continued supply of fibrogenic cells beyond the normal fibroblast pool. EndMT is a plausible contributor to this abnormal signal, as it introduces new collagen-secreting cells even after the scar should have matured [63].

MicroRNAs (miRNAs) provide more mechanistic information about EndMT. miR-21 promotes EndMT by suppressing the inhibitory regulator Smad7, while miR-29 normally represses collagen transcripts but is downregulated after MI. Thus, a miR-21↑/miR-29↓ profile is characteristic of fibrogenic activity driven by EndMT [45,64]. Measuring these levels in circulation reflects not only the presence of active fibrosis but also its mechanistic basis. Adding miR-126, which is enriched in endothelial cells, further improves specificity: a fall in circulating miR-126 signals endothelial cell injury or loss, which aligns with the depletion of endothelial cells through EndMT [65]. Together, these signatures allow clinicians to infer that fibrosis is being driven not solely by resident fibroblasts, but also by endothelial-derived cells.

Beyond miRNAs, noncoding RNAs (ncRNAs) such as long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs) are increasingly recognized as key regulators of endothelial plasticity [66]. Unlike miRNAs, which primarily repress gene expression post-transcriptionally, lncRNAs and circRNAs act through diverse mechanisms including miRNA sponging, scaffolding transcription factors, modulating chromatin states, and stabilizing specific mRNAs [66]. These regulatory functions allow them to adjust signaling pathways that drive endothelial cells toward a mesenchymal phenotype. Several lncRNAs have already been implicated in EndMT-related signaling. For instance, MALAT1 has been shown to promote EndMT by activating TGF-β/Smad signaling [70], while H19 can act as a competitive endogenous RNA (ceRNA) to sequester antifibrotic miRNAs, thereby enhancing mesenchymal transition [71]. CircRNAs often function as miRNA sponges, binding to pro- or anti-fibrotic miRNAs to alter the balance of gene expression. For example, circRNAs such as circACR have been linked to vascular remodeling and myocardial injury by regulating endothelial apoptosis, autophagy, and differentiation pathways [72]. Because lncRNAs and circRNAs are stable in circulation, circRNAs in particular resist exonuclease degradation due to their covalently closed loop structure, they represent attractive candidates for biomarker development [66]. Unlike collagen peptides, which reflect general matrix turnover, or miRNAs, which highlight a narrow regulatory axis, lncRNAs and circRNAs could provide multi-level insight into the transcriptional and post-transcriptional networks that sustain EndMT. Measuring their circulating levels could therefore extend current biomarker panels, improving specificity for endothelial-derived fibrosis and potentially distinguishing EndMT activity from fibroblast activation alone [66,70–72].

The most direct, but still experimental, approach for detecting EndMT activity is the identification of circulating endothelial–mesenchymal hybrid cells. These cells co-express

endothelial markers such as CD31 or VE-cadherin together with mesenchymal markers including α-smooth muscle actin (α-SMA) or vimentin, reflecting their transitional state. Early feasibility assays using multicolor flow cytometry have detected such dual-marker populations in peripheral blood samples, while more recent advances in single-cell RNA sequencing have confirmed their hybrid transcriptomic profiles in patients with ischemic cardiomyopathy [35,73]. Although not yet validated for clinical practice, these methods demonstrate that EndMT-derived cells can be directly traced in human circulation. If optimized for sensitivity and standardization, circulating hybrid cells could serve as a unique and specific biomarker of ongoing EndMT, distinguishing it from fibroblast activation and providing real-time insight into the dynamics of post-MI remodeling.

Clinical and Functional Indicators

Clinical phenotyping provides real-world evidence of excessive EndMT. Patients who develop ventricles that are disproportionately stiff compared to their infarct size, detectable with echocardiographic markers of diastolic dysfunction or invasive pressure–volume analysis, are showing signs of diffuse interstitial fibrosis [53]. When fibrosis extends beyond the infarct zone, ventricular compliance falls, filling pressures rise, and diastolic function deteriorates. This is the clinical manifestation of excessive EndMT: an expanded pool of fibroblast-like cells laying down diffuse extracellular matrix in regions that should remain compliant.

Another clue is the early appearance of heart failure with preserved ejection fraction (HFpEF) in post-MI patients who did not suffer large infarcts. HFpEF reflects a mismatch between preserved systolic function and impaired diastolic filling, often due to diffuse fibrosis and vascular rarefaction. Because EndMT both stiffens myocardium and reduces endothelial cell density, its signature phenotype is exactly this combination of compliance loss and perfusion impairment [54,68]. Longitudinal studies in HFpEF consistently show that patients with microvascular rarefaction and diffuse fibrosis have worse exercise capacity and prognosis [69]. In this way, functional testing is not specific for EndMT, but when combined with imaging and biomarkers, it highlights which patients have progressed beyond adaptive scarring to maladaptive fibrosis.

Toward an "EndMT Activity Score"

Individually, none of these methods definitively identify EndMT. Imaging captures structure and activity, biomarkers reflect molecular drivers, and clinical indices demonstrate physiological consequences. By integrating them, however, it becomes possible to construct an EndMT activity score that stratifies patients based on the likelihood of ongoing maladaptive remodeling. A prototypical high-risk patient would show an elevated ECV on CMR disproportionate to infarct size, persistent FAP-PET uptake beyond the expected healing phase, and a circulating profile of miR-21↑/miR-29↓ with reduced miR-126. This constellation strongly suggests excessive EndMT activity and would identify candidates for selective, short-term therapy during the proliferative phase (weeks 1–3 post-MI). Patients lacking this profile could avoid unnecessary treatment.

The strength of this framework lies in its structured integration of tools across different stages of clinical development. Validated clinical markers such as CMR-derived ECV and serum collagen peptides provide a robust framework that can already be implemented in routine care. Translational tools, including FAP-PET and circulating microRNA panels, add mechanistic specificity that connects imaging findings to the biological processes of EndMT. Finally, experimental approaches, such as tracers designed to identify endothelial–mesenchymal hybrids and noncoding RNA signatures, represent future refinements that could elevate the specificity of the score once standardized.

Unlike traditional fibrosis metrics that simply quantify scar size or global collagen burden, an EndMT activity score would offer a mechanism-based index that links structural remodeling with the molecular and cellular processes driving it. This approach would advance precision cardiology in two major ways: first, by providing clinicians with a decision-making tool to individualize treatment based on biology rather than anatomy alone; and second, by serving as a trial enrichment strategy to ensure that novel EndMT-targeted therapies are tested in the subset of patients most likely to benefit. By shifting from descriptive endpoints to mechanistically informed risk stratification, the EndMT activity score has the potential to transform post-MI care and accelerate the development of targeted antifibrotic interventions.

Figure 4: Translational framework for an EndMT Activity Score.

ECV mapping, FAP-PET imaging, and circulating biomarkers are combined into a composite score to stratify post-MI patients. High scores identify candidates for short, selective anti-EndMT therapy, enabling a precision medicine approach.

Future Directions

Precision Timing of Therapy

EndMT activity peaks during the proliferative phase of MI healing (weeks 1–3), coinciding with fibroblast expansion and extracellular matrix deposition [36]. Early suppression, before fibroblasts complete scar stabilization, risks rupture; late intervention, after collagen cross-linking, cannot reverse mature scar tissue [1]. The therapeutic window for EndMT is therefore narrow and defined. Monitoring tools such as CMR-derived extracellular volume, FAP-PET imaging, and circulating microRNAs [3,6,45,55] may allow clinicians to identify when EndMT activity is excessive, enabling time-limited therapies that intervene precisely during this window. Such biomarker-guided approaches would resolve the "temporal paradox" that has defeated global antifibrotic strategies.

Therapeutic Strategies

A range of therapeutic approaches has been proposed to selectively target EndMT. The most direct involve inhibition of canonical developmental pathways such as TGF-β/Smad, Notch, and Wnt/β-catenin, using small-molecule inhibitors, neutralizing antibodies, or ligand traps. While these interventions are mechanistically straightforward, their lack of specificity raises concerns about systemic side effects, as these pathways regulate a wide variety of tissues. More refined strategies focus on microRNA modulation. In particular, anti-miR-21 therapy or miR-29 replacement may restore balance within the regulatory axis that drives EndMT and fibrotic collagen deposition. Another promising line of investigation seeks to preserve or restore endothelial identity. Experimental interventions such as glycocalyx stabilizers, vascular endothelial growth factor analogues, and bone morphogenetic protein 7 (BMP-7) have demonstrated the ability to reverse EndMT and reestablish endothelial phenotype. Beyond suppression of mesenchymal transition, pro-endothelial and angiogenic strategies, including endothelial progenitor cell therapy and the delivery of angiogenic growth factors, aim to counteract the microvascular rarefaction induced by EndMT and restore tissue perfusion.

Delivery Systems and Localization

Because systemic blockade of pathways such as TGF-β and Wnt is likely to produce off-target effects, localized delivery systems are increasingly viewed as essential for safe and effective EndMT-directed therapy. Nanoparticle-based systems, injectable hydrogels, microneedle patches, and drug-eluting stents provide promising platforms. Hydrogels allow localized, sustained release of anti-EndMT agents directly into the infarct border zone, reducing systemic exposure. Microneedle cardiac patches can deliver small molecules, RNA therapeutics, or antibodies directly to the epicardial surface in a minimally invasive fashion. Similarly, bioresorbable stents coated with anti-EndMT compounds could modulate endothelial plasticity in coronary arteries while maintaining vascular patency. These approaches allow high local drug concentrations while minimizing systemic exposure, an important consideration given the multifunctional roles of these signaling pathways in development, immunity, and tissue homeostasis. The combination of localized delivery with biomarker-guided timing offers the potential to suppress EndMT only when and where it is pathogenic, thereby maximizing therapeutic efficacy while minimizing risk.

Biomarker-Guided Personalization

The integration of imaging, molecular, and clinical measures into a composite "EndMT activity score" may enable real-time personalization of therapy. Patients who exhibit persistently elevated extracellular volume on CMR, abnormal FAP-PET uptake beyond the expected healing window and circulating biomarkers consistent with a miR-21-high/miR-29-low profile could be classified as high risk for maladaptive EndMT. These individuals might then be considered candidates for short-course EndMT-targeted therapy delivered during the proliferative phase, while patients without this profile would be spared unnecessary intervention. This paradigm exemplifies precision cardiology, in which therapeutic decisions are guided not only by anatomy and symptoms but also by dynamic biomarkers that reflect ongoing cellular processes.

Research Priorities

Looking ahead, progress will require a clear roadmap that spans preclinical discovery to clinical application. Preclinical studies should focus on mechanistic dissection of key pathways (TGF-β, Notch, Wnt/β-catenin, mechanosensitive channels) and the validation of ncRNA regulators such as MALAT1, H19, and circRNAs as therapeutic targets. Translational efforts should then refine biomarker panels, including miR-21/miR-29, endothelial miRNAs, collagen peptides, and emerging lncRNAs/circRNAs, to identify reliable markers of EndMT activity in patients. Once validated, these biomarkers can be incorporated into early-phase clinical trials that test EndMT-directed therapies delivered through hydrogels, microneedle patches, or targeted nanoparticles. Finally, large-scale randomized trials can use the EndMT activity score both to select high-risk patients and to monitor response, ensuring that outcomes are tied directly to the biology of interest.

Conclusion

Fibrosis after myocardial infarction is crucial for scar stability yet becomes maladaptive when excessive, driving ventricular stiffening, impaired perfusion, and heart failure. Broad antifibrotic therapies have failed because they suppress essential fibroblast activity, whereas EndMT represents a non-essential, maladaptive source of fibrosis that simultaneously depletes vascular networks. Evidence from lineage tracing, human biopsies, and single-cell analyses confirms its role in adverse remodeling, while advances in imaging and circulating biomarkers now make its activity clinically measurable. With defined molecular drivers, partial reversibility, and a clear

therapeutic window, EndMT emerges as a uniquely targetable process. Selective, time-limited modulation of EndMT could reduce pathological scarring, preserve microvascular integrity, and shift post-MI care toward precision antifibrosis therapy.

Declarations

Conflict of Interest

The author declares no conflicts of interest.

Ethical Approval

Not applicable.

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Research Article

Higher levels of Monocyte Distribution Width as a potential flagging parameter of HIV progression: results of a monocentric observational study

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Abstract

Objectives: Monocyte Distribution Width (MDW) is the standard deviation of the mean volume of monocytes and may indicate innate immune activation. We investigated the possible association between MDW values and late HIV diagnosis in consecutive patients.

Methods: We retrospectively enrolled newly diagnosed HIV patients admitted to our clinical center. Demographic and clinical characteristics were analyzed.

Results: A total of 97 patients were enrolled. Of these, 63% were late presenters and 43% fulfilled the criteria for advanced HIV disease. Continuous measures showed a significant inverse correlation between CD4 T-cell count and MDW. Multivariate analysis showed that MDW≥21.1 (OR:7.45, 2.13-30.54), HIV viral load >5 log (10) c/mL (OR:3.62, 1.04-13.30), blood lymphocytes<2 x10^3/µL (OR:14.82, 3.19-111.8) and HIV testing without symptoms (OR:0.21, 0.05-0.82) were independently associated with late presentation. Similarly, adjusted ORs for MDW≥22.5 (OR:4.03, 1.28-13.17), blood lymphocytes<1 x10^3/µL (OR:9.67, 2.19-57.57), age (OR:1.05, 1.00-1.10) and HIV testing without symptoms (OR:0.16, 0.04-0.52) were significantly associated with advanced HIV disease.

Conclusions: Our results suggest that MDW may be a potential flagging parameter of innate immune activation in HIV infection. Continuous measurements of MDW showed a significant inverse correlation with CD4 T-cell count. Patients with increased MDW values were more likely to be diagnosed late.

Introduction

Monocytes and macrophages are pivotal cells of innate immunity, playing a relevant role in HIV transmission and viral spread in the early phases of HIV infection [1-3].
Their recognition as a reservoir for HIV raises interest on investigating the role of specific subsets of monocytes and macrophages [1-5].
HIV patients losing CD4-T lymphocytes during disease progression usually exhibit markers of monocyte activation and a chronic proinflammatory response that is hallmark of poor clinical outcome [5].
Several markers of monocyte activation have been characterized [6-9]. Monocyte Distribution Width (MDW), a measure of the size of monocytes in the bloodstream, has been recently introduced as a tool to support the diagnosis of sepsis in Emergency Departments and Intensive Care Units, based upon its rapid variation upon bloodstream invasion by pathogens and the convenient availability of routine blood tests [10-13].
The aim of the present study was to investigate the possible association between increased values of MDW and late HIV diagnosis, adjusting for known major risk factors in consecutive patients first referred to our unit.

Methods

We conducted a retrospective study, including all consecutive newly diagnosed cases of HIV-infected patients referred for antiviral treatment to the Infectious Diseases Unit of the Pescara General Hospital, Italy, between January 2020 and June 2024. The study plan was approved by the local Health Administrative Board.
We considered two main outcomes: late presentation and advanced HIV disease. The former was defined as a person first diagnosed with HIV with a CD4 T-cell count < 350 cells/μL or with an AIDS-defining event, regardless of the CD4 T-cell count. The latter was defined as a person first diagnosed with HIV with a CD4 T-cell count<200 cells/μL [14]. Symptoms, clinical signs, HIV CDC class and AIDS-defining condition(s) were duly recorded, while MDW measurements were available among routine CBC parameters measured using DxH 900 Analyser (Beckman Coulter Inc., Miami, FL).
In addition to clinical and demographics variables, we considered the way of access to HIV testing as a binary variable (1 vs 0), depending on whether it was performed with or without prior symptoms (taking screening as a reference category).
To aid clinical interpretation, we transformed MDW and HIV viremia into binary variables, by using optimal cut-offs based on the Youden index separately for each outcome, to compute odds ratios (ORs) in univariate and multivariate logistic regression [10, 12, 15]. We did not use the total number of lymphocytes as a continuous explanatory variable, due to its collinearity with the outcome, as both contain CD4 T-cells [14].
The level of association between each categorical risk factor and the outcome was tested using the chi-square test for univariate odds ratios (ORs) [15]. Student's t-test and non-parametric Kruskal–Wallis rank tests were used for normal and non-normal continuous variables, respectively. Multivariate logistic regression was used to compute adjusted ORs and 95%CI, taking into account all risk factors [11, 12].
Backward elimination was used to keep only significant variables in the model, to avoid overfitting due to the low number of cases [16]. Age and gender were tested as potential confounders in all models, before deciding on their exclusion.
All models were estimated by allowing at least 10 events per variable.

Table 1: General characteristics of study population.

Variable (Category)	Late Presentation		Advanced Disease		Overall
	No	**Yes**	**No**	**Yes**	
N**	36 (37.1)	61 (62.9)	55 (56.7)	42 (43.3)	97 (100.0)
Age*	40.7 (13.5)	47.2 (13.8)	41.7 (14.0)	48.7 (13.1)	44.7 (14.0)
Gender**					
F	8 (22.2)	14 (23.0)	11 (20.0)	11 (26.2)	22 (22.7)
M	28 (77.8)	47 (77.0)	44 (80.0)	31 (73.8)	75 (77.3)
HIV asymptomatic screening**					
No	14 (38.9)	46 (75.4)	24 (43.6)	36 (85.7)	60 (61.9)
Yes	22 (61.1)	15 (24.6)	31 (56.4)	6 (14.3)	37 (38.1)
Heterosexual factor risk**					
No	22 (61.1)	22 (36.1)	29 (52.7)	15 (35.7)	44 (45.4)
Yes	14 (38.9)	39 (63.9)	26 (47.3)	27 (64.3)	53 (54.6)

Years of diagnosis**					
2020	9 (25.0)	10 (16.4)	11 (20.0)	8 (19.0)	19 (19.6)
2021	3 (8.3)	14 (23.0)	10 (18.2)	7 (16.7)	17 (17.5)
2022	15 (41.7)	8 (13.1)	17 (30.9)	6 (14.3)	23 (23.7)
2023	3 (8.3)	21 (34.4)	7 (12.7)	17 (40.5)	24 (24.7)
2024	6 (16.7)	8 (13.1)	10 (18.2)	4 (9.5)	14 (14.5)
Country**					
Africans	2 (5.6)	3 (4.9)	4 (7.3)	1 (2.4)	5 (5.2)
European	33 (91.7)	49 (80.3)	49 (89.1)	33 (78.6)	82 (84.5)
South Americans	1 (2.8)	9 (14.8)	2 (3.6)	8 (19.0)	10 (10.3)
MDW[1], ***	20.4 (15.0, 34.3)	23.6 (18.0, 45.0)	20.8 (15.0, 34.3)	24.2 (18.0, 45.0)	22.3 (20.4, 24.8)
Lymphocytes, x10^3 cell/µL[2],***	1.9 (0.8, 5.2)	1.1 (0.30, 600)	1.8 (0.8, 5.2)	0.9 (0.3, 600)	1.4 (1.0, 1.9)
Viremia, Log (10), c/mL[3]	4.4 (2.1, 6.3)	5.3 (1.5, 7.0)	4.5 (1.5, 7.0)	5.5 (2.5, 7.0)	5.0 (4.4, 5.6)

* Continuous, mean (SD); ** n (%); ***Continuous, median (IQR)

[1]Monocyte Distribution Width; [2]Total number of peripheral lymphocytes; [3]Viremia: blood HIV RNA

Results

The study included 97 consecutive patients with a confirmed new diagnosis of HIV infection (19 in 2020, 17 in 2021, 23 in 2022, 24 in 2023, 14 in 2024).

The general characteristics of the study population are shown in Table 1.

A percentage of 84.5% was of European nationality, and 77.3% were males. The mean age was 44.7 (s.d.=14.0). A total of N=61 (62.9%) were classified as late presenters, while N=42 (43.3%) matched the criteria of advanced disease.

Continuous measures were significantly different between late and early presenters in terms of higher MDW (23.6 vs 20.4, p<0.001) and HIV-RNA viral loads (5.3 vs.
4.4 Log(10) cp/mL, p<0.001), while lower for peripheral blood lymphocytes (1.1 vs. 1.9 x10^3/µL, p<0.001).

Similarly, for advanced disease, values were significantly higher for MDW (24.2 vs. 20.8, p<0.001), HIV-DNA viral loads (5.5 vs. 4.5 Log(10) c/mL, p<0.001), and lower for peripheral blood lymphocytes (0.9 vs. 1.80 x10^3/µL, p<0.001).

The results of univariate analysis are shown in Table 2.

Among categorical variables, the risk of a late HIV diagnosis was significantly higher for MDW≥21.1 (OR: 10.33, 95%CI: 3.94-27.08, p<0.001), blood lymphocytes <2x10^3/µL (OR: 9.17, 95%CI: 3.16-26.63, p<0.001), and higher HIV viral loads ≥5 Log(10) cp/mL (OR: 11.81, 95%CI: 4.00-34.85, p<0.001).

An almost 80% decreased risk was found for those testing HIV without symptoms (OR: 0.21, 95%CI: 0.09-0.5, p<0.001), while the risk was significantly increased for age (OR: 1.03, 95%CI: 1.00-1.07; p=0.028) and heterosexual risk of transmission (OR: 2.79, 95%CI: 1.19-6.52; p=0.02).

Table 2: Results of univariate analysis.

Variable (Category)	Late Presentation		Advanced Disease	
	O.R. (95%C.I.)*	p>χ2	O.R. (95%C.I.)**	p>χ2
Age, years*	1.03 (1.00-1.07)	0.03	1.04 (1.01-1.07)	0.01
Males (r.c. = females)	0.96 (0.36-2.57)	0.93	0.70 (0.27-1.83)	0.47
HIV asymptomatic screening (r.c. = no)	0.21 (0.09-0.50)	<0.001	0.13 (0.05-0.36)	<0.001
Heterosexual (r.c. = no)	2.79 (1.19-6.52)	0.02	2.01 (0.88-4.58)	0.09
European Nationality (r.c. = no)	0.37 (0.10-1.42)	0.12	0.45 (0.15-1.38)	0.16
Year of diagnosis (r.c. = 2000)				
2021	4.20 (0.90-19.56)	0.05	0.96 (0.26-3.63)	0.96
2022	0.48 (0.14- 1.67)	0.24	0.49 (0.13-1.78)	0.27
2023-2024	2.90 (0.90- 9.35)	0.07	1.70 (0.56-5.17)	0.35

MDW[1]				
≥21.1 (r.c. = <21.1)	10.33 (3.94-27.08)	<0.001		
≥22.5 (r.c. = <22.5)			9.56 (3.26-28.01)	<0.001
Lymphocytes, x10³ cell/µL[2]				
<2 (r.c. = ≥2)	9.17 (3.16-26.63)	<0.001		
<1 (r.c. = ≥1)			13.33 (2.92-60.91)	<0.001
Viremia, Log (10), c/mL[3]				
≥5 (r.c. = <5)	**11.81 (4.00-34.85)**	**<0.001**	**6.67 (2.73-16.31)**	**<0.001**

* Continuous; r.c.: Reference Category

[1]Monocyte Distribution Width; [2]Total number of peripheral lymphocytes; [3]Viremia: blood HIV RNA

No association was found for gender and years of diagnosis.
The scatter plot showed a significant inverse correlation between MDW and CD4-T values (Spearman Rho = - 0.61; p < 0.0001, see Figure 1).
Considering advanced disease as the outcome, the risk was significantly higher for: MDW ≥22.5 (OR: 9.56, 95%CI: 3.26-28.01, p<0.001), HIV viral loads ≥ 5 (OR: 6.67, 95%CI: 2.73-16.31, p<0.001), and blood lymphocytes <1 x10³ µL (OR: 13.33, 95%CI: 2.92-60.91, p<0.001).
An almost 87% decreased risk was found for those testing HIV without symptoms (OR: 0.13, 95%CI: 0.05-0.36, p<0.001).
The results of multivariate logistic regression are shown in Table 3.
For late presentation, six variables were left in the model out of 61 events.
Age and gender were initially not retained in the model by backward elimination. However, the estimates were radically different from those obtained with their inclusion. This indicated that age and gender were confounders of the relation between explanatory variables and late presentation. Consequently, age and gender were included in the final model, even if not significant.
Adjusted ORs were significant for MDW≥21.1 (OR:7.45, 95%CI: 2.13-30.54, P<0.01), HIV viral loads ≥5 log(10) c/mL (OR:3.62, 95%CI: 1.04-13.30, p<0.05), blood lymphocytes <2 x10³/µL (OR:14.82, 95%CI: 3.19-111.8, p<0.01) and HIV testing without symptoms (OR:0.21, 95%CI: 0.05-0.82, p=0.03).
For advanced disease, four variables were left in the model out of 42 events.
The risk was significantly increased for: age (OR:1.05, 95%CI:1.00-1.10, p=0.04), MDW≥22.5 (OR:4.03, 95%CI: 1.28-13.17, p=0.02), blood lymphocytes <1 x10³/µL (OR:9.67, 95%CI: 2.19-57.57, p<0.01). The risk was significantly decreased for HIV screening without symptoms (OR:0.16, 95%CI: 0.04-0.52, p<0.01). In this case, excluding gender did not substantially change the estimated coefficients.

Figure 1: Scatter plot of MDW (X axis) by bloodstream CD4-T cell count (Y axis). Overlap is the least squares regression line with slope equal to the Spearman correlation, along with its 95% confidence intervals.

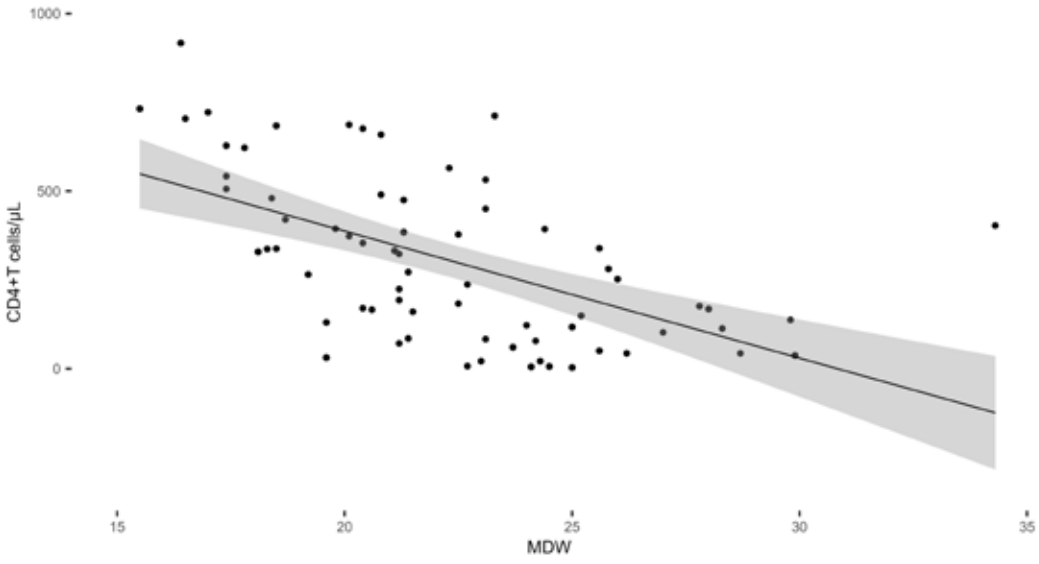

Table 3: Results of multivariate logistic regression analysis.

Variable (Category)	Late Presentation N=97 (Events=61)"		Advanced Disease N=97 (Events=42)	
	O.R. (95%C.I.)	**p**	**O.R. (95%C.I.)**	**p**
Age, years*	1.03 (0.98-1.08)	0.22	1.05 (1.00-1.10)	0.04
Males (r.c. = females)	0.44 (0.09-1.93)	0.29		
HIV asymptomatic screening (r.c. = no)	0.21 (0.05-0.82)	0.03	0.16 (0.04-0.52)	<0.01
MDW[1]				
≥21.1 (r.c. = <21.1)	7.45 (2.13-30.54)	<0.01		
≥22.5 (r.c. = <22.5)			4.03 (1.28-13.17)	0.02
Lymphocytes, x10^3 cell/µL[2]				
<2 (r.c. = ≥2)	14.82 (3.19-111.8)	<0.01		
<1 (r.c. = ≥1)			9.67 (2.19-57.57)	<0.01
Viremia, Log (10) c/mL[3]				
≥5 (r.c. = <5)	3.62 (1.04-13.30)	0.04		

Discussion

Although higher values of MDW are associated with sepsis, limited information is available about changes in viral diseases. This study found that patients with a value of MDW≥21.1 were over seven times more likely with late presentation, independently from age and sex. Similarly, values of MDW≥22.5 were more likely among patients with advanced HIV disease.

As for other European countries, the number of cases presenting late in Abruzzo region remains of concern, despite national programs to achieve earlier diagnosis [17-18].

Our findings confirm that patients presenting late are more frequently males, older, migrants, and exposed through heterosexual contact [19]. However, multivariate regression failed to show any significant association, independently from HIV asymptomatic screening and laboratory parameters e.g. MDW, number of lymphocytes and viremia. Possible explanations are the limited sample size and the high predictive value of biological parameters.

In terms of temporal trends, the scientific literature reports an increase in the percentage of persons with late HIV diagnosis, from 58.2% in 2022 to 60.1% in 2023 [17-18]. However, our study did not confirm a significant increase over four consecutive years.

In terms of progression of HIV over time, several studies reported monocyte activation as a potential correlate [2,5,6,20,21].

A study of flow cytometry showed that HIV patients losing CD4-T cells, both in early and chronic infection, are at higher risk of developing a heightened proinflammatory status [19]. Percentages of blood subsets of intermediate (CD14++CD16+) and non-classical monocytes (CD14+CD16++), which generally increase during inflammatory reactions, were significantly higher in chronic HIV-1-infected cART-naïve patients [22]. Soluble plasma CD14 (sCD14) and CD163 (sCD163), two further biomarkers of monocyte activation, may play a role in the inflammatory response during HIV progression. sCD14 have higher blood concentration in chronically HIV-infected than healthy subjects [23], being inversely associated with CD4-T cell count recovery during ART [20]. Similarly, sCD163 levels were higher compared to healthy subjects, decreasing after the start of ART [24].

The above results support the adoption of MDW as a potential flag of innate immune activation in HIV infection. Higher MDW values may be associated with the pro-inflammatory state of the innate immune system occurring during HIV progression and the subsequent loss of lymphocytes. Therefore, in HIV patients with disease progression, MDW could help identifying bacterial infections related to the immune activation during the loss of CD4+ T cells.

For instance, monocyte indicators may change rapidly in gut-associated lymphoid tissue (GALT) due to CD4+ T-cell depletion and fibrosis, which only improves slowly with antiretroviral therapy. Disruption to the integrity of the gut mucosa and its immunity may result in high plasma levels of bacterial lipopolysaccharide (LPS) and bacterial translocation, leading to an increase in MDW results and the activation of other biomarkers frequently found in this condition, such as HLA-DR and CD69 on monocytes, CD38 on lymphocytes, and CRP [25, 26].

Additionally, MDW may serve as a pro-inflammatory indicator in non-infectious conditions, as in the case of chronic inflammation during antiretroviral therapy (ART) for HIV immunological non responders (INR). However, this represents currently only a research hypothesis that needs to be proven. The cause of chronic inflammation observed in HIV infection during ART has been addressed by many recent studies, due to its role as the underlying cause of increased risk of SNAEs (serious non-AIDS events: CVD, HIV-associated neurocognitive dementia, and aging).

The analysis of MDW values in treated people living with HIV may be a reasonable target of future research, given that monocyte activation related to a heightened proinflammatory status has been described mainly in immunological non-responders (INR), where it was found associated with an increased risk of developing SNAEs [5, 27].
Macrophages take up high amounts of cholesterol-rich low-density lipoprotein (LDL) in vessels [28]. This accumulation leads to macrophage necrosis, leading to the growth of a cholesterol-rich necrotic core and cholesterol accumulation [28,29,30]. Consequently, monocyte indicators such as CD11b and CX3CR1 or blood non-classical monocyte subsets (CD14+CD16+, CD14-CD16+) are associated with subclinical atherosclerosis in HIV patients. However, many studies are ongoing and more results are needed to shed light on this relationship [29].
Additionally, MDW may be investigated in HIV-Associated Neurocognitive Disorders (HAND) and aging. It is widely known that monocytes traversing the blood-brain barrier are a major source of HIV infection in the brain, and that HIV-DNA viral load in monocytes, but not in plasma or in CD4-T cells, is associated with HAND, suggesting that they may contribute to brain injury [31]. However, more studies are needed to support this hypothesis [32].
Finally, similar to CVD risk and HAND, premature immunosenescence is associated with HIV-1 infection, due to the persistent immune activation and chronic inflammation. Evidence suggests that monocytes contribute to pathogenesis. For instance, immunosenescence is associated with a heightened activation of the proinflammatory status of monocytes, with an increase in the blood levels of intermediate or non-classical monocytes [33] and an increased production of monocyte indicators e.g. neopterine, sCD163, and CD11b [34]. Monitoring monocyte activation through MDW may warrant further research in immunological non-responders (INR) to ART, who are known to be at increased risk of developing SNAEs (e.g. cerebral and cardiac acute events, a leading cause of mortality and morbidity in HIV patients) [27].
Relevant limitations of our study are worth to be outlined. Firstly, the observational design is prone to a series of known limitations, including missing data and potential recall bias. Further, the results obtained from a small sample size drawn from a single clinical center need to be validated through additional samples from multiple sites.
Secondly, the automated choice of cut-offs may lead to unstable results, which may not be confirmed by other data collections. In the absence of gold standards, we used the Youden index to find an optimal cut-off for both late presentation and advanced disease.
The cut-off point of MDW=21.1 for late presentation may seem close to normal values, undermining its accuracy for large samples. However, normal MDW values for the general population range between 14 and 18, meaning that values that are substantially higher may be considered appropriate to rule out innate immune activation.
The cut-off value found for MDW in advanced disease is equal to 22. This is consistent with other studies, including the FDA-registered MDW cut-off point for identifying sepsis in the emergency department (ED).
By all means, in this study we did not aim to encourage monitoring MDW to diagnose advanced HIV. Instead, we meant to describe MDW modifications in response to lymphocyte deterioration during HIV infection. Consequently, our results should be only interpreted as exploratory findings that need to be confirmed by future evidence.
Thirdly, we were unable to compare MDW to other biomarkers of monocytes during innate immune activation e.g. sCD14 and sCD163. Since MDW has been only recently introduced in clinical practice, no direct comparisons with sCD14 and sCD163 currently exist. Further studies are needed to address this comparison.
In conclusion, we found that MDW values are inversely correlated with CD4-T cell counts in HIV patients. HIV patients with elevated MDW are more likely to be diagnosed late, suggesting that MDW may be used as a potential flagging parameter of innate immune activation in HIV infection, that can be easily measured using peripheral blood counts.

Ethical approval and Informed consent
The local Health Administrative Board in Pescara reviewed in detail the study plan, set up by the Infectious Diseases Staff in Pescara General Hospital.
Informed consent for participating into the study was not required, because we used archival data, which were anonymized. There was no intervention beyond standard clinical procedures (measurement of blood cell volumes and indices). Moreover, written informed consent for the use of anonymized clinical and laboratory data for institutional research purposes was provided by all patients upon admission. This study was conducted in accordance with the ethical principles for medical research involving human subjects, as reported in the Declaration of Helsinki.

Author contributions
EP, FC, and GP wrote the manuscript; FS and GA retrieved patient data; EP and FC performed the statistical analysis. The corresponding author is responsible for the accuracy of the descriptions. All authors have accepted responsibility for the entire content of this manuscript and approved its submission. All authors declare that they have no conflicts of interest.

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Data availability
The datasets are available from the corresponding author upon reasonable request.

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Research Article

Correlations of parathormone and biochemical parameters in chronic kidney disease

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Keywords

Calcium, CKD, eGFR, phosphorus, PTH

Abstract

Introduction: Hyperparathyroidism due to chronic kidney disease (CKD) is a common complication characterized by elevated parathyroid hormone (PTH) levels secondary to derangements in the homeostasis of calcium and phosphorus. The aim of our study was to figure out PTH, calcium, phosphorus, and magnesium levels in CKD and the possible correlations between them and the stages of CKD.

Materials and Methods: We performed a prospective study including 217 outpatients with levels of serum creatinine out of reference range from February 2023 to July 2024. We calculated the glomerular filtration rate (eGFR) with the 2021 CKD-Epi equation using serum creatinine. We used Jamovi Statistical Software version 2.3.28. A "p" value equal to or less than 0.05 was considered statistically significant.

Results: We had 105 females (48%) and 112 males (52%), median age 72 years (35-94yrs). We found differences in PTH and phosphorus levels between distinct stages of CKD. For PTH the differences were found between stages IIIa and IV ($p<0.001$), IIIb and IV ($p=0.001$), while for phosphorus between stages IIIa and IIIb ($p=0.003$), IIIa and IV ($p<0.001$), IIIb and IV ($p=0.01$). We found correlation between eGFR and PTH ($r= -0.360$, $p=0.001$), eGFR and calcium ($r=0.169$, $p=0.015$), eGFR and magnesium ($r= -0.153$, $p=0.028$), eGFR and phosphorus ($r= -0.336$, $p<0.001$).

Conclusions: We concluded that there is a statistically significant correlation between PTH and stages of CKD, but the strength of correlation is low, it cannot be generalized, therefore each patient with CKD must be assessed individually.

Introduction

Chronic kidney disease (CKD) is a progressive condition that affects more than 10% of the general population worldwide, amounting to over 800 million individuals [1].

One crucial complication of CKD is secondary hyperparathyroidism (SHPT), marked by elevated parathyroid hormone levels due to hyperphosphatemia, hypocalcemia, and low active vitamin D from impaired renal function [2].

According to the kidney disease: Improving Global Outcomes (KDIGO) guidelines, SHPT screening should begin at CKD stage III-that is, when the eGFR drops below 60 mL/min/1.73 m^2 [3].

In our daily practice, we have noticed that PTH as a biomarker in CKD is slightly overestimated by clinicians.

The aim of our study was to measure levels of PTH, calcium, phosphorus, and magnesium and assess their correlation with CKD stages.

Materials and methods

We performed a descriptive and prospective study. We identified patients with an elevated serum creatinine level more than 1.02 mg/dL for females and more than 1.3 mg/dL for males, from all patients subjected to blood sampling for chemistry analyses at Polyclinic Father Luigi Monti and Catholic Hospital "Our Lady of Good Counsel" Tirana, Albania during the period February 2023 through July 2024.

To keep the confidentiality of participants' data, we coded the identity of each participant in the study, according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Peripheral venous blood was collected in BD Vacutainer SST II Advance 3.5 mL tubes and processed within 2 h from collection. Creatinine, calcium, magnesium, phosphorus, were analyzed on an integrated chemistry system Dimension EXL 200 (SIEMENS) according to the routine methods and the manufacturer's instructions. We stored 1 mL of serum for each patient, including in our study at -20 degrees Celsius and measured PTH on ADVIA Centaur XPT (SIEMENS) with chemiluminescence.

We calculated the estimated glomerular filtration rate (eGFR) with the 2021 CKD-Epi equation using serum creatinine.

Statistics

All data management and statistical analyses were performed using Jamovi Statistical Software version 2.3.28. We performed basic descriptions for all variables, and we have studied frequencies. We used the Shapiro-Wilk test for normal distribution. We tested differences with Kruskal-Wallis for non-parametric variables between more than two groups. We performed Pearson's correlations; a two-sided p-value of 0.05 or less was considered statistically significant. We did linear regression analysis, and we used coefficient "t" to measure the statistical significance of an independent variable in explaining the dependent variable, and r^2 (coefficient of determination) to measure the percentage of the variation in the dependent variable that is explained by variation in the independent variable.

Results

From February 2023 to July 2024, 4731 patients performed a serum creatinine test in our laboratory, of which 217 patients had abnormal serum creatinine levels. The prevalence of chronic kidney disease for our patients was 4.6%. Our sample had 105 females and 112 males, with a median age of 72 years old (35-94 years old).

The key characteristics of the patients according to age group classification are summarized in Table 1.

Table 1: The key characteristics of the patients according to age group classification.

	Age group	eGFR (mL/min/1.73 m2)	PTH (pg/mL)	Calcium (mg/dL)	Magnesium (mg/dL)	Phosphorus (mg/dL)
Reference ranges*		≥ 90 G1 60-89 G2 45-59 G3a 30-44 G3b 15-29 G4 < 15 G5	18.5-88	8.5-10.1	1.8-2.4	2.6-4.7
Number of patients	≤ 65 yrs	47	47	47	47	47
	66–75 yrs	82	82	82	82	82
	> 75 yrs	88	88	88	88	88
Median	≤ 65 yrs	49	66	9.3	2	3.6
	66–75 yrs	44	74	9.4	1.9	3.4
	> 75 yrs	42	96	9.3	2.1	3.6

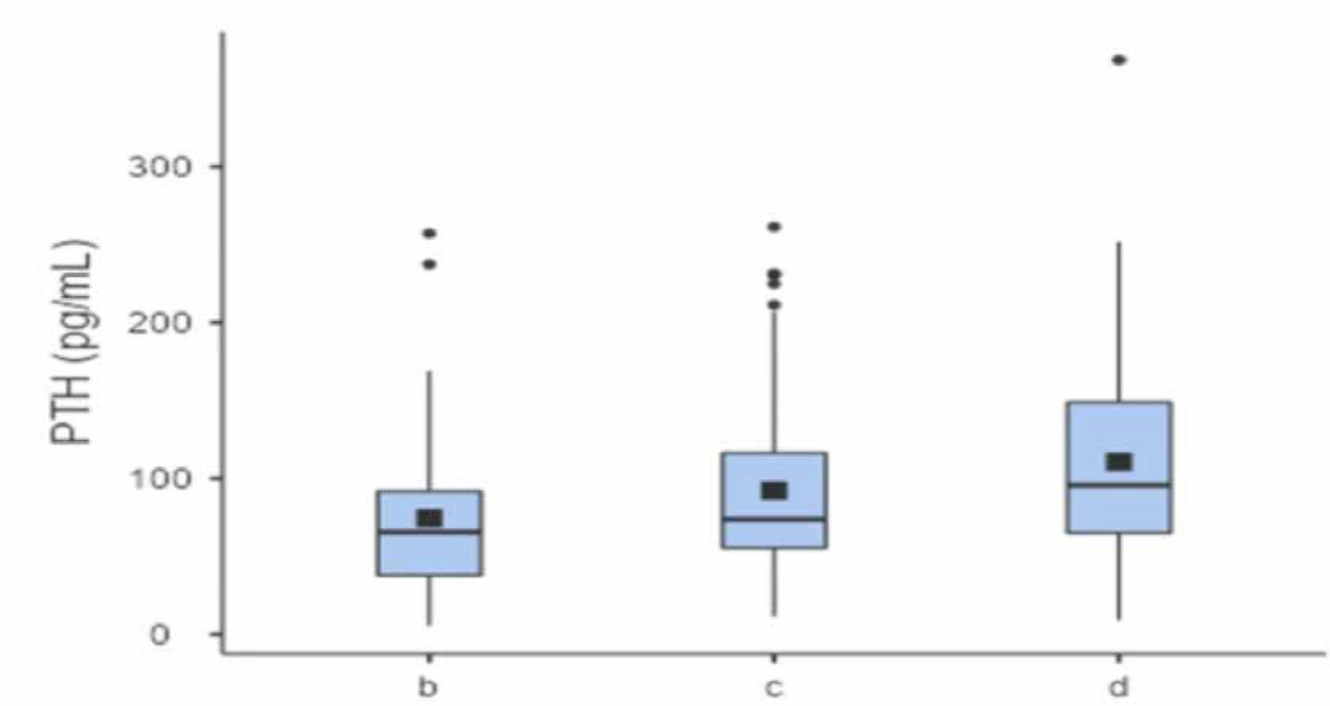
Minimum	≤ 65 yrs	21	6	6.9	1.3	2.6
	66–75 yrs	20	11	8.2	1.3	1.6
	> 75 yrs	7	9	6.6	1.3	1.8
Maximum	≤ 65 yrs	73	257	12.5	3.2	5.9
	66–75 yrs	59	261	12	2.9	5.4
	> 75 yrs	58	368	12.2	4	6.3
IQR	≤ 65 yrs	41-58	38-92	8.8-9.6	1.8-2.3	3.3-4.1
	66–75 yrs	37-52	55-116	9.0-9.8	1.7-2.2	3.1-3.8
	> 75 yrs	34-47	65-149	9.0-9.7	1.9-2.3	3.2-4.0

*The reference range for the eGFR was based on the KDIGO 2012 clinical practice guidelines, while the reference range for the PTH, calcium, magnesium and phosphorus were based on instructions for use papers provided by manufacturers.

Patients were divided into three groups: ≤ 65 years old, 66-75 years old, and older than 75 years old. The groups had more than 30 outpatients, but the data was not normally distributed. We used the median instead of the mean to show the central tendency and the IQR (interquartile range) instead of standard deviation to measure the variability.

We found that the dominant age group was > 75 years, with 40%, while only 22% of outpatients were ≤ 65 years old. It is noticeable that, except for PTH (Figure 1), in the age group over 75 years old where median was higher than the reference, all the other variables have a median within the range of reference values. The maximum value for all variables was present in the age group > 75 years, except for calcium, which was found in patients ≤ 65 years old. We found that for all age groups the IQR was within the range of reference values for all variables except for PTH which had an upper limit of IQR outside the reference value.

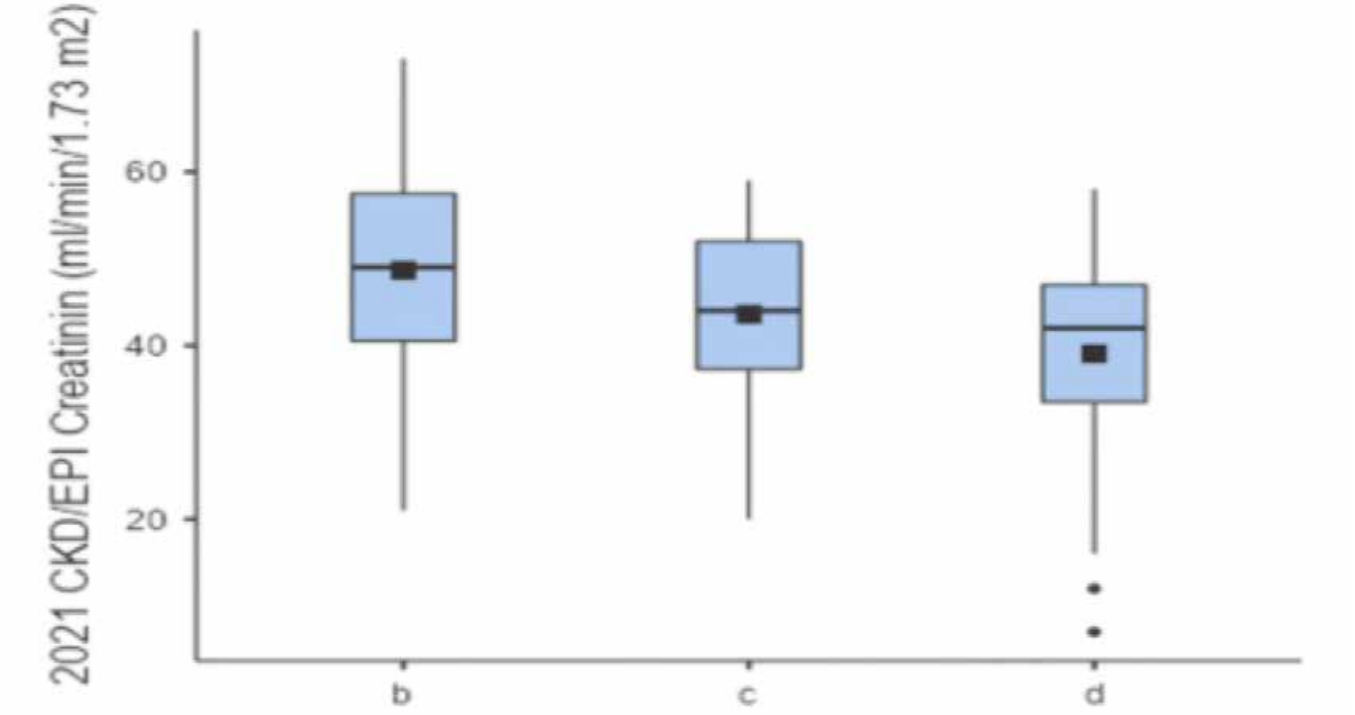
Figure 1: Distribution of PTH levels according to age groups (b is ≤ 65 yrs, c is 66-75yrs and d is > 75 yrs).



We noticed that about the eGFR (Figure 2), the minimum value in patients over 75 years old was 7 mL/min/1.73 m2, stages G5, kidney failure, while patients ≤ 65 years old and 66–75 years old had the minimum value 21 mL/min/1.73 m2 and 20 mL/min/1.73 m2 respectively, stages G4, severely decreased.

Regarding the maximum value, age group ≤ 65 years old was in stages G2, mildly decreased, and age groups 66-75 years old and > 75 years old were in stages G3a, mildly to moderately decreased.

Figure 2: Distribution of CKD stages according to age groups.



The age group classification helped us also to find the differences of biochemical parameters between them. For this reason, we used the Kruskal-Wallis test for non-parametric variables in Table 2.

We found age-related differences in PTH, and magnesium levels as follows: PTH was significantly higher in patients over 75 years old compared to younger groups, and magnesium levels differed significantly between the 66–75 years old and >75 years old age groups.

Table 2: One-Way ANOVA (Non-parametric) Kruskal-Wallis for different age groups.

	χ^2	df	p	ε^2
PTH	15.302	2	< .001	0.07084
Calcium	1.793	2	0.408	0.0083
Magnesium	8.738	2	0.013	0.04046
Phosphorus	6.632	2	0.056	0.03071

About the distinct stages of chronic kidney disease, we found that G3a predominated with 44% followed by G3b with 38%. This distribution allowed us to examine biochemical trends across a range of CKD severities, as shown in Table 3.

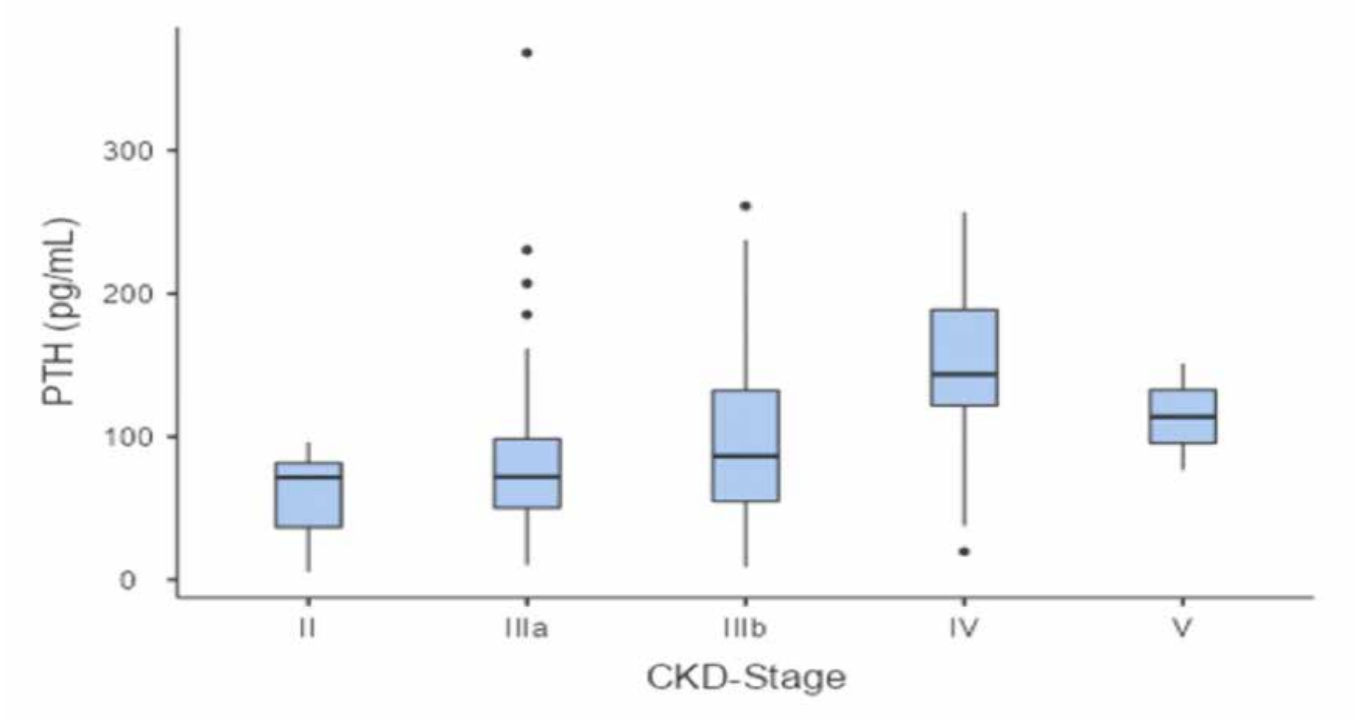
Table 3: Biochemical trends across distinct stages of chronic kidney disease.

	CKD-Stages	PTH (pg/mL)	Calcium (mg/dL)	Magnesium (mg/dL)	Phosphorus (mg/dL)
Number of patients	G2	9	9	9	9
	G3a	96	96	96	96
	G3b	83	83	83	83
	G4	27	27	27	27
	G5	2	2	2	2
Median	G2	72	9.5	2	3.6
	G3a	72	9.4	2	3.4
	G3b	87	9.3	2.1	3.6

	G4	144	9.2	2.1	4
	G5	114	8.7	2.7	5.8
Minimum	G2	6	8.4	1.6	2.9
	G3a	11	6.9	1.3	2.3
	G3b	9	6.6	1.3	1.8
	G4	20	7.3	1.7	1.6
	G5	77	8.2	2.4	5.3
Maximum	G2	96	12.5	2.6	4.7
	G3a	368	12.2	2.8	5.9
	G3b	261	11.9	3.2	5.4
	G4	257	10.7	4	5.8
	G5	151	9.1	2.9	6.3
IQR	G2	37-82	8.7-10.6	2-2.4	3.2-3.9
	G3a	50-99	9.0-9.8	1.7-2.3	3.0-3.6
	G3b	55-132	9.1-9.7	1.8-2.2	3.3-4.0
	G4	122-189	8.8-9.5	1.9-2.3	3.7-4.3
	G5	95-133	8.4-8.9	2.5-2.8	5.6-6.1

PTH median value and the IQR were out of the reference range only in G4 and G5 stages of CKD (Figure 3). In stages of G3a and G3b, only the upper limits of IQR were beyond the reference range.

Figure 3: Distribution of PTH across distinct stages of CKD.



For calcium, magnesium, and phosphorus, the median values were inside the reference range, except in stages G5 where for magnesium and phosphorus they were higher than the reference (Figure 4,5,6). The IQR was within the reference for all variables in all CKD stages, except in G5 where calcium trended to be lower, and magnesium and phosphorus trended to be higher.

Figure 4: Distribution of calcium across distinct stages of CKD.

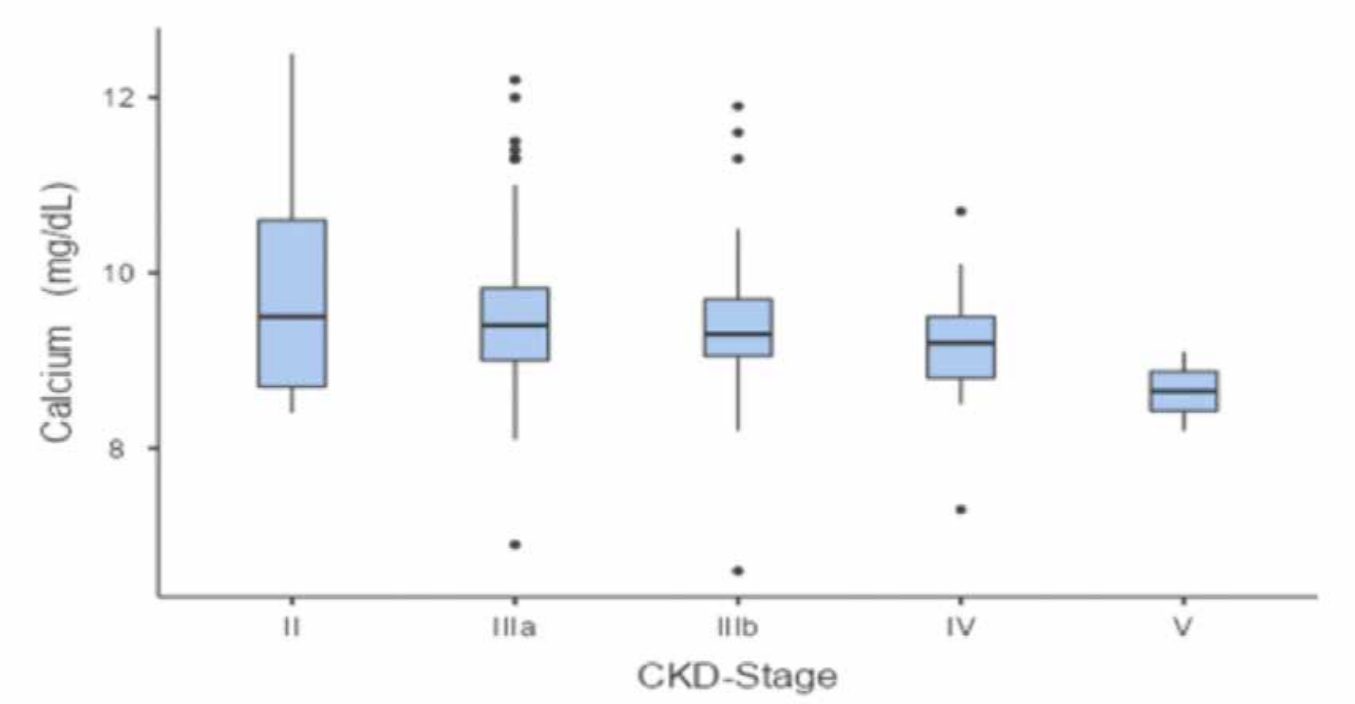


Figure 5: Distribution of magnesium across distinct stages of CKD.

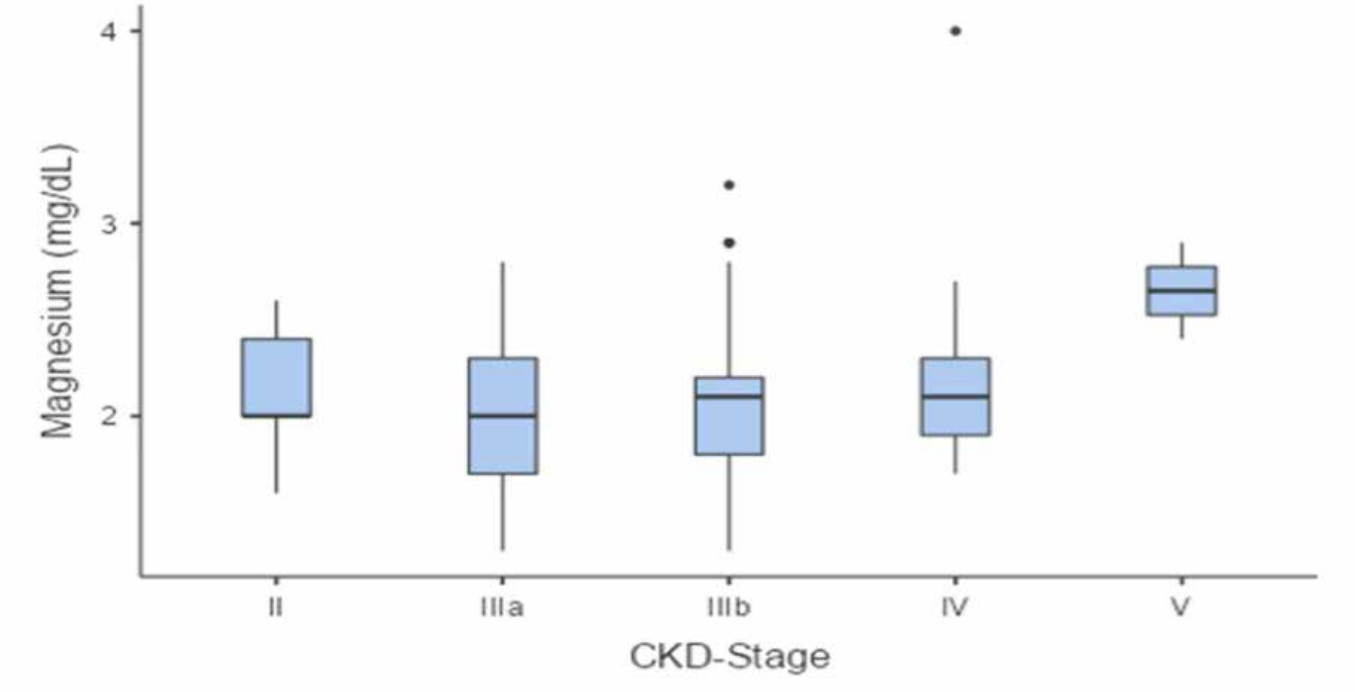
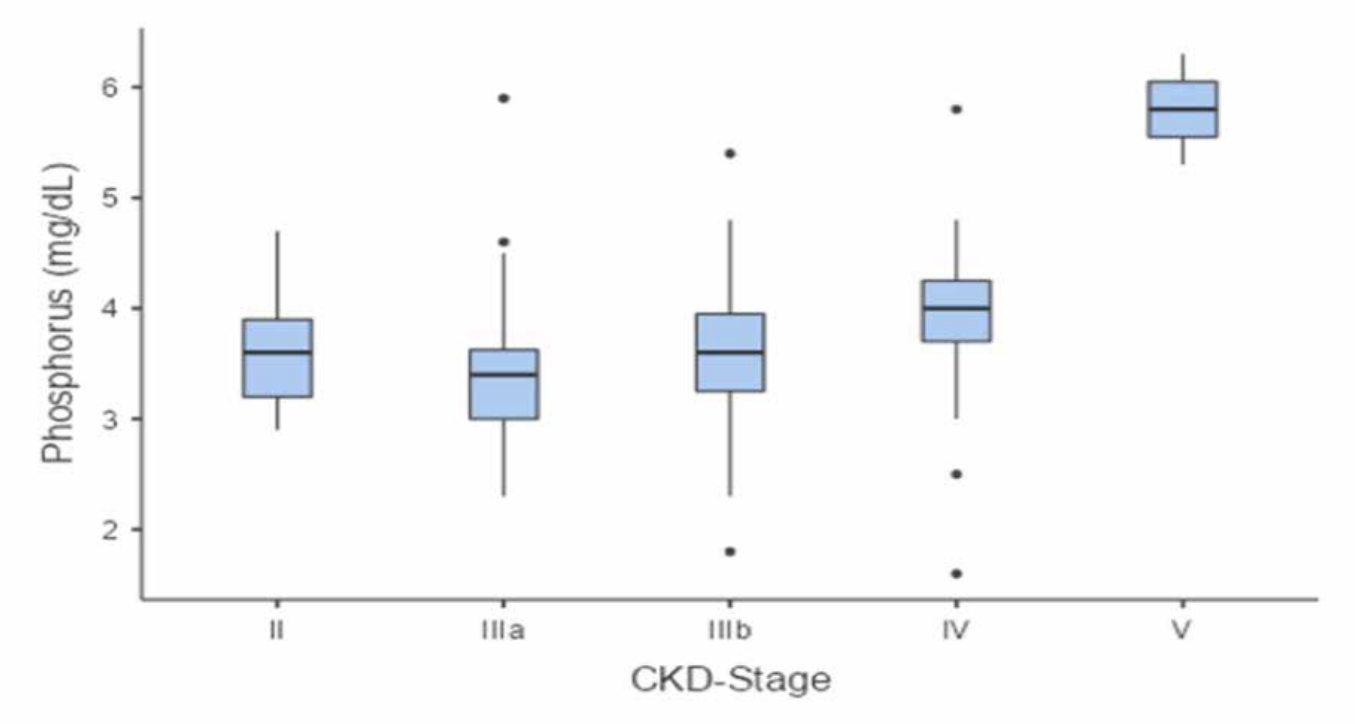


Figure 6: Distribution of phosphorus across distinct stages of CKD.



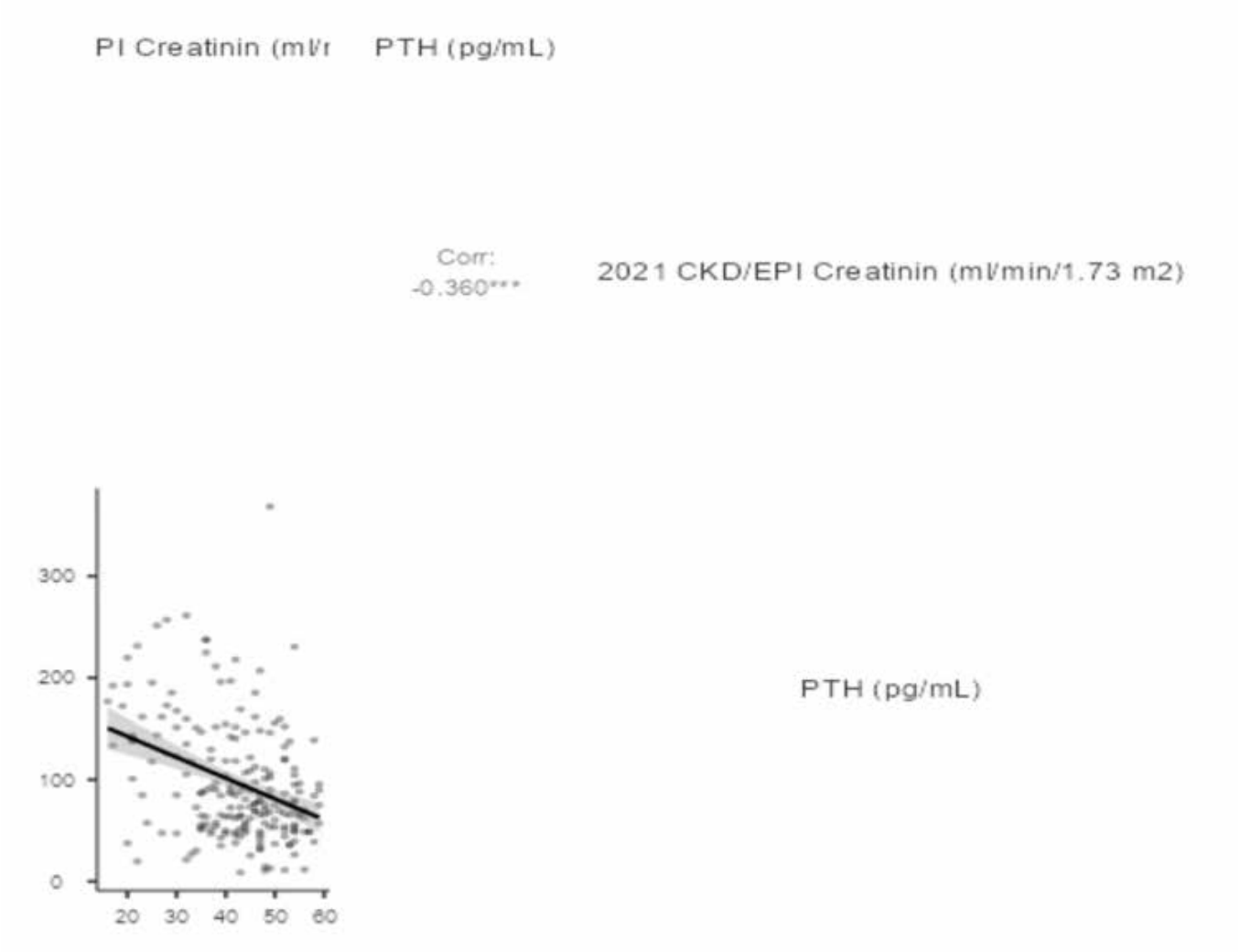
Because the number of patients in stages of G2 and G5 is exceedingly small, with 9 and 2 patients respectively, in the following statistical tests these patients were excluded. According to the Kruskal-Wallis test for non-parametric variables (Table 4), we saw significant differences in PTH levels between stages G3a and G4 ($p<0.001$), and G3b and G4 ($p=0.001$). For phosphorus, we noted differences between stages G3a and G3b ($p=0.003$), as well as G3a and G4 ($p<0.001$), and G3b and G4 ($p=0.01$). These differences reinforce the biochemical complexity of CKD progression.

Table 4: One-Way ANOVA (Non-parametric) Kruskal-Wallis for different CKD- stages.

	χ^2	df	p	ε^2
PTH	23.34	2	< .001	0.1138
Calcium	4.11	2	0.128	0.0201
Magnesium	3.14	2	0.208	0.0153
Phosphorus	26.14	2	< .001	0.1275

After performing the Pearson correlation (Table 5), we can state the following: eGFR has a negative correlation with PTH ($r= -0.360$, $p<0.001$), magnesium ($r= -0.153$, $p=0.028$), and phosphorus ($r= -0.336$, $p<0.001$). The magnitude, or strength, of the association, is weak ($0.3<|r|<0.5$) between both eGFR and PTH, eGFR and phosphorus, and very weak ($0.1<|r|<0.3$) between eGFR and magnesium. EGFR also has positive correlation with calcium ($r=0.169$, $p=0.015$), and the magnitude, or strength, of the association, is very weak ($0.1<|r|<0.3$).

Figure 7: Correlation plot of eGFR and PTH.



Similarly, magnesium shares a positive correlation with PTH (r=0.229, p<0.001), calcium (r=0.205, p=0.003), and phosphorus (r=0.260, p<0.001). The magnitude, or strength, of the association, is very weak (0.1<|r|<0.3).

Table 5: Pearson correlation.

		eGFR	PTH	Calcium	Magnesium	Phosphorus
eGFR	Pearson' r	1	-0.36	0.169	-0.153	-0.336
	df	204	204	204	204	204
	p-value		<0.001	0.015	0.028	<0.001
PTH	Pearson'r	-0.36	1	-0.1	0.229	0.002
	df	204	204	204	204	204
	p-value	<0.001		0.154	<0.001	0.982

Calcium	Pearson' r	0.16	-0.1	1	0.205	0.004
	df	204	204	204	204	204
	p-value	0.015	0.154		0.003	0.949
Magnesium	Pearson's r	-0.153	0.229	0.205	1	0.26
	df	204	204	204	204	204
	p-value	0.028	<0.001	0.003		<0.001
Phosphorus	Pearson's r	-0.336	0.002	0.004	0.26	1
	df	204	204	204	204	204
	p-value	<0.001	0.982	0.949	<0.001	

For all the correlations of eGFR that turned out to be statistically significant, we performed the regression analysis procedure to see if the variables have a predictive effect on each other (Table 6).

Table 6: Regression analysis.

Independent variable	b	m	t	r^2	p	Dependent variable
PTH	183.32	-2.04	-5.52	0.13	<.001	eGFR
Calcium	8.88	0.013	2.45	0.029	<.001	eGFR
Magnesium	2.27	-0.006	-2.33	0.023	<.001	eGFR
Phosphorus	4.39	-0.02	-5.09	0.113	<.001	eGFR

From the table we see that PTH as an independent variable can serve as a predictor of eGFR (t=-5.52, p<0.001). Estimating r^2 we had quantified the percentage of this predictive effect. This means that, if we find an increased value of PTH, the probability of finding a decreased value of eGFR is calculated to be 13%. This predictive effect for magnesium is 2.3% and for phosphorus is 11.3%.

Meanwhile, if we find a decreased value of calcium, the probability of finding a decreased value of eGFR is 2.9%.

Linear regression also gives an equation that can be used to predict the value of a response variable based on the predictor variable.

The formula for simple linear regression is Y = mX + b, where Y is the response (dependent) variable, X is the predictor (independent) variable, m is the estimated slope, and b is the estimated intercept.

Using linear regression, we modeled PTH, calcium, magnesium and phosphorus as a function of eGFR:

PTH = (-2.04 × eGFR) + 183.32

Calcium = (0.013 × eGFR) + 8.88

Magnesium = (-0.006 × eGFR) + 2.27

Phosphorus = (-0.02 × eGFR) + 4.39

Examples:

eGFR = 60 ml/min/1.73 m2 (G2)

PTH = [(-2.04) x 60] + 183.32 = 60.92 pg/mL, (95% CI, 14.59 - 136.4 pg/mL)

eGFR = 40 ml/min/1.73 m2 (G3b)

PTH= [(-2.04) x 40] + 183.32 = 101.7 pg/mL, (95% CI, 40.8-162.6 pg/mL)

These examples illustrate how renal decline directly influences PTH secretion.

Discussion

In our daily work in the laboratory, we have noticed that nephrologists often claim that their patients with chronic kidney disease have high parathormone levels. Considering the pathophysiology of chronic kidney disease and the involvement of the parathyroid gland and phospho-calcium metabolism [2], we undertook this study to discover possible correlations between parathormone, calcium, magnesium and phosphorus in patients with chronic kidney disease.

The prevalence of CKD in our study resulted in 4.6% which compared with global prevalence,>10% is lower [1]. The interpretation of this result is related to the fact that most of the patients with CKD live in low-income and lower-middle-income countries, and a sizable proportion of these individuals lack access to kidney disease diagnosis, prevention, or treatment [4]. Albania is classified as an upper-middle-income country according to the World Bank, and our prevalence is inside the interval of the Europe region, where the adjusted CKD stages 1-5 prevalence varied between 3.31% (95% confidence interval [95% CI], 3.30% to 3.33%) in Norway and 17.3% (95% CI, 16.5% to 18.1%) in northeast Germany, and the adjusted CKD stages 3-5 prevalence varied between 1.0% (95% CI, 0.7% to 1.3%) in central Italy and 5.9% (95% CI, 5.2% to 6.6%) in northeast Germany [5].

Our sample has a slightly predominance of males 5.5% compared to females 3.9%. The higher CKD prevalence described in men go in the same line with experimental data

showing the protective effects of estrogen and potential deleterious effects of testosterone on nondiabetic CKD [6], as well as data that indicate a higher incidence of kidney failure in men [7-8].

Distribution by age results in 40% older than 75 years and only 22% were ≤65 years old, coinciding with literature reports which stated that chronic kidney disease stage 3-5 is common, especially in the elderly [9]. Indeed, the meta-analysis by Hill et al. assessed the impact of age on CKD prevalence and reported a linearly higher prevalence for CKD stages 1–5 associated with advancing age, ranging from 13.7% in the 30-40-years old group to 27.9% in patients aged >70 to 80 years [10]. Similar trends were reported in the United States during 2015 to 2016, where the prevalence of CKD stages 1–4 was 5.6% among individuals aged 20 to 39 years and 44% among those aged >70 years [11].

Evaluating the descriptive analysis of the variables taken in the study, we concluded that their median is within the reference range, and in terms of distribution we noted that the biochemical parameters of patients have normal values, except for PTH which had an upper limit of IQR outside the reference range. These findings of biochemical parameters in CKD are reported also on Journal of Nephrology on September 2018, by Raman, M et al as an analysis of the Salford Kidney Study, a prospective, longitudinal, observational study of 2,667 patients with eGFR < 60 ml/min/1.73 m2 [12]. Most patients with CKD Stages 3–5 already have PTH values above the upper reference limit [13], which is confirmed by a more recent study of 2021 by a cohort from Stokholm, Sweden [14].

Previous articles working on the establishment of age-adjusted reference intervals for parathyroid hormone in healthy individuals observed higher upper limit values of PTH in the elderly [15-16] corroborating our findings of age-related differences in PTH levels.

Regarding the age-related differences for magnesium this is supported by several articles as aging seems to be a risk factor for inadequate magnesium levels due to reduced intestinal absorption, and this could be related to the decrease in vitamin D levels [17]. Another reason that supports this statement is the increased urinary excretion of Mg [18], because with advanced age, renal function and tubular reabsorption decline [19].

Most of our patients were in stage G3a and G3b with 44% and 38%, respectively. The same trend was reported from a United Kingdom retrospective laboratory audit study [20], and from a systematic review and meta-analysis of observational studies estimating CKD prevalence in general populations was conducted through literature searches in 8 databases [10].

The trend of biochemical parameters in distinct stages of CKD highlights an increase in the median and IQR values for PTH in stage G4. The same findings were previously reported in Spain [21] [22], later in Taiwan [23], and most recently in India [24].

In the present study, the PTH and phosphorus levels progressively increased with the advancing stages of CKD, like that observed by Natikar et al. in 2020 [25], and Kumari et al in 2024 [24]. The authors compared the level of PTH in patients of various stages of CKD. As the disease progressed, there was a progressive increase in the PTH level.

In the current study we found a weak negative correlation between PTH and eGFR, which is in contrast with our Indian colleagues who had found a strong correlation of PTH and eGFR [24]. These differences are because they studied only stage 4 and 5 of CKD. Their results suggest that patients with CKD Grades 4 and 5 are at an 8.6 times higher risk of having increased serum PTH levels, and the prevalence of SHPT is amplified as the stage of CKD increases.

Correlations found by us between eGFR and phosphorus are related in epidemiological studies with adverse outcomes in patients with CKD [26].

Conclusions

We concluded that there are statistically significant correlations between the CKD stage and PTH, calcium, magnesium, and phosphorus levels. However, the strength of these correlations is low, so results cannot be generalized. Therefore, individualized assessment is crucial in managing CKD patients. In addition, the results of this survey should be helpful for improving patient education, the importance of which is also endorsed by international experience [27-28].

We expect local health caregivers to treat their patients more individually and in general, to focus more on chronic kidney disease-mineral and bone disorder issues, which have been seen internationally.

Collectively, we believe we have a correct and careful assessment of SPTH that includes clinical assessment, patient symptoms, and laboratory results beyond PTH levels that justify the type and timing of treatment initiation.

Disclosure

The authors have nothing to declare.

Ethical Approval

Our study involved human subjects and is following the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki.

Authors contributions

Hamide Shllaku-Sefa contributed to conceptualization, data curation, formal analysis, investigation, validation, methodology, visualization, writing – original draft. Ndok Marku, role in conceptualization, data curation, supervision, and project administration.

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Data Availability Statement
Data will be provided on request.

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Research Article

Metabolic Dysfunction - Associated Fatty Liver Disease (MAFLD), and Lipid-Based Insulin Resistance Markers in Hepatitis C Virus Infection (HCV)

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Keywords

Insulin Resistance. Fatty Liver, Hepatitis C, Metabolic Syndrome, Liver Fibrosis, Metabolic Dysfunction Associated Fatty Liver Disease (MAFLD), Lipid Profile

Abstract

Aim: Hepatitis C Virus (HCV) infection promotes insulin resistance, and metabolic dysfunction-associated fatty liver disease (MAFLD). HCV per se leads to impairment in host lipid metabolism and causes a deranged lipid profile. This study aims to analyze the prevalence of MAFLD and determine the levels of lipid profile parameters, surrogate markers of insulin resistance, liver fibrosis, and steatosis in patients with HCV infection.

Methods: This study used data from the Centers for Disease Control – National Health and Nutritional Examination Survey (CDC-NHANES) 2017-2020. Those who tested positive on HCV RNA PCR were included in HCV group (n=89). Propensity score-based age- and gender-matching was done among those tested negative for HCV to select controls (n=89). Homeostatic Model Assessment of Insulin Resistance (HOMAIR), Homeostatic Model Assessment of Beta-cell Function (HOMAB), and the lipid-based insulin resistance markers such as Visceral Adiposity Index (VAI), Lipid Accumulation Product (LAP), Triglyceride-Glucose Index (TyG) were calculated using the standard formulae.

Results: Serum triglycerides, total cholesterol and low-density lipoprotein cholesterol were significantly lower in HCV. HOMAIR, HOMAB were similar, and the lipid-based insulin resistance markers such as VAI, LAP and TyG index were significantly lower in HCV. FibroScan showed less steatosis, but increased fibrosis in the HCV patients. The surrogate markers of insulin resistance showed a significant association with the presence of MAFLD.

Conclusion: HCV patients showed hypocholesterolemia, hypotriglyceridemia and the levels of lipid-based insulin resistance markers were significantly lower. TyG index showed a strong positive association with the presence of MAFLD. These observations could be due to association between HCV replication and host lipid metabolism.

Introduction

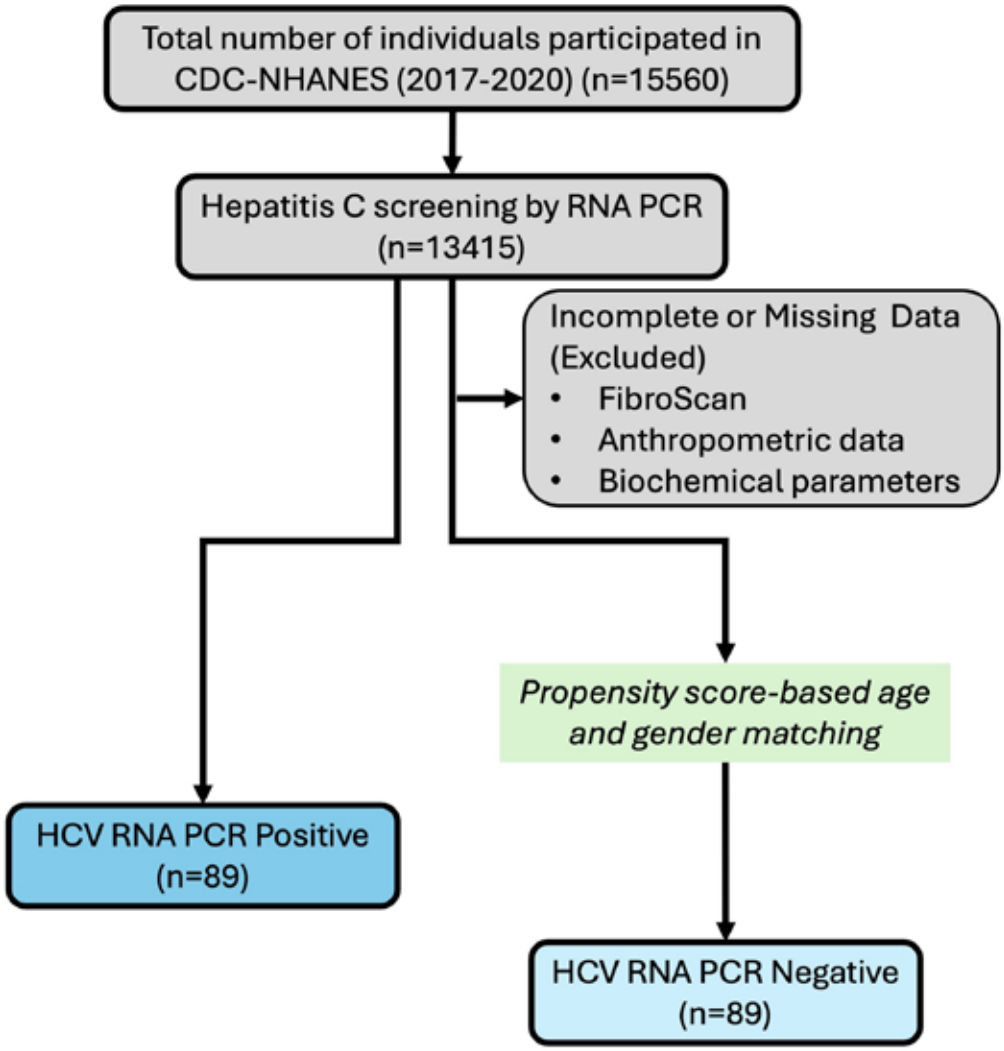
Hepatitis C virus (HCV) infection is one of the leading causes of chronic liver disease, with global estimates indicating that around 50 million people have chronic HCV infection [1]. The prevalence of HCV is 0.5% in India, affecting 4.7 to 10.9 million people [2], with 54-86% of the infected population progressing to chronic HCV infection [3]. Persistent chronic inflammation caused by HCV predisposes to insulin resistance, especially in non-diabetic and non-obese patients, suggesting that HCV per se can induce insulin resistance [4]. HCV has been demonstrated to downregulate the hepatic expression of peroxisome proliferator-activated receptor – alpha (PPAR-α), a nuclear receptor mainly involved in regulating the genes encoding for enzymes of peroxisomal, microsomal, and mitochondrial β oxidation of fatty acids [5]. PPAR-α is known to regulate the import of fatty acids into the mitochondria by modulating the expression of carnitine palmitoyl transferase 1A (CPT1A), a crucial enzyme necessary for mitochondrial beta-oxidation [5]. HCV is also shown to activate the expression of sterol regulatory element-binding protein (SREBP)-1c, a transcription factor that controls fatty acid synthase, an enzyme responsible for lipid synthesis in the endoplasmic reticulum [6,7]. These observations suggest that HCV causes derangement of fatty acid metabolism, leading to the release of free fatty acids, promoting the formation of triacylglycerol in the liver, which results in steatosis. HCV is also reported to cause dyslipidemia, as it perturbs the metabolism of lipoproteins. HCV particles physically associate with liver-derived lipoproteins like very low-density and low-density lipoproteins (VLDL and LDL) to form highly infective lipo-viro particles (LVPs). Formation of LVPs confers protection to the virus against antibody-mediated immunity, thereby enhancing its infectivity [8] and replication in the liver. This also causes a reduction in LDL and VLDL levels in chronic HCV infection [9]. However, despite this reduction in atherogenic lipoproteins, the risk of insulin resistance, hepatic steatosis, and hepatic fibrosis persists.

Hepatic steatosis is commonly encountered in chronic HCV infection, as it is observed in nearly half of the patients [10], in which genotype 3a was predominant. In India, the prevalence of hepatic steatosis is 55.54% in patients with HCV infection, with genotype 3 being predominant (40-80%) [11]. Hepatic steatosis induces insulin resistance, which predisposes them to MAFLD. HCV is also reported to cause impairment of the insulin receptor substrate (IRS)-PI3 kinase signaling pathway, leading to insulin resistance [12]. HCV patients with concomitant MAFLD have poor clinical outcomes, as there is an increased risk of liver fibrosis [13]. The new definition for MAFLD as per the International Consensus Statement includes hepatic steatosis (determined by elastography or histology or biomarker-based fatty liver index) as a major criterion [14]. The studies employing this latest diagnostic criteria for MAFLD are limited. Hence, this study was done in an attempt to analyze the levels of lipid profile parameters, markers of insulin resistance, and the presence of MAFLD, liver steatosis, and fibrosis in patients with HCV infection.

Materials and Methods

This study was done using the Centers for Disease Control and Prevention – National Health and Nutritional Examination Survey (CDC-NHANES-2017-2020) data available in the public domain [15]. Informed consent was obtained from all the participants. Those who tested positive for HCV RNA by PCR were included in the HCV group (n=89). Propensity score-based age- and gender-matching was done among those tested negative for HCV RNA PCR to select age- and gender-matched controls (n=89) (Figure 1).

Figure 1: Flow chart depicting the screening and selection of participants from the survey data.



The chart describes the screening and selection of participants from CDC-NHANES survey data. Participants who tested positive for HCV RNA by PCR were included in the HCV group (n=89). Propensity score-based age and gender matching was done among those tested negative for HCV RNA PCR to select age- and gender-matched controls (n=89)

The participants were categorized as prediabetic or diabetic based on the HbA1c and fasting plasma glucose levels, using the American Diabetes Association criteria [16]. FibroScan-derived Controlled Attenuation Parameter (CAP) and Liver Stiffness Measurement (LSM) were used as markers of liver steatosis and fibrosis, respectively [17].

Homeostatic Model Assessment of Insulin Resistance (HOMAIR), Homeostatic Model Assessment of Beta-cell Function (HOMAB) were calculated using fasting plasma glucose and insulin values [18]. Using lipid profile and anthropometric measures, we calculated the Lipid Accumulation Product (LAP) [19], Visceral Adiposity Index (VAI) [20] and Triglyceride-Glucose Index (TyG) [21].

Statistical Analysis

All statistical analyses were done using R software version 4.3.1. The continuous data were represented as median and interquartile range. The distribution of the data was analyzed by Kolmogorov-Smirnov/Shapiro-Wilk test. The normally distributed data were compared using Independent t-test and non-normally distributed data were compared using Mann Whitney U test. Pearson's Chi-Square test was done to compare the categorical variables. Multivariate logistic regression was carried out to determine the association of covariates with the presence of MAFLD in a model adjusted for age and gender. The odds ratio (OR) was estimated by exponentiating the regression estimate. A p value of < 0.05 was considered statistically significant.

Criteria for diagnosis of Metabolic Syndrome and MAFLD

The guidelines proposed by American Heart Association-National Heart Lung Blood Institute (AHA-NHLBI) [22] and international expert consensus statement [14] were used for the diagnosis of metabolic syndrome and MAFLD respectively. These criteria were multiple and comprehensive and varies with gender, race, BMI and applying these manually is prone to error and misclassification. Hence, the standard R packages such as "MetabolicSyndrome" [23] and "MAFLD" [24] which employs the above mentioned criteria were used to establish accurate diagnosis in this study.

Results

Age and gender distribution were similar between the two groups, as controls were matched for age and gender using propensity score. The prevalence of diabetes was significantly lower in HCV group compared to controls (n (%), 7(8%) vs. 20(22%)); however, there was no difference regarding the presence of prediabetes. The proportion of participants with metabolic syndrome was similar between control and HCV groups (n (%), 33(37%) vs. 31(35%)). Presence of MAFLD were similar between HCV and controls (n (%), 25(60%) vs 39(71%)). Genotype 1 (n (%), 64(72%) was predominant among the HCV patients (Table 1).

Table 1: Baseline characteristics of participants of the study.

Parameter	Control (n=89)	Hepatitis-C (n=89)	p value
Age in years	60 (51-65)	60 (51-65)	1
Gender (male/female), n(%)	64/25 (72/28)	64/25 (72/28)	1
Diabetes/prediabetes, n (%)			<0.0001
Normoglycemia	26 (29)	41 (46)	
Prediabetes	41 (46)	41 (46)	
Diabetes Mellitus	20 (22)	7 (8)	
Metabolic syndrome, n (%)			0.87
Yes	33 (37)	31 (35)	
No	56 (63)	58 (65)	
MAFLD, n (%) #			0.333
Yes	39 (71)	25 (60)	
No	16 (29)	17 (40)	
HCV Genotype, n (%)			NA
Genotype 1	NA	64 (72)	
Genotype 2	NA	9 (11)	
Genotype 3	NA	10 (12)	
Genotype 4	NA	1 (1)	
Genotype 6	NA	1 (1)	
Genotype (undetermined)	-	2 (3)	

The baseline characteristics were compared between controls and those with Hepatitis-C infection. #MAFLD diagnosis was done in both the groups based on international expert consensus criteria [14]. Pearson's Chi-Square test was done to compare the difference in proportions of diabetes, metabolic syndrome and gender between two groups. $p < 0.05$ is considered statistically significant.

Hemoglobin and fasting plasma glucose levels were similar between the two groups. Liver function tests were deranged in HCV patients with significantly higher levels of globulins, AST, ALT, GGT, and total protein. Serum albumin levels were lower in patients with HCV. Serum ferritin levels were higher in patients with HCV; however, serum CRP levels were similar (Table 2).

Table 2: Hematological and Biochemical parameters.

Parameter	Control (n=89)	Hepatitis-C (n=89)	p value
Hematological parameters			
Hb, g/dL	14.1 (13.5-15)	14.6 (12.7-15.4)	0.87
Biochemical parameters			
Fasting plasma glucose, mg/dL	105 (101-114)	100 (93-115)	0.120
Total bilirubin, mg/dL	0.4 (0.3-0.6)	0.5 (0.3-0.62)	0.298
Total protein, g/dL	7.1 (6.9-7.3)	7.4 (7.2-7.8)	<0.0001
Albumin, g/dL	4 (3.8-4.3)	3.8 (3.6-4.1)	0.0002
Globulins, g/dL	3 (2.8-3.3)	3.6 (3.3-4.1)	<0.0001
Aspartate Transaminase (AST), IU/L	20 (17-25.5)	41 (29-66)	<0.0001
Alanine Transaminase (ALT), IU/L	19 (14.5-26.5)	41.5 (26-63.5)	<0.0001
Alkaline Phosphatase (ALP), IU/L	75 (65-89.5)	80 (67.5-96.8)	0.41
Gamma Glutamyl Transferase (GGT), IU/L	24 (17-42)	53.5 (28-141)	<0.0001
CRP, mg/L	1.74 (0.95-4.63)	1.67 (0.51-4.1)	0.171
Serum ferritin, ng/mL	138.5 (62.4-246.7)	184 (74-375)	0.051

The hematological and biochemical parameters were compared between controls and those with Hepatitis-C infection. Mann Whitney U test was done to compare the biochemical parameters between the two groups. $p < 0.05$ is considered statistically significant.

The HCV group had an improved lipid profile, with significantly lower serum total cholesterol (TC), triglycerides (TG), and LDL-C and VLDL-C compared to controls. (Table 3).

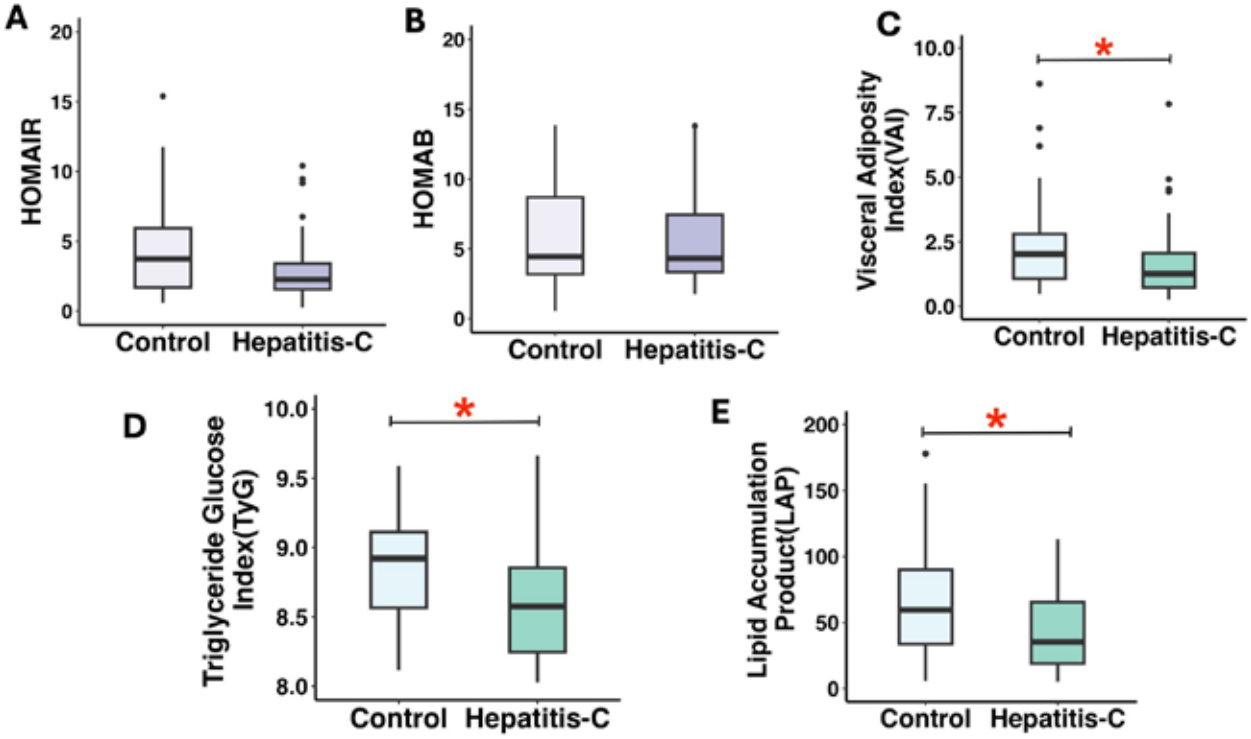
Table 3: Lipid profile parameters.

Parameter	Control (n=89)	Hepatitis-C (n=89)	p value
Lipid Profile			
Total cholesterol, mg/dL	190 (167-219)	162 (138-185)	<0.0001
Triglycerides, mg/dL	145 (99-193)	93 (71-124)	<0.0001
High-density lipoprotein cholesterol (HDL-C), mg/dL	48 (40-62)	50.5 (41-64)	0.368
Low-density lipoprotein cholesterol (LDL-C), mg/dL	117 (94-141)	92 (78-113)	0.01
Very low-density lipoprotein cholesterol (VLDL-C), mg/dL	24 (17-34)	15 (11-20)	<0.0001

The lipid profile parameters were compared between controls and those with Hepatitis-C infection. Mann Whitney U test was done to compare the lipid profile parameters between the two groups. $p<0.05$ is considered statistically significant.

The levels of HOMAIR, HOMAB were similar between the groups, (Figure 2A and 2B) and the lipid-based insulin resistance markers such as VAI, LAP and TyG index were significantly lower in the HCV group (Figure 2C-2E).

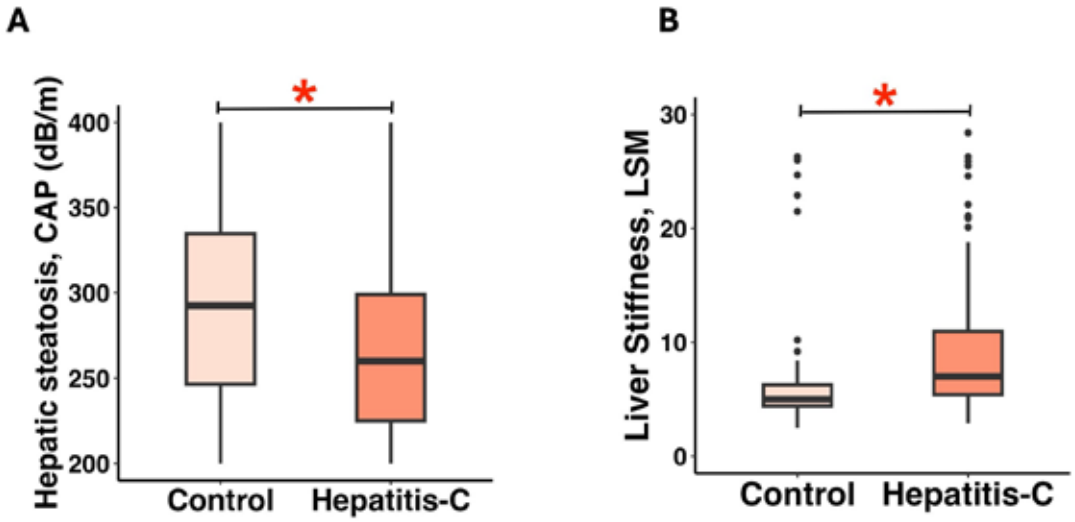
Figure 2: Markers of inuslin resistance.



Insulin resistance markers such as HOMAIR (2A), HOMAB (2B), VAI (2C), TyG (2D) and, LAP (2E) were compared between controls and those with Hepatitis-C infection. Mann Whitney U test was done to compare the insulin resistance markers between the two groups. *$p < 0.05$ is considered statistically significant.

Patients with HCV showed a significant decrease in liver steatosis (Figure 3A); however, liver stiffness (fibrosis) is increased (Figure 3B) compared to the control group.

Figure 3: Liver steatosis and fibrosis determined by elastography.



Controlled attenuation parameter (CAP in dB/m) and median liver stiffness (LSM in kPa) determined by elastography were used as markers for hepatic steatosis (3A) and fibrosis (3B). Mann Whitney U test was done to compare between the two groups. *$p < 0.05$ is considered statistically significant.

Using MAFLD status as the dependent variable, a multivariate logistic regression was done adjusting for age and gender. TyG index, VAI, LAP, HOMAIR, and HOMAB had a significant positive association with the presence of MAFLD. The odds ratio of TyG was 61.8, indicating its strong positive association with the presence of MAFLD (Table 4).

Table 4: Logistic regression to determine the association of covariates with the presence of MAFLD.

Parameter	Estimate	Odds ratio (adjusted)	p-value
HOMAIR	0.38	1.45	0.000807
HOMAB	0.08	1.08	0.0393
TyG index	4.13	61.8	0.0005
VAI	0.31	1.36	0.00619
LAP	0.018	1.02	0.000856
Serum Ferritin	0.0004	1.0004	0.696
Serum CRP	-0.01196	0.988	0.651

Logistic regression model adjusted for age and gender with MAFLD as the dependent variable. HOMAIR, HOMAB, VAI, TyG, LAP, serum ferritin and CRP were the covariates. *$p < 0.05$ is considered statistically significant.

Discussion

This study examined the metabolic alterations in those with HCV infection. HCV patients had lower levels of total cholesterol, triglyceride, and LDL (Table 3), suggesting a close link between HCV replication and host lipid metabolism. Similar findings have been reported previously in chronic HCV infection [9], and it was found that treatment with antivirals has shown improvement [25]. Despite the lipid-lowering effect observed in chronic HCV, cardiovascular disease is still higher compared to the HCV-negative individuals with higher TC and LDL levels [26]. The lipid-lowering effect is attributed to the fact that HCV replication in the liver alters the genes involved in fatty acid metabolism, promoting the synthesis of fatty acids and triacylglycerols [5–7].

In this study, patients with HCV infection showed significantly decreased liver steatosis compared to propensity-matched controls (Figure 3A). This could be because insulin resistance per se is a major determinant of liver fibrosis, irrespective of the genotype and extent of liver damage [27]. There are contrasting reports on genotype-specific effects on hepatic steatosis and fibrosis. In genotype 3, hepatic steatosis is related to viral load and hypolipidemia but not to fibrosis. However, in genotypes 1,2,4, insulin resistance and metabolic steatosis are associated with liver fibrosis [28]. This aligns with the observations of the current study, as genotype -1 was predominant (72%) and liver fibrosis was higher in HCV group.

Persistent chronic inflammation caused by HCV predisposes to insulin resistance, and studies have reported increased levels of HOMAIR in patients with HCV compared to controls [29]. However, in this study, HOMAIR and HOMAB were similar between groups. This could be because of the smaller sample size and missing data on fasting plasma insulin and glucose values. In addition, the proportion of people with diabetes is significantly lower in the HCV group compared to controls.

In this study, other surrogate markers of insulin resistance were studied, and it was found that VAI, LAP, and TyG were significantly lower in the HCV group. This could be attributed to the lipid-lowering effect of HCV, as evidenced by lowered levels of serum triglycerides, total cholesterol, and low-density lipoprotein cholesterol.

Liver function tests were deranged in HCV group, indicating liver dysfunction. In the current study, serum ferritin levels were higher in HCV group but did not show any association with the presence of MAFLD. This is contrast to the studies that has shown increased ferritin levels are significantly associated with the presence of MAFLD [30,31]. This difference could be because of smaller sample size of the current study.

In this study, 60% of the HCV subjects had concomitant MAFLD diagnosed as per the International Consensus Statement [14]. HCV with concomitant MAFLD was shown to increase the risk of advanced liver fibrosis compared to controls [13]. It was found that markers of insulin resistance, such as HOMAIR, HOMAB, VAI, TyG, and LAP, showed a significant positive association with the presence of MAFLD. The odds ratio of TyG for MAFLD was high despite its levels being significantly lower in HCV group. Previous studies have reported that the TyG index was independently associated with liver steatosis and viral load in HCV genotype 1 patients. [32]. The LAP index was shown to be associated with steatosis in MAFLD [33]; however it utility in HCV with concomitant MAFLD has not been explored.

Limitations

The temporal relationship between HCV and lipid profile derangements could not be determined as this study was a cross-sectional study. Therefore, further large-scale cohort and mechanistic research is required to establish the role of HCV in MAFLD.

Conclusion

Patients with HCV presented with hypolipidemia and increased liver fibrosis. Routinely used inuslin resistance markers such as HOMAIR and HOMAB levels did not differ between groups; however, lipid-based insulin resistance markers, such as VAI, TyG, and LAP, were significantly lower in HCV patients. TyG index showed a strong positive association with the presence of MAFLD.

Statements and Declarations

Ethics Approval

The survey is approved by National Center for Health Statistics (NCHS) Ethics Review Board (ERB) (Protocol #2018-01, Continuation of Protocol #2011-17, effective through October 26, 2017). Informed consent was obtained from all participants.

Conflict of interests

The author does not have any conflict of interest to declare in this study.

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Data availability

This data is in the public domain and is available online.

Author contributions

JR was involved in data retrieval, acquisition, analysis and interpretation of the data, and wrote the manuscript. GB, AD and VM contributed to analysis and interpretation of data. MM was involved in data, analysis and interpretation of the data, and wrote the manuscript. JR conceptualized and designed the study, supervised the study in its entirety, analysed and interpreted the data, and wrote the manuscript. All authors reviewed the manuscript and approved its final version, and agree to be accountable for all aspects of the work in ensuring its accuracy and integrity.

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Research Article

Evaluation of urinary albumin/creatinine ratio strip assay: a reliable screening alternative to replacing the quantitative biochemical methods

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Keywords

albumin/creatinine ratio, chronic kidney disease, urinary strip.

Abstract

Background: Clinical laboratories play a crucial role in the diagnosis and monitoring of chronic kidney disease. Quantitative measurement of urinary albumin, expressed as the albumin/creatinine ratio (ACR), is the most commonly used biomarker for this purpose. This study evaluates the feasibility of using urinary strips as a screening tool for ACR, compared with conventional biochemical methods. Specifically, we assessed the diagnostic performance of the urinary strip and the potential economic impact of implementing this screening approach.

Materials and methods: This study included 1,257 samples obtained in primary care, with systematic assessment of requests for urinary strip tests and biochemical quantification of urinary albumin and creatinine. Semi-quantitative measurements were performed using Unamax autoanalyzer and quantitative determinations were conducted using AU5800 autoanalyzer. Diagnostic indicators for ACR were calculated for different albumin and creatinine levels. Economic effects were analyzed based on the costs of both testing methods.

Results: Results at different cut-off values for albumin and creatinine showed optimal performance at 10 mg/L and above 100 mg/dL, respectively. 666 biochemical quantification tests (53.85% screening) for urinary albumin and creatinine could have been avoided during the study period, resulting in total savings of 522.81€.

Conclusions: The present study supports the use of Unamax autoanalyzer for ACR measurement as a screening tool, avoiding unnecessary quantitative measurement, as well as allowing early identification of patients with pathological albuminuria levels. The economic impact was significant, demonstrating effective optimisation of financial resources and workflow efficiency in clinical laboratories.

Introduction

Urinalysis is the third most commonly requested test in clinical laboratories and is often included in annual medical check-ups requested from patients as a control measure [1-2]. Untreated proteinuric nephropathies can cause irreversible damage to the renal parenchyma, chronic kidney disease (CKD), and ultimately lead to end-stage renal failure. CKD is considered a major global public health problem worldwide due to the high associated comorbidities, poor prognosis, and high resource consumption for the healthcare system [3]. Therefore, the early detection of CKD in at-risk patients should be considered a top health priority, as this could allow the implementation of strategies to slow disease progression to advanced stages and mitigate associated complications [4].

CKD usually has an insidious onset and therefore may go unnoticed in its earliest phases, remaining asymptomatic or silent until advanced phases of the disease. However, this disease can initially be suspected through routine laboratory tests, such as glomerular filtration rate (GFR) or the detection of markers of renal damage. Therefore, the clinical laboratory plays a crucial role in the diagnosis, prognosis, and follow-up of CKD [5].

The presence of elevated protein or albumin concentrations in urine, along with GFR, forms the basis of the current diagnostic and staging criteria for CKD [6]. Albumin is the most abundant and common protein in the urine of normal individuals, which is why albuminuria, and more specifically the albumin/creatinine ratio (ACR), is considered to be the most frequently used biomarker, as it is the most sensitive and early for the assessment and detection of kidney damage and CKD [7]. Moreover, this test is also a predictor of the development and progression of both diabetic and non-diabetic kidney disease, as well as being a systemic marker of endothelial dysfunction, arterial remodelling and a predictor of incident hypertension and cardiovascular mortality [8-9]. It is worth noting that patients with diabetes and hypertension, two of the three most prevalent diagnoses in primary care (PC), are at significant risk of nephropathy [10]. Current guidelines for the correct use of albuminuria testing are clear for both patient groups: the test should be used diagnostically, prognostically, and then annually for follow-up [11-12].

The normal ACR value for healthy adults is less than 30 mg/g. ACR values between 30 and 300 mg/g indicate moderately increased albuminuria, while values exceeding 300 mg/g represent severely increased albuminuria [6]. A persistent ACR ≥30 mg/g over three months, confirmed by three different determinations spaced within those months, is indicative of renal damage [3, 13].

The development of tools that allow mass screening of CKD in the general population could be highly valuable. In recent years, several manufacturers have incorporated the semi-quantitative estimates of albumin, creatinine, and ACR concentrations into their urinalysis reagent strips. This provides laboratories with a more economical, efficient, and widely applicable screening tool to the general population particularly for patients at risk of CKD.

The hypothesis of this study is that the semiquantitative determination of ACR using Labusticks 14F strips could serve as an effective screening tool for urine samples from PC patients. The objective was to compare these measurements with those obtained using the biochemistry autoanalyzer and to assess the feasibility, cost-effectiveness, and impact on laboratory workflow of implementing dipstick testing as a routine screening method. This approach could help avoid unnecessary quantitative testing, optimize resource utilization, and reduce turnaround times, particularly in samples with negative results.

Material and Methods

Ethics statement

This study was approved by the Institutional Research Ethics Committee of Valencia General University Hospital, Valencia, Spain. The need for written informed consent was waived due to the nature of the study and the anonymity of the data. All personal information, such as patient name, hospital registration number, date of birth, and national resident registration number, was deleted after assigning a research subject number to ensure anonymity. The study was performed in accordance with the Declaration of Helsinki.

Setting and patients

The clinical laboratory is located in a 503-bed urban university hospital serving an assigned population of 383.162 inhabitants within the Valencia-West Interdepartmental Health Area. The laboratory processes samples from urgent care, hospitalized patients, outpatient clinics, and PC from 24 different collection centres. The most common clinical indications for laboratory tests in PC within our health district are pathologies associated with cardiovascular risk such as dyslipidemia, essential hypertension, and diabetes mellitus.

Study design

A retrospective and unicentric cross-sectional study were designed from 24-31 July 2024.

Laboratory methods

A total of 1.257 samples were processed over one week. For urine strip tests, Labusticks 14F strips (Menarini Diagnostics®, Badalona, Spain) analyzed with the Unamax device (Menarini Diagnostics®, Badalona, Spain) were used. For quantitative biochemical determinations of urinary albumin and creatinine, the AU5800 autoanalyzer (Beckman Coulter®, California, USA) was employed.

The Unamax analyzer utilizes the technique of reflectance spectrophotometry to provide semi-quantitative measurements of albumin, creatinine, and ACR based on these two parameters. The detection system uses a contact image sensor. The possible results provided include: for albumin, ≤10, 30, 80, ≥150 mg/L;

and for creatinine, 10, 50, 100, 200, 300 mg/dL.
The AU5800 autoanalyzer determined urinary albumin using the immunoturbidimetric method. This involves the specific reaction of human serum albumin with anti-human serum albumin antibodies to form insoluble complexes. The absorbance of these complexes is proportional to the albumin concentration in the sample.
Creatinine determination in AU5800 autoanalyzer used the technique of kinetic colourimetry (Jaffé method) traceable to the reference method of isotope dilution mass spectrometry. In this method, creatinine forms a yellow-orange compound with picric acid in an alkaline medium. The rate of absorbance change at 520/800 nm is proportional to the creatinine concentration in the sample.
Conventional reagents for the AU5800 (Beckman Coulter®, California, USA) were used for both determinations.

Cross-sectional study

This retrospective study included 1,257 first-morning urine samples from PC patients. The inclusion criterion was the simultaneous request for both semiquantitative ACR testing using urine strips and quantitative biochemical analysis. Semiquantitative measurements were performed using an automated urine strip analyzer (Unamax), while quantitative determinations of albumin, creatinine, and ACR were conducted on the AU5800 biochemistry platform. Both methods were applied to each sample, allowing for direct comparison of diagnostic performance.
Patient demographic data from PC, along with quantified values of urinary albumin and creatinine, and the three determinations provided by urine strips (ACR, albumin, and creatinine), were collected using the Laboratory Information System (LIS) (Modulab®).
Calculations were conducted under four different scenarios depending on urine strip results: in the first one, no data adjustments were made, and only ACR values ≤30 mg/g (normal or non-pathological) were considered. In the second scenario, data selection was based on the criteria of albumin = 10 mg/L and creatinine ≥10 mg/dL. The third scenario applied the criteria of albumin = 10 mg/L and creatinine ≥50 mg/dL. The fourth scenario used the criteria of albumin = 10 mg/L and creatinine ≥100 mg/dL. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and screening percentage were calculated.
The number of immunoturbidimetric tests potentially avoided was estimated, and an economic estimate was made taking into account that for negative results it would no longer be necessary to analyze them by the reference method (quantification). An economic analysis was conducted based on the costs of the two tests (€0.98 for quantitative albumin and creatinine tests and €0.195 for a urine strip). The costs considered in this study are limited to reagent expenses, without accounting for personnel costs or time invested.

Statistical analysis

Data were statistically processed and analyzed using RStudio (Version 4.3.2) and Microsoft Excel. Results from the Unamax analyzer were cross-referenced with those from the AU5800 autoanalyzer, considering only requests containing both tests. The VLOOKUP function in Excel was used to search matches, and contingency tables were populated using the COUNTIFS function. Subsequently, data were extracted for sensitivity, specificity, PPV, NPV, and screening percentages.
For the cost-benefit analysis, we estimated potential savings by comparing the cost of quantitative albumin and creatinine measurements with that of the labusticks 14F strips.

Results

A total of 1.257 samples were analyzed by urine strip and biochemical quantification. Their demographic characteristics and percentage of negative quantified ACR values (ACR < 30 mg/g) are shown in Table 1.

Table 1: Study population characteristics.

Population characteristics	
Patients, n	1257
Men, n (%)	608 (48.4%)
Women, n (%)	649 (51.6%)
Age, years (mean ± SD) [range]	59 ± 17 [7–95]
Negative results of ACR (<30 mg/g)	
Patients, n (%)	1023 (81.4%)

Table 2 shows the diagnostic indicators and potential cost savings across the four different scenarios. The greatest cost savings were achieved for "normal or non-pathological" urine strip results (ACR ≤30 mg/g) (Table 2, Columns I). Significant savings were also observed for urine analysis with strip albumin values of 10 mg/L and creatinine strip results exceeding 10 mg/dL (Table 2, Columns II), though this came at the expense of a 5.40% false-negative (FN) rate. For urinalysis with strip albumin values of 10 mg/L and creatinine strip results exceeding 50 mg/dL (Table 2, Column III), the FN rate dropped below 2.62%, and sensitivity improved. However, the best results were observed in the fourth scenario

(Table 2, Column IV), which showed a high NPV of 98.38% and a sensitivity of 93%.

Based on the results of the best scenario (Table 2, Column IV), 666 biochemical quantification tests for albumin and creatinine in urine could have been avoided during the study period (53.85% screening), corresponding to a total cost savings of 522.81€. Considering that the results pertain exclusively to one-week, systematic application of this strategy over a year (52 weeks) could hypothetically save 34.632 biochemical tests and 27.186.12€.

Table 2: Diagnostic indicators and potential savings, considering negative values when the ACR value of the strip is normal (column I), and when the albumin value = 10 mg/L, with creatinine values above 10, 50, and 100 mg/dl, respectively (column II, II and IV).

Results	I ACR≤30 mg/g	II Albumin=10 mg/L Creatinine≥10 mg/dl	III Albumin=10 mg/L Creatinine≥50 mg/dl	IV Albumin=10 mg/L Creatinine≥100mg/dl
Negatives	1023	1001	954	666
False negatives	36 (2.86%)	68 (5.40%)	33 (2.62%)	11 (1.65%)
NPV (%)	96.60%	93.64%	96.66%	98.38%
PPV (%)	63.70%	50%	47.76%	27.90%
Screening (%)	84.24%	85.04%	78.52%	53.85%
Specificity (%)	93.50%	91.50%	87.20%	60.88%
Sensitivity (%)	77.60%	58%	80%	93%
Savings (€)	803.05€	785.78€	748.89€	522.81€

Discussion

The epidemiological significance of CKD lies not only in its high prevalence but also in the significant reduction in quality of life, the elevated morbidity and mortality rates, and the associated healthcare and social costs. It is estimated that more than 850 million people (10% of the adult population) worldwide suffer from kidney diseases, the majority of which have CKD [14]. The estimated global prevalence of CKD ranges from 8% to 16% [15]. Internationally, CKD was responsible for 1.2 million deaths and 35 million years lived with disability (YLD) in 2016 [16]. The global incidence, prevalence, mortality, and YLD rates of CKD have risen dramatically since 1990, driven by population growth, aging, and the increasing number of individuals with diabetes and hypertension [16], which, along with glomerulonephritis, are the main causes of CKD [16-17]. Additionally, common non-renal complications of CKD, such as myocardial infarction, stroke, heart failure, and infections, further contribute to its burden [18]. This is an important issue, as the prevalence of stages 3-5 of CKD among adults over 20 years of age is 4.7% in men and 5.8% in women, and more than 50% of CKD cases going undetected if albuminuria is ignored [6, 19].

Proteins are excreted variably throughout the day depending on hydration status, physical activity, or protein intake. This makes 24-hour urine collection the reference specimen for measuring proteinuria or albuminuria [6]. However, the preanalytical challenges and collection issues associated with this method have led to the search for alternative specimens, such as single-void urine samples. In adults, most guidelines recommend evaluating proteinuria by determining the ACR preferably in the first-morning urine [3, 6, 20].

Proteinuria may increase in certain situations, such as the presence of fever, stress, high protein intake, heart failure, or intense physical exercise, and this can be corrected after the causative factor disappears. Similarly, urinary tract infections or menstruation can lead to false positive results. Therefore, it is advisable to avoid urine collection for albuminuria/proteinuria assessment under these circumstances. Moreover, smoking and obesity have also been associated with the presence of albuminuria, and up to 25% of individuals over 80 years old show this condition. In this scenario, PC plays a crucial role in the early detection of CKD, managing patients in these situations, controlling progression factors, and addressing early-stage complications [3].

Our data show good concordance and a NPV close to 98.3%, which facilitated the screening of ACR results below 30 mg/g in Unamax, without the need for quantitative determination in AU5800. In our study, this corresponded to 53.85% of the samples. Considering the sensitivity (93%) and NPV (98.3%) indicators, ACR testing using urine strips can be considered an effective screening method for patients who require regular monitoring of this parameter, such as those with diabetes, hypertension, and renal disease. For patients with a semi-quantitative positive ACR result, it would be useful to extend further quantitative determination, as this would aid in the early diagnosis of patients at risk of renal damage.

Our best scenario indicates that the screening method using reagent strips has a sensitivity of 93% and a specificity

of 60.88%, with PPV and NPV of 27.9% and 98.38%, respectively. In previous studies that evaluated others strip tests, the sensitivity, specificity, PPV and NPV for detecting albuminuria was 97–97.5%, 44–67%, 22–70.3% and 97.1–99% respectively. These studies have demonstrated positive correlations between urinary albumin and ACR determinations using urine test strips, compared to biochemical assays employing immunonephelometric and immunoturbidimetric methods, emphasizing both their cost-effectiveness and potential for automation [12, 21-23]. The variability may be attributed to differences in study populations, inclusion/exclusion criteria, methodologies used for urine strip tests and ACR quantitative determination and differences in the definition of the cut-off. To date, our study is the first to evaluate and validate the use of Unamax autoanalyzer specifically, thus providing novel evidence supporting their applicability and reliability for clinical screening purposes.

The implementation of the findings from this study in clinical laboratories could lead to significant savings in both economic resources and personnel time, mainly due to the high screening percentage achieved (53.85%). Additionally, the low cost, speed, and ease of use allow it feasible for analyzing many samples, reducing the need for quantitative determinations and thus lowering overall costs. This workflow has the potential to generate substantial resource savings, as previously described in other studies [12]. In addition to financial benefits, the time and effort saved by staff can be redirected toward more critical tasks, enhancing overall laboratory efficiency [24].

The high prevalence of pathologies such as diabetes, hypertension, and CKD itself, in which ACR is used as an early marker of kidney damage, along with other renal injury markers and GFR, has led to a significant increase in the number of requests for these ratios from PC. Therefore, clinical laboratories play a crucial role in the diagnosis and subsequent monitoring of these conditions [12]. Additionally, they are responsible for adequate and efficient demand management, which requires laboratories to establish good workflows to accommodate this increase in healthcare demand while reducing associated costs. This management could be solved by implementing an ACR screening system using test strips in urine autoanalyzers.

In conclusion, the present study demonstrated that semi-quantitative determinations of albuminuria and creatinuria in urine strips using Unamax are a valid and cost-effective method for identifying patients without pathological albuminuria values, thus preventing unnecessary subsequent quantification. According to our analysis, with this strategy we can obviate the quantitative measurement of approximately 53.85% of the requested urinary albumin test, resulting in significant economic savings. Moreover, this method helps exclude patients without renal risk and can be used as a screening tool due to its lower cost, high sensitivity, and ease of application across the population. By applying only a simple condition in the LIS, screening results significantly improve while maintaining a high screening percentage. Implementing the findings of this study can lead to substantial savings in both economic costs and time, thus producing an improvement in laboratory workflow.

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No.

Author Contributions

The corresponding author takes full responsibility that all authors on this publication have met the following required criteria of eligibility for authorship: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved. Nobody who qualifies for authorship has been omitted from the list.

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Abbreviations

ACR: Albumin/Creatinine Ratio
CKD: Chronic Kidney Disease
FN: False Negative
GFR: Glomerular Filtration Rate
LIS: Laboratory Information System
NPV: Negative Predictive Value
PC: Primary Care
PPV: Positive Predictive Value

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Research Article

Synovial Fluid Cell Counts Remain Stable for 72 Hours Regardless of Storage Temperature: Implications for PJI Diagnosis

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Periprosthetic Joint Infections, Synovial fluid, Cells stability

Abstract

Background: Synovial fluid analysis plays a crucial role in the diagnosis of periprosthetic joint infection (PJI). However, the stability of leukocyte counts and the percentage of polymorphonuclear neutrophils (PMN%) under different storage conditions remains uncertain, and many institutions lack immediate access to on-site laboratories. We investigated whether storage temperature (room temperature (RT) vs 4 °C) influences synovial fluid white blood cell (WBC) count and PMN%, and if these parameters are stable for up to 72 hours after aspiration.

Methods: We prospectively analysed 106 synovial fluid samples obtained during revision arthroplasty for suspected PJI. Assuming that the population was homogenous according to the inclusion criteria, patient's samples were randomly allocated either to be stored at RT or at 4°C, in order to obtain two different set of samples. In both set of samples WBC count and PMN% were measured at baseline, 6, 12, 24, 48, and 72 hours using an automated haematology analyser after pre-treatment with hyaluronidase. Changes over time in the same patient synovial fluid and between different storage temperature groups were assessed with independent T test.

Results: Both WBC count and PMN% remained stable for up to 72 hours in samples stored at either RT or 4 °C. Mean WBC counts were slightly higher in refrigerated samples, but differences were minimal and not statistically significant. No variation led to reclassification of samples across the ICM 2018 diagnostic thresholds for PJI.

Conclusion: Synovial fluid WBC and PMN% remain stable for up to 72 hours regardless of storage temperature. These findings challenge the assumption that immediate analysis is required and support greater flexibility in clinical workflows, particularly in institutions without immediate on-site laboratory availability.

Introduction

Periprosthetic joint infection (PJI) is one of the most devastating complications of hip and knee arthroplasty, associated with high morbidity, mortality, and healthcare costs. Diagnosis remains challenging because symptoms are often subtle, and no single gold standard exists [1–3]. To address this, the 2018 International Consensus Meeting (ICM) incorporated synovial fluid white blood cell (WBC) count and polymorphonuclear neutrophil percentage (PMN%) as key minor diagnostic criteria [4-14]. These two parameters alone account for five of the six points required for achieving a definitive diagnosis and are also used to guide the timing of second stage reimplantation [15, 16]. Traditionally, WBC counts have required timely analysis, as delays or suboptimal storage conditions were thought to compromise accuracy [17–19]. To prevent protein precipitation, saline is often used as a diluent [20]. Early studies also described a rapid decline in WBCs after aspiration due to poor preservation, leading to their lysis. Conversely, subsequent reports indicated that storage at 4 °C might preserve cell counts for short periods [19, 21-23]. However, prior work has been limited to ≤24 hours or to comparisons of anticoagulants, leaving uncertainty about the stability of WBC and PMN% beyond the first day. The purpose of this study was to evaluate the effect of storage time (up to 72 hours) and temperature (room temperature (RT) vs 4 °C) on synovial fluid WBC count and PMN%. We aimed to determine whether delays in analysis alter these diagnostic parameters and affect classification according to the 2018 ICM thresholds for PJI.

Materials and Methods

One hundred and eleven consecutive patients with failed or painful joint arthroplasty of hip or knee seeking orthopaedic consultation between June and November 2023 were included. Inclusion criteria were age >18 years; diagnosis of PJI (knee or hip) based on ICM 2018 criteria and acute or delayed infection. The exclusion criteria were chronic inflammatory joint diseases; samples that were grossly haemolysed, or when the leftover was insufficient; antibiotic therapy not suspended at least 14 days before the procedure.

Residual samples from individual patients (106 patients) were used for the study, and a pseudo-anonymization protocol was applied immediately after routine examinations. All patients underwent a standardized diagnostic protocol, including clinical assessment and arthrocentesis; total WBC count and percentage of PMN were performed on SF ("best practice and according to the study protocol: T0). An experienced orthopaedic surgeon collected the SF during preoperative evaluation of joint disorder symptoms, suggesting a failure of uncertain origin of the knee prosthesis. Insufficient amount of SF (< 1.0 mL) was considered as an exclusion criterion. The SF was collected directly into K3EDTA tubes (Becton Dickinson, Franklin Lakes, NJ) and conveyed to the central laboratory within 2 hours at RT.

Automated leukocyte counting on SF was performed using a Sysmex XN-2000 (Sysmex, Inc. Kobe, Japan) equipped with a dedicated body fluid analysis module (XN-BF from Sysmex, Inc. Kobe, Japan). Specific quality control "XN check" (from Sysmex, Inc. Kobe, Japan) was used daily to assess imprecision and bias.

According to the laboratory consolidated routine, upon arrival at the laboratory, the SF was pre-treated a with hyaluronidase solution (Sigma Chemical Co., St. Louis, MO, USA), prepared by dissolving 2.5 mg hyaluronidase in 5 mL 0.1 mol/L phosphate-buffered saline at pH 7.4 (final concentration, 0.5 mg/mL). Pre-treatment consisted of adding 20 µL of hyaluronidase to 1 mL of SF, followed by incubation for 5 min at RT [24]. After routine analysis, the patients were divided into two groups and leftover samples were handled according to the following protocol: the first group of patients was maintained at RT, while the second was stored refrigerated at 2–8 °C. All the samples were then reanalyzed at specific time intervals, i.e., after 6, 12, 24, 48, 72 hours, i.e. T6, T12, T24, T48 and T72. Immediately after each measurement, the samples were returned to their original temperature conditions.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics (version 29.0, IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages. The Shapiro–Wilk test was used to assess the normality of continuous data distribution. Between-group comparisons of mean WBC counts and %PMN at each time point (baseline, 6, 12, 24, 48, and 72 hours) were performed using independent samples t-tests, with equality of variances assessed by Levene's test. Effect sizes were calculated using Cohen's d, Hedges' correction, and Glass's delta. Categorical variables based on diagnostic cut-off thresholds for WBC count and %PMN (according to the 2018 ICM criteria) were compared between storage conditions using the Pearson χ^2 test or Fisher's exact test, as appropriate. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using binary logistic regression to evaluate the association between storage condition and exceeding diagnostic cut-off values. A two-tailed p-value <0.05 was considered statistically significant.

Results

A total of 106 synovial fluid samples were analysed, with 52 stored at 4 °C and 54 kept at RT. All samples, as expected from the clinical situation, yielded a high number of WBC Across all subsequent time points (6, 12, 24, 48, and 72 h), WBC counts showed a progressive decline in both patients stored at Rt and at 4°C, with slightly higher values consistently observed in refrigerated samples, suggesting slightly better storage condition. Nevertheless, differences between storage conditions did not achieve statistical significance at any time point (p >

0.25 for all). In every patient, %PMN remained stable over time in both storage conditions, no statistically significant differences were found for %PMN at any time point ($p > 0.39$). A detailed summary of mean values, SD, and p-values for WBC and PMN% across all time points and storage conditions is provided in Table 1, complementing the graphical trends shown in Figures 1 and 2. Mean WBC values represent all samples assigned to each storage group at each time point (RT, n=54; 4 °C, n=52). Because each sample contributed to only one storage condition, per-sample trajectories between RT and 4 °C could not be shown.

Table 1: Synovial Fluid WBC and %PMN over Time (RT vs 4 °C).

Time	WBC RT (mean±SD)	WBC 4°C (mean±SD)	p-value WBC (RT vs 4°C)	PMN% RT (mean±SD)	PMN% 4°C (mean±SD)	p-value PMN (RT vs 4°C)
T0	5417.8 ± 10243.6	8496.4 ± 16712.1	0.94	49.8 ± 28.7	50.4 ± 28.8	0.87
T6	5334.6 ± 10241.2	8259.5 ± 16503.3	0.72	50.3 ± 27.2	51.8 ± 27.2	0.54
T12	5276.4 ± 10224.6	8340.4 ± 16647.5	0.81	51.2 ± 28.0	50.6 ± 28.3	0.61
T24	5327.7 ± 10416.0	8130.0 ± 16170.3	0.65	50.1 ± 28.0	53.9 ± 26.7	0.42
T48	5349.0 ± 10723.9	7884.2 ± 15622.3	0.33	50.2 ± 29.0	52.3 ± 27.2	0.21
T72	5176.5 ± 10628.5	7525.4 ± 15313.8	0.18	49.9 ± 28.1	56.6 ± 24.6	0.09

Figure 1: Mean (±SD) synovial fluid WBC counts over time in samples stored at room temperature or 4 °C.

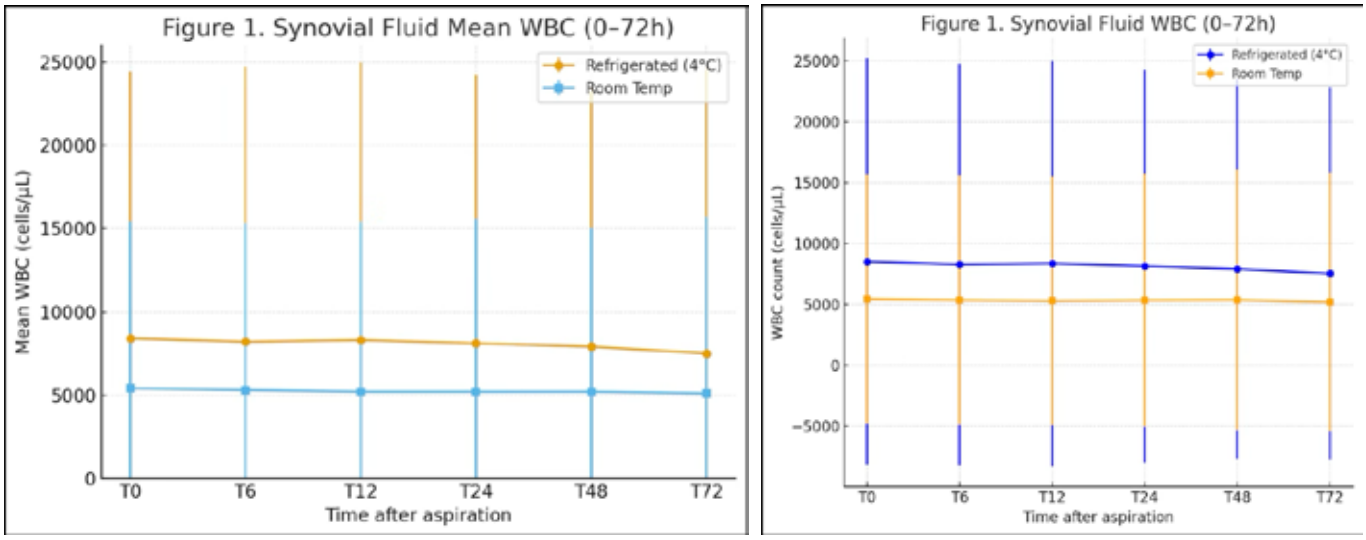
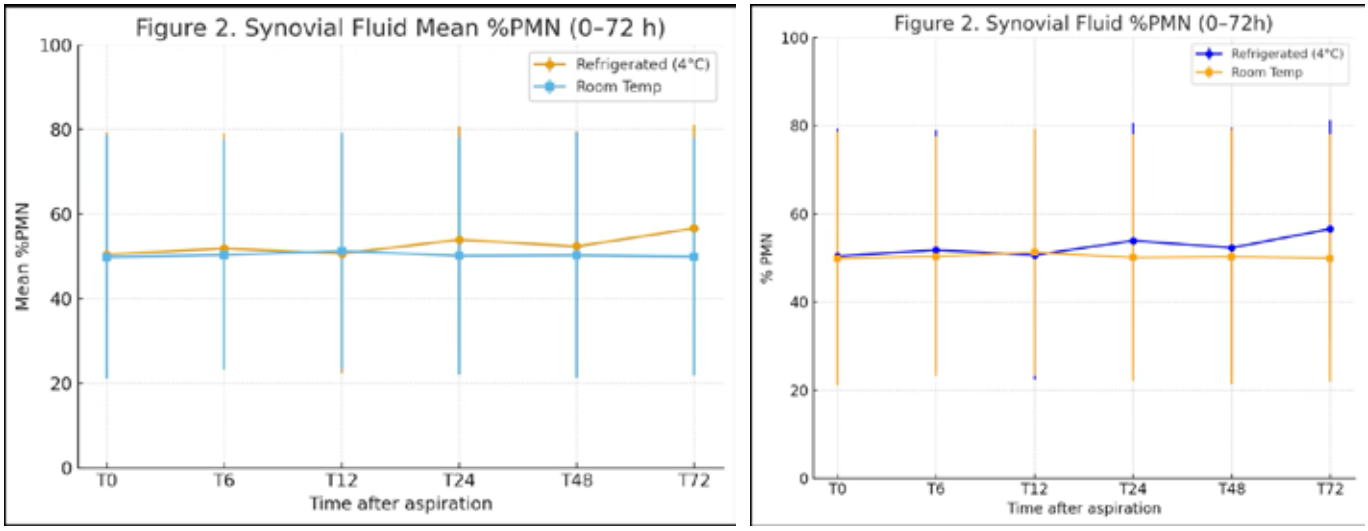


Figure 2: Mean (±SD) synovial fluid PMN% over time in samples stored at room temperature or 4 °C.



Effect size estimates (Cohen's d) for WBC counts ranged from 0.18 to 0.22 across time points, indicating minor, non-clinically relevant differences between groups. Effect sizes for %PMN were close to zero. Post-hoc power analysis was performed only for WBC counts, as this parameter highly exhibited distributions and very wide SDs. Using the observed absolute mean differences between storage conditions (≈2,300–3,100 cells/μL) and the pooled standard deviation at each time point, statistical power remained low (0.15–0.21), indicating that even moderate between-group differences would likely remain undetected. When applying the diagnostic cut-off values defined by the 2018 ICM criteria for periprosthetic joint infection, the proportion of samples above threshold was similar between groups at all time points. Pearson's χ^2 and Fisher's exact tests confirmed the lack of significant association between storage condition and exceeding the diagnostic cut-offs for either WBC count or %PMN ($p > 0.45$ for all). Binary logistic regression analyses showed no significant effect of storage temperature on the likelihood of exceeding WBC or %PMN diagnostic thresholds at any time point (all ORs close to 1, $p > 0.90$).

Discussion

Synovial fluid analysis has long been recognized as one of the most valuable diagnostic tests for PJI and for guiding the decision to proceed to a second-stage procedure in patients with a spacer [21]. Early evidence by Schumacher et al. showed that leukocyte counts may decrease as early as one hour after aspiration, leading the authors to consider synovial fluid analysis an emergency procedure [25]. However, subsequent studies have shown that reliable test results can still be obtained after 48–72 hours [26]. Manual cell count in synovial fluid has traditionally been considered the gold standard [22], but concerns about reproducibility and inter-observer variability have prompted attempts to automate the process. Vincent et al. [27] initially discouraged the use of automated counters due to artifacts such as fat droplets and cell damage. Conversely, Sugiuchi et al. [28] demonstrated that pre-treatment with hyaluronidase enabled the reliable and automated determination of leukocyte counts, with results comparable to those obtained through manual analysis. Although the limitations in obtaining hyaluronidase supplies in middle- and low-income countries need to be acknowledged, automated methods may offer advantages of higher precision, reproducibility, and time efficiency [22]. In our study, we employed automated leukocyte counting to evaluate the effect of storage temperature on synovial fluid WBC count and %PMN over 72 hours, comparing the effect of storing the same sample at 4 °C versus RT. Our findings indicate that refrigeration did not significantly influence either parameter at any time point, with both protocols showing similar temporal trends. Although mean WBC counts were generally higher in refrigerated samples, as predictable, these differences were minor and not statistically significant. This suggests that synovial fluid cellular parameters remain relatively stable over short-term storage, regardless of the temperature. When compared with earlier literature, some critical differences emerge. Koolvisoot et al. [29] first demonstrated that storage had a minimal impact on the accuracy of leukocyte counts, although their analysis was limited to short observation periods and manual methods. Later, Salinas et al. [26] investigated the role of anticoagulants and found that EDTA preserved leukocytes more effectively than heparin, even if a progressive decline in WBC could still be observed at 24 hours, emphasizing the importance of prompt processing.

In contrast, our 2023 data show that both WBC count and %PMN remain stable for up to 72 hours, independent of storage temperature, with no significant effect on ICM 2018 diagnostic thresholds. Taken together, these findings suggest a shift in perspective: while earlier studies highlighted the vulnerability of synovial samples to pre-analytical factors, our results support the view that synovial fluid may be less

vulnerable than previously assumed, and that short delays in processing, even up to three days, do not apparently compromise the diagnostic accuracy for PJI.
To this end, our findings extend current knowledge by demonstrating that short-term delays in synovial fluid analysis do not compromise the accuracy of WBC or %PMN measurements, regardless of whether samples are stored at RT or refrigerated. This challenges the traditional assumption that immediate analysis or mandatory refrigeration is required to preserve diagnostic reliability. From a clinical perspective, these results suggest that institutions without immediate access to on-site laboratories can safely delay the analysis up to 72 hours, thereby allowing for more flexible workflows without jeopardizing diagnostic accuracy for PJI.
Nevertheless, the absence of statistically significant differences should be interpreted with caution. Our study was not powered to detect minimal effects, and subtle changes in cellular morphology not reflected in quantitative counts may still occur. Furthermore, synovial WBC counts are highly heterogeneous, resulting in wide SDs. Across all timepoints, between-group differences were small (Cohen's d = 0.18–0.22), and post-hoc power analyses using observed mean differences and pooled SDs showed low statistical power (<0.30), indicating that subtle effects may have remained undetected. Although we complemented mean-based comparisons with categorical analyses aligned with ICM 2018 thresholds, this variability remains a limitation. Moreover, we focused exclusively on WBC and %PMN parameters, without evaluating other biomarkers (e.g., alpha-defensin, C-reactive protein) that may be more sensitive to storage-related degradation. Future studies should validate these findings in larger, multicenter cohorts using standardized protocols and incorporating a broader panel of synovial biomarkers. Such research could inform evidence-based recommendations for synovial fluid storage and transport, streamlining PJI diagnostic workflows. while maintaining accuracy.

Conclusion

Our findings indicate that the percentages of synovial fluid WBCs and PMNs remain stable for up to 72 hours, regardless of the storage temperature. This challenges the need for immediate analysis or mandatory refrigeration, suggesting that short delays do not significantly compromise the diagnostic accuracy for PJI. If confirmed by the stability of other parameters included in the Parvizi score, our results may help streamline workflows, especially in healthcare facilities without on-site laboratories.

Ethics Approval

The study was approved by the independent ethics committee on June 7, 2023. The research was conducted in accordance with the Declaration of Helsinki and national and institutional standards.

Conflict of interests

The authors declare no conflicts of interest for the conduction of the study.

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Research Article

Sustainability Practices and Green Lab Initiatives in Clinical Laboratories in Pakistan: A National e-Survey-Based Analysis

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Sustainability, Green Lab, survey, Pakistan

Abstract

Introduction: Clinical laboratories play a vital role in healthcare but contribute significantly to environmental challenges through high energy consumption, water usage, and waste generation. Pakistan's healthcare sector faces challenges, including limited funding and inadequate awareness of sustainable practices. There is little data on the extent to which clinical laboratories in Pakistan have implemented green practices, making it crucial to assess current efforts and identify barriers to adoption. This study aims to assess the adoption of sustainability and green lab practices in clinical laboratories across Pakistan.

Methods: A cross-sectional survey was conducted by the Chemical Pathology section at Aga Khan University (AKU) using a structured questionnaire. The survey comprised 13 sections to evaluate sustainability practices, covering demographics, current green practices (energy efficiency, water conservation, waste management, etc.), barriers to implementation, environmental and cost impacts, and future goals. It assessed laboratories' existing efforts, challenges, and aspirations for improving sustainability. The survey was distributed via Google Forms to major laboratories across Pakistan via WhatsApp and email. Data was analyzed using Excel (Microsoft Corporation, 2018) software.

Results: A total of 12 laboratories across the country, from the capital Islamabad and all provincial capitals participated in the survey. Key findings include widespread adoption of energy-efficient lighting (75%) and electronic reporting (91.7%), but limited use of water-saving technologies (8.3%) and renewable energy (0%). Barriers like limited resources (58.3%), lack of staff awareness (50%), and financial constraints (41.7%) hindered green practices, though 41.7% reported moderate cost savings. Future goals focused on green certifications (58.3%), recycling programs (50%), and energy-efficient upgrades (41.7%).

Conclusion: Our findings underscore the urgent need for structured sustainability policies, financial incentives, and educational programs to enhance green laboratory practices in Pakistan. While some progress has been made, significant gaps remain in energy efficiency, waste management, and regulatory compliance

Introduction

Clinical laboratories play a critical role in healthcare delivery, providing essential diagnostic services that inform patient care and treatment decisions [1]. However, the operations of these laboratories often come with significant environmental costs, including high energy consumption, water usage, and the generation of hazardous and non-hazardous waste [2]. As global awareness of environmental sustainability grows, there is an increasing need for healthcare institutions, including clinical laboratories, to adopt practices that minimize their ecological footprint while maintaining high standards of patient care [2,3].

Sustainability in clinical laboratories encompasses a wide range of practices, from energy-efficient lighting and equipment to waste reduction, water conservation, and the use of environmentally friendly chemicals [4]. These practices not only contribute to environmental preservation but can also lead to operational cost savings and improved efficiency [5–7]. Despite these potential benefits, the adoption of sustainable practices in clinical laboratories remains uneven, particularly in low- and middle-income countries where resource constraints and competing priorities often take precedence [8–10].

In Pakistan, the healthcare sector faces numerous challenges, including limited funding, inadequate infrastructure, and a lack of awareness about sustainable practices [11]. While there is growing recognition of the importance of sustainability in healthcare, there is a paucity of data on the extent to which clinical laboratories in Pakistan have embraced green practices. Understanding the current state of sustainability in these laboratories is essential for identifying gaps, addressing barriers, and developing strategies to promote environmentally responsible operations.

This study aims to assess the adoption of sustainability practices in clinical laboratories across Pakistan. Through a comprehensive survey, we evaluated key areas such as energy and water consumption, waste management, and the use of environmentally friendly chemicals. The survey also explored the barriers laboratories face in implementing sustainable practices and the potential cost savings associated with these initiatives. By providing a snapshot of the current state of sustainability in Pakistani clinical laboratories, this study seeks to inform policymakers, healthcare administrators, and laboratory professionals about the opportunities and challenges in promoting greener laboratory practices.

The findings of this study are expected to contribute to the growing body of literature on sustainability in healthcare, particularly in resource-constrained settings. Furthermore, they will provide a foundation for future research and interventions aimed at reducing the environmental impact of clinical laboratories while enhancing their operational efficiency and cost-effectiveness.

Methods

Study Design

This study employed a cross-sectional, survey-based design to assess sustainability practices in clinical laboratories in Pakistan. The survey aimed to evaluate current green practices, identify barriers to implementation, and explore future goals for improving sustainability. Data was collected through an online survey administered over a two-week period.

Participant Recruitment

The target population for this study included clinical laboratories in Pakistan. A total of 12 large-scale clinical laboratories were invited to participate in the survey, and all 12 responded (100% response rate). Inclusion criteria required laboratories to be operational and actively engaged in diagnostic activities. Recruitment was conducted via WhatsApp and email invitations sent to Consultant Pathologists and Lab Directors.

Survey Instrument

The survey consisted of 13 sections designed to comprehensively assess sustainability practices:

Section I: Demographics (e.g., laboratory size, type, and location).

Section II: Current Green Practices, divided into subsections:

- II-A: Energy Efficiency
- II-B: Water Conservation
- II-C: Material and Resource Optimization
- II-D: Waste Management
- II-E: Digital and Paperless Operations
- II-F: Chemical and Biological Sustainability
- II-G: Green Certifications and Policies
- II-H: Sustainability Training and Awareness
- II-I: Additional Practices

Section III: Barriers to Implementing Green Practices
Section IV: Environmental and Cost Impact
Section V: Future Goals and Improvements
The survey included a mix of closed ended (e.g., Likert scale, multiple-choice) and open-ended questions. The instrument was pretested for clarity and relevance with a small group of laboratory professionals prior to distribution. The full questionnaire is provided in Supplementary File.

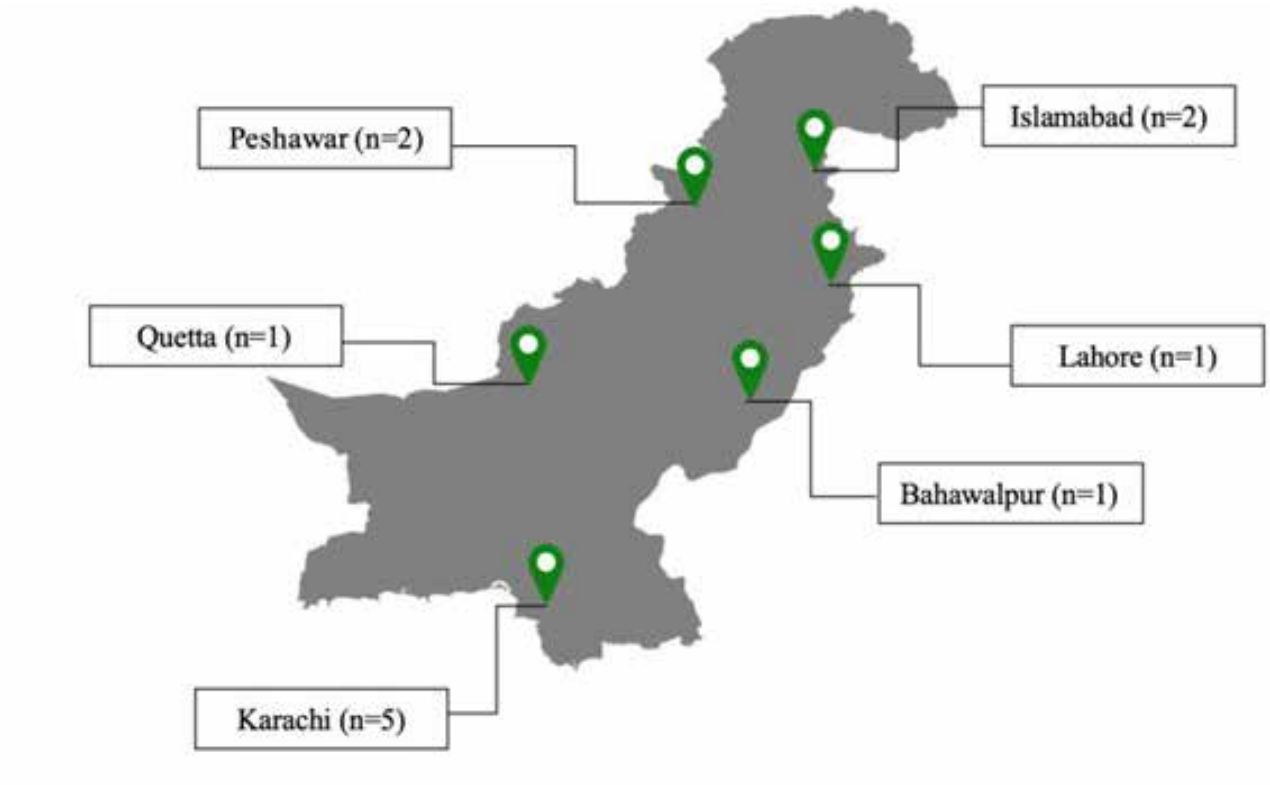
Data Collection
The survey was administered online using a secure survey platform (Google Forms). Participants were provided with a unique link to access the survey. The survey remained open for two weeks, and reminders were sent to non-responders after the first week. Electronic informed consent was obtained from all participants before they could proceed to the survey questions. Participation was voluntary, and responses were anonymized to ensure confidentiality.

Data Analysis
Descriptive statistics were used to summarize demographic data and responses related to current green practices, barriers, and future goals.

Ethical Considerations
All participants provided electronic informed consent prior to participating. The survey was designed to ensure anonymity, and no personally identifiable information was collected. Data were stored securely on password-protected servers, and access was restricted to the research team. Approval was sought from the institutional ethical review committee of the Aga Khan University (2025-11186-33468).

Results
Laboratory Size, Type, and Location
The survey collected responses from clinical laboratories of varying sizes, with the majority being large laboratories (> 50 staff members) (10/12; 83.3%). A smaller proportion comprised medium-sized laboratories (10–50 staff members) (2/12; 16.7%). Laboratories from multiple cities participated, as depicted in Figure 1, with the highest number of responses coming from Karachi (5/12; 41.6%), followed by Islamabad (2/12; 16.7%), Peshawar (2/12; 16.7%), and others from Lahore, Quetta, and Bahawalpur (1/12 each; 8.3%). Most participating laboratories were affiliated with private hospitals (5/12; 41.7%), while public hospitals (4/12; 33.3%), diagnostic centers (2/12; 16.7%), and independent laboratories (1/12; 8.3%) also contributed.

Figure 1: Map of Pakistan highlighting the cities from which responses were received, along with their respective frequencies.

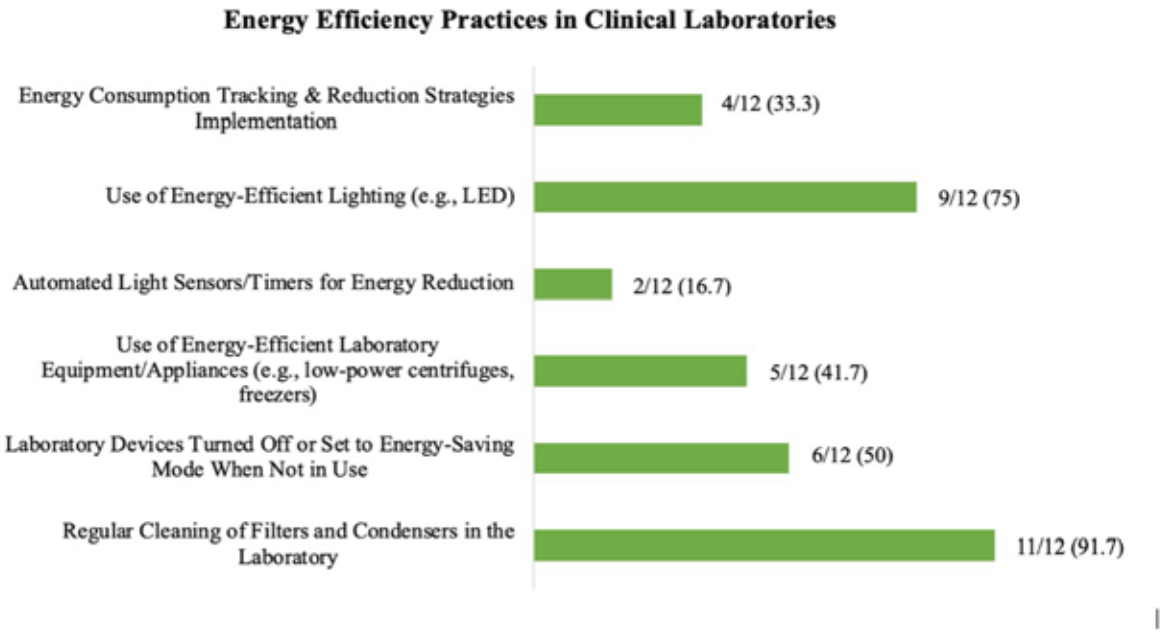


Current Green Practices

Energy Efficiency

Energy consumption tracking was implemented in 8/12 (66.7%) laboratories; however, only 4/12 (33.3%) actively employed strategies to reduce energy use. Energy-efficient lighting was widely adopted, with 9/12 (75.0%) laboratories utilizing LED lighting throughout their facilities and an additional 3/12 (25.0%) incorporating LED lighting in some areas. However, automated light sensors or timers were either fully or partially installed in only 2/12 (16.7%) laboratories. Furthermore, 7/12 (58.3%) laboratories lacked energy-efficient equipment, such as low-power centrifuges and freezers. While half of the laboratories (6/12, 50.0%) actively minimized unnecessary energy consumption through scheduled equipment shutdowns, a notable 11/12 (91.7%) prioritized regular maintenance by either frequently cleaning filters and condensers, or as needed. Findings have been summarized in Figure 2.

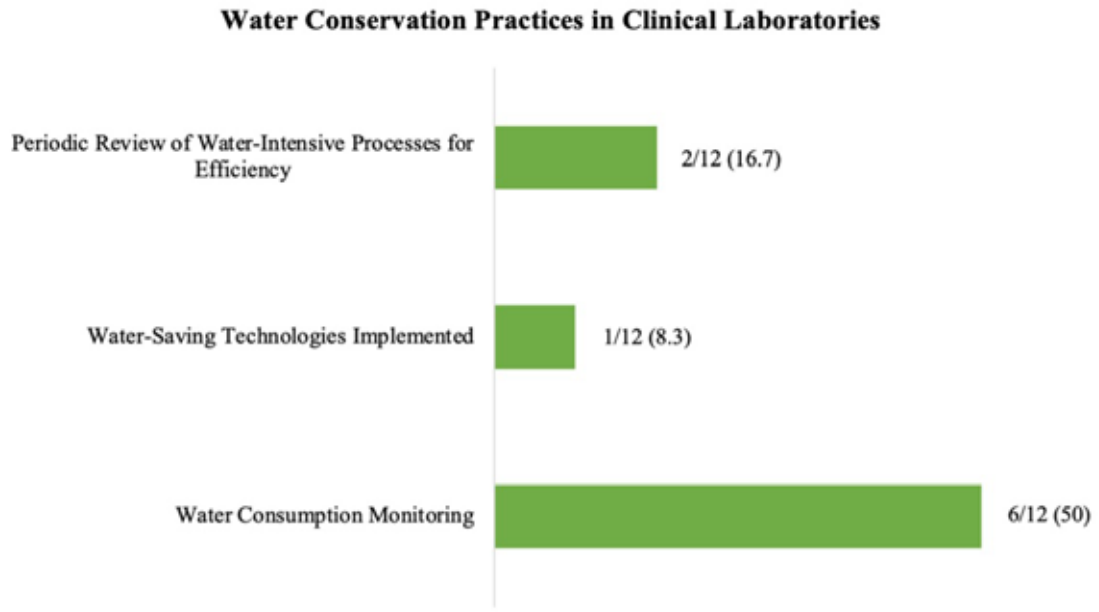
Figure 2: Adoption of energy efficiency practices in clinical laboratories, showing the percentage of laboratories implementing various sustainability measures.



Water Conservation

Water consumption (either all or only water-intensive processes) was actively monitored in 6/12 (50.0%) laboratories, while only 1/12 (8.3%) reported implementing water-saving technologies such as reusing wastewater from their reverse osmosis plant for flushing toilets. A majority, 10/12 (83.3%), did not conduct routine reviews of their water-intensive processes for efficiency improvements. Findings have been summarized in Figure 3.

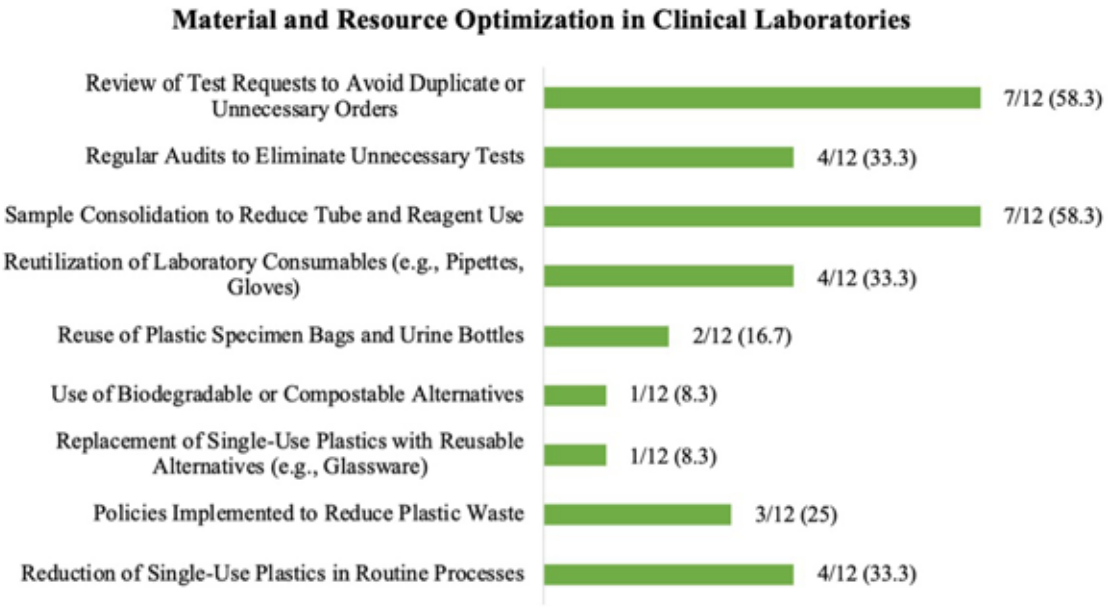
Figure 3: Adoption of water conservation practices in clinical laboratories, showing the percentage of laboratories implementing various sustainability measures.



Material and Resource Optimization

The survey results highlight varying levels of commitment to reducing single-use plastics in laboratories. While 4/12 (33.3%) laboratories have taken steps to limit the use of single-use plastics in routine processes, an equal proportion, 4/12 (33.3%), reported that no actions have been taken, indicating a gap in sustainability efforts. 3/12 (25.0%) laboratories have implemented policies aimed at reducing plastic waste, reflecting a structured approach toward sustainability. However, the adoption of alternative materials remains low, with only 1/12 (8.3%) replacing single-use plastics with reusable alternatives such as glassware, and another 1/12 (8.3%) switching to biodegradable or compostable options. 2/12 (16.7%) laboratories identified and reused plastic specimen bags and 24-hour urine bottles within safe limits to minimize plastic waste. Additionally, 4/12 (33.3%) implemented the reutilization of laboratory consumables, such as pipettes and gloves, where safety permitted. The consolidation of samples to reduce collection tube and reagent use was reported by 7/12 (58.3%), ensuring resource optimization and minimizing unnecessary consumption. Regular audits to identify and eliminate outdated or unnecessary tests were conducted in 4/12 (33.3%) laboratories, helping to prevent the excessive use of reagents and consumables. Furthermore, 7/12 (58.3%) laboratories reviewed test requests to reject unnecessary or duplicate test orders, improving efficiency and reducing excess material usage. Findings have been summarized in Figure 4.

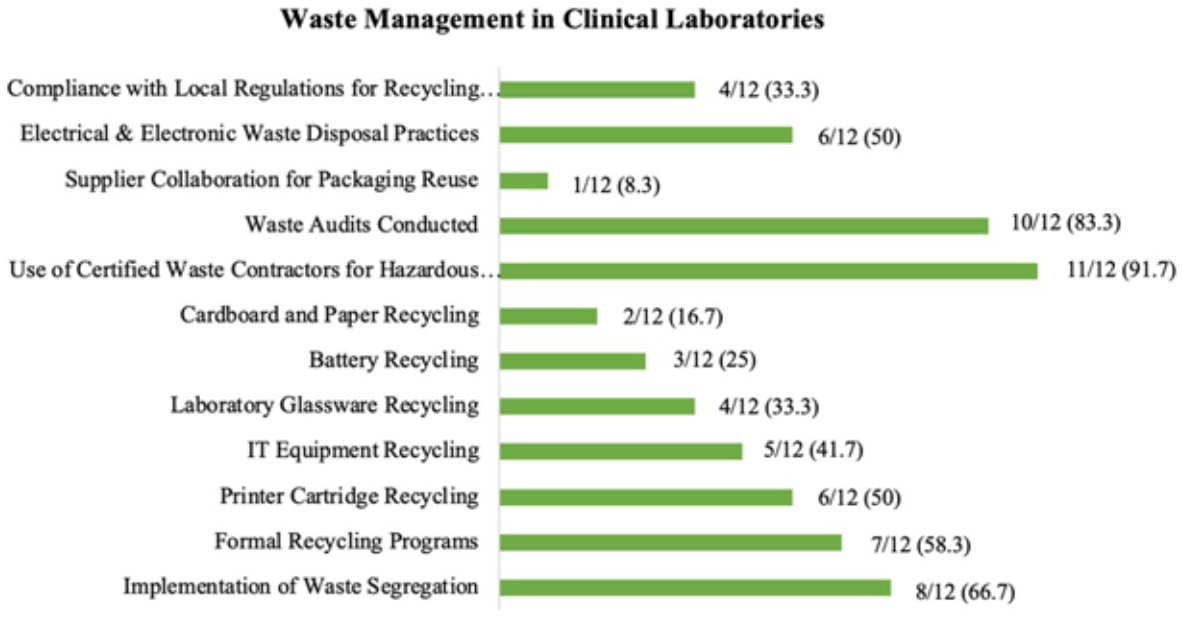
Figure 4: Material and resource optimization in clinical laboratories, showing the percentage of laboratories implementing various sustainability measures.



Waste Management

Waste segregation was implemented in 8/12 (66.7%) laboratories, ensuring the separation of hazardous, recyclable, and general waste. Despite this, 5/12 (41.7%) laboratories had no formal recycling programs in place. Among those actively engaged in recycling, printer cartridge recycling was reported by 6/12 (50.0%), IT equipment recycling by 5/12 (41.7%), and laboratory glassware recycling by 4/12 (33.3%), followed by battery recycling in 3/12 (25.0%) and cardboard/paper recycling in 2/12 (16.7%). For hazardous waste disposal, a majority of 11/12 (91.7%) laboratories relied on certified waste contractors to handle hazardous materials safely. Additionally, 10/12 (83.3%) laboratories conducted waste audits to evaluate and improve their waste separation practices, with 8/12 (66.7%) conducting them on a regular basis. However, only 1/12 (8.3%) laboratories worked with suppliers to return and reuse packaging materials such as Styrofoam, paper, and cardboard. Half of the laboratories (6/12, 50.0%) adopted electrical and electronic waste disposal practices, but only 4/12 (33.3%) reported compliance with local regulations for recycling items such as fluorescent tubes, batteries, phones, and computers. Findings have been summarized in Figure 5.

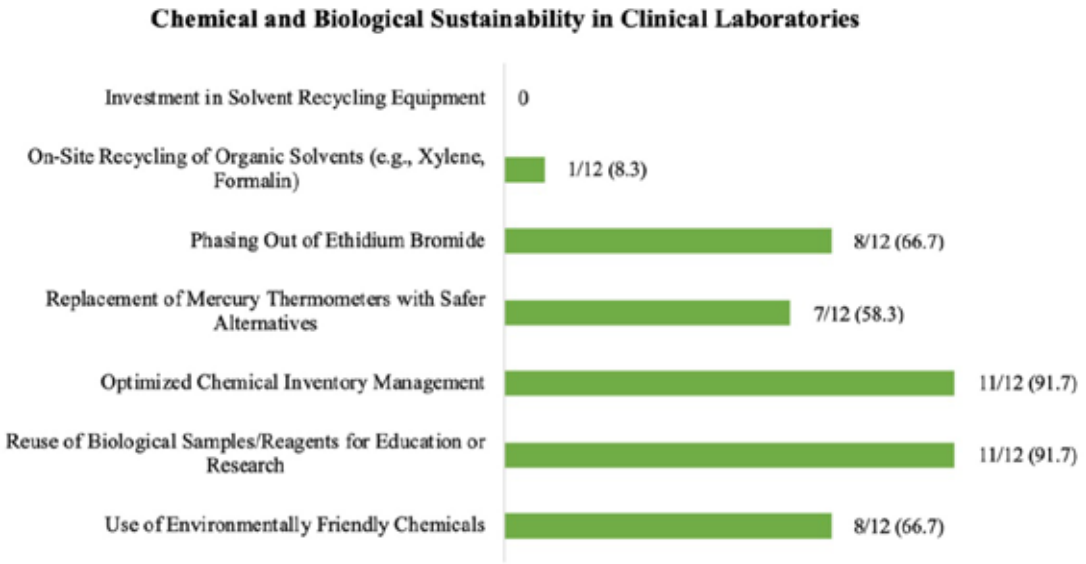
Figure 5: Waste management practices in clinical laboratories, showing the percentage of laboratories implementing various sustainability measures.



Digital and Paperless Operations
Paper reduction strategies were widely adopted, with 11/12 (91.7%) laboratories implementing electronic reporting to minimize paper use. Furthermore, 6/12 (50.0%) had fully transitioned to electronic medical records (EMRs). Moreover, 9/12 (75.0%) laboratories reported encouraging their staff to practice double-sided printing or avoid printing when necessary.

Chemical and Biological Sustainability
Environmentally friendly chemicals were used in 8/12 (66.7%) laboratories, primarily replacing toxic solvents with safer alternatives such as xylene substitutes, and 11/12 (91.7%) were involved in the reuse of biological samples or reagents for educational or research purposes where applicable. Additionally, 11/12 (91.7%) laboratories had optimized their chemical inventory management to minimize overstocking and reduce waste. Mercury thermometers were completely replaced with safer alternatives in 7/12 (58.3%) laboratories, while 8/12 (66.7%) had either completely or almost completely phased out ethidium bromide for safer nucleic acid staining methods. Only 1/12 (8.3%) laboratories recycled certain organic solvents like xylene and formalin on-site, while none of the laboratories had invested in a solvent recycler. Findings have been summarized in Figure 6.

Figure 6: Chemical and biological sustainability practices in clinical laboratories, showing the percentage of laboratories implementing various sustainability measures.



Green Certifications and Policies
5/12 (41.7%) of the laboratories had either a formal (2/12, 16.7%) or informal (3/12, 25.0%) sustainability and environmental policy in place. None of the laboratories had green purchasing policies, and only 3/12 (25.0%) reported that their suppliers were aware of their green policies, though these had not been formally communicated. 7/12 (58.3%) of the laboratories expressed that their suppliers provided eco-friendly reagents and materials, but this was not consistent. Additionally, 9/12 (75.0%) laboratories expressed that they had no interest in obtaining any green certifications such as ISO 14001 to align sustainability efforts with industry standards.

Sustainability Training and Awareness
Staff training on sustainability practices was implemented in 4/12 (33.3%) laboratories, with 1/12 (8.3%) conducting regular workshops or awareness sessions. Moreover, 9/12 (75.0%) laboratories reported ongoing initiatives or campaigns to promote environmental awareness among staff, with 2/12 (16.7%) conducting regular campaigns and initiatives.

Additional Practices
11/12 (91.7%) of the laboratories expressed that they did not use any carbon offset programs or any kind of renewable energy sources.

Barriers to Implementing Green Practices
The most cited barriers to sustainability adoption, as depicted in Figure 7, included limited resources (space, equipment, etc.) in 7/12 (58.3%) laboratories; lack of awareness or training among staff in 6/12 (50.0%); and financial constraints in 5/12 (41.7%). Additionally, resistance to change by staff was reported by 4/12 (33.3%), and regulatory or legal constraints by 4/12 (33.3%). Lack of support from leadership was cited by 1/12 (8.3%) laboratories. In terms of perceived significance, 7/12 (58.3%) respondents described these barriers as somewhat significant, while 5/12 (41.7%) considered them very significant.

Figure 7: Key barriers to sustainability adoption in laboratories.

Environmental and Cost Impact

The adoption of green practices resulted in a moderate reduction in operational costs for 5/12 (41.7%) laboratories, while 1/12 (8.3%) reported a significant reduction in operational costs (e.g., energy savings, reduced material usage, waste management cost reduction). However, 3/12 (25.0%) laboratories reported no change in operational costs. In terms of environmental benefits, 9/12 (75.0%) laboratories reported a reduction in waste production, and 5/12 (41.7%) reported reduced water usage, an increased recycling rate, and improved air quality (e.g., reducing chemicals or emissions). 4/12 (33.3%) noted a decrease in carbon footprint as well as improved resource efficiency. Additionally, 5/12 (41.7%) experienced cost savings, but only in specific areas (e.g., energy savings from energy-efficient equipment).

Future Goals and Improvements

When asked about desired future sustainability practices, respondents identified key areas for improvement. The most frequently suggested initiatives included obtaining green certifications and water-saving technologies in 7/12 (58.3%) laboratories; enhancing recycling programs in 6/12 (50.0%); investing in energy-saving equipment upgrades and comprehensive waste reduction programs in 5/12 (41.7%); and collaborating with environmentally conscious suppliers in 4/12 (33.3%).

Discussion

This study provides a comprehensive assessment of sustainability practices in clinical laboratories across Pakistan, shedding light on current green initiatives, obstacles to implementation, and potential areas for improvement. Although the sample size was limited to 12 laboratories, these represented major diagnostic centers located in the federal capital and all four provincial capitals of Pakistan. Given the centralized nature of diagnostic services in Pakistan-where provincial capitals and national referral centers serve as hubs for high-volume testing-this cohort provides a meaningful reflection of current practices and barriers to sustainability in the country. While not exhaustive, the geographic breadth and inclusion of both public and private sector laboratories strengthens the representativeness of our findings. While there is growing recognition of environmental responsibility, many laboratories face significant challenges in fully integrating sustainability into their operations. Stronger institutional commitment, clearer policy guidelines, and widespread awareness efforts are essential to making meaningful progress [12].

Although some laboratories have adopted sustainability measures, their implementation remains inconsistent. Energy efficiency strategies, such as LED lighting and regular equipment maintenance, indicate progress in reducing energy consumption; however, the adoption of automated light sensors and energy-efficient equipment remains limited, largely due to financial constraints and a lack of technical guidance. Many laboratories struggle to justify the initial investment despite the potential for long-term cost savings. To overcome these financial barriers, several mechanisms could be explored, including government-led subsidies, preferential tax benefits for eco-friendly equipment, targeted funding programs, and external support through international sustainability grants or public-private partnerships. The economic case for sustainability is strong: for instance, an Australian laboratory implementing ISO 14001 reported over $500,000 in savings by adopting digital reporting, energy-efficient upgrades, and optimized air conditioning use [7]. Such examples highlight the substantial return on investment (ROI) that can be

achieved when sustainability is integrated strategically [13]. Demonstrating these long-term cost savings may also help secure stronger leadership commitment-an obstacle identified by 8.3% of respondents-and emphasizes the importance of aligning financial incentives with sustainability outcomes to overcome both economic and institutional resistance to change.

Water conservation is another area where sustainability efforts remain inadequate. Most laboratories do not prioritize water-saving technologies, despite Pakistan's ongoing water scarcity crisis. Simple measures, such as installing low-flow fixtures, optimizing water-intensive processes, and recycling water where possible, could significantly reduce water waste. Greater regulatory oversight and awareness campaigns could encourage laboratories to adopt these measures [5,14].

Efforts to optimize material and resource use also vary widely. Some laboratories have reduced reagent use by consolidating samples, but the shift away from single-use plastics remains slow. While reducing single-use plastics is a key step toward minimizing environmental impact and long-term operational costs [15], many laboratories hesitate to make this transition due to short term cost concerns and limited supplier options. Collaboration with suppliers to provide affordable biodegradable alternatives, along with incentives for sustainable procurement, could facilitate greater adoption. Waste segregation is practiced in some laboratories, but the absence of formal recycling programs and the lack of supplier partnerships for reusing packaging materials highlight gaps in waste management. Establishing structured recycling programs, engaging suppliers in sustainable packaging solutions, and enforcing waste reduction policies could significantly improve these practices [4]. Additionally, digital documentation and electronic reporting have played a crucial role in reducing paper waste in majority of the clinical laboratories, demonstrating that sustainability efforts can be both feasible and impactful when integrated into routine workflows [16].

The adoption of environmentally friendly chemicals and safer alternatives reflects a positive shift toward greener laboratory practices. The replacement of toxic solvents and the phasing out of hazardous substances like mercury thermometers and ethidium bromide indicate growing awareness of chemical safety and sustainability. Additionally, the widespread reuse of biological samples and optimized inventory management highlight efforts to minimize waste. However, the limited adoption of solvent recycling, with no laboratories investing in dedicated recycling equipment, suggests that cost and infrastructure barriers remain. Addressing these challenges through financial incentives, supplier partnerships, and regulatory support could enhance sustainable chemical management in laboratories [17].

A lack of awareness and training among laboratory staff further exacerbates the problem. Many professionals are unfamiliar with best practices in sustainability or lack the motivation to implement them. Regular training programs, workshops, and incentive-based participation in sustainability initiatives could help bridge this knowledge gap and create a culture of environmental responsibility. Additionally, resistance to change and limited regulatory enforcement contribute to slow progress. Without strong leadership commitment and clearly defined sustainability policies, laboratories are unlikely to prioritize green initiatives [18].

The absence of formal green policies and certifications presents another significant challenge. Few laboratories have implemented sustainability policies, and most do not follow established green purchasing guidelines. This lack of commitment was further underscored by our finding that 75.0% of laboratories reported no interest in pursuing ISO 14001 certification. Voluntary uptake alone appears insufficient in this context, highlighting the need for stronger regulatory intervention. Regulatory bodies should therefore introduce mandatory sustainability frameworks, accompanied by technical support, training, and clear implementation guidelines to help laboratories achieve compliance. Regular audits, coupled with incentives such as preferential accreditation or funding for compliant laboratories, would promote accountability and encourage integration of sustainability into routine operations [5]. Strengthening regulatory oversight in this way would help laboratories align with international best practices and ensure that sustainability becomes an institutional priority rather than an optional initiative.

Despite the challenges, adopting sustainable practices offers significant environmental and financial benefits. Laboratories that have implemented waste reduction strategies report noticeable decreases in waste production and water usage. However, the financial impact varies, with some institutions experiencing cost savings while others see no immediate economic benefits. The financial feasibility of sustainability initiatives depends on factors such as the scale of implementation, initial investment costs, and access to government incentives. Cost-benefit analyses and long-term financial planning could help laboratories better understand and maximize the economic advantages of sustainable practices [19,20].

Supplier engagement also emerged as a critical area requiring improvement, with only 8.3% of laboratories reporting collaboration with suppliers on packaging reuse. Strengthening these partnerships is essential to reduce upstream waste and encourage the use of eco-friendly reagents and materials. Establishing return-and-reuse programs for packaging, promoting biodegradable or recyclable alternatives, and incorporating sustainability criteria into supplier contracts

could facilitate more responsible waste management [20]. Such initiatives, alongside investments in energy-efficient equipment, water conservation technologies, and structured waste management systems, would not only minimize laboratory-generated waste but also drive broader changes in the healthcare supply chain, embedding sustainability across the lifecycle of laboratory operations and ensuring long-term environmental and financial benefits [5].

To advance sustainability in clinical laboratories, a multifaceted approach is necessary. Education, policy enforcement, and financial incentives must work together to create meaningful change. Training programs tailored for laboratory staff can increase awareness and encourage practical implementation of green practices, while regulatory bodies should take a more active role in enforcing sustainability policies and offering incentives to help laboratories transition smoothly [5]. Recognized sustainability certifications such as ISO 14001 should be actively promoted to establish a structured framework for green practices. Adopting these certifications would not only standardize sustainability efforts but also help laboratories align with global environmental standards. Additionally, fostering collaborations between government institutions, healthcare providers, and environmental organizations could create a more cohesive and effective approach to sustainability. By working together, these stakeholders can ensure that clinical laboratories contribute to a greener and more efficient healthcare sector [5].

This study has several limitations that should be acknowledged. The most important is the small sample size (n = 12), which limits the generalizability of the findings. While the participating laboratories included major institutions from the federal capital and all four provincial capitals, the results cannot be considered fully representative of all clinical laboratories in Pakistan. The low response rate may reflect factors such as survey fatigue among laboratory professionals or limited institutional interest in sustainability, both of which could have influenced participation. Consequently, the sample may be biased toward laboratories already engaged in sustainability initiatives, potentially overestimating the adoption of green practices. Despite these limitations, the study provides valuable initial insights and highlights critical areas for policy development and future research.

While this study provides important baseline insights, future research should focus on developing context-specific strategies for implementing sustainability initiatives in Pakistan. Practical next steps could include pilot programs in large public and private laboratories to test the feasibility of water-saving technologies, energy-efficient equipment upgrades, and structured waste recycling systems. Establishing multi-institutional collaborations between laboratories, government agencies, and environmental organizations could facilitate shared learning and resource optimization. Research should also explore the cost-effectiveness of different green interventions in the Pakistani context, helping to build an economic case for sustainability. Finally, incorporating sustainability metrics into laboratory accreditation frameworks and national health policies would provide a structured pathway for scaling up successful initiatives across the country.

Conclusion

This study underscores the urgent need for structured sustainability policies, financial incentives, and educational programs to enhance green laboratory practices in Pakistan. While some progress has been made, significant gaps remain in energy efficiency, waste management, and regulatory compliance. Overcoming these challenges requires strong institutional leadership, stakeholder collaboration, and supportive regulatory frameworks. By integrating sustainable practices into routine laboratory operations, the healthcare sector can contribute to environmental conservation while improving long-term operational efficiency and cost-effectiveness.

Competing interests

None declared.

Ethical Approval

Approval was sought from the institutional ethical review committee of the Aga Khan University (2025-11186-33468).

Author Statement

Sibtain Ahmed conceived the idea, designed the study, and wrote the first draft. Alizeh Sonia Fatimi contributed to writing, data compilation, and questionnaire development. Imran Siddiqui conceived the idea, assisted with study design, questionnaire development, and final draft review. Ghazanfar Abbas, Sahar Iqbal, Mohsin Shafi, Khushbakht Arbab, Rizwan Uppal, Asma Shaukat, Muhammad Dilawar Khan, Muhammad Qaiser Alam Khan, Adnan Mustafa Zubairi, Syed Haider Nawaz Naqvi, and Junaid Mahmood Alam were involved in data collection, assisted with figures, reviewed the first draft, and suggested revisions. Tomris Ozben contributed to questionnaire design, reviewed the final draft, and provided improvements.

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Supplementary

Survey Instrument

Sustainability Practices in Clinical Laboratories

This survey aims to gather insights into the current sustainability practices, barriers, and future goals of clinical laboratories. Your valuable input will help identify trends and opportunities for improving environmentally friendly practices in laboratory settings. The survey is designed to take approximately 10–15 minutes to complete. All responses will remain confidential and will be used solely for research purposes.

Thank you for considering participation in this survey. By proceeding, you acknowledge that:

1. Your participation is voluntary.
2. The information you provide will remain confidential and be used only for research purposes.
3. No identifiable data will be collected or shared.

Section 1 of 13

I. General Information

This section collects basic details about your laboratory, including its size, location, and organizational affiliation.

- What is the size of your laboratory? *
- Which city is your laboratory located in? *
- What type of organization is your laboratory part of?
- If your answer was "Other," please specify:

Section 2 of 13

II-A. Current Green Practices – Energy Efficiency

The following sections (II-A – II-I) explore the sustainability measures currently in place at your laboratory, covering energy efficiency, water conservation, waste management, and more. Your responses will provide valuable insights into the implementation of green practices and areas for potential improvement.

- Does your laboratory track energy consumption and implement strategies to reduce it? *
- Does your laboratory use energy-efficient lighting (e.g., LED)? *
- Are automated light sensors or timers installed to reduce energy usage? *
- Are energy-efficient laboratory equipment or appliances used? (e.g., low-power centrifuges, freezers) *
  - If your answer was "Yes," please specify which energy efficient equipment is used:
- Are laboratory devices turned off or placed in energy-saving modes when not in use? *
  - If your answer was "Yes," please specify which devices are turned off or placed in energy-saving modes when not in use:
- Does your laboratory frequently clean filters and condensers? *

Section 3 of 13
II-B. Current Green Practices – Water Conservation

- Does your laboratory monitor water consumption? *
- Have any water-saving technologies been implemented? (e.g., low-flow faucets, closed-loop systems) *
  - If your answer was "Yes," please specify which water-saving technologies have been implemented:
- Are water-intensive processes periodically reviewed for efficiency improvements? *

Section 4 of 13
II-C. Current Green Practices – Material and Resource Optimization

- What actions has your laboratory taken to reduce single-use plastics? (Select all that apply) *
- If you selected "Replacing single-use plastics with reusable alternatives," please specify which items have been replaced and with what alternatives:
- Does your laboratory identify and reuse items such as plastic specimen bags or 24-hour urine bottles within safe limits? *
- Are laboratory consumables, such as pipettes and gloves, reused where safety permits? *
- Does your laboratory consolidate samples to minimize the use of collection tubes and reagents? *
- Does your laboratory conduct regular audits to identify and eliminate outdated or unnecessary tests? *
- Are test requests reviewed to reject unnecessary or duplicate test orders? *

Section 5 of 13
II-D. Current Green Practices – Waste Management

- Does your laboratory separate waste into categories such as hazardous, recyclable, and general waste? *
- Are recycling programs in place for the following items? (Select all that apply) *
- If your answer was "Other," please specify:
- Are hazardous wastes disposed of by certified waste contractors? *
- Does your laboratory conduct regular waste audits to evaluate and improve separation practices? *
- Does your laboratory collaborate with suppliers to return and reuse packaging materials like Styrofoam, paper, or cardboard? *
- Are electrical and electronic wastes (e.g., fluorescent tubes, batteries, phones, computers) recycled in compliance with local regulations? *

Section 6 of 14
II-E. Current Green Practices – Digital and Paperless Operations

- Does your laboratory use electronic reporting to reduce paper consumption? *
- Are staff encouraged to use double-sided printing or avoid printing altogether? *
- Have digital technologies, such as electronic medical records (EMRs), been implemented to streamline processes? *

Section 7 of 13
II-F. Current Green Practices – Chemical and Biological Sustainability

- Are environmentally friendly or less hazardous chemicals used in your laboratory? *
  - If your answer was "Yes," please specify which environmentally friendly or less hazardous chemicals are used:
- Are chemical inventories optimized to minimize overstocking and waste? *
- Are biological samples or reagents reused for educational or research purposes where applicable? *
- Has your laboratory replaced mercury thermometers with safer alternatives? *
- Has your laboratory replaced the use of ethidium bromide for gels? *
- Does your laboratory recycle organic solvents like xylene and formalin on-site? *
- Has your laboratory invested in a solvent recycler? *

Section 8 of 13
II-G. Current Green Practices – Green Certifications and Policies

- Does your laboratory have green certifications (e.g., ISO 14001)? *
- Is there an internal sustainability or environmental policy guiding operations? *
- Does your laboratory have a green purchasing policy in place? *
- Are suppliers and contractors informed of the laboratory's green purchasing policies? *
- Do your suppliers provide environmentally friendly reagents or equipment (e.g., energy-efficient, water-saving, or

biodegradable)? *

Section 9 of 13

II-H. Current Green Practices – Sustainability Training and Awareness

- Are staff trained on sustainability practices and their importance? *
- Are there ongoing initiatives or campaigns to promote environmental awareness among staff? *

Section 10 of 13

II-I. Current Green Practices – Additional Practices

- Does your laboratory use carbon offset programs or renewable energy sources? *
- Have any innovative green practices been adopted that are unique to your laboratory?

Section 11 of 13

III. Barriers to Implementing Green Practices

- What are the main barriers your laboratory faces in adopting more green practices? (Select all that apply) *
- If your answer was "Other," please specify:
- How significant are these barriers in preventing further adoption of sustainable practices? *

Section 12 of 13

IV. Environmental and Cost Impact

- The implementation of sustainability initiatives in your laboratory has led to: (Select all that apply) *
- What environmental impact have you observed due to sustainability practices? (Select all that apply) *
- If your answer was "Other," please specify:
- Has your laboratory experienced any measurable cost savings from green practices? *

o If yes, please specify which areas you have experienced cost savings:

Section 13 of 13

V. Future Goals and Improvements

- What additional sustainability practices would you like to see implemented in your laboratory? (Select all that apply) *
- If your answer was "Other," please specify:
- Are there any other sustainability challenges or ideas you would like to share regarding your laboratory's operations?

Research Article

Temporal changes of liver function tests in relation to adiposity in the community: The CoLaus|PsyCoLaus Study

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Keywords
liver function tests, obesity, age, gender

Abstract

Background: Liver function test (LFT; including alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, and alkaline phosphatase) results are modulated by multiple factors, but their temporal changes have been insufficiently explored, especially in relation to aging and adiposity. First, we assessed the trends of LFTs levels over time across different age groups and sexes. Second, we tested the cross-sectional and longitudinal associations between levels of LFTs and anthropometric measurements capturing various degree of adiposity.

Methods: 5171 participants (2393 males), aged 35-75 years at baseline (2003-2006), from a prospective population-based cohort (CoLaus|PsyCoLaus study), were included and followed up until 2019-2023. Anthropometric measurements included body mass index, waist-to-height ratio, waist-to-hip ratio, relative fat mass, body shape index, body roundness index, waist-to-weight ratio and body surface area. Boxplots presented changes of LFTs across age groups. Multiple linear regressions and multilevel mixed models were used to analyze the cross-sectional and longitudinal associations between levels of LFTs and anthropometric measurements, adjusting for a large range of variables.

Results: LFTs values showed distinct temporal changes between age groups and sexes. Anthropometric measurements capturing various degree of adiposity demonstrated a strong and significant association ($p<0.001$) with all four LFTs in both cross-sectional and longitudinal analyses. These associations remained robust even after adjusting for multiple covariates.

Conclusion: In a population-based study, LFTs changed over time according to age and sex. These changes were independently associated with markers of adiposity, showing the importance of interpreting LFTs based on the clinical context, especially in presence of overweight or obesity.

Introduction

Liver function tests (LFTs; including alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, and alkaline phosphatase) are frequently performed in clinical practice [1-6], reflecting either hepatocyte integrity or cholestasis [2, 7-10]. Obesity and closely linked metabolic dysfunction-associated fatty liver disease (MAFLD) are widespread public health issues globally [3, 8-9, 11-14]. MAFLD arises from lipid accumulation in liver tissue, in the absence of significant chronic alcohol consumption, viral infection, or other chronic liver disease causes [11-12, 15]. MAFLD encompasses two entities that are: i) metabolic dysfunction-associated fatty liver (MAFL), characterized by steatosis; and ii) metabolic dysfunction-associated steatohepatitis (MASH), representing an inflammatory phase which involves various degrees of steatohepatitis and fibrosis potentially leading to cirrhosis and hepatocellular carcinoma [9, 11-13, 15-17]. However, MAFLD does not necessarily present with abnormal liver tests [12, 18], with 80% of affected people having normal alanine aminotransferase (ALT) levels [11]. A large body of evidence shows an increase in liver enzyme levels associated with the rising prevalence of obesity [4-6, 8, 11-13, 18], but there is still a lack of comprehensive understanding regarding their change over a prolonged period of time and their correlation with different clinical measures of obesity (such as body mass index [BMI], body shape index [BSI], body surface area [BSA], relative fat mass [RFM], weight-adjusted-waist index [WWI], waist-to-hip ratio [WHR] and waist-to-height ratio [WHtR]) [8, 13, 18-20].

Additionally, the relationship between LFTs and age and sex, which can blur any interpretation of longitudinal change of LFTs with another factor, remains unclear [19, 21-22]. While age was usually not considered as impacting LFTs levels, recent studies have shown ambiguous results [22]. A study by Dong et al showed a reduction of LFTs with increasing age [22]. Moreover, in the US National Health and Nutrition Examination Surveys, older age was associated with lower ALT values than at a younger age [5, 9, 12]. Regarding sexes, another study concluded that there exists a significant age-related correlation in ALT values among males, with higher levels of ALT observed in males aged 25-34 and 65-74 years [21]. The age-related correlation in ALT values among females was notably weaker, with only a slight increase observed around age 50. The levels of gamma-glutamyl transferase (GGT) rose until the age of 60 in males, while in females, they continue to increase throughout life [21]. However, most of these studies have been carried out in people with active medical conditions that can affect the interpretation of the evolution of liver tests [5, 9, 12, 21-22]. In general, most studies clearly show that ALT levels are higher in males than in females, regardless of age or BMI [4-6, 12, 18].

In this study, we first aimed to assess the changes of LFTs levels over time across different age groups and sexes. Second, we investigated the cross-sectional and longitudinal associations between levels of LFTs and anthropometric measurements capturing various degree of adiposity.

Methodology

CoLaus|PsyCoLaus Study

The CoLaus|PsyCoLaus study is an ongoing population-based prospective study conducted in the city of Lausanne, Switzerland, aiming to assess the biological and genetic determinants of cardiovascular disease, together with psychiatric disorders [23]. Briefly, a random sample of 6733 individuals aged 35-75 years from the population of Lausanne, Switzerland, was recruited between 2003 and 2006. Subjects were included if they consented to participate in the study. The first follow-up was performed between April 2009 and September 2012, the second follow-up between May 2014 and April 2017 and the third follow-up between April 2019 and September 2023. The information collected at follow-ups was the same as that collected during the baseline examination. For this study, data from the baseline (2003-2006), first (2009-2012), second (2014-2017) and third (2019-2023) follow-ups were used. The cantonal Ethics Commission of the Canton of Vaud approved the CoLaus|PsyCoLaus study ((http://www.cer-vd.ch) project number PB_2018-00038, reference 239/09), and all participants provided written informed consent.

Selection of participants

Participants were excluded based on missing data at baseline and follow-up and if high-sensitivity C-reactive protein (hs-CRP) level was ≥ 10 mg/L, indicative of an ongoing inflammatory process that might modify levels of LFTs.

Liver function tests

Blood analyses were conducted using fasting venous blood samples drawn from patients [23]. LFTs, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP), were measured at Lausanne University Hospital clinical laboratory.

Anthropometric measurements and LFT body weight and height were measured with participants barefoot and in light indoor clothes. Body weight was measured in kilograms to the nearest 100 g using a Seca® scale (Hamburg, Germany). Height was measured to the nearest 5 mm using a Seca® (Hamburg, Germany) height gauge. Body Mass Index (BMI) was calculated as the ratio of weight (in kilograms) to height squared (in meters). Waist-to-Height Ratio (WHtR) was determined by dividing waist circumference (in meters) by height (in meters). Waist-to-Hip Ratio (WHR) was obtained by dividing waist circumference (in meters) by hip circumference (in meters). Relative Fat Mass (RFM) was calculated using the formula [64 - (20 × height / waist circumference) + (12 × sex)], where sex is coded as 0 for males and 1 for females. Body Shape Index (BSI) was measured as

[waist circumference / weight$^{(-2/3)}$ × height $^{(5/6)}$]. Body Roundness Index (BRI) was calculated with the formula [364.2 - 365.5 × (1 - (0.5 × waist circumference / π)^2 / (0.5 × height)2)$^{0.5}$]. Waist-to-Weight Ratio (WWR) was calculated by dividing waist circumference by body weight. Body Surface Area (BSA) was calculated using the formula [weight$^{0.425}$ × height$^{0.725}$ × 0.007184]. Weight-adjusted waist index (WWI) was calculated by dividing waist circumference (cm) by the square root of weight (kg).

Covariates

Blood pressure and heart rate were measured thrice on the left arm, with an appropriately sized cuff, after at least 10 minutes' rest in the seated position using an Omron® HEM-907 automated oscillometric sphygmomanometer (Matsusaka, Japan). The average of the last two measurements was used for analyses. Serum lipids were measured using enzymatic colorimetric assays. Hs-CRP was assessed by immunoassay. Information on age, lifestyle, medical history of diabetes, alcohol consumption was obtained through a questionnaire. Alcohol consumption was obtained by asking if participants regularly consumed alcohol and their weekly consumption of wine, beer, and spirits in units per week. Smoking was categorized as never, former, and current.

Statistical analysis

Statistical analyses were performed using Stata version 17. Baseline characteristics of the study population are described as frequencies and percentages for categorical variables, mean and standard deviation, or median and 25th–75th percentile for continuous variables. The normality of continuous variables was assessed through histogram visualization and the Shapiro-Wilk test. Variables that exhibited skewness, such as LFTs, anthropometric measurements and hs-CRP, were log-transformed to approximate a normal distribution.

Cross-sectional analysis

For the cross-sectional analysis of baseline LFTs, confounders and anthropometric measurements were included. We compared LFTs, anthropometric measurements, sociodemographic characteristics, blood pressure levels (systolic blood pressure (SBP) and diastolic blood pressure (DBP)), lipid status (total cholesterol (TC), low-density lipoprotein cholesterol (LDL)), and hs-CRP levels between males and females using independent-samples t-tests. T-test or Mann–Whitney U test were used for continuous variables, and the chi-squared test for categorical variables. Multiple linear regression models were used to investigate the association of LFTs levels (each as independent variables) with anthropometric measurements (as dependent variables). Two models were used: 1) adjusting for age and sex; and 2) adjusting additionally for smoking, alcohol use, prevalence of diabetes, SBP, DBP, LDL, TC and hs-CRP. Results were expressed as beta coefficient and 95% confidence interval. As a sensitivity analysis, we stratified all analyses by sex.

Longitudinal analysis

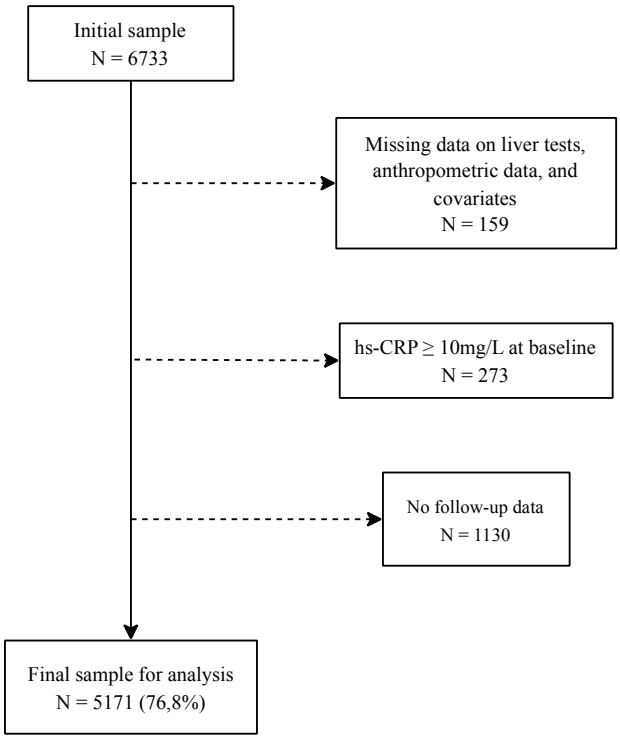
To explore the temporal changes in LFTs values across different age groups, boxplots were used to visually compare the patterns of values for each LFT in males and females. The participants were categorized into four age groups: < 40 years, 40-54 years, 55-69 years, and ≥ 70 years. For each age group, LFTs values were plotted for each survey.

To investigate the longitudinal association between LFTs and anthropometric measurements, we used a multilevel mixed-model approach for baseline, first, second and third follow-ups, including the same baseline confounders as in the cross-sectional analysis. Our model incorporated both fixed and random effects to comprehensively account for individual variability and potential confounding factors. The fixed effects included anthropometric measurements, follow-up time, and their interaction term, together with the same baseline confounders as in the cross-sectional analysis. The fixed effects elucidated how changes in LFTs were associated with anthropometric measurements over time. The random effects comprised random intercepts and random slopes, capturing individual-level variability in baseline LFTs levels and their rates of change over time. As a sensitivity analysis, we stratified all analyses by sex.

Results

Out of 6733 participants who completed the baseline survey, 159 (2.4%) were excluded due to missing information on LFTs and anthropometric data, and 273 (4%) due to a hs-CRP level ≥ 10 mg/L. Additionally, 1130 participants (16.8%) were excluded due to absence of follow-up data. The final sample size eventually comprised 5171 participants (76.8%) (Figure 1).

Figure 1: Enrolment flow chart for study population.



hs-CRP, high-sensitivity C-reactive protein

Baseline characteristics of participants

Baseline characteristics of participants indicated that males were slightly younger than females (mean age: 51.57 vs 52.7 years, p = 0.003). Males showed higher levels of LFTs (ALT, AST, ALP, and GGT) compared to females, highlighting sex-specific patterns in LFTs. Additionally, males had higher anthropometric measurements of adiposity, a higher prevalence of diabetes, a higher blood pressure (systolic and diastolic), higher LDL-cholesterol and alcohol consumption. In contrast, females exhibited higher HDL-cholesterol and hs-CRP levels (Table 1). The comparison between included and excluded participants showed that the included participants were younger, smoked less, had a higher education, drank more alcohol, were less likely to have diabetes and had a higher HDL-cholesterol. In excluded participants, their BMI was higher, and they presented higher hs-CRP and blood pressure levels. Total cholesterol, LDL-cholesterol, waist-to-hip ratio and the body surface area were similar between the two groups. All four LFTs, ALT, AST, ALP, and GGT, were significantly higher in the excluded people than included ones (24 vs 23 IU/L, p-value 0.001; 28 vs 27 IU/L, p-value 0.003; 68.2 vs 61.9 IU/L, p-value 0.002; 24 vs 20 IU/L, p-value 0.005, respectively) (Supplementary Table 1).

Table 1: Baseline characteristics of participants.

Variable	Total	Males	Females	P value*
	N = 5171	N = 2393	N = 2778	
Age (years)	52.1 (10.6)	51.57 (10.5)	52.7 (10.5)	0.003
Smoking status (n, %)				<0.001
Never	2126 (41.1)	835 (34.8)	1291 (46.4)	
Former	1729 (33.4)	920 (38.4)	809 (29.1)	
Current	1316 (25.4)	638 (26.6)	678 (24.4)	
Education level (n, %)				<0.001
High	1102 (21.3)	623 (26.03)	479 (17.2)	
Middle	1321 (25.5)	577 (24.1)	744 (26.7)	
Low	2748 (53.1)	1193 (49.8)	1555 (55.9)	

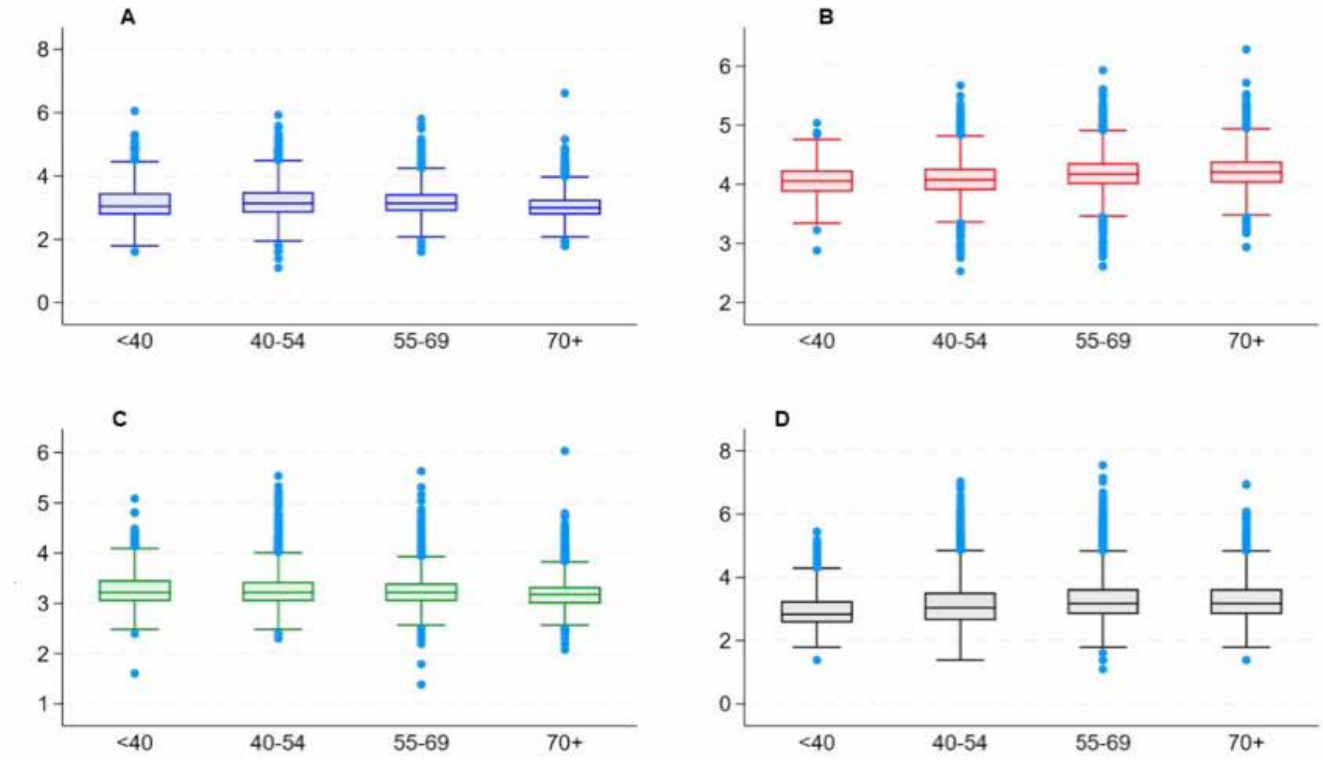
Alcohol drinker (weekly consumption)	4 (0-10)	7 (2-14)	2 (0-6)	0.04
Excessive alcohol consumers (n, %)	1029 (19.9)	566 (23.6)	463 (16.6)	0.02
BMI (kg/m2)	24.4 (4.23)	26.3 (3.81)	24.7 (4.45)	<0.001
BSI (m11/6kg-2/3)	0.078 (0.074; 0.082)	0.081 (0.078; 0.083)	0.075 (0.072; 0.079)	0.004
BSA (m0.725kg0.425)	0.006 (0.005; 0.007)	0.006 (0.006; 0.007)	0.0059 (0.0056;0.0063)	<0.03
RFM	31.02 (22.9; 38.35)	38.69 (36; 41.38)	23.49 (19.49; 27.57)	<0.001
WWI (m/kg2)	10.34 (9.82; 10.87)	10.55 (10.11; 11.01)	10.10 (9.58, 10.71)	0.002
WHtR (ratio)	0.51 (0.46; 0.56)	0.53 (0.5; 0.57)	0.49 (0.44; 0.54)	0.001
WHR (ratio)	0.86 (0.80-0.92)	0.92 (0.88-0.96)	0.81 (0.77- 0.86)	<0.001
hs-CRP (mg/l)	1.18 (0.6-2.4)	1.17 (0.6-2.1)	1.2 (0.6-2.5)	0.01
Diabetes (yes/no)	274 (5.3)	191(7.9)	83 (2.9)	0.03
SBP (mmHg)	126.8 (17.3)	130.8 (16.2)	123.4 (17.51)	<0.001
DBP (mmHg)	78.8 (10.7)	80.92 (10.7)	77.09 (10.48)	<0.001
HDL-C (mmol/L)	1.6 (0.4)	1.4 (0.35)	1.82 (0.42)	<0.001
TC (mmol/L)	5.51 (1)	5.53 (0.99)	5.57 (1.01)	0.05
LDL (mmol/L)	3.3 (0.9)	3.4 (0.8)	3.2 (0.91)	<0.001
Liver tests (IU/L)				
Alanine aminotransferase	23 (17-32)	29 (29-39)	15 (15-24)	<0.001
Aspartate aminotransferase	27 (23-33)	30 (25-37)	24 (21-29)	<0.001
Alkaline phosphatase	61.9 (51-75)	64 (54-75.6)	60.9 (49.3-75.6)	<0.001
Gamma-glutamyl transpeptidase	20 (14- 32)	27 (19-43)	16 (12-23)	<0.001

BMI, body mass index; BSA, body surface area; BSI, body shape index; RFM, relative fat mass; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; WWI, weight-adjusted-waist index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; CRP, C-reactive protein; HDL-C, High-Density Lipoprotein; TC, Total Cholesterol; LDL, Low-Density Lipoprotein Cholesterol. Continuous variables shown as mean (SD) with p according to t-test; categorical variables as % with p according to $\chi 2$, median (25th-75th percentile) with p according to Mann–Whitney U-test (§). *Comparing males and females.

Distribution of liver function tests according to age

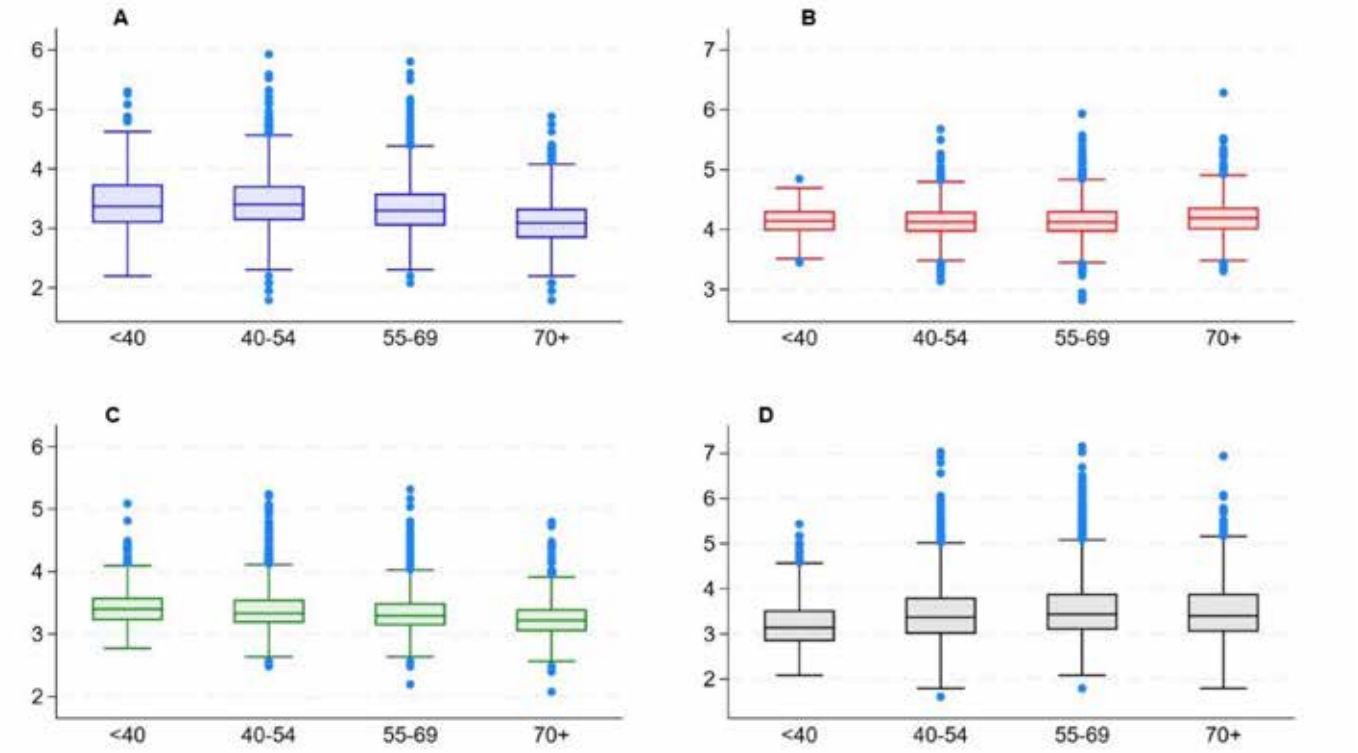
To analyze the trend in LFTs values based on age, boxplots were generated for different age groups (< 40 years, 40-54 years, 55-60 years, > 70 years) across all follow-up periods. Figure 2 illustrates that the progression of liver test values varies with age for both males and females, with distinct patterns observed for each LFT. Concisely, ALT levels either slightly increased or remained stable in middle-aged people (40-70 years) and decreased after 70 years. AST and GGT levels showed a consistent increase with age, while ALP levels remained relatively stable but exhibit a slight decrease after 70 years. The prospective change of LFTs levels by age and sex is depicted in Figures 3 and 4.

Figure 2: Distribution of liver function tests according to age ranges in total population.



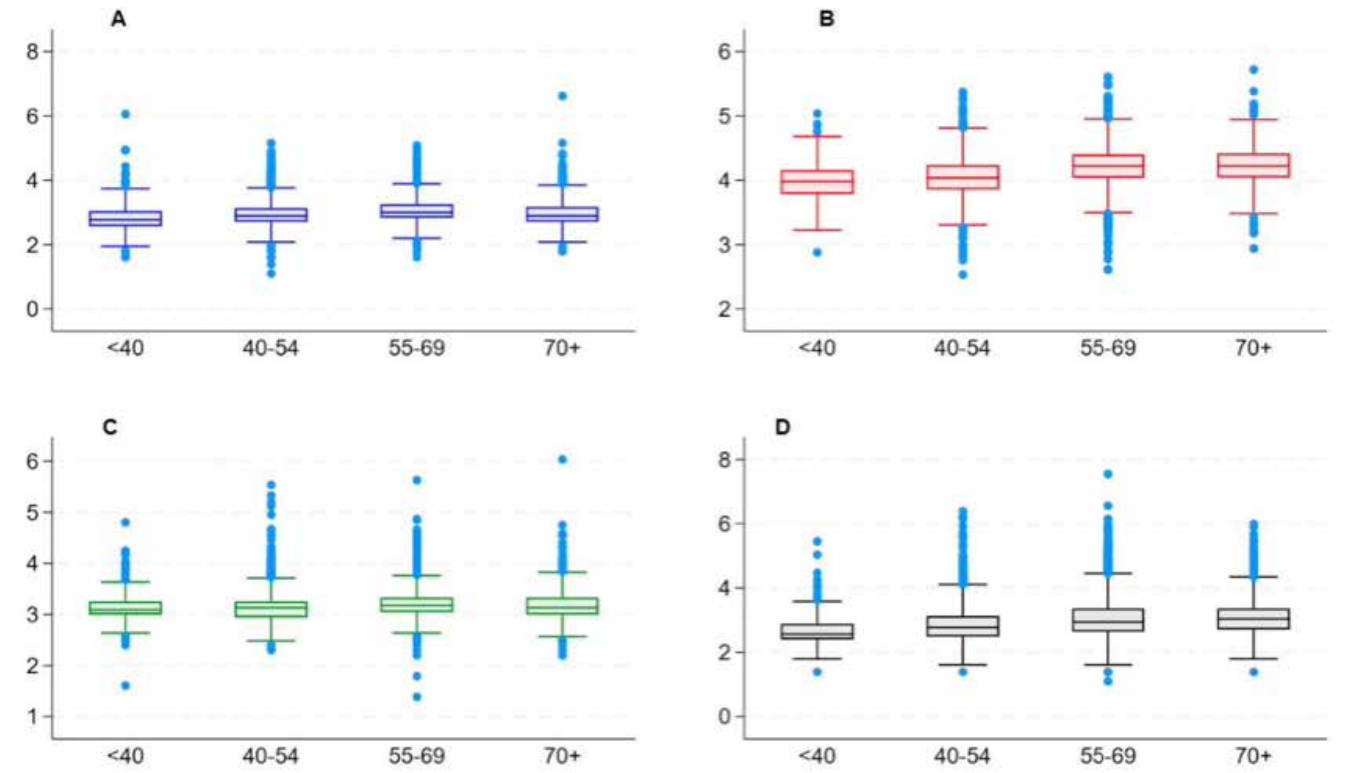
Panels A, B, C and D show the distribution of log-transformed alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase, respectively, by age groups.

Figure 3: Distribution of liver function tests according to age ranges in males.



Panels A, B, C and D show the distribution of log-transformed alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase, respectively, by age groups.

Figure 4: Distribution of liver function tests according to age ranges in females.



Panels A, B, C and D show the distribution of log-transformed alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase, respectively, by age groups.

Association between liver function tests and anthropometric variables of adiposity

The associations between LFTs and anthropometric variables capturing adiposity at baseline are presented in Table 2. BMI, BSI, BSA, RFM, WWI, WHtR, and WHR were positively associated with all four LFTs, ALP, AST, ALP and GGT (p-value < 0.001). These associations remained stable after adjusting for a broad range of potential confounders (Model 2).

Table 2: Associations between log-transformed liver function tests and anthropometric variables at baseline (2003-2006).

	Total Model 1		**Total Model 2**	
	Coefficient (95% CI)	**P-value**	**Coefficient (95% CI)**	**P-value**
	N = 5171		N = 5171	
Alanine aminotransferase (IU/L)				
BMI (kg/m2)	0.04 (0.03; 0.04)	<0.001	0.02 (0.02; 0.03)	<0.001
BSI (m11/6kg-2/3)	2.002 (1.81; 2.19)	<0.001	1.47 (1.28; 1.66)	<0.001
BSA (m0.725kg0.425)	1.83 (1.72; 1.93)	<0.001	1.52 (1.40; 1.64)	<0.001
RFM	0.74 (0.70; 0.78)	<0.001	0.67 (0.63; 0.72)	<0.001
WWI (m/kg2)	2.01 (1.83; 2.19)	<0.001	1.49 (1.30; 1.67)	<0.001
WHtR (ratio)	1.42 (1.32; 1.51)	<0.001	1.19 (1.08; 1.30)	<0.001
WHR (ratio)	2.26 (2.13; 2.40)	<0.001	1.02 (0.8; 1.20)	<0.001
Aspartate aminotransferase (IU/L)				
BMI (kg/m2)	0.015 (0.014; 0.019)	<0.001	0.009 (0.007; 0.01)	<0.001
BSI (m11/6kg-2/3)	1.03 (0.91; 1.15)	<0.001	0.80 (0.67; 0.92)	<0.001
BSA (m0.725kg0.425)	0.84 (0.78; 0.91)	<0.001	0.73 (0.65; 0.81)	<0.001
RFM	0.35 (0.33; 0.38)	<0.001	0.34 (0.32; 0.37)	<0.001
WWI (m/kg2)	0.91 (0.79; 1.02)	<0.001	0.69 (0.57; 0.81)	<0.001
WHtR (ratio)	0.58 (0.52; 0.65)	<0.001	0.50 (0.43; 0.58)	<0.001

WHR (ratio)	1.08 (0.99; 1.17)	<0.001	0.40 (0.28; 0.51)	<0.001
Alkaline phosphatase (IU/L)				
BMI (kg/m2)	0.013 (0.011; 0.016)	<0.001	0.006 (0.004; 0.009)	<0.001
BSI (m11/6kg-2/3)	0.59 (0.47; 0.70)	<0.001	0.50 (0.39; 0.62)	<0.001
BSA (m0.725kg0.425)	0.38 (0.31; 0.48)	<0.001	0.29 (0.22; 0.34)	<0.001
RFM	0.18 (0.16; 0.20)	<0.001	0.16 (0.13; 0.19)	<0.001
WWI (m/kg2)	0.75 (0.64; 0.86)	<0.001	0.52 (0.41; 0.64)	<0.001
WHtR (ratio)	0.48 (0.42; 0.54)	<0.001	0.33 (0.26; 0.40)	<0.001
WHR (ratio)	0.47 (0.39; 0.57)	<0.001	0.24 (0.13; 0.35)	<0.001
Gamma-glutamyl transpeptidase (IU/L)				
BMI (kg/m2)	0.043 (0.039; 0.048)	<0.001	0.02 (0.01; 0.03)	<0.001
BSI (m11/6kg-2/3)	2.96 (2.71; 3.21)	<0.001	2.02 (1.78; 2.26)	<0.001
BSA (m0.725kg0.425)	2.15 (2.01; 2.45)	<0.001	1.57 (1.41; 1.72)	<0.001
RFM	0.90 (0.85; 0.95)	<0.001	0.72 (0.66	

Results express variations in log-transformed LFTs per a 1-unit increase in log-transformed anthropometric measure. BMI, body mass index; BSI, body shape index; BSA, body surface area; RFM, relative fat mass; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; WWI, weight-adjusted-waist index. Model 1 was adjusted for age, sex. Model 2 was adjusted for age, sex, smoking, and alcohol use, education levels, prevalence of diabetes, SBP, DBP, LDL, TC, hs-CRP.

Table 3 presents the associations between LFTs and anthropometric variables (BMI, BSI, BSA, RFM, WWI, WHtR and WHR) at baseline, stratified by sex. Among males, ALT and GGT showed a positive association with all anthropometric variables. However, AST and ALP presented a few exceptions: AST was positively associated with all anthropometric variables except BSI, while GGT demonstrated a positive association only with BSI and WWI. In females, all LFTs were positively associated with all anthropometric variables.

Table 3: Associations between log-transformed liver function tests and anthropometric variables at baseline (2003-2006), stratified by sex.

	Males		**Females**	
	Coefficient (95% CI)	**P-value**	**Coefficient (95% CI)**	**P-value**
	N = 2393		N = 2778	
Alanine aminotransferase (IU/L)				
BMI (kg/m2)	0.04 (0.03; 0.04)	<0.001	0.02 (0.01; 0.02)	<0.001
BSI (m11/6kg-2/3)	0.59 (0.22; 0.96)	0.002	0.31 (0.08; 0.54)	<0.001
BSA (m0.725kg0.425)	0.89 (0.68; 1.11)	0.000	0.61 (0.43; 0.80)	<0.001
RFM	1.30 (1.13; 1.45)	<0.001	0.29 (0.21; 0.36)	<0.001
WWI (m/kg2)	1.49 (1.16; 1.82)	<0.001	0.63 (0.42; 0.84)	<0.001
WHtR (ratio)	1.28 (1.10; 1.46)	<0.001	0.64 (0.50; 0.75)	<0.001
WHR (ratio)	1.72 (1.42; 2.02)	<0.001	0.67 (0.47; 0.86)	<0.001
Aspartate aminotransferase (IU/L)				
BMI (kg/m2)	0.01 (0.009; 0.015)	<0.001	0.007 (0.004; 0.01)	<0.001
BSI (m11/6kg-2/3)	0.043 (-0.23; 0.28)	0.728	0.25 (0.10; 0.40)	<0.001
BSA (m0.725kg0.425)	0.35 (0.21; 0.50)	<0.001	0.12 (0.009; 0.24)	<0.001
RFM	0.38 (0.26; 0.50)	<0.001	0.11 (0.06; 0.15)	<0.001
WWI (m/kg2)	0.34 (0.11; 0.59)	<0.001	0.35 (0.22; 0.49)	<0.001
WHtR (ratio)	0.38 (0.26; 0.50)	<0.001	0.63 (0.50; 0.75)	<0.001
WHR (ratio)	0.54 (0.34; 0.74)	<0.001	0.31 (0.18; 0.44)	<0.001
Alkaline phosphatase (IU/L)				
BMI (kg/m2)	-0.001 (-0.004; 0.002)	0.231	0.01 (0.008; 0.01)	<0.001
BSI (m11/6kg-2/3)	0.46 (0.24; 0.68)	<0.001	0.35 (0.19; 0.51)	<0.001

BSA (m0.725kg0.425)	-0.04 (-0.17; 0.08)	0.532	0.38 (0.22; 0.48)	<0.001
RFM	0.08 (-0.02; 0.19)	0.139	0.20 (0.15; 0.25)	<0.001
WWI (m/kg2)	0.33 (0.13; 0.53)	0.002	0.47 (0.33; 0.62)	<0.001
WHtR (ratio)	0.06 (-0.4; 0.17)	0.231	0.38 (0.29; 0.47)	<0.001
WHR (ratio)	0.14 (-0.03; 0.32)	0.112	0.28 (0.14; 0.42)	<0.001
Gamma-glutamyl transpeptidase (IU/L)				
BMI (kg/m2)	0.03 (0.02; 0.03)	<0.001	0.01 (0.01; 0.02)	<0.001
BSI (m11/6kg-2/3)	0.95 (0.47; 1.43)	<0.001	0.87 (0.58; 1.16)	<0.001
BSA (m0.725kg0.425)	0.73 (0.45; 1.02)	<0.001	0.52 (0.28; 0.76)	<0.001
RFM	1.28 (1.04; 1.51)	<0.001	0.24 (0.15; 0.33)	<0.001
WWI (m/kg2)	1.73 (1.30; 2.16)	<0.001	0.90 (0.64; 1.17)	<0.001
WHtR (ratio)	1.27 (1.04; 1.50)	<0.001	0.26 (0.17; 0.34)	<0.001
WHR (ratio)	1.93 (1.51; 2.33)	<0.001	0.93 (0.68; 1.18)	<0.001

Results express variations in log-transformed LFTs per a 1-unit increase in log-transformed anthropometric measure. BMI, body mass index; BSI, body shape index; BSA, body surface area; RFM, relative fat mass; WWI, weight-adjusted-waist index; WHtR, waist-to-height ratio; WHR, waist-to-hip ratio.

Longitudinal association between liver tests and anthropometric variables

The longitudinal associations between LFTs and anthropometric variables (BMI, BSI, BSA, RFM, WWI, WHtR and WHR) are presented in Table 4. Model 1, adjusting for age and sex, showed a positive association between all LFTs and anthropometric variables. These associations remained significant even after further adjustment for additional factors (including smoking, alcohol use, education levels, diabetes, blood pressure, cholesterol, and hs-CRP), although the magnitude of the associations slightly decreased.

Table 4: Longitudinal associations between log-transformed liver function tests and anthropometric variables.

	Total Model 1		**Total Model 2**	
	Coefficient (95% CI)	**P-value**	**Coefficient (95% CI)**	**P-value**
	N = 5171		N = 5171	
Alanine aminotransferase (IU/L)				
BMI (kg/m2)	0.027 (0.025; 0.029)	<0.001	0.028 (0.024; 0.03)	<0.001
BSI (m11/6kg-2/3)	0.16 (0.07; 0.26)	0.001	0.19 (0.09; 0.29)	<0.001
BSA (m0.725kg0.425)	1.25 (1.15; 1.34)	<0.001	1.04 (0.94; 1.14)	<0.001
RFM	0.53 (0.49; 0.56)	<0.001	0.46 (0.42; 0.60)	<0.001
WWI (m/kg2)	0.62 (0.60; 0.83)	<0.001	0.57 (0.54; 0.60)	<0.001
WHtR (ratio)	0.88 (0.82; 0.95)	<0.001	0.79 (0.72; 0.88)	<0.001
WHR (ratio)	1.11 (1.01; 1.22)	<0.001	0.98 (0.87; 1.09)	<0.001
Aspartate aminotransferase (IU/L)				
BMI (kg/m2)	0.009 (0.007; 0.01)	<0.001	0.007 (0.005; 0.009)	<0.001
BSI (m11/6kg-2/3)	0.27 (0.20; 0.34)	<0.001	0.26 (0.19; 0.33)	<0.001
BSA (m0.725kg0.425)	0.24 (0.17; 0.31)	<0.001	0.16 (0.09; 0.23)	<0.001
RFM	0.19 (0.17; 0.22)	<0.001	0.15 (0.12; 0.18)	<0.001
WWI (m/kg2)	0.40 (0.33; 0.46)	<0.001	0.35 (0.28; 0.42)	<0.001
WHtR (ratio)	0.36 (0.31; 0.40)	<0.001	0.30 (0.25; 0.35)	<0.001
WHR (ratio)	0.31 (0.24; 0.49)	<0.001	0.27 (0.19; 0.35)	<0.001
Alkaline phosphatase (IU/L)				
BMI (kg/m2)	0.01 (0.009; 0.014)	<0.001	0.007 (0.006; 0.009)	<0.001
BSI (m11/6kg-2/3)	0.16 (0.10; 0.21)	<0.001	0.12 (0.06; 0.17)	<0.001

BSA (m0.725kg0.425)	0.44 (0.37; 0.51)	<0.001	0.31 (0.24; 0.38)	<0.001
RFM	0.24 (0.22; 0.27)	<0.001	0.15 (0.12; 0.18)	<0.001
WWI (m/kg2)	0.29 (0.24; 0.35)	<0.001	0.17 (0.12; 0.22)	<0.001
WHtR (ratio)	0.36 (0.32; 0.41)	<0.001	0.29 (0.23; 0.35)	<0.001
WHR (ratio)	0.17 (0.11; 0.23)	<0.001	0.06 (0.001; 0.12)	0.004
Gamma-glutamyl transpeptidase (IU/L)				
BMI (kg/m2)	0.03 (0.031; 0.037)	<0.001	0.025 (0.023; 0.029)	<0.001
BSI (m11/6kg-2/3)	0.39 (0.23; 0.50)	<0.001	0.36 (0.25; 0.47)	<0.001
BSA (m0.725kg0.425)	1.60 (1.46; 1.70)	<0.001	1.40 (1.001; 1.27)	<0.001
RFM	0.56 (0.50; 0.63)	<0.001	0.41 (0.36; 0.46)	<0.001
WWI (m/kg2)	0.78 (0.67; 0.89)	<0.001	0.63 (0.52; 0.73)	<0.001
WHtR (ratio)	1.07 (0.97; 1.25)	<0.001	0.86 (0.77; 0.94)	<0.001
WHR (ratio)	1.42 (1.22; 1.55)	<0.001	1.23 (1.10; 1.35)	<0.001

Results express variations in log-transformed LFTs per a 1-unit increase in log-transformed anthropometric measure. BMI, body mass index; BSI, body shape index; BSA, body surface area; RFM, relative fat mass; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; WWI, weight-adjusted-waist index. Model 1 was adjusted for age, sex. Model 2 was adjusted for age, sex, smoking, and alcohol use, education levels, prevalence of diabetes, SBP, DBP, LDL, TC, hs-CRP.

The same analyses stratified by sex are presented in Table 5. Among males, only AST showed a positive association with all anthropometric variables. ALT and GGT were positively associated with nearly all anthropometric variables, except for BSI. Lastly, ALP did not demonstrate a positive association with BMI, BSA or WHR. Among females, almost all LFTs showed a positive association with anthropometric variables, with the sole exception of the association between AST and BSI.

Table 5: Longitudinal associations between log-transformed liver function tests and anthropometric variables, stratified by sex.

	Males		**Females**	
	Coefficient (95% CI)	**P-value**	**Coefficient (95% CI)**	**P-value**
	N = 2393		N = 2778	
Alanine aminotransferase (IU/L)				
BMI (kg/m2)	0.034 (0.029; 0.037)	<0.001	0.018 (0.015; 0.020)	<0.001
BSI (m11/6kg-2/3)	0.05 (-0.13; 0.24)	0.565	0.204 (0.089; 0.320)	<0.001
BSA (m0.725kg0.425)	1.30 (1.14; 1.46)	<0.001	0.85 (0.73; 0.98)	<0.001
RFM	1.15 (1.03; 1.26)	<0.001	0.30 (0.26; 0.35)	<0.001
WWI (m/kg2)	0.74 (0.56; 0.92)	<0.001	0.45 (0.34; 0.56)	<0.001
WHtR (ratio)	1.13 (1.02; 1.25)	<0.001	0.56 (0.48; 0.64)	<0.001
WHR (ratio)	1.48 (1.29; 1.67)	<0.001	0.65 (0.52; 0.78)	<0.001
Aspartate aminotransferase (IU/L)				
BMI (kg/m2)	1.11 (1.03; 1.26)	<0.001	0.004 (0.002; 0.006)	<0.001
BSI (m11/6kg-2/3)	0.25 (0.11; 0.39)	<0.001	0.03 (-0.05; 0.11)	0.465
BSA (m0.725kg0.425)	0.32 (0.20; 0.43)	<0.001	0.08 (0.05; 0.11)	<0.001
RFM	0.42 (0.33; 0.50)	<0.001	0.27 (0.19; 0.35)	<0.001
WWI (m/kg2)	0.47 (0.33; 0.60)	<0.001	0.42 (0.35; 0.49)	<0.001
WHtR (ratio)	0.48 (0.37; 0.56)	<0.001	0.18 (0.12; 0.23)	<0.001
WHR (ratio)	0.46 (0.32; 0.51)	<0.001	0.12 (0.03; 0.21)	<0.001
Alkaline phosphatase (IU/L)				
BMI (kg/m2)	0.0001 (-0.002; 0.002)	0.921	0.01 (0.009; 0.016)	<0.001
BSI (m11/6kg-2/3)	0.22 (0.12; 0.31)	<0.001	0.04 (0.02; 0.11)	<0.001
BSA (m0.725kg0.425)	-0.05 (-0.15; 0.04)	0.271	0.54 (0.44; 0.60)	<0.001

RFM	0.11 (0.09; 0.18)	<0.001	0.15 (0.10; 0.18)	<0.001
WWI (m/kg2)	0.22 (0.13; 0.31)	<0.001	0.16 (0.11; 0.26)	<0.001
WHtR (ratio)	0.12 (0.05; 0.19)	<0.001	0.27 (0.23; 0.32)	<0.001
WHR (ratio)	-0.08 (-0.18; 0.01)	0.115	0.08 (0.007; 0.17)	0.003
Gamma-glutamyl transpeptidase (IU/L)				
BMI (kg/m2)	0.039 (0.033; 0.044)	<0.001	0.018 (0.014; 0.022)	<0.001
BSI (m11/6kg-2/3)	0.13 (-0.07; 0.34)	0.221	0.45 (0.32; 0.57)	<0.001
BSA (m0.725kg0.425)	1.48 (1.26; 1.71)	<0.001	0.92 (0.75; 1.09)	<0.001
RFM	1.11 (0.96; 1.25)	<0.001	0.31 (0.25; 0.36)	<0.001
WWI (m/kg2)	0.67 (0.47; 0.88)	<0.001	0.61 (0.48; 0.73)	<0.001
WHtR (ratio)	1.27 (1.17; 1.45)	<0.001	0.66 (0.55; 0.76)	<0.001
WHR (ratio)	1.84 (1.61; 2.07)	<0.001	0.93 (0.78; 1.08)	<0.001

Results express variations in log-transformed LFTs per a 1-unit increase in log-transformed anthropometric measure. BMI, body mass index; BSI, body shape index; BSA, body surface area; RFM, relative fat mass; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; WWI, weight-adjusted-waist index.

Factors influencing liver function tests longitudinally

Several factors (diabetes, smoking, blood pressure, cholesterol and alcohol) that can influence LFTs levels over time are presented in Supplementary Table 2. ALT was positively associated with all these factors. There were positive associations between AST and GGT with nearly all factors, except for smoking. ALP, on the other hand, demonstrated a positive association with all the factors, with the exception of diabetes.

Discussion

Based on a prospective and contemporaneous cohort of > 5000 middle-aged community-dwellers, our findings showed that LFTs progression varied by age. In general, ALT levels remained stable, while AST, ALP and GGT levels showed an increase with age. Whereas LFTs were higher in males at all age groups, patterns of changes over time were not exactly similar in both sexes, with ALP levels decreasing with age in males and increasing in females. Both at baseline and longitudinally, adiposity-related measures were strongly associated with LFTs, a relationship that remained robust after adjustment, though some sex-specific variations were observed. Additionally, LFTs changes correlated positively and independently with most traditional cardiovascular risk factors and markers of inflammation. However, smoking was associated only with changes in ALT and ALP, while diabetes showed no association with ALP changes.

We first analyzed the distribution of LFTs across different age groups in the total population, followed by a sex-specific analysis in males and females. A study by Leclerc et al, conducted on volunteer blood donors, aligns with our results, showing that ALT levels increased with age up to the fifth decade [5]. This study also reported an age-related increase in ALT levels in females, while in males, they rose until around 50 years old before declining [5]. Similarly, a study on 1673 community-dwelling males found a 30% decrease in ALT levels between those aged 70 to 74.9 years and those over 90 years [8]. Petroff et al also observed comparable trends with a moderate increase in AST values until around 60 followed by stabilization in females, and a continuous increase in GGT values up to the age of 60 in males, and throughout life in females [21]. However, their findings, suggesting a decline in ALT levels with age in males, differ from ours [21]. A cross-sectional study of 2364 participants [19] and data from the US National Health and Nutrition Examination Survey [5, 9, 12] also reported a decline in ALT levels with age. Similarly, a study by Chen et al found decreasing ALT levels with age in males but an increase in females [13]. Conversely, a cross-sectional study performed in 934 male blood donors showed a negative relation between ALT and AST, and age [20].

Further, we assessed how traditional cardiovascular factors and inflammation (such as diabetes, smoking, blood pressure, total cholesterol and LDL-cholesterol, hs-CRP, and alcohol consumption) associate with LFTs over time. Nearly all factors were associated with LFTs. A comparative study by Teshom et al reported a significant association between ALT, AST, ALP and various risk factors, including blood pressure, fasting blood sugar, triglycerides, total cholesterol and LDL-cholesterol, which aligns closely with our findings [2]. A cross-sectional study on 500 health-check examinees found that both cigarette smoking and alcohol consumption independently elevate GGT values but do not influence ALT or AST [24]. However, this is not enterily consistent with our findings, as smoking showed no association with GGT, while alcohol consumption was positively associated with all LFTs. Kim WR et al analyzed the available scientific data and concluded that cholesterol and triglycerides were positively associated with ALT, whereas smoking showed a negative association [6].

We investigated how several clinical anthropometric measures capturing adiposity, namely BMI, BSI, BSA, RFM, WWI, WHtR, and WHR, associated with LFTs changes. At baseline, our findings demonstrated that adiposity-related measures were consistently associated with LFTs, which was robust to adjustment. Most studies reported a strong positive association

between LFTs and BMI [3-6, 11-13, 18, 20]. However, only a few studies analyzed additional adiposity parameters over a prolonged period. One cross-sectional study, conducted on 5724 participants, found a positive association between ALT ant two measures of adiposity, that is BMI and WHR [12]. Similarly, a cross-sectional study, on 934 male blood donors aged 18 to 68 years, reported associations between ALT, AST, and GGT with BMI, central adiposity, as well as waist and hip circumference [20]. Expanding on this, a cross-sectional study of patients with type 2 diabetes highlighted waist circumference, BMI, AST levels, and educational background as key clinical predictors of significant and advanced fibrosis in primary care [25]. Physicians should take these factors into account and integrate this understanding into their clinical decision-making and patient management.

Strengths and limitations

The main strengths of the present study included a population-based prospective design using both cross-sectional and longitudinal data. With a large sample size of over 5000 participants, the study provided robust statistical power. It also accounted for a variety of confounders, such as cardiovascular risk factors, inflammation, and lifestyle factors. Furthermore, we were able to assess associations between LFTs and a wide range of anthropometric measures, with consistent findings. The inclusion of various adiposity indices offered for a more nuanced understanding of body composition's impact on LFTs. Some limitations should be acknowledged. First, a sizable portion of the baseline sample was excluded from the analyses. Participants who were excluded due to missing follow-up data or elevated hs-CRP levels had significantly different baseline characteristics, including higher BMI and LFTs. This exclusion may as well have favored the selection of the most motivated individuals (with complete data and follow-ups), potentially causing selection bias. Second, the study was conducted with a middle-aged population from the city of Lausanne, Switzerland, which may limit its generalizability to other populations. Finally, the observed elevation in LFTs could be influenced by various factors, including ethnicity, comorbidities, patient medications [7], dietary habits, physical activity, and genetic predispositions, among others, which were not accounted for.

Conclusion

Our study highlights the role of adiposity-related clinical markers that are independently associated with changes in LFTs. Temporal variations in LFTs should then be interpreted in the context of the clinical context, comprising age, sex, and cardiometabolic factors.

From a clinical perspective, the elevation of LFTs in individuals with overweight and obesity represents an indirect sign of potential MASH and, consequently, significant liver fibrosis development. Our study highlights the importance of LFTs monitoring in these individuals.

Data availability

The data of CoLaus|PsyCoLaus study used in this article cannot be fully shared as they contain potentially sensitive personal information on participants. According to the Ethics Committee for Research of the Canton of Vaud, sharing these data would be a violation of Swiss legislation with respect to privacy protection. However, coded individual-level data that do not allow researchers to identify participants are available upon request to researchers who meet the criteria for data sharing of the CoLaus|PsyCoLaus Datacenter (CHUV, Lausanne, Switzerland). Any researcher affiliated to a public or private research institution who complies with the CoLaus|PsyCoLaus standards can submit a research application to research.colaus@chuv.ch or research.psycolaus@chuv.ch. Proposals requiring baseline data only, will be evaluated by the baseline (local) Scientific Committee (SC) of the CoLaus and PsyCoLaus studies. Proposals requiring follow-up data will be evaluated by the follow-up (multicentric) SC of the CoLaus|PsyCoLaus cohort study. Detailed instructions for gaining access to the CoLaus|PsyCoLaus data used in this study are available at www.colaus-psycolaus.ch/professionals/ how-to-collaborate/.

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Declaration of Conflict of interests

The authors of this article declare that they have no conflicts of interest related to the content of this manuscript.

Credit author statement

Noushin Ahanchi Sadat was responsible for the methodology, software development, and contributed to the supervision of the project. Professor Julien Vaucher conceived the project and provided supervisory oversight. Dr. Montserrat Fraga served as a reviewer and advisor, offering her expertise as a hepatologist. Manon Scyboz conducted the literature review and was the primary author of the introduction, results, discussion, conclusion, and abstract.

Use of AI

During the preparation of this article, ChatGPT was used as a language assistance tool to help refine the English phrasing of certain sections. This support was particularly helpful in improving sentence structure, ensuring the use of appropriate scientific vocabulary, and enhancing overall clarity and coherence.

Abbreviations

ALP, alkaline phosphatase. ALT, alanine aminotransferase.

AST, aspartate aminotransferase. BMI, body mass index. BRI, body roundness index. BSA, body surface area. BSI, body shape index. DBP, diastolic blood pressure. GGT, gamma-glutamyl transferase. HCC, hepatocellular carcinoma. Hs-CRP, high-sensitivity C-reactive protein. LDL, low-density lipoprotein cholesterol. LFT, liver function test. MAFL, metabolic dysfunction-associated fatty liver. MAFLD, metabolic dysfunction-associated fatty liver disease. Mash, metabolic dysfunction-associated steatohepatitis. RFM, relative fat mass. SBP, systolic blood pressure. TC, total cholesterol. WHR, waist-to-hip ratio. WHtR, waist-to-height ratio. WWI, weight-adjusted-waist index. WWR, waist-to-weight ratio.

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Supplementary Tables

Table 1: Comparison between Included and Excluded.

Variable	Included	Excluded	P value*
Sample size	5171	1562	
Age (years)	52.1 (10.4)	54.3 (11.8)	0.004
Smoking status (n, %)			0.002
Never	2126 (41.1)	612 (38.4)	
Former	1729 (33.4)	454 (29.1)	
Current	1316 (25.4)	496 (31.8)	
Education level (n, %)			0.02
High	1102 (21.3)	218 (14.1)	
Middle	1321 (25.5)	304 (19.6)	
Low	2748 (53.1)	1040 (66.2)	
Alcohol drinker (units/week)	4 (0-10)	3 (0-10)	0.02
Excessive alcohol consumers (n, %)	1029 (19.9)	330 (21.1)	0.003
BMI (kg/m2)	24.4 (4.23)	26.8 (5.1)	0.04
BSI (m11/6kg-2/3)	0.078 (0.07; 0.082)	0.079 (0.07; 0.08)	0.04
BSA (m0.725kg0.425)	0.006 (0.005; 0.007)	0.006 (0.005; 0.006)	0.05
RFM	31.02 (22.9; 38.35)	34.11 (26.06; 40.25)	0.002
WWI (m/kg2)	10.34 (9.82; 10.87)	10.41 (9.8; 10.95)	0.01
WHtR (ratio)	0.51 (0.46; 0.56)	0.52 (0.47; 0.57)	0.04
WHR (ratio)	0.86 (0.80-0.92)	0.89 (0.83-0.95)	0.06
hs-CRP (mg/L)	1.18 (0.6-2.4)	1.92 (0.9-5.4)	0.02
Prevalence of diabetes	274 (5.3)	162 (10.4)	0.003
SBP (mm Hg)	126.8 (17.3)	131.5 (18.9)	0.006
DBP (mm Hg)	78.8 (10.7)	80.4 (11.1)	0.04
HDL-C (mmol/L)	1.6 (0.4)	1.5 (0.43)	0.03

TC (mmol/L)	5.5 (1.00)	5.6 (1.1)	0.06
LDL (mmol/L)	3.32 (0.9)	3.33 (0.9)	0.07
Liver tests (IU/L)			
Alanine aminotransferase	23 (17-32)	24 (17-35)	0.001
Aspartate aminotransferase	27 (23-33)	28 (23-35)	0.003
Alkaline phosphatase	61.9 (51-75)	68.2 (56-82)	0.002
Gamma-glutamyl transpeptidase	20 (14- 32)	24 (16-42)	0.005

BMI, body mass index; BSA, body surface area; BSI, body shape index; RFM, relative fat mass; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; WWI, weight-adjusted-waist index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; CRP, C-reactive protein; HDL-C, High-Density Lipoprotein; TC, Total Cholesterol; LDL, Low-Density Lipoprotein Cholesterol. Continuous variables shown as mean (SD) with p according to t-test; categorical variables as % with p according to $\chi 2$, median (25th-75th percentile) with p according to Mann–Whitney U-test * Compare the characteristics of participants who were included versus those who were excluded.

Table 2: Factors longitudinally influencing liver function tests.

	Coefficient (95% CI)	**P-value**
	N = 5171	
Alanine aminotransferase (IU/L)		
Diabetes	0.09 (0.07; 0.12)	<0.001
Smoking status	-0.01 (-0.02; -0.003)	0.008
SBP	0.002 (0.001; 0.003)	<0.001
DBP	0.005 (0.003; 0.008)	<0.001
TC	0.05 (0.04; 0.06)	<0.001
hs-CRP	0.005 (0.002; 0.007)	<0.001
LDL	0.04 (0.02; 0.05)	<0.001
Alcohol use	0.004 (0.003; 0.005)	<0.001
Aspartate aminotransferase (IU/L)		
Diabetes	0.03 (0.01; 0.05)	<0.001
Smoking status	-0.005 (-0.01; 0.001)	0.13
SBP	0.002 (0.001; 0.003)	<0.001
DBP	0.003 (0.002; 0.004)	<0.001
TC	0.04 (0.03; 0.05)	<0.001
hs-CRP	0.003 (0.002; 0.40)	<0.001
LDL	0.02 (0.01; 0.03)	<0.001
Alcohol use	0.004 (0.003; 0.005)	<0.001
Alkaline phosphatase (IU/L)		
Diabetes	0.003 (-0.011; 0.018)	0.62
Smoking status	0.011 (0.009; 0.013)	<0.001
SBP	0.0008 (0.0006; 0.0021)	<0.001
DBP	0.001 (0.001; 0.27)	<0.001
TC	0.025 (0.021; 0.030)	<0.001
hs-CRP	0.013 (0.012; 0.014)	<0.001
LDL	0.017 (0.01; 0.02)	<0.001
Alcohol use	-0.002 (-0.003; -0.001)	<0.001
Gamma-glutamyl transpeptidase (IU/L)		
Diabetes	0.13 (0.10; 0.16)	<0.001
Smoking status	0.012 (-0.001 ; 0.02)	0.07

SBP	0.0025 (0.0023; 0.0028)	<0.001
DBP	0.006 (0.005; 0.007)	<0.001
TC	0.08 (0.06; 0.09)	<0.001
hs-CRP	0.02 (0.01; 0.03)	<0.001
LDL	0.03 (0.01; 0.04)	<0.001
Alcohol use	0.011 (0.010; 0.012)	<0.001

Results express variations in log-transformed LFTs per a 1-unit increase in log-transformed anthropometric measure. BMI, body mass index; BSI, body shape index; BSA, body surface area; RFM, relative fat mass; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio; WWI, weight-adjusted-waist index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; hs-CRP, high-sensitivity C-reactive protein; TC, Total Cholesterol; LDL, Low-Density Lipoprotein Cholesterol.

Research Article

Lead Toxicity Testing in the Asia-Pacific - Practices, Challenges, and Policy Insights: An APFCB Communication and Publications Committee Survey report

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Keywords

Lead toxicity, Lead testing, APFCB C-CP, Asia-Pacific, Occupational exposure, Environmental contamination, Policy recommendations

Abstract

Background: Lead exposure remains a major health concern in the Asia-Pacific, particularly affecting children. Despite its significance, lead toxicity testing is underutilized because of limited awareness, resources, and policy support. On December 16, 2024, the APFCB C-CP (Asia-Pacific Federation for Clinical Biochemistry and Laboratory Medicine -Communication and Publications Committee) conducted Webcast & eLearning Program Webinar themed as "Protecting Health in Asia-Pacific: Laboratory Advances and Lead Exposure Prevention", aimed to address these issues and acknowledge need based solutions. An online survey was conducted during webinar in real time to assess the current lead-testing practices, common exposure sources, testing challenges, and policy changes.

Methods: A seven-question survey was distributed to webinar participants, covering testing frequency, methodologies, exposure sources, information sources, challenges, and policy needs. A total of 66 professionals attended the session and 22 complete surveys were collected from Nepal, India, Indonesia, Japan, and Australia.

Results: Lead testing was infrequent in the region, with 58.6% of the respondents reporting rare or no testing. Weekly testing has been reported in 20.7% of cases. The most commonly used methodology was point-of-care testing via anodic stripping voltammetry (37.5%) followed by electrothermal atomic absorption spectrometry (25%). Occupational exposure (39.1%) was the leading source of lead poisoning, followed by dietary sources (26.1%) and environmental contamination (21.7%). Academic journals (47.5%) were the primary educational resources. Key challenges included low awareness among healthcare providers (43.5%) and resource shortage (39.1%). The most recommended policy change was to increase government support (61.5%).

Conclusion: In conclusion, lead testing remains infrequent across many settings, with limited routine implementation and heavy reliance on point-of-care methodologies. Occupational exposure emerged as the predominant source of lead poisoning, underscoring the need for targeted interventions. Strengthening government support is identified as the most critical policy change to enhance lead testing and management efforts.

Background

Lead exposure remains a major public health concern in many regions, particularly in the Asia-Pacific, where its effects on vulnerable populations especially children are profound [1]. Despite its significance, lead toxicity testing is often underutilized because of limited awareness, resource constraints, and insufficient policy frameworks [1, 2]. Biomonitoring practices and identification of lead exposure pathways vary widely across countries. High-income nations have implemented comprehensive regulations addressing legacy lead sources, such as lead-based paint, water plumbing systems, and other environmental hazards [3]. While several countries have established regulatory frameworks that have contributed to reduced lead exposure, only a few have developed robust policies for laboratory diagnosis and routine blood lead level (BLL) testing [4]. There is a pressing need for international harmonization of stepwise laboratory diagnostic protocols to ensure consistency in the detection and management of lead poisoning.

Current lead testing and detection methods may not fully align with the evolving demographics and exposure patterns [5]. According to the Centers for Disease Control and Prevention (CDC), initial screening can be performed using a point-of-care device to analyze a capillary blood sample [6]. If the screening result is negative, further testing is typically unnecessary, unless clinically indicated. However, if a positive result is obtained; confirmatory testing using venous blood lead level measurement is required for definitive diagnosis. Venous samples are analyzed in laboratories using validated methods, such as graphite furnace atomic absorption spectroscopy (GFAAS), atomic absorption spectroscopy (AAS), and inductively coupled plasma mass spectrometry (ICP-MS). Strengthening biomonitoring efforts and improving data collection on exposure pathways would enhance our understanding of lead sources and enable more targeted prevention strategies.

One of the critical challenges is the lack of public awareness of low-level lead exposure. Although public health experts stress the importance of minimizing population-wide lead exposure, this issue remains under-recognized by laboratory professionals and the general public. Bridging these gaps through increased advocacy, improved diagnostic capabilities, and stronger policies is essential for effective lead exposure mitigation.

To address these pressing concerns, the Asia-Pacific Federation for Clinical Biochemistry and Laboratory Medicine Communication and Publications Committee (APFCB C-CP) conducted a Webcast & eLearning Program Webinar on December 16, 2024, themed as "Protecting Health in Asia-Pacific: Laboratory Advances and Lead Exposure Prevention" [7]. As part of the initiative, a survey questionnaire was developed to gather insights from the participants. The online survey aimed to assess the current lead testing methodologies used in laboratories across the region, identify common causes of elevated blood lead levels, highlight challenges in testing, and determine necessary policy changes recommended to improve the lead exposure prevention and management.

Methods

A structured questionnaire comprising seven closed ended questions was developed to gather insights from webinar participants regarding lead toxicity testing and related challenges in clinical laboratories across the Asia-Pacific region. Participants could select multiple options. The questionnaire was designed to be concise and focused to ensure clarity and encourage higher response rates [7]. These questions were developed based on a review of the relevant literature and consultation with relevant experts and Team APFCB C-CP. The questionnaire was then reviewed and approved by an independent expert in laboratory medicine and expert feedback was incorporated to refine the questionnaire before its release. The survey questions (Supplementary 1) focused on the following key areas.

1. Geographical distribution: Participants were asked to specify their country of practice to assess regional representation in the responses.
2. Lead testing Frequency – Respondents were asked to provide details on how often their laboratory conducts lead level testing, helping to evaluate testing accessibility and demand.
3. Testing methodologies: The survey gathered information on the laboratory techniques used for lead analysis, including atomic absorption spectrometry, inductively coupled plasma mass spectrometry, CLIA-waived point-of-care devices, and other methods to examine variations in testing practices.
4. Common Sources of lead exposure: Participants were asked to identify the most frequently observed sources of lead exposure among their patients, including occupational exposure, environmental contamination, dietary sources such as herbal remedies, and household items such as toys, to gain a broader understanding of regional exposure patterns.
5. Sources of Information on lead poisoning: Respondents were required to indicate how they acquired knowledge about lead poisoning, such as through academic literature, webinars and workshops, government guidelines, or news and social media, to assess key information dissemination channels.
6. Challenges in implementing lead testing: The questionnaire explored the barriers laboratories face in conducting lead toxicity testing, including resource limitations, regulatory challenges, and lack of awareness.

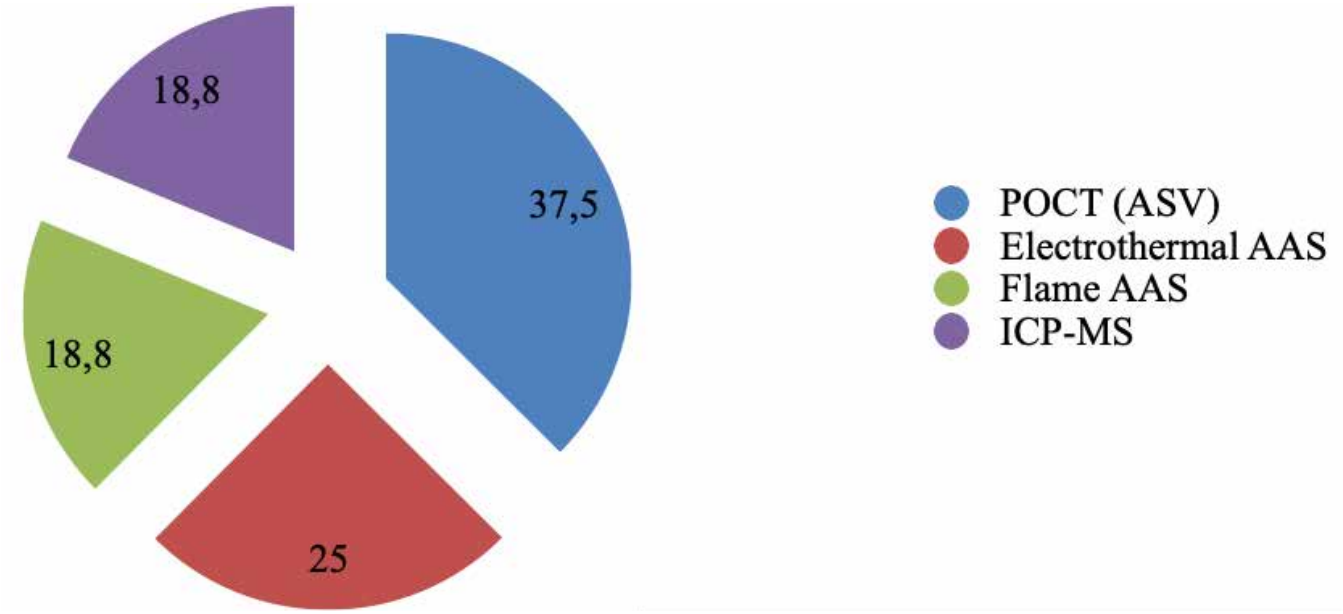
7. Policy recommendations: Participants were asked to share their perspectives on the policy changes needed to enhance lead screening, diagnosis, and management. Suggested improvements include increased government support for testing, stricter regulations on lead exposure, better training for healthcare workers, and greater assistance from international organizations.

The questionnaire was disseminated electronically during the webinar, allowing for real-time participation. A total of 66 professionals attended the session, representing various laboratories across the Asia-Pacific region. Of these, 22 participants from five countries Nepal, India, Indonesia, Japan, and Australia submitted complete responses. This study ensured strict anonymity and confidentiality of the collected data. Data were summarized using descriptive statistics, and all analyses were performed using Microsoft® Excel® 2019

Results

Lead testing is infrequent in the region, with 58.6% of laboratories reporting that they rarely or never perform it. Weekly testing was the second most common method (20.7%). The most commonly used methodology is point-of-care testing via ASV (anodic stripping voltammetry), followed by electrothermal atomic absorption spectrometry (Figure 1).

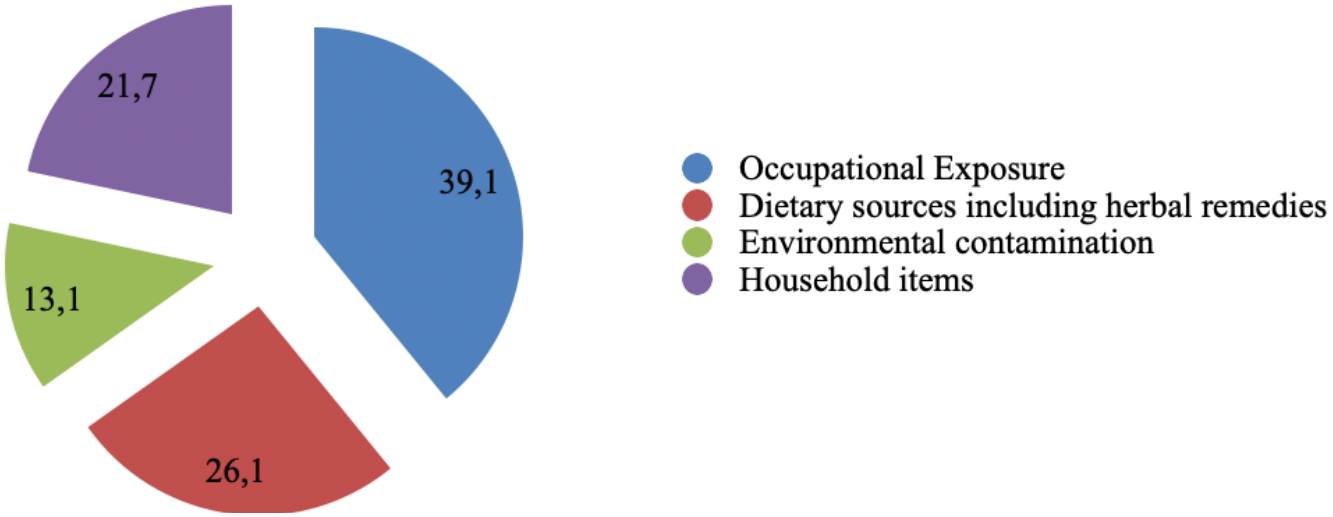
Figure 1: Commonly used methodologies to analyze blood lead level in Asia Pacific region.



Abbreviation: ASV-Anodic Stripping Voltammetry, POCT- Point of Care Testing, AAS- Atomic Absorption Spectrometry, ICP-MS- Inductively Coupled Plasma Mass Spectrometer

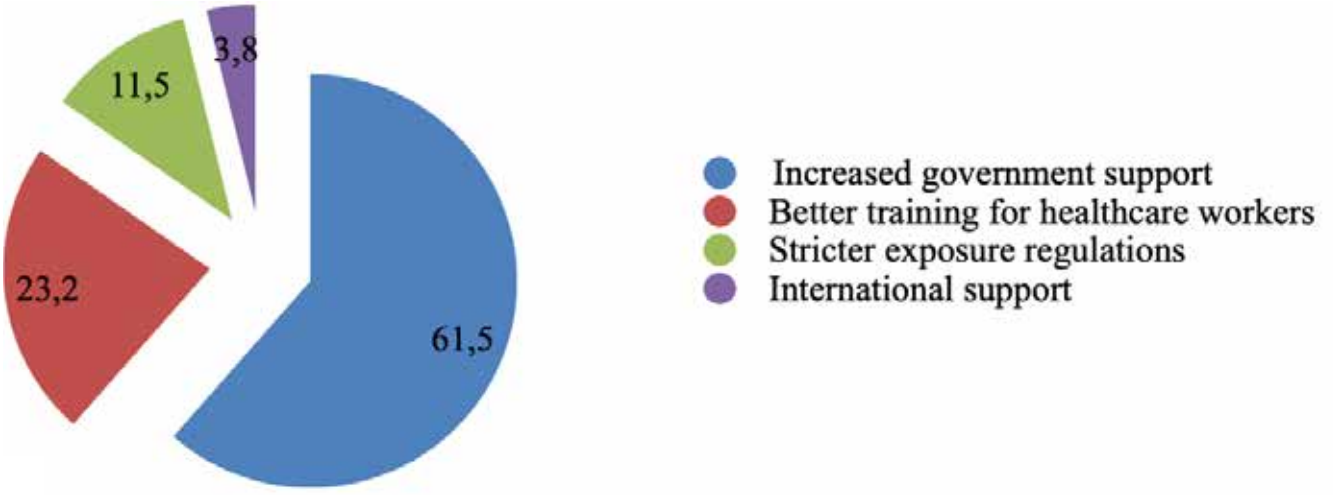
Occupational exposure was the most common cause of lead toxicity, as indicated by the participants, followed by dietary sources, including herbal remedies (26.1%) (Figure 2).

Figure 2: Commonest cause of lead toxicity in Asia Pacific region as pointed by the participants.



Academic journals were the primary source of information on lead poisoning (47.5%), followed by workshops/webinars and government health resources (15% each). News articles and social media were less utilized. Key challenges in lead testing included lack of awareness among healthcare providers (43.5%) and resource shortages (39.1%). The most commonly suggested policy improvement included increased government support (61.5%) (Figure 3).

Figure 3: Participants perspectives on policy changes needed to enhance lead screening, diagnosis, and management.



Discussion

The findings of this survey highlight several critical challenges in the implementation of lead toxicity testing across the Asia-Pacific region. One of the most notable issues is the limited frequency of lead testing, which suggests that many healthcare facilities either lack the necessary infrastructure or do not prioritize lead-level screening as part of routine diagnostics. This gap in testing accessibility may lead to under diagnosis and missed opportunities for early intervention in cases of lead exposure. An estimated one-third of children worldwide have elevated BLL, contributing to cognitive impairment and increased cardiovascular risk [8]. The lack of widespread BLL testing prevents accurate assessment of exposure and limits targeted interventions. Evidence from Georgia highlights the impact of population-level testing, which has revealed previously underestimated lead poisoning rates and led to policy action [9]. Expanding BLL testing is essential for identifying high-risk populations, driving policy changes, and implementing effective lead-exposure prevention strategies. A major barrier for lead testing identified in this survey was a lack of resources, including access to appropriate testing equipment and reagents. Advanced techniques, such as Graphite Furnace atomic absorption spectrometry and inductively coupled plasma mass spectrometry, which are considered gold standards for lead testing, are not widely available in all laboratories, which might be due to high costs

and technical requirements. Some laboratories may rely on alternative methods, such as point-of-care devices; however, the reliability and standardization of such approaches remain a concern [10, 11]. A study conducted in Japan found that point-of-care lead level analyzers showed a significant positive bias compared to ICP-MS at levels above 45 µg/dL [12]. Nevertheless, POCT devices based on anodic stripping voltammetry (ASV) offer rapid and accessible BLL testing, providing results in approximately three minutes from a small capillary blood sample [13]. These portable devices are essential in physician-office laboratories, regulatory-waived settings, and resource-limited areas where traditional laboratory testing is unavailable [13, 14]. Their ease of use and pre-calibrated disposable components make them effective for initial screening, though elevated results should be confirmed with laboratory-based ICP-MS or Graphite Furnace AAS testing. Portable testing devices have been essential in humanitarian crises such as the 2010 lead poisoning outbreak in Zamfara, Nigeria, where these devices allowed health workers to quickly test people on-site, leading to faster diagnosis and treatment, which helped save many lives [15]. Their ability to provide immediate results enhances their early detection and intervention, making them invaluable tools for lead exposure management in both clinical and emergency settings.

Awareness among healthcare providers regarding lead testing has emerged as a significant limitation. Clinicians and laboratory professionals may not routinely consider lead exposure in differential diagnoses, particularly in regions where lead poisoning is not commonly recognized as a public health threat. [16]. This lack of awareness can delay diagnosis and appropriate management, allowing continued exposure and worsening of health outcomes. Greater efforts are needed to educate healthcare workers about the importance of lead testing, potential sources of exposure, and the clinical manifestations of lead poisoning. Healthcare workers can be educated through targeted training programs, workshops, and continuing medical education sessions. Collaboration with public health agencies, professional associations, and laboratories can facilitate guideline dissemination and case-based learning. Additionally, hospitals and clinics can implement screening protocols and provide quick reference materials to aid diagnosis and management. Raising awareness through conferences, newsletters, and digital platforms further reinforces knowledge and encourages proactive detection and prevention of lead poisoning.

The survey also shed light on the primary sources of lead exposure reported by the participants. Occupational exposure was identified as the most common cause and this is seen particularly among workers in industries, such as battery manufacturing, metal recycling, and construction. Additionally, dietary sources, including the consumption of herbal remedies and environmental contamination, such as lead in water supplies, air pollution, and exposure to lead-based paints, have also been reported as significant contributors. A systematic review of 520 studies identified major sources of lead exposure, including informal lead-acid battery recycling and manufacturing, metal mining and processing, electronic waste, and lead adulteration in food, particularly in spices [17]. Lead exposure comes from different sources depending on the country. Cosmetics and traditional medicines are common sources in India and Nepal [18, 19]. China also has issues with electronic waste, traditional medicines, and industrial pollution [20]. Australia faces risks from paint, dust, imported toys, and traditional medicine [21]. In wealthier countries, past lead use continues to pose risks, while in lower-income countries, weak or poorly enforced regulations worsen the problem. These findings emphasize the need for targeted interventions such as workplace safety regulations, stricter monitoring of food and herbal products, and improved environmental policies to reduce lead contamination.

Although the survey included only 22 participants, they were laboratory experts specializing in heavy metal toxin analysis and research. Their insights offer a meaningful snapshot of lead toxicity testing in their regions and are valuable for guiding policy development. The limited number of respondents, even in an international webinar setting, also highlights how often this critical issue is overlooked.

To ensure effective lead toxicity testing across the Asia-Pacific region, targeted solutions are needed to address key gaps in accessibility, standardization, and awareness. Strengthening testing capacity, resources, and policies will enhance early detection, safeguard public health, and reduce lead exposure risks. Priority actions include:

1. Expanding regional representation by involving more countries.
2. Improving access to regular testing through increased funding and lab support.
3. Standardizing testing methodologies across the region.
4. Educating communities about common lead sources and promoting awareness.
5. Enhancing government guidelines and launching public awareness campaigns.
6. Streamlining regulatory processes for more efficient implementation.
7. Developing a quality assurance framework for point-of-care blood lead level testing.

Conclusion

This survey highlights the urgent need for stronger government support and policy action on lead testing. Wider access to advanced testing methods, and inclusion of laboratory professionals in decision making are essential. Beyond testing, laboratory experts play a vital role in interpretation, clinician guidance, and quality assurance and their involvement in policy development can strengthen screening programs, standardize protocols, and build laboratory capacity.

Conflict of Interest
None.

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Authors Contribution
VP - Conceptualization, Preparation of survey questionnaire, writing manuscript
VP, DP, PKD, MU- Conducted online survey, collected results and analyzed findings
DP, MU, RO, MLS- Scientific Review
PKD- Review and approval of final version

Ethics approval and consent to participate
This study is in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki. Formal ethical approval was not required, as the survey involved anonymized and voluntary responses collected during a webinar with participant consent. Surveys of this nature are exempt from ethics approval at the institution where it was conducted.

Data Availability Statements
The data generated and analyzed in the presented study are available from the corresponding author on request.

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Supplementary
Survey Questionnaire

1. Name of Country:

2. How frequently does your laboratory test for lead levels in patients?
a) Daily
b) Weekly
c) Monthly
d) Rarely/Never

3. What methodologies does your laboratory use for lead testing? (Select all that apply)
a) Flame atomic absorption Spectrometry
b) Inductively Coupled Plasma Mass Spectrometry (ICP-MS)
c) Electro thermal atomic absorption Spectrometry
d) CLIA waived POCT device based on Anodic stripping voltametry

4. What is the most common source of lead exposure identified in your region?
a) Occupational exposure
b) Environmental contamination
c) Dietary sources including herbal remedies
d) Household items including toys

5. Which of the following sources have you used to learn about lead poisoning? (Select all that apply)
a) Academic journals
b) News articles
c) Social media
d) Workshops/Webinars
e) Government health resources
f) Community organizations

6. What challenges do you face in implementing lead toxicity testing in your practice or region?
a) Lack of resources (equipment, reagents)
b) Limited awareness among healthcare providers
c) Policy barriers
d) Non-compliance from patient

7. What policy changes are needed to improve lead screening and management in the Asia-Pacific?
a) More government support for testing
b) Stricter rules on lead exposure
c) Better training for healthcare workers
d) Support from international organizations

Research Article

Risk Management in a Clinical Biochemistry Laboratory

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Keywords
risk management, laboratory, quality, FMEA, patient safety

Abstract

Introduction: To ensure compliance with new laboratory standards, it is imperative to adopt risk-based thinking, which involves a systematic examination of the functions, procedures, and activities associated with risks and opportunities. This article aims to explore the implementation of risk-based thinking in medical biology laboratories and to highlight the challenges inherent in this approach.

Materials and Methods: This descriptive study was conducted in the biochemistry laboratory of the Main Military Teaching Hospital of Tunis during the first half of 2024. A risk analysis was performed by a working group to identify failures by analyzing non-conformities recorded during the study period. The group adopted the Failure Mode and Effects Analysis (FMEA) methodology, an inductive approach well-suited to process analysis and mastered by all participants. Subsequently, a corrective action plan was developed for each process phase.

Results: Across the entire laboratory workflow, 33 distinct failure modes were identified and cataloged for each step, followed by a criticality analysis. The distribution of these failures was 36.36% in the pre-analytical phase, 33.34% in the analytical phase, and 30.3% in the post-analytical phase. A review of the severity of their effects revealed that a significant portion constituted major risks.

Conclusion: In response to the major risks identified at each stage of the laboratory workflow, a corrective action plan has been proposed. This plan outlines specific actions designed to reduce the criticality of these risks and enhance patient safety and quality of service.

Introduction

Risk management is a concept with ancient roots, first appearing around 3200 BC in the Tigris-Euphrates valleys under the guidance of the Asipu, who are considered among the earliest risk consultants [1, 2]. Following the Second World War, large corporations with diversified physical asset portfolios began to develop self-insurance mechanisms to cover the financial consequences of adverse events or accidental losses [3]. Modern risk management was implemented after 1955, initially within the insurance sector [4].

The concept of risk is not new to clinical laboratories, as it was implicitly addressed in previous versions of the ISO 9001 standard through preventive measures aimed at eliminating potential non-conformities and preventing their recurrence (ISO 9001:2008 [5], ISO/IEC 17025:2005 [6]). However, in the latest versions of standards such as ISO 9001:2015 [7], ISO/IEC 17025:2017 [6], and particularly ISO 15189:2022 [8], risk-based thinking is more pronounced and has become a mandatory requirement. Furthermore, the ISO 31000:2018 standard [9] defines risk management as the coordinated set of activities that an organization undertakes to direct and control risk.

Consequently, for a laboratory to achieve and maintain compliance with current standards, it is essential to understand and implement risk-based thinking by systematically examining its functions, procedures, and activities in relation to risks and opportunities. To address this need, this article explores the implementation of a risk-based framework in a medical biology laboratory and highlights the challenges posed by this approach.

Materials and Methods

Study Description

This descriptive study was conducted within the biochemistry laboratory of the Main Military Teaching Hospital of Tunis during the first half of 2024. A working group, composed of members from the laboratory's quality unit, was formed. The group convened on multiple occasions to analyze non-conformities recorded within the laboratory. The objective was to conduct a rigorous analysis of these failures to determine their root causes, evaluate their criticality, and implement preventive measures to mitigate associated risk factors.

Study Protocol

The working group first conducted a risk analysis by identifying failures encountered through the review of non-conformity records from the first half of 2024 related to the laboratory's core processes. The group selected the Failure Mode and Effects Analysis (FMEA) methodology, which is an inductive, process-oriented approach well-understood by all participants. In practice, the FMEA method was executed in five distinct steps:

- Step 1: Establish a working group. This crucial step involved forming a team of biologists and medical laboratory technicians who had received training in quality management.
- Step 2: Define the scope of the study. The scope was confined to the three core phases of the clinical biochemistry laboratory's workflow: pre-analytical, analytical, and post-analytical.
- Step 3: Describe the process. All steps within the workflow, from the pre-analytical phase to the final reporting of results, were mapped using flowcharts, specifying the personnel, documentation, and equipment required for each stage.
- Step 4: Analyze risks across the pre-analytical, analytical, and post-analytical phases. For each phase, the working group identified potential failure modes through brainstorming sessions and investigated their possible root causes. These causes were categorized using the 5M (Manpower, Method, Machine, Material, Milieu) framework and presented in Ishikawa (fishbone) diagrams. The group then assigned Severity (S) and Frequency (F) scores to each failure mode based on established quantification grids. The criticality of each failure was calculated using the formula:

Criticality (C) = Severity (S) × Frequency (F)

- Step 5: Define the action plan. For each significant failure mode, one or two risk-reduction actions were identified and compiled into a comprehensive improvement plan.

Results

Risk Management

Across the entire process, 33 distinct failure modes were identified, for which a criticality analysis was performed. Table 1, 2, and 3 summarize the results of the FMEA conducted on the three phases of the laboratory workflow.

Table 1: Failure Modes and Associated Criticality in the Preanalytical Phase.

Step/Phase	Failure Modes	Effects / Impact	Potential Causes	S	F	C
Sample Registration & Labeling	Patient identification error	Result linked to the wrong patient identity	- Non-compliant test request form - Lack of concentration	4	2	8
	Failure to observe fasting conditions	Falsely elevated results for glucose and lipid panel	- Lack of patient information	3	3	9
	IT system failure	- Delay in registration - Congestion at the Central Specimen Reception	- Mismatch between workload and IT system capacity	3	1	3
Blood Collection	Collection by an unauthorized trainee	- Non-compliant collection - Risk of needlestick injury	- Non-adherence to trainee supervision protocols	3	4	12
	Expired collection tube	Ineffective anticoagulant	- Poor stock management	2	2	4
	Hemolyzed sample	- Sample rejection and re-collection - Delayed results	- Tourniquet applied for >1 min - Vigorous mixing	3	3	9
	Coagulated sample	- Delayed results	- Insufficient mixing of tubes - Incorrect blood-to-anticoagulant ratio	3	4	12
Sample Transport	Sample contaminated by anticoagulants	Certain parameters will be erroneous	- Incorrect order of draw	3	3	9
	Broken tube	- Risk of contamination from blood - Loss of sample	- Poor quality of tubes	4	2	8
	Tube soiled with blood	Occupational exposure to blood	- Incomplete tube closure before pneumatic transport	3	4	12
Hygiene and Safety	Inadequate cleaning of facilities/restrooms	Occupational exposure to pathogens	- Disproportion between high patient volume and sanitary facilities	3	3	9
	Improper waste management	Risk of sharps injuries for cleaning staff	- Lack of staff awareness on waste sorting protocols	4	2	8

SIL: Laboratory Information System

S = Severity, F = Frequency, C = Criticality

Table 2: Failure Modes and Associated Criticality in the Analytical Phase.

Step/Phase	Failure Modes	Effects / Impact	Potential Causes	S	F	C
Analyzer Maintenance	Missed maintenance	Erroneous calibration and/or controls	- Unauthorized personnel performing maintenance - Non-adherence to maintenance procedure	3	1	3
	Analyzer breakdown	Delayed results	- Mechanical or electronic failure	3	1	3
	Poor water quality	Erroneous calibration and/or controls	- Uncontrolled water quality - Damaged water station filters	3	1	3
Execution of Calibrations	Incorrect calibration	Erroneous quality control results	- Lack of personnel training - Expired or degraded calibrators	3	1	3
QC Execution & Validation	Unacceptable QC results	- Erroneous QC results - Delayed sample analysis - Incorrect Levey-Jennings charts	- Lack of personnel training - Poor organization - Expired or degraded control solutions	3	2	6
Analysis by Analyzer	Barcode reading error	- Analysis not performed - Delayed results	- IT network failure - Poor quality of barcode labels	3	1	3

	Mismatch between barcodes and requested tests					
	Sample/reagent pipetting error (e.g., air bubble)		- Blockage in the analyzer's pipetting system	2	2	4
	No automated transfer of results		- IT network failure	2	2	4
Technical Validation of Results	Failure to check patient's previous results	Validation of a result inconsistent with patient history	- High workload - Omission	4	3	**12**
	Delayed or absent validation	Delay in patient management		4	2	8

S = Severity, F = Frequency, C = Criticality

Table 3: Failure Modes and Associated Criticality in the Post-analytical Phase.

Step/Phase	Failure Modes	Effects / Impact	Potential Causes	S	F	C
Biological Validation	Lack of clinical information for interpretation	Erroneous interpretation	- Lack of a standardized, easy-to-use request form - High workload in clinical services	2	4	8
	Absent or delayed biological validation	Delayed patient management	- Lack of an on-call system for biologists at night	2	4	8
Electronic Result Reporting	IT network failure	Delayed result transmission	- Faulty or under-maintenance IT network	3	1	3
	Missing test method information in report	Misinterpretation of certain parameters	- Lack of a detailed procedure for communicating this information	2	4	8
	Insufficient reference values for interpretation	Misinterpretation of certain parameters		2	4	8
	No procedure for delayed results	Patients not informed	- Lack of a relevant procedure	2	2	4
	Issue with automated results distributor	Congestion at manual distribution counters	- Distributor out of service - Paper shortage	1	1	1
Critical Result Reporting	Non-communication of a critical result	Delayed patient management	- Lack of awareness - Omission	3	1	3
	Delayed communication of a critical result		- Lack of training	3	1	3
	Lack of communication traceability on log		- Omission of transcription on the register	1	1	1
Sample Storage	Non-compliance with storage conditions (temp, time)		- Lack of a detailed procedure for sample storage - Lack of dedicated storage areas	1	4	4

S = Severity, F = Frequency, C = Criticality

Discussion

This study aimed to implement a comprehensive and integrated risk management approach within our biochemistry laboratory to align with quality standards and foster a culture of risk mitigation. The FMEA methodology was applied across the pre-analytical, analytical, and post-analytical stages of the laboratory workflow. Our analysis identified 33 distinct failure modes.

The distribution of these failures revealed that 36.36% occurred in the pre-analytical phase, 33.34% in the analytical phase, and 30.3% in the post-analytical phase. This finding is consistent with a large body of literature demonstrating that the pre-analytical phase is responsible for 60% to 70% of laboratory errors. This is partly due to the involvement of multiple

stakeholders (physicians, nurses, trainees, phlebotomists, technicians) in this phase [10]. Our results align with a study in Morocco on pre-analytical risks in hemostasis, which reported a rate of 39.58% [11], and another FMEA study in Lyon, which found that 36.36% of risks (48 out of 132) in hemostasis testing were pre-analytical [12].

Indeed, the majority of non-conformities affect the pre-analytical phase, the mastery of which is strongly recommended by the ISO 15189 standard. It is increasingly evident that quality improvement efforts must be directed toward this phase, especially since many pre-analytical variables are not under the direct control of the laboratory. Regarding the severity of these failures, our study found that over 58.33% of failure modes had a high criticality score ($C \geq 9$). The combination of FMEA with Ishikawa cause-and-effect analysis led to the conclusion that human factors ('Personnel') are the primary root cause of the identified issues. This highlights the critical role of human intervention in pre-analytical errors. Implementing a robust quality assurance system requires the laboratory to be fully aware of the risks inherent in this phase.

The primary solution, as outlined in paragraph 5.4.1 of the ISO 15189 standard, is for the laboratory to "have documented procedures and information for pre-examination activities to ensure the validity of the results" [8]. The standard requires not only the creation of these procedures but also their dissemination to internal and external collectors and prescribing physicians. To address this, our laboratory has developed and maintains a comprehensive, up-to-date phlebotomy manual. This document contains specific instructions for sample collection and handling, conforming to best practice recommendations. Despite the availability of this manual in both paper and digital formats, failures associated with high criticality persist.

To further mitigate these risks, continuous training sessions on best practices for the pre-analytical phase are included in the hospital's professional development program, along with periodic reviews of phlebotomists' certifications. A second major improvement has been the implementation of a pneumatic tube system for transporting blood samples, which helps control and reduce transport times. While this system has resolved many issues related to transport delays, it remains unsuitable for certain tests, such as blood gases and cerebrospinal fluid analysis. Moreover, the system can be a source of occupational exposure to blood if tubes are not hermetically sealed. In this regard, ISO 15189 (paragraph 5.4.5) mandates that samples be transported within a suitable timeframe and at an appropriate temperature to ensure their integrity and the safety of all personnel [8].

To ensure robust control over the pre-analytical process, written criteria for sample acceptance and rejection must be defined. Any sample not meeting these criteria must be rejected, and the non-conformity must be formally documented.

(The full action plans derived from this study are detailed in Table 4, 5, and 6.)

Table 4: Action Plan for the Preanalytical Phase.

Failure Mode	Corrective/Preventive Action	Responsible Party	Resources/Tools
Patient identification error	Scan barcodes before collection, verifying patient ID	Supervisor of Collection Unit	Barcode scanner
Non-adherence to fasting	Educate staff responsible for registration		Awareness sessions
Collection by unauthorized trainee	Prohibit trainees from performing collection without direct supervision		Meetings, Protocols
Expired tube	Implement stock management training		Deploy stock management software
Hemolyzed sample	"Update procedures and instructions for blood collection		
Ensure continuous training for phlebotomists"	"Laboratory Biologist		
Phlebotomist"	Procedures, Instructions, Training		
Coagulated sample			
Contaminated sample			
Broken tube	Prioritize use of high-resistance materials	Laboratory Biologist	Call for tenders
Soiled tube	Do not transport tubes that are not hermetically sealed via pneumatic system	Technicians	Instructions
Poor waste management	Perform daily cleaning of all facilities	Cleaning Staff	Cleaning Procedure

Table 5: Action Plan for the Analytical Phase.

Failure Mode	Corrective/Preventive Action	Responsible Party	Resources/Tools
Missed maintenance	Adhere to manufacturer's instructions for maintenance Apply the pre-established maintenance schedule	Planning Manager, Technical Staff Biologists	Technical Docs, Planning Schedule
Analyzer breakdown	Apply the pre-established maintenance schedule Draft a procedure for IT-related failures	Technical Staff	Planning, Procedure
Poor water quality	Change filters periodically	Technical Staff	Procedures, Instructions
Incorrect calibration	Respect calibration procedure Respect instructions for preparation and storage	Technicians Biologists	Procedures, Instructions
Barcode reading error	Improve the print quality of labels	IT Service	High-quality printer and labels
Pipetting system error	Implement metrological control of pipetting systems	Technical Staff Biologists	
Delayed/absent validation	Sensitize technicians on the need for proper organization	Biologists	SIL

Table 6: Action Plan for the Post-analytical Phase.

Failure Mode	Corrective/Preventive Action	Responsible Party	Resources/Tools
Lack of clinical information	Implement a standardized request form to be completed by clinicians	Clinician-Pharmacist Collaboration, Hospital Admin	Update hospital's IT system
Absent/delayed biological validation	"Improve biologist involvement in on-call system		
Sensitize biologists to the impact of validation speed"	Head of Service, Biologists	Awareness meeting	
IT network failure	Create a documented procedure for communicating results during system downtime	Quality Unit	Procedure
Insufficient reference values	Conduct studies to adapt reference values to the local population	Biologists	
Non-communication of critical result	Sensitize biologists on the importance of rapid communication of critical results	Biologists, IT Service	Awareness meeting

Conclusion

In a medical biology laboratory, mastering the three phases of the workflow is an essential requirement to limit non-conformities that compromise not only the analytical process but also patient and clinician satisfaction.

Based on the major risks identified at each stage of the laboratory workflow, corrective actions have been proposed in an action plan. These actions, such as continuous training, staff sensitization, and the creation and dissemination of communication procedures, are designed to reduce the criticality of major risks. The implementation and monitoring of these measures must be part of a continuous improvement cycle to yield satisfactory results. Consequently, during subsequent evaluations, some risks may remain priorities while

their criticality is reduced, others may be eliminated, and new ones may emerge. This underscores the dynamic nature of risk management and the necessity for ongoing vigilance and adaptation.

Conflicts of interest

We have no conflicts of interest to disclose, and all authors have approved the manuscript for submission.

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Ethical considerations

This study protocol was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Military Hospital of Tunis.

Data Availability Statements

Data sharing is not applicable to this article as no new data were created or analyzed in this study. All sources analyzed are cited in the references.

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Research Article

Linking Glomerular Endothelial Dysfunction with Urinary KIM-1, sFlt-1, Serum IL-10, and Regulatory T Cells in Preeclampsia

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Keywords

Regulatory T cells, Serum sFlt[1], IL-10, Urinary KIM-1, Preeclampsia

Abstract

Background: Preeclampsia (PE) usually presents after 20 weeks of pregnancy with high blood pressure and protein levels in the urine. An imbalance between the body's pro-inflammatory and anti-inflammatory responses has been suggested to be a key issue in the pathophysiology of the disease. Some important factors, such as soluble fms like tyrosine kinase -1 (sFlt-1), T regulatory cells (Tregs), and Interleukin-10 (IL-10) molecules are thought to be involved as mediators in a systematic response affecting the blood vessel lining. Proteinuria is an essential feature of preeclampsia suggesting the involvement of the kidneys in the disease.

Objective: Our study aimed to explore how Tregs, IL-10 and sFlt-1 correlate with Kidney Injury Molecule-1(KIM-1) protein levels in urine to better understand preeclampsia-induced renal endothelial dysfunction in better way.

Methodology: 36 normal pregnant women and 29 women with preeclampsia were enrolled in this cross-sectional study. Tregs, IL-10, sFlt-1 and KIM-1 levels were analysed and correlated between both the groups.

Results: Our findings revealed that the levels of CD4+FOXP3+ Treg cells and serum IL-10 were much higher and the levels of serum sFlt-1 and urinary KIM-1 were lower in normal pregnant women than in those with preeclampsia. ROC curve showed that serum sFlt-1 was a strong marker for diagnosing preeclampsia with a sensitivity of 93% and specificity of 92%, followed by urinary KIM-1 with a sensitivity of 76% and specificity of 58%, implying at ongoing kidney injury in preeclampsia.

Conclusion: Our study elucidates preeclampsia and supports better biomarker use and treatments, aiming to improve health outcomes for mothers and babies.

Introduction

Preeclampsia (PE) is a hypertensive disorder of pregnancy characterized by new-onset hypertension after 20 weeks of gestation, defined as a systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg on two occasions at least 4 hours apart, in a previously normotensive woman, accompanied by either proteinuria (≥300 mg per 24 hours or a protein-to-creatinine ratio ≥ 0.3) or, in the absence of proteinuria, new-onset maternal organ dysfunction such as thrombocytopenia, renal insufficiency, impaired liver function, pulmonary edema, or cerebral or visual symptoms [1]. This condition affects approximately 3-5% of pregnant women worldwide and it's one of the key causes of perinatal mortality and morbidity.

If not properly managed, preeclampsia can lead to severe complications such as eclampsia, pulmonary edema, or kidney failure in the mother. Additionally, the baby may face risks like premature birth or impaired growth (fetal growth restriction). Due to these risks, it is crucial to promptly detect and manage preeclampsia to ensure the well-being of both the mother and the baby. At present, there are no specific treatments available for this condition. The primary aim is to control symptoms and delay childbirth until approximately 34 weeks to enhance outcomes for both mothers and their infants [2].

Despite extensive research, the exact etiology of PE remains incompletely elucidated and is likely multifactorial [3,4]. Preeclampsia is often observed in two phases. The first phase, early placental phase, involves problems with how the placenta develops and is due to poor trophoblast invasion into the spiral arteries, resulting in reduced blood flow to the fetus. In the later phase of preeclampsia, the clinical manifestations arise from the maternal syndrome, which reflects the maternal response to placental dysfunction rather than a distinct late-onset stage of disease. The ischemic placenta releases excess anti-angiogenic factors, particularly soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng), into the maternal circulation. These molecules antagonize the effects of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), leading to widespread endothelial dysfunction, vasoconstriction, and increased vascular permeability. The resulting systemic effects manifest as hypertension, proteinuria, and multi-organ involvement, which together constitute the maternal syndrome of preeclampsia [1,5]. One of these substances is sFlt-1. This protein can block the actions of important growth factors such as Vascular Endothelial Growth factor (VEGF) and Placental Growth Factor (PlGF)-thereby preventing their binding to the endothelial lining of blood vessels in the placenta ultimately causing an increase in maternal blood pressure during preeclampsia [6]. Moreover, increased sFlt-1 levels have been shown to accelerate proteinuria by lowering the expression of the podocyte protein nephrin as demonstrated in rat models of crescentic glomerulonephritis [7]. Thus, findings like these point out the importance of antiangiogenic proteins like sFlt-1 in understanding preeclampsia.

From early pregnancy, the maternal immune system undergoes adaptive changes to promote tolerance toward the semi-allogenic fetus. A critical component of this immunological adaptation involves regulatory T cells (Tregs), which actively suppress excessive T cell–mediated immune responses and maintain maternal–fetal immune tolerance. These cells help prevent the maternal immune system from recognizing and attacking fetal antigens. In healthy pregnancies, the balance between pro-inflammatory and anti-inflammatory responses is tightly regulated. However, in preeclampsia, impaired Treg function and an increase in inflammatory T helper (Th1 and Th17) responses disturb this delicate equilibrium, contributing to abnormal placentation and endothelial dysfunction[8,9]. Studies in mice have shown that having sufficient healthy Tregs is important for supporting changes in blood vessels during pregnancy thereby preventing the development of inflammatory problems related to the placenta[10].

Interleukin-10 (IL-10) is another key player; -it helps to reduce inflammation by inhibiting pro-inflammatory T helper 1 (Th1) cell activities and promoting healthy development of the placenta and its blood vessels-making IL-10 a crucial factor for successful pregnancy [11]. The lack of IL-10 can lead to serious problems such as premature birth or even miscarriage [12,13].

KIM-1 is a special trans-membrane glycoprotein mainly synthesized by proximal tubular cells in the renal system; when it is found in blood or urine, it usually means that kidney tubular damage is occurring [14].Therefore high levels of KIM-1 could provide an early warning about potential kidney injury related to preeclampsia.

Therefore, Regulatory T cells, serum IL-10, sFlt-1, and urinary KIM-1 which have shown the potential to be key clinical mediators in the pathogenesis of glomerular endothelial injury in preeclampsia, have been studied. Dysregulated levels of these markers contribute to immune imbalance, angiogenic disruption, and renal glomerular damage, thereby exacerbating disease severity. Current study hypothesised that these markers act through interrelated pathways, and their interactions could provide deeper insights into the mechanisms underlying preeclampsia. This hypothesis further suggests that a comprehensive understanding of these biomarkers could facilitate the development of advanced diagnostic tools and targeted therapeutic strategies for effective management of preeclampsia.

The current study was conducted to examine how Tregs, IL-10, sFlt-1 correlate with KIM-1 protein levels found in the urine of patients with preeclampsia compared with normal pregnancies. This comparison could help us to better understand the relationship between kidney damage and preeclampsia a lot better.

Materials and methods

It is a cross-sectional study done at All India Institute of Medical Sciences, Bhubaneswar after getting ethical clearance from the Institute (Ref no: IEC/AIIMS BBSR/ PG THESIS/ 2022-23/50). We included 29 women diagnosed

with preeclampsia and 36 healthy pregnant women as case (Group-A) and control (Group-B) groups from the Department of Obstetrics and Gynaecology. Participants provided informed consent to join this study, during which we adhered to the ACOG guidelines for diagnosing PE. Healthy pregnant women were included if they were over 20 weeks of gestation. The gestational age of each participant was noted at the time of blood and urine collection.

The ACOG (American College of Obstetricians and Gynecologists) guidelines for diagnosing preeclampsia include pregnant women with two separate blood pressure readings at ≥ 140/90 mmHg with proteinuria of either 300 mg within 24 hours or protein/creatinine ratio of 0.3 mg/dL or more, or a dipstick test showing +2 for protein. This is done only if other quantitative methods unavailable [1].

In Group A, all participants had early-onset preeclampsia that is PE occurring before 34 weeks gestation.

Women diagnosed with Hemolysis, Elevated Liver Enzymes, and Low Platelet Count (HELLP) syndrome, eclampsia, diabetes mellitus, other inflammatory diseases, autoimmune disorders or prior kidney disease were excluded from the study.

Method: Five millilitres of blood was collected from each participant; 2 ml in Ethylenediaminetetraacetic acid (EDTA) containing vacutainers and the other 3 ml in plain vacutainers. The EDTA sample was used immediately for analysis of Tregs by flow cytometry (Beckman Coulter Navios and Dx flex cytometers) while serum was isolated from plain vacutainers via centrifugation and stored at -20 C for estimation of sFlt-1 and IL-10 levels by Enzyme Linked Immunosorbent Assay (ELISA) (ELK biotechnology human sFlt-1 ELISA Kit and EliKine™ Human IL-10 ELISA Kit). Urine was collected from the enrolled participants and stored at -80°C for the assessment of KIM-1 levels using ELISA (ELK Biotechnology Human Urinary KIM-1 ELISA Kit). All three ELISA kits employed a sandwich ELISA technique. Complete blood counts, liver function tests (TBIL, DBIL, AST, ALT, ALP, TP, albumin, globulin, A:G) and kidney function tests (Blood urea, serum creatinine, uric acid, Sodium, Potassium, Chloride) were performed for all study participants.

Flow cytometric analysis

After collecting blood samples in EDTA vials, samples were processed following Beckman Coulter's DURAClone IM Treg tubes instruction manual for estimation of Tregs. The processed samples were analyzed using a Beckman Coulter Navios flow cytometer and Beckman Coulter Dx Flex flow cytometer. The computer system connected to the flow cytometer archives the data for each individual cell, which are subsequently analyzed using the built-in software, CXP. The DURAClone IM Treg panel facilitated the detection and characterization of FoxP3+ T regulatory cells through a rapid permeabilization protocol. These tubes incorporate eight markers in various fluorochrome combinations that support the reliable identification of cell populations, including CD3, CD4, CD25, FoxP3, CD39, CD45, CD45RA, and Helios.

Tregs were defined as CD4+CD25+FOXP3+ using flow cytometry. Gating strategies were standardized and applied equally across groups. Only co-expressing cells were analyzed. Markers such as Helios and CD45RA were collected but not statistically explored due to sample size limitations.

Statistical Analysis

Data were anonymously collected and electronically stored for analysis. Data analysis was performed using IBM Statistical Package for the Social Sciences (SPSS) v26. Data were checked for normal distribution, and all data were reported accordingly. Continuous data are expressed as median (IQR), and discrete data as counts (percentage). Comparisons between groups were performed using the Mann Whitney U test. Spearman's correlation coefficient was used to determine the correlation between the studied parameters. Receiver operating characteristic (ROC) analysis was utilized to assess the diagnostic capability of biomarkers at the time of sampling. The area under the curve (AUC) and its 95% confidence intervals were determined and the optimal threshold for each biomarker was established through Youden's J index, calculated as (sensitivity + specificity – 1). At this determined threshold, sensitivity and specificity were recorded. ROC analyses were conducted to differentiate between preeclampsia and control groups, but they do not offer predictions before the disease develops. A p-value of less than 0.05 was considered statistically significant.

Results

Of all the 65 participants included (29 in Group-A and 36 in Group-B) we could not collect urine samples from three normal pregnant women because of patient unavailability. Owing to technical issues, we were unable to perform flowcytometry analysis for the two samples in the preeclampsia group. Hence, the flow cytometric analysis included 27 women with PE and 36 normal pregnant women, and urinary KIM-1 was estimated in 29 cases and 33 control women. Among anthropometric measurements as shown in Table 1, height, weight, pulse and systolic and diastolic blood pressure (BP) were significantly higher in patients than in controls ($p<0.05$). No significant difference was found between the cases and controls with respect to the various parameters of the complete blood picture (shown in Table 2). Among all Liver function tests (LFT's) (Table 3), Aspartate Transaminase (AST) was significantly higher in case group than in the control group while total protein and albumin levels were significantly higher in the control group. Among the Renal Function Tests (RFT's) (shown in Table 3), creatinine levels were significantly higher in the case group than in the control group while serum sodium levels were significantly higher in the control group than in the case group. Binary logistic regression was performed to account for the baseline anthropometric characteristics of the individuals, and none of the parameters were found to be significant in the model.

Among the immunological parameters (shown in Table 4), the percentages (%) of CD4+CD25+ and CD 4+FOX P3+ Tregs (in % of lymphocytes) were significantly higher in the control group than in the case group. The serum sFlt-1 and urinary KIM-1 levels were significantly higher in the case group than in the control group. The serum IL-10 levels were significantly higher in the control group than cases group. According to the correlation statistics (shown in Table 5), within the overall population, a significant negative correlation was found between the following parameters: a) CD4+ FOXP3+ and serum sFLT-1 b) CD4+ FOX P3+ & Urinary KIM-1, and c) serum sFlt-1 and serum IL-10. Within the case group, a significant negative correlation existed between CD4+ FOXP3+ and urinary KIM-1, whereas in the control group no significant correlation was found between any of the parameters. The correlation heatmap of Regulatory T Cells and serum and urine parameters in the overall population is shown in Figure 1. According to the Receiver Operating Characteristic (ROC) curve as shown in Figure 2, serum sFlt-1 served as the best marker with a higher sensitivity and specificity of approximately 93% and 92% respectively (Area Under Curve (AUC) =0.955), followed by urinary KIM-1 with a sensitivity and specificity of 76% and 58 % respectively (AUC=0.671). The AUC curve analysis data with cut-off values for different parameters are shown in Table 6.

The flowcytometry flow page of T-regs of preeclampsia women (Group A) is shown in Figures 3A, 3B, 3C and normal pregnant women (Group B) are shown in 4A, 4B and 4C respectively.

Table 1: Demographic anthropometric characteristics of cases and controls.

Parameters		Cases [n=29] Median (IQR)	Controls [n=36] Median (IQR)	p-value MW
Age (in years)		28 (25-30)	28 (25.5-29.5)	0.942
Primi Gravida#	No	13 (44.83)	14 (38.89)	0.800χ
	Yes	16 (55.17)	22 (61.11)	
Gestational age <37 weeks#	No	9 (31.03)	8 (22.22)	0.571χ
	Yes	20 (68.97)	28 (77.78)	
Height (in cms)		153 (150-156)	150.5 (149-154)	0.048*
Weight (in kgs)		66 (62-68.5)	61 (56.65-66)	0.021*
BMI (kg/m2)		27.63 (26.77-28.19)	27.02 (25.85-27.63)	0.066
Weight Gained During Pregnancy (kgs)		7.5 (6.5-9)	7.95 (7-9)	0.735
Pulse (in bpm)		96 (88-100)	88 (81.5-93)	0.003*
Systolic Blood Pressure (in mm of Hg)		144 (139-150)	110 (105.5-115.5)	0.001*
Diastolic Blood Pressure (in mm of Hg)		92 (90-96)	71 (67-76)	0.001*

#Count (%). MW: p-value based on Mann Whitney U test. χ : Chi-Square test. *Significant difference-value <0.05.

Table 2: Showing Complete blood picture of cases and controls.

Parameter	Cases (n=29)	Controls (n=36)	p- valueMW
	Median(IQR)	Median (IQR)	
Hb (in g/dl)	11.1 (10.5-11.6)	11.35 (10.35-12.2)	0.44
RBC count(in millions/ cu mm)	4.15 (3.8-4.58)	4.365 (3.875-4.695)	0.44
PCV (in %)	34.7 (32.5-38.6)	36 (32.4-38.9)	0.319
MCV (in fl)	82.7 (76.5-86.7)	82.45 (73.15-90.05)	0.995
MCH(in pg)	26.7 (23.9-29.4)	25.75 (22.2-28.15)	0.295
MCHC(in g/dl)	31.6 (29.9-32.7)	30.25 (29.15-31.85)	0.122
RDW CV (in %)	14.8 (13.5-16.8)	15.6 (14-16.5)	0.191
WBC count (10^3/cu mm)	10.74 (8.81-12.61)	10.68 (9.125-12.19)	0.522
Neutrophils (in %)	74.9 (70.5-78.8)	77.45 (73.85-83.05)	0.251
Lymphocytes (in %)	18 (15.3-20.25)	16.75 (13.7-19.5)	0.402
Eosinophils (in %)	1.2 (0.6-1.8)	1.15 (0.6-1.95)	0.716
Monocytes (in %)	2.8 (2.4-3.1)	3 (2.5-3.5)	0.253

Basophils (in %)	0.3 (0.1-0.4)	0.3 (0.2-0.45)	0.459
Platelet count(in 10^3/cu mm)	238 (185-274)	232.5 (184-269.5)	0.874

Table 3: shows liver function tests and renal function tests among the cases and controls.

Parameter	Cases (n=29)	Controls (n=36)	p- valueMW
	Median(IQR)	Median (IQR)	
Total Bilirubin (T.Bil) (in mg/dl)	0.6 (0.4-0.92)	0.72 (0.455-0.895)	0.761
Direct Bilirubin (D.Bil) (in mg/dl)	0.1 (0.1-0.15)	0.1 (0.08-0.13)	0.771
Indirect bilirubin (IBil) (in mg/dl)	0.43 (0.3-0.72)	0.61 (0.375-0.8)	0.209
Aspartate Transaminase (AST) (in U/L)	30 (21.1-43)	20.25 (16-30.55)	0.017*
Alanine Transaminase (ALT) (in U/L)	28 (15-39)	18.95 (10.5-35)	0.409
Alkaline Phosphatase (ALP) (in U/L)	165 (127-196)	145 (109.5-173)	0.162
Total Protein (TP) (in g/dl)	6.2 (5.8-6.3)	6.51 (6.15-6.75)	0.001*
Albumin (in g/dl)	3.12 (3-3.3)	3.45 (3.2-3.6)	0.001*
Globulin (in g/dl)	3 (2.8-3.1)	3 (2.8-3.3)	0.31
Albumin to Globulin Ratio (A:G)	1.06 (1.03-1.09)	1.11 (1.03-1.27)	0.109
Urea (in mg/dl)	19 (17-24)	18.2 (15.3-23.5)	0.667
Creatinine (in mg/dl)	0.83 (0.79-0.91)	0.76 (0.665-0.835)	0.006*
Uric acid (in mg/dl)	4.8 (3.92-6.2)	4.4 (3.9-5.65)	0.366
Sodium (in mEq/L)	132 (129-134)	134.5 (131.5-138.5)	0.005*
Potassium (in mEq/L)	3.87 (3.51-4.4)	3.98 (3.675-4.29)	0.501
Chloride (in mmol/L)	101 (100-104)	102 (101-104)	0.328

MW: p-value based on Mann Whitney U test. *: Significant difference-value <0.05.

Table 4: Immunological parameters and serum sFlt-1, IL-10 and urinary KIM-1 among the two study groups.

Parameter	Cases (n=29)	Controls (n=36)	p- valueMW
	Median(IQR)	Median (IQR)	
CD4+ CD 25+ (in % of lymphocytes)	3.5 (2.15-4.67)	5.83 (4.48-6.64)	0.001 *
CD4+ FOX P3+ (in % of lymphocytes)	1.92 (1.3-2.8)	4.35 (3.455-5.08)	0.001 *
Serum sFlt-1 (in ng/ml)	7.84 (5.6-9.96)	2.031 (1.41-3.345)	0.001 *
Serum IL-10 (in pg/ml)	1.69 (1.51-15.4)	11.185 (1.845-27.9)	0.021 *
Urinary KIM-1 (in pg/ml)	560.47 (481.07-723.37)	445.42 (387.7-536.7)	0.008 *

MW: p-value based on Mann Whitney U test. *: Significant difference-value <0.05.

Table 5: Correlation between Regulatory T-Cells and Serum & Urine parameters related to Preeclampsia.

Compared Parameter Pair	Overall Population		Cases		Controls	
	Coeff.	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**
CD4+ FOX P3+ & Serum sFlt-1	**-0.664**	**0 .001***	-0.026	0.882	-0.181	0.346
CD4+ FOX P3+ & Serum IL-10	0.164	0.193	-0.25	0.142	0.023	0.904
CD4+ FOX P3+ & Urinary KIM-1	**-0.351**	**0.005***	**-0.357**	**0.041***	0.074	0.701
Serum sFlt-1 & Serum IL-10	**-0.268**	**0.031***	-0.071	0.683	0.025	0.898
Serum sFlt-1 & Urinary KIM-1	0.25	0.05	0.023	0.06	-0.037	0.847
Serum IL-10 & Urinary KIM-1	-0.097	0.451	0.009	0.9	0.066	0.734

Significant correlation is known to exist when p value <0.05, denoted by *.

*p value <0.05, **- p value <0.01, ***- p value 0.001

Figure 1: Correlation heatmap of Regulatory T Cells and Serum and Urine Parameters in overall population.

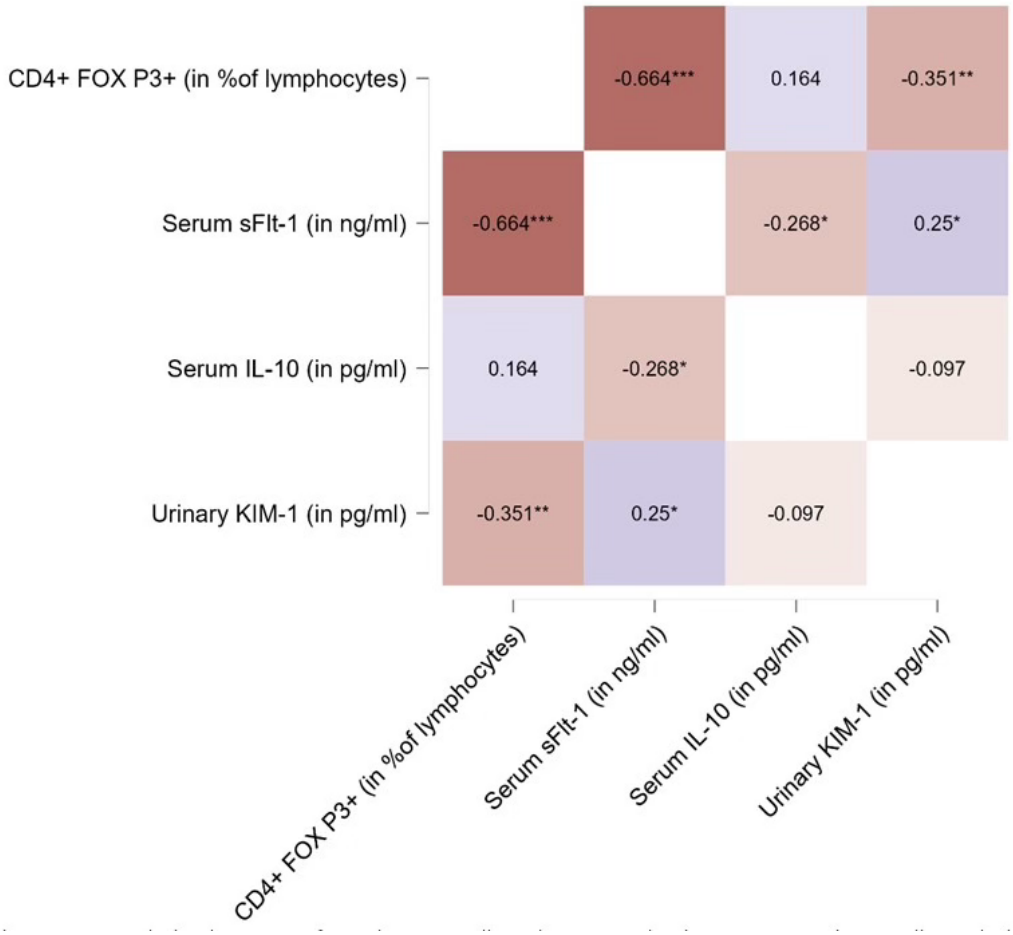

Figure 2: ROC Curve Analysis for prediction of preeclampsia.

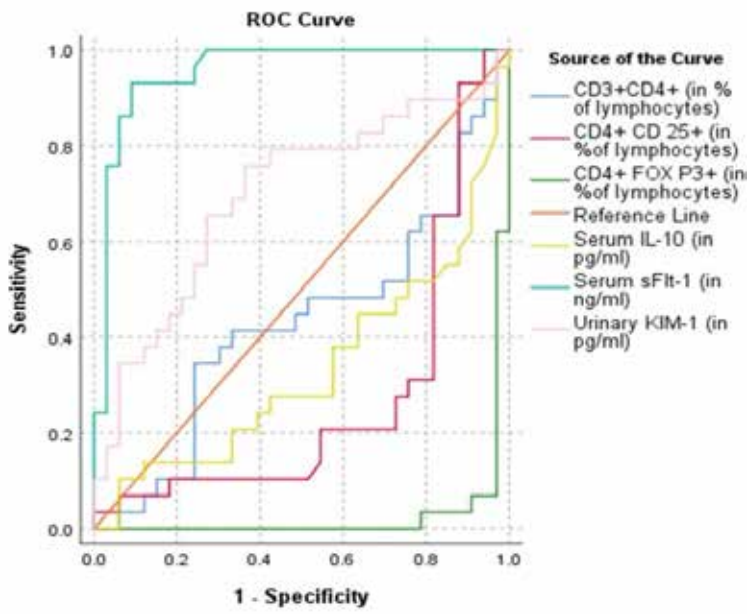

Table 6: Area Under Curve analysis for Prediction of Preeclampsia with Cut-Off estimation by Youden's Index.

Parameters	AUC	p-value	Cutoff	Sensitivity	Specificity
CD3+CD4+ (in %of lymphocytes)	0.581	0.344	> 39.35	34%	75%
CD4+ CD 25+ (in %of lymphocytes)	0.733	0.001	< 0.60	100%	6%
CD4+ FOX P3+ (in % of lymphocytes)	0.973	0.001	> 6.60	0%	100%
Serum sFlt-1 (in ng/ml)	0.955	0.001	> 3.96	93%	92%
Serum IL-10 (in pg/ml)	0.667	0.021	< 0.96	97%	6%
Urinary KIM-1 (in pg/ml)	0.671	0.005	< 481.07	76%	58%

Figure 3: Flowcytometry flow page of T-regs of preeclampsia women (Group A).

3A: CD3+ CD4+ **3B:** CD4+CD25+ **3C:** CD4+FOXP3+

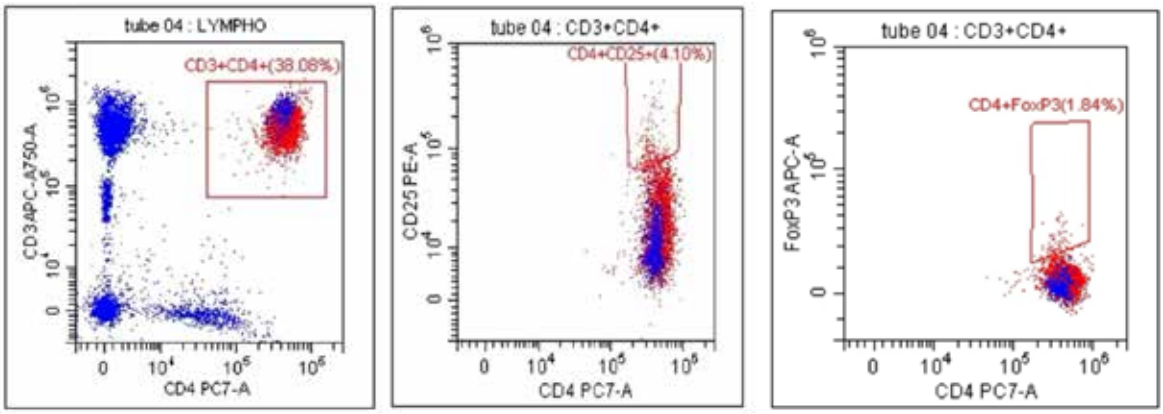

Figure 4: Flowcytometry flow page of T-regs of normal pregnant women (Group B).

4A: CD3+CD4+ **4B:** CD4+CD25+ **4C:** CD4+FOXP3+

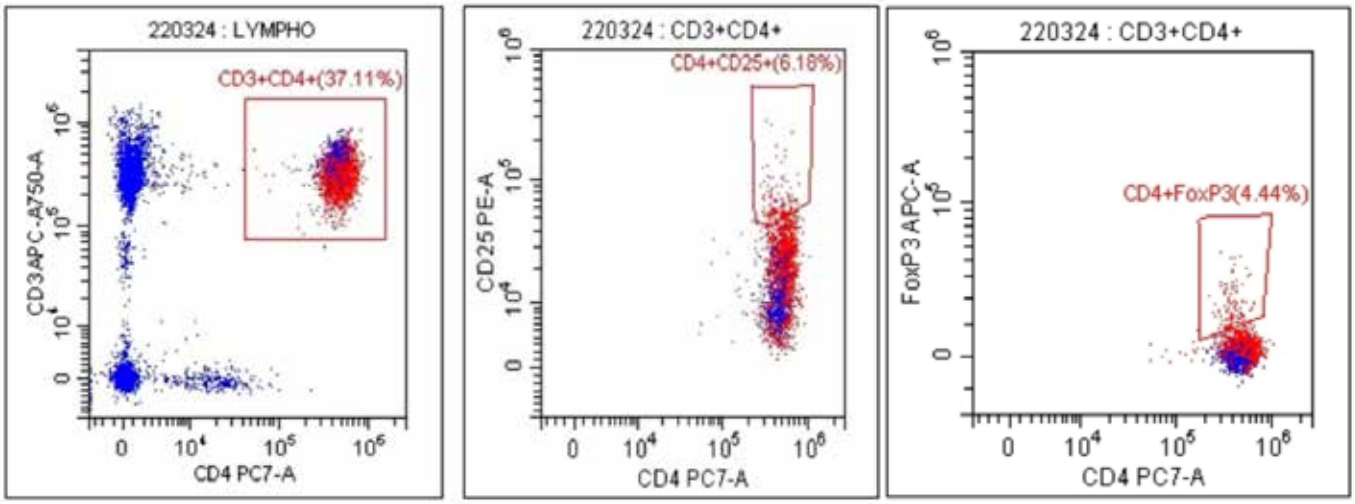

Table 7: Correlation of Novel Biomarkers with routine anthropometric, clinical and biochemical parameters.

Parameter	$CD3^+CD4^+$	$CD4^+CD25^+$	$CD4^+FOXP3^+$	Serum sFlt-1	Serum IL-10	Urinary KIM-1
Age (yrs)	−0.172 (0.171)	0.035 (0.784)	0.049 (0.697)	0.102 (0.419)	−0.116 (0.358)	0.081 (0.534)
Height (cm)	0.070 (0.582)	−0.108 (0.392)	−0.092 (0.466)	0.110 (0.382)	−0.032 (0.801)	0.034 (0.794)
Weight (kg)	0.151 (0.228)	−0.137 (0.277)	−0.057 (0.654)	0.117 (0.353)	−0.089 (0.482)	0.065 (0.616)
Pulse (bpm)	0.167 (0.183)	−0.192 (0.125)	−0.271 (0.029)	0.358 (0.003)	−0.096 (0.446)	0.310 (0.014)
SBP (mm Hg)	−0.007 (0.953)	−0.254 (0.041)	−0.653 (<0.001)	0.658 (<0.001)	−0.311 (0.012)	0.284 (0.025)
DBP (mm Hg)	−0.105 (0.406)	−0.313 (0.011)	−0.687 (<0.001)	0.704 (<0.001)	−0.161 (0.201)	0.186 (0.147)
AST (U/L)	0.025 (0.842)	−0.227 (0.069)	−0.278 (0.025)	0.077 (0.544)	−0.192 (0.125)	0.104 (0.421)
ALT (U/L)	0.143 (0.256)	−0.177 (0.158)	−0.148 (0.238)	−0.057 (0.653)	−0.107 (0.398)	0.219 (0.087)
ALP (U/L)	0.016 (0.901)	−0.371 (0.002)	−0.106 (0.399)	0.085 (0.503)	−0.262 (0.035)	0.173 (0.179)
Total Protein (g/dl)	0.161 (0.201)	0.272 (0.028)	0.324 (0.008)	−0.453 (<0.001)	0.055 (0.666)	0.208 (0.105)
Albumin (g/dl)	0.069 (0.585)	0.304 (0.014)	0.381 (0.002)	−0.413 (0.001)	0.106 (0.402)	0.106 (0.411)
Globulin (g/dl)	0.155 (0.217)	0.029 (0.818)	0.076 (0.546)	−0.197 (0.116)	−0.005 (0.968)	0.237 (0.063)
A/G Ratio	−0.040 (0.755)	0.183 (0.144)	0.195 (0.120)	−0.103 (0.414)	0.064 (0.610)	−0.070 (0.588)
Urea (mg/dl)	0.090 (0.475)	−0.083 (0.510)	−0.035 (0.781)	−0.032 (0.797)	−0.030 (0.813)	−0.005 (0.972)
Creatinine (mg/dl)	−0.145 (0.249)	−0.094 (0.456)	−0.289 (0.020)	0.232 (0.063)	−0.181 (0.149)	0.226 (0.077)
Uric acid (mg/dl)	0.073 (0.562)	−0.059 (0.638)	0.012 (0.924)	0.048 (0.701)	−0.037 (0.767)	0.243 (0.057)
Sodium (mEq/L)	−0.012 (0.925)	0.170 (0.176)	0.311 (0.012)	−0.148 (0.240)	0.077 (0.542)	−0.008 (0.949)
Potassium (mEq/L)	0.064 (0.614)	0.050 (0.691)	0.012 (0.927)	0.005 (0.967)	0.136 (0.280)	0.040 (0.755)
Chloride (mmol/L)	−0.110 (0.382)	0.045 (0.721)	0.116 (0.357)	0.088 (0.485)	0.169 (0.178)	0.028 (0.826)
Urine protein	−0.140 (0.265)	−0.402 (0.001)	−0.815 (<0.001)	0.783 (<0.001)	−0.288 (0.020)	0.340 (0.007)

Spearman's rank correlation analysis was performed to explore the relationship between immunological markers ($CD3^+CD4^+$, $CD4^+CD25^+$, $CD4^+FOXP3^+$, sFlt-1, IL-10, and urinary KIM-1) and clinical-biochemical variables. A strong negative correlation was observed between $CD4^+FOXP3^+$ T-regulatory cell frequency and both systolic ($\rho = -0.653$, $p < 0.001$) and diastolic blood pressure ($\rho = -0.687$, $p < 0.001$), as well as urinary protein levels ($\rho = -0.815$, $p < 0.001$). Conversely, serum sFlt-1 showed a strong positive correlation with systolic ($\rho = 0.658$, $p < 0.001$) and diastolic pressures ($\rho = 0.704$, $p < 0.001$), and with urine protein excretion ($\rho = 0.783$, $p < 0.001$). $CD4^+CD25^+$ cells demonstrated modest inverse associations with blood pressure parameters ($\rho \approx -0.25$ to -0.31, $p < 0.05$) and alkaline phosphatase ($\rho = -0.371$, $p = 0.002$), but positive correlations with total protein and albumin levels. In contrast, serum IL-10 correlated negatively with systolic pressure ($\rho = -0.311$, $p = 0.012$) and ALP ($\rho = -0.262$, $p = 0.035$). Urinary KIM-1, a marker of tubular injury, showed a significant positive correlation with systolic pressure ($\rho = 0.284$, $p = 0.025$) and urinary protein ($\rho = 0.340$, $p = 0.007$). Collectively, these findings indicate that rising anti-angiogenic activity and renal injury markers are associated with higher blood pressure and proteinuria, whereas reduced T-regulatory cell populations reflect immune dysregulation contributing to the pathophysiology of preeclampsia.

Discussion

In this study, the relationship between regulatory T cells (Tregs), serum IL-10, sFlt-1, and urinary KIM-1 with glomerular endothelial dysfunction in preeclampsia was explored. The results suggest possible biomarker and immunological connections to endothelial damage. However, the cross-sectional nature of the study restricts the ability to draw causal conclusions and to validate the underlying mechanisms functionally. As our findings are observational, mechanistic studies (e.g., animal models, prospective cohorts) are warranted.

Although height and weight were significantly higher in the case group than controls, BMI and weight gain showed no differences between groups, nullifying these differences. This helps exclude anthropometric measurements interference in concluding that observed changes in T-regulatory cells, IL-10, sFlt-1 and KIM-1 are primarily due to preeclampsia. The findings of the current study gain credibility when these possible confounding variables are eliminated. The case group showed significantly higher pulse rate and blood pressure than controls, confirming the diagnostic criteria of PE.

Our findings revealed a marked decrease in the presence of CD4+CD 25+ Treg cells, CD4+FOXP3+ Treg cells and lower levels of serum IL-10 in PE women. This observation aligns with previous research that associates reduced Treg function with compromised maternal–fetal immune tolerance and increased inflammation [15] [16].

Extensive clinical and experimental evidence indicates a reduction in CD4+CD25+FOXP3+ Tregs and diminished IL-10 levels in PE, which compromises maternal–fetal tolerance [11,15]. Mechanistically, the lack of Treg/IL-10 intensifies pro-inflammatory Th1/Th17 responses, hinders placental angiogenesis, and triggers endothelial activation, leading to glomerular damage [17,18]. Hu et al. also discovered a decrease in fetal thymic Tregs in PE, which can be reversed with maternal acetate supplementation [19].

New approaches are being investigated to enhance Treg function, such as adoptive Treg transfer, IL-10 analogues, and Short Chain Fatty Acid (SCFA) supplementation, or to create Treg-based biomarkers for assessing PE risk [20]. Gaining insight into the disruptions of the Treg-IL-10 axis is essential for precise immunomodulation in PE.

Consistent with the findings of Daneva et al [21] and Nath et al [11], our study identified a marked reduction in serum IL-10 levels in PE cases when compared to healthy pregnant controls, which tilts the cytokine balance towards pro-inflammatory mediators [11,22]. The lack of IL-10 facilitates endothelial activation, leading to the increased expression of adhesion molecules like Vascular cell adhesion molecule-1 (VCAM-1) and the attraction of leukocytes to the glomerular endothelium [23]. A decrease in IL-10 also diminishes antioxidant capacity, thereby elevating oxidative stress and causing direct harm to the endothelium [24].

Insufficient IL-10 may allow for unchecked complement activation, resulting in endothelial damage through membrane attack complex pathways [25]. Furthermore, the absence of IL-10 intensifies angiotensin II–driven vasoconstriction, further compromising renal blood flow [25]. Together, these processes-cytokine imbalance, complement activation, oxidative stress, and Renin–Angiotensin–Aldosterone System (RAAS) sensitization-hinder endothelial repair mechanisms, leading to glomerular damage in PE.

As reported by Maynard et al [26], De vivo et al [27] and Moghaddas et al [28], our findings also indicate a significant rise in sFlt-1 levels in PE women when compared to normotensive women. This protein binds to VEGF/PlGF, causing a disruption in glomerular endothelial fenestrations and podocyte function, which leads to proteinuria [6,26]. The reduction of VEGF due to sFlt-1 also exacerbates oxidative stress and triggers complement activation, further impairing endothelial repair and lowering Glomerular filtration rate (GFR) [24,29]. These interconnected mechanisms are central to the renal dysfunction associated with sFlt-1 in PE.

Another novel marker that we have studied is KIM-1 protein in urine. In our study we found that urinary KIM-1 levels were significantly higher in the preeclampsia group than in the normal pregnant women.

KIM-1, a proximal tubular injury marker, is elevated in PE and correlates with glomerular endothelial damage, reflecting hypertension- and oxidative stress–mediated tubular injury [30,31]. Elevated KIM-1 augments vascular permeability, and its degree of upregulation parallels glomerular injury severity

[31]. Exploring the relationship between KIM-1 and injury to glomerular endothelial cells could deepen our understanding of preeclampsia's pathophysiology. This insight might pave the way for the development of specific therapeutic interventions and enhanced diagnostic techniques, ultimately leading to better health outcomes for both mothers and their infants.

The Receiver Operating Characteristic (ROC) curve analysis revealed that serum sFlt-1 is the most effective marker for diagnosing preeclampsia, with impressive sensitivity (93%) and specificity (92%). This finding highlights the potential of sFlt-1 as a diagnostic biomarker for preeclampsia. However, our data were not adjusted for gestational age, and all preeclampsia cases were early-onset, occurring before 34 weeks. As a result, although sFlt-1 levels were elevated in these cases, the small sample size and lack of gestational age normalization limit the predictive conclusions we can draw. Previous literature emphasizes the variability of sFlt-1 levels with gestational age and disease severity, which should be considered in future prospective studies assessing its predictive utility (Matin et al., 2020 [32]; Vogtmann et al., 2021[6]]. Urinary KIM-1 also showed promise as a marker, albeit with lower sensitivity and specificity. It is important to emphasize that these ROC analyses illustrate diagnostic differentiation at the point of sample collection, rather than predicting when the disease will begin.

The study revealed negative correlations between CD4+FOXP3+ Tregs and both serum sFlt-1 and urinary KIM-1, suggesting their interplay in preeclampsia pathogenesis. One hypothesis explaining this negative correlation was that increased sFlt-1 levels during PE are associated with oxidative stress and inflammation, which reduces Treg cell numbers and thereby impair their suppressive function [33]. The negative correlation between Tregs and urinary KIM-1 can be explained by inflammatory state of PE where KIM-1 acts as protective factor by suppressing inflammation during acute kidney injury [34]. The negative correlation between serum sFlt-1 and IL-10 indicates an imbalance between pro- and anti-inflammatory factors. A rodent study by Lai et al found that hypoxic conditions as observed in preeclampsia result in increased sFlt-1 expression and this expression increases further especially in IL-10 deficient IL-10-/- mice suggesting a possible negative correlation between serum sFlt-1 and IL-10, as noted in our study [35].

By simultaneously assessing Tregs, IL-10, sFlt-1, and urinary KIM-1, this study offers new insights into the interrelated roles of immunological dysregulation, angiogenic imbalance, and renal damage in preeclampsia. Being one of the first Indian studies to examine this combination in a clinical context, the integrated analysis supports the use of these indicators for early diagnosis and monitoring and points to a possible immune-vascular-renal axis in disease etiology.

However, this study has certain limitations, including its cross-sectional design, which precludes the establishment of causal relationships. The relatively small sample size may limit the generalizability of the findings. Future longitudinal studies with larger cohorts could provide more robust evidence and potentially elucidate the temporal relationships between these markers and the development of preeclampsia. Another key limitation is the variability of immune and angiogenic mediators across pregnancy and the lack of precise gestational-age matching, which may have influenced biomarker levels.

To conclude, this study reveals that preeclampsia is associated with decreased levels of Tregs and IL-10, alongside increased concentrations of sFlt-1 and urinary KIM-1. These consistent relationships suggest their potential as important biomarkers for clinical diagnosis and risk assessment. Future prospective research is essential to confirm these findings and to explore their role in informing management strategies. These findings may contribute to the development of improved diagnostic tools and targeted therapeutic strategies (immune modulators) for managing preeclampsia, potentially leading to better outcomes for both mothers and babies affected by this condition.

Novelty of the study

This research differs from earlier studies that examined angiogenic, immune, or renal markers separately by combining all three pathways within a single cohort. It reveals that a decrease in Tregs and IL-10 is associated with an increase in sFlt-1 and urinary KIM-1. This finding establishes an immune–angiogenic–renal axis in preeclampsia and introduces new evidence from an Indian population, emphasizing urinary KIM-1 as a potential non-invasive indicator of renal involvement. It also supports the clinical value of using multi-marker panels for diagnosis and risk assessment.

Funding

The study did not receive any external funding.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical statement

After receiving approval from the Institutional Ethics Committee (Ref no: IEC/AIIMS BBSR/PG THESIS/2022-23/50), a cross-sectional study was executed at the All India Institute of Medical Sciences (AIIMS), Bhubaneswar. The research included 29 women diagnosed with preeclampsia (Group A) and 36 healthy pregnant women (Group B), all sourced from the Department of Obstetrics and Gynaecology. Written informed consent was obtained from each participant before collecting blood and urine samples for

research purposes. The study was conducted in alignment with the ethical standards of the Declaration of Helsinki.

Data Availability Statement

The data presented in this study is available on request from the corresponding author. The data is not publicly available due to privacy and intellectual property reasons.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used AI tools in order to reformulate some sentences. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Credit authorship contribution statement

- Kasala Farzia: Conceptualization, Data curation, Investigation, Writing – original draft.
- Prakruti Dash: Supervision, Methodology, Writing – review & editing, Project administration.
- Gautom Kumar Saharia: Resources, Data collection, Validation.
- Saubhagya Kumar Jena: Patient recruitment, Clinical support, Validation.
- Saurav Nayak: Formal analysis, Visualization, Writing – review & editing.

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Research Article

Professional Misconduct Discipline in New York State Clinical Laboratories, 2006-2024

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Abstract

Since its implementation in 2006, the New York State Clinical Laboratory Practice Act has licensed 30,287 professionals. As of 2024, 56 disciplinary actions (0.18%) have been recorded, mostly due to criminal convictions (64.3%), particularly DWI, followed by fraudulent applications (23.2%) and workplace violations (12.5%). Males comprised 57% of the disciplined population, 66.7% of those fined, and 78.6% of the total fines paid. Most significant disciplinary outcome included 22 individuals (39.2%) that lost their licenses through annulment (n=13), revocation (n=2), surrender (n=5), and actual suspension (n=2). Laboratory disciplinary outcomes include 3 license surrenders and 1 actual suspension of at least two years (57.1% of workplace actions). Among cases triggered by criminal conviction, 2 licenses were surrendered and 2 revoked (11.1%). The data indicate rare but patterned misconduct, with notable gender disparities in penalties and license terminations.

Background

The New York State (NYS) Clinical Laboratory Practice Act (CLPA), signed by Governor George Pataki on January 30, 2005, and effective September 1, 2006, established licensure for Clinical Laboratory Technologists (CLT), Certified Clinical Laboratory Technicians (CTN), and Cytotechnologists (CT), later expanding to Histotechnicians, Histotechnologists, and CLTs-Restricted [1]. The law followed a 15-year campaign by the Professional Standards Coalition for Clinical Laboratory Personnel (PSCCLP), a consortium of 21 organizations advocating for education-based licensure and standardized competency testing [2]. With only 20 months for implementation, the State Education Department (SED) built regulatory infrastructure, appointed leadership, and issued initial licenses by September 15, 2006.

The State Board (SB) for the Professions, appointed by the Board of Regents (BOR), advises the SED on regulations, discipline, and standards. SB members serve as Public Officers and participate in panels and policy development [3]. The SED's Office of the Professions (OP) oversees licensure for 63 professions via 36 SBs; 33 require continuing education, and 18 mandate child abuse/maltreatment training [4]. The Office of Professional Discipline (OPD) investigates misconduct, with final decisions made by the BOR in consultation with SB members [5].

Licensure defines entry-level qualifications, scope of practice, and title protection. Licenses are permanent unless revoked, while registration permits practice within a set period [6]. Ethical conduct and public welfare are core to licensure [7]. Applicants must show good moral character, verified through background checks at initial application and triennial renewal [8]. False or incomplete submissions may invoke perjury laws [9]. Hearings may be convened before SB and OPD panels, and post-licensure complaints or court findings may trigger investigations under Part 29 of Title VIII, Unprofessional Conduct [10].

This observational study analyzes anonymized, publicly available data to identify trends in professional misconduct discipline (PMD) within the Clinical Laboratory Technology Profession (CLTP) in NYS from September 15, 2006, through 2024.

Methods

Data was retrieved from the publicly available final disciplinary actions from the NYS-SED OP website's Enforcement navigation tab [11]. Further information was retrieved from the Verification & Certifications navigation tab [12]. Data on all PMD within the CLTP was collected from September 15, 2006, to December. 31, 2024. Gender was inferred from first names. All gender determinations were validated against two online tools, yielding 100% concordance across methods [13-14]. No appeals were made to the courts regarding the disciplinary cases [15].

The data outlines anonymous demographic categorization of PMD cases by gender and professional title, forming the basis for a statewide profile.

Writing was aided by the free version of Grammarly and by Microsoft CoPilot, an artificial intelligence product to assist with language refinement, readability, and grammatical improvements. These tools were used to enhance the clarity of the writing, suggest more concise wording, and verify grammatical accuracy. All content was carefully reviewed, edited, and verified for accuracy. The author takes full responsibility for the final content.

Results

Between 2006–2024, 30,287 CLTP licenses were issued across six title codes: 56 faced discipline (0.18%). CTN (Title code (TC) 094) had the highest rate (0.57%), followed by CLT (TC 090/092) at 0.13%, and CT (TC 093) at 0.10%. No cases were reported for Histotechnicians (TC 091) or Histotechnologists (TC 095) (Table 1).

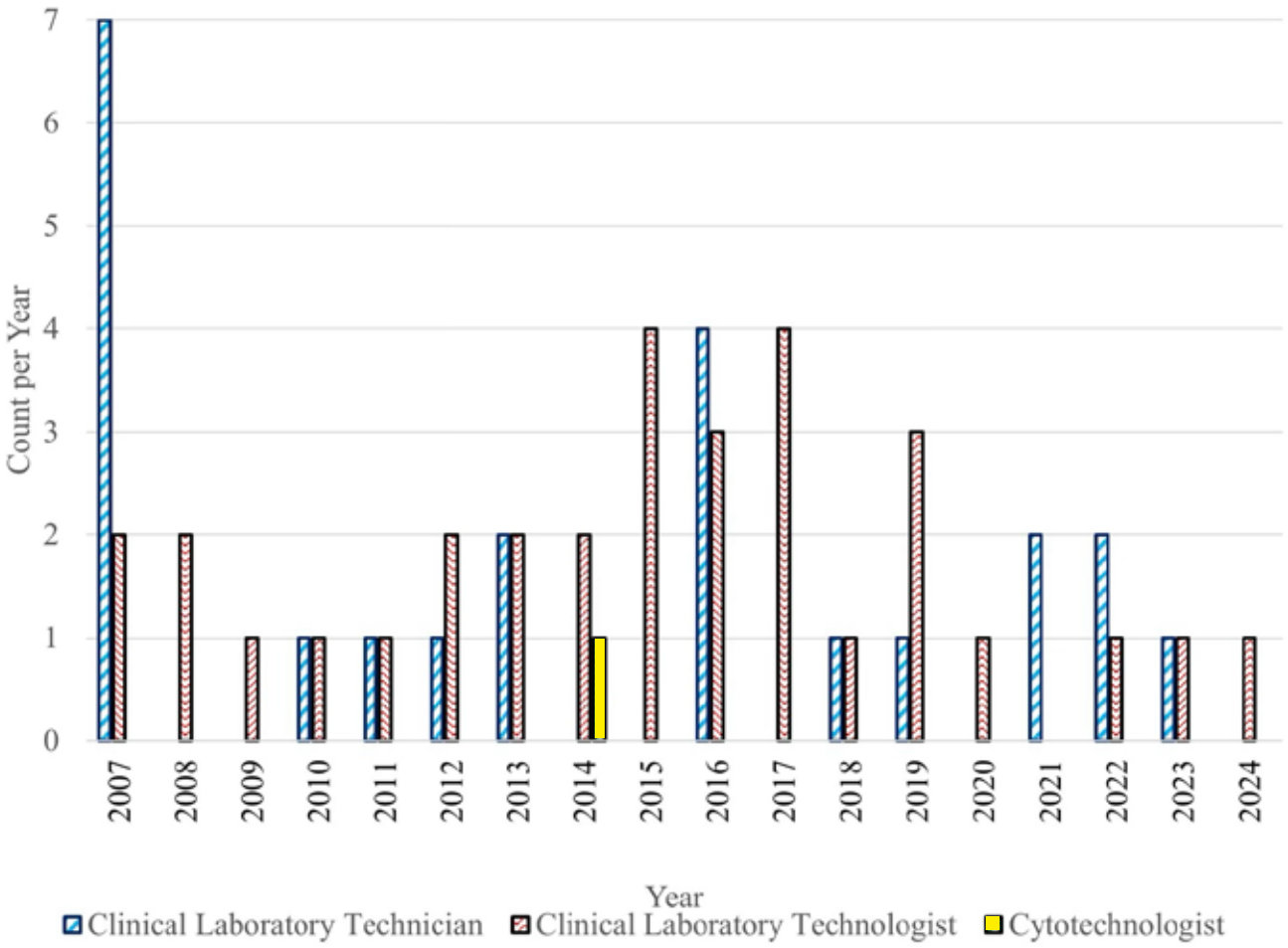
Table 1: Disciplinary Cases as a Percent of Total Clinical Laboratory Licenses Issued from 2006-2024.

Title Code: License Title	Date First Issued	Last License Number through 2024	Number of Disciplinary cases 2006-2024	Percent of Discipline cases by title
091: Histotechnicians	9/15/2006	1136	0	0.00%
090: Clinical Laboratory Technologist-Restricted*	6/15/2006	23095	32	0.13%
092: Clinical Laboratory Technologist	4/15/2020	951		
093: Cytotechnologist	9/15/2006	984	1	0.10%
094: Clinical Laboratory Technician	9/15/2006	4065	23	0.57%
095: Histotechnologists	2/13/2024	56	0	0.00%
Total	-	30287	56	0.18%

*Restricted Clinical Laboratory Technologist licenses (title code 090) are not distinguished in disciplinary reports from Clinical Laboratory Technologist (title code 092).

PMD peaked in 2007 (9 cases) and 2016 (7 cases) (Figure 1). In 2007, BOR annulled licenses tied to fraudulent experience claims, verified by the laboratory director's signature. In 2016, cases stemmed from criminal convictions. Across 2006–2024, PMD averaged 3.1 cases/year. Of 56 total, 64.3% (n=36) involved criminal convictions; 23.2% (n=13) were fraudulent applications; 12.5% (n=7) stemmed from workplace misconduct. One individual with dual CTN/CLT licenses was disciplined in 2023 for weapon possession and reckless endangerment listed on both licenses. A CLT received separate sanctions for DWIs in 2012 and 2020 - the only repeat case of PMD.

Figure 1: Count of Disciplinary Cases by year.



*Year 2023 shows one Clinical Laboratory Technician and one Clinical Laboratory Technologist license disciplined. This is the same person. Years 2012 and 2020 show a Clinical Laboratory Technologist disciplined for repeated DWI.

Penalty

Of 56 PMD cases, 36 (64.3%) stemmed from criminal convictions (Table 2). DWIs accounted for 19 (33.9%), comprising 52.7% of conviction-related infractions. Severe penalties - including suspensions - were issued for DWI, felony bank fraud, weapon possession with reckless endangerment, and nondisclosure of prior convictions. Licenses were voluntarily surrendered in cases of petit larceny and failing to disclose a conviction on the application. One CLT disciplined for DWI received censure, reprimand, and probation; however, the license status is revoked. Another CLT's license was involuntarily revoked following convictions for grand larceny, forged instruments, and identity theft.

Table 2: Discipline due to Criminal Convictions with Penalty by Title and Gender*.

		Profession Abbreviation/Gender					
		CLT		CT	CTN		
Infraction	Penalty	Female	Male	Female	Female	Male	Total
Attempted Arson	Indefinite AS >=1Y, 2Y prob.					1	1
Attempted Burglary	3M AS, 21M SS, 24M prob.				1		1
Attempted Grand Larceny	1M AS 23M SS, 2Y prob., $500		1				1
Criminal Contempt	Indef.AS, 2Y prob., $250	1					1
Criminal Obstruction of Breathing	2Y SS, 2 yr Prob. $500		1				1
Criminal Possession of a Controlled Substance	$1,250					1	1
Criminal Possession of a weapon, reckless endangerment	6M AS 18M SS, 2Y Prob.					1	1
Criminal Possession of a Weapon; Reckless Endangerment	6M AS 18M SS, 2Y Prob.		1				1
Criminal Possession of a Controlled Substance	6M AS 18M SS, 2Y Prob.				1		1
DWI	1M AS 23M SS, 2Y prob.		1				1
DWI	1Y SS, 1Y Prob., $500	2	1		1		4
DWI	1Y SS, 1Y Prob.				1		1
DWI	2Y SS, 1Y Prob., $500	2	3	1			6
DWI	2Y SS, 2Y Prob., $1000					1	1
DWI	2Y SS, 2Y Prob., $750		1				1
DWI	Revocation	1					1
DWI and Computer Intrusion	1Y SS, 1Y Prob., $500		1				1
DWI and Criminal Contempt	2Y SS, 2Y Prob., $250					1	1
DWI; Aggravated DWI; Operating a Motor Vehicle without an Ignition Interlock Device; Criminal Impersonation	6M AS 18M SS, 2Y Prob.					1	1
DWID	2Y SS, 2Y Prob., $500		1				1
Failed to disclose a conviction	6M AS 18M SS, 2Y Prob.	1					1
Felony: Bank Fraud	6M AS 18M SS		1				1
Felony: False Statements	2Y SS, 2Y Prob., $1000		1				1
Grand Larceny; ten counts of Criminal Possession of a Forged Instrument, Identity Theft.	Revocation	1					1
Lied on the application- failed to disclose a conviction	Surrendered					1	1
Petit Larceny	Surrendered				1		1
Reckless Endangerment	1M AS 23M SS, 2Y prob., $500					1	1
Unlawful Surveillance	1M AS 23M SS, 2Y prob., $1500		1				1
Criminal Conviction Total Count:	**-**	***8***	***14***	***1***	***5***	***8***	***36***

*Abbreviations: CLT, Clinical Laboratory Technologist; CT, Cytotechnologist; CTN, Clinical Laboratory Technician; DWI, Driving While Impaired; DWID, driving while impaired by drugs; Indef, indefinite; AS, actual suspension; Y, year; M, month; SS, Stayed Suspension. Prob., Probation

Table 3 details workplace PMD penalties. Though unrelated to lab duties, application fraud cases are included. Seven CLTPs (12.5%) were disciplined for lab-related misconduct, including 3 surrendered licenses (42.9%); and 1 indefinite suspension of at least 2 years.

Table 3: Penalties for Application Fraud or Lab Triggers by Title and Gender*.

		Profession Abbreviation/Gender				
		CLT		CTN		
Infraction	Penalty	Female	Male	Female	Male	Total
Application Fraud	Annulment	2	3	6	2	13
Lab Workplace Total:		1	4	2		7
Alcohol on Duty	Surrendered		1			1
Falsifying Test Results	Surrendered			1		1
Gross negligence on a particular occasion	Surrendered		1			1
QC Error	2Y SS 2Y prob. $1000	1				1
QC Error	2Y SS 2Y prob. $250		1			1
QC Fraud	2Y SS 2Y prob. $250			1		1
Video Recording Device in Unisex Restroom	Indef. AS >=2Y, 5Y prob., $5000		1			1

*Abbreviations: CLT, Clinical Laboratory Technologist; CTN, Clinical Laboratory Technician; Indef, indefinite; AS, actual suspension; Y, year; M, month; SS, Stayed Suspension.

Following license annulment (23.2%), the most frequent penalty of the disciplined cohort was 2-year stayed suspension with 2 years' probation and fines ranging from $250–$1000 (n=15; 26.8%). Seven licenses (12.5%) were lost-either revoked (n=2; 3.6%) or surrendered (n=5; 8.9%). Six individuals (10.7%) received 6 months' actual suspension with 18 months stayed; two also had 2 years' probation, and one was fined $1000. Another six (10.7%) received a 1-year stayed suspension with 1 year probation; five of these were fined $500.

Gender

During the disciplinary period, 53.7% of the PMD population were male (n=30) and 44.4% female (n=24). Among CLT licensees, 64.5% were male (n=20), while CTNs showed a slight female majority at 52.2% (n=12). One male held both CLT and CTN licenses and was counted under each; another male CLT was a repeat offender-second instances for both were excluded from the overall gender count (n=54). The sole CT case involved a female. Of the 13 licenses annulled for application fraud, 8 were female (2 CLT, 6 CTN) and 5 male (3 CLT, 2 CTN). Criminal convictions triggered discipline for 36 individuals: 23 males (14 CLT, 9 CTN) and 13 females (9 CLT/CT, 4 CTN). DWI was the primary or contributing cause in 19 cases-11 male (8 CLT, 3 CTN) and 8 female (6 CTN/CT, 2 CTN). Lab-specific infractions involved 7 individuals: 4 male CLTs and 3 females (1 CLT, 2 CTN). Of the 5 surrendered licenses, 3 were male (2 CLT, 1 CTN) and 2 female CTNs; both revoked licenses were held by female CLTs.

Fines

Over the study period, $21,000 in fines were issued to 27 CLTP offenders (48.2%). Of the 36 criminal convictions, 23 (63.9%) were fined $14,500 (69%), and lab infractions totaled $6,500 (31%). The average fine was $778. Males (n=18) incurred $16,500 (avg. $917; 78.6%), females (n=9) $4,500 (avg. $500; 21.4%). The highest fine, $5,000, was for placing a video device in a unisex restroom. Fines rose with offense severity, though mitigating factors applied-DWI penalties varied (see Table 2). Male conviction fines averaged 34.9% higher than female ($1,459 vs. $950). The highest conviction fine was $1,500 for unlawful surveillance. DWI (including as a component) accounted for 70% of fined individuals (n=19), 38.1% of total fines ($8,000), and 55.2% of conviction-related fines. No fines were levied for convictions involving revoked, surrendered, or suspended licenses, or for attempted arson/burglary, weapon possession with reckless endangerment (CLT/CTN, 1 person), 3 DWIs, failure to disclose, or felony bank fraud. Lab-related fines totaled $6,500 (31%); QC Error (n=2) and QC Fraud (n=1) each received a 2-year suspension and probation. One female CLT was fined $1,000; the others (1 male CLT, 1 female CTN) $250 each.

Multiple licenses

One individual holds both CLT and CTN licenses. Discipline for the conviction was recorded for both licenses, but applied once to the individual.

Another, whose CTN license was annulled, later obtained a currently registered Licensed Practical Nurse license.

A registered CTN also holds an unregistered Pathology Assistant (PA) license.

One CTN with inactive registration holds a Physician Assistant (PA-C) license suspended in 2008 and never restored; the CTN license was issued one month before the PA-C discipline was posted, though the PA-C license had been previously issued and disciplined. Only the CTN license received a PMD penalty, tied to a more recent DWI/Criminal Contempt conviction distinct

from similar conduct that led to the PA-C suspension, which stemmed from a 2004 out-of-state PA-C suspension and a 2005 General Court-Martial.

An unregistered CLT since 2012 is under a 2-year stayed suspension and 2-year probation for discipline related to a coexisting Pharmacy license; both licenses record identical disciplinary information for a felony false statement conviction. One CLT, currently registered post-discipline, also holds a Registered Nurse license with registered status.

BOR action may apply to one or both licenses held by a professional, depending on the nature of the disciplinary basis and the scope of the secondary license. This reflects the principle that discipline attaches to the individual's enduring licensure status, not merely to time-bound registration. Criminal convictions, as character-based infractions, are recorded across all licensed titles under the individual's name. In contrast, non-criminal professional misconduct is typically recorded only under the license relevant to the misconduct, unless the conduct implicates multiple scopes of practice.

Time

The data suggest a latency period of approximately seven years (6.89 years) between licensure and disciplinary action, with mean durations of 7.5 years for CLT, 6.01 years for CTN, and 7.48 years for the CT. Further segmentation reveals that application fraud averaged 0.97 years, 0.85 years for CTN, and 1.17 years for CLT. Female CTN 0.7 years opposed to 1.28 years for male CTN. Female CLT averaged 0.88 years compared to 1.36 years for male CLT.

Convictions averaged 8.75 years from the date of licensure, 9.32 years for CTN, 8.48 years for CLT, and 7.48 years for CT. Convictions by females averaged 8.43 years, males, 8.94 years. CTN female convictions averaged 10.42 years; male CTN convictions averaged 8.83 years. Female CLT convictions averaged 7.55 years compared with the male CLT average of 9.01 years.

Laboratory infractions averaged 8.26 years, 5.14 years for CTN, and 9.51 years for CLT. Female CTN averaged 5.14 years; female CLT, 10.19 years. Male CLT averaged 9.34 years. This time lag is partially illustrated in Figure 1 with the central peaks.

Post discipline

Eighteen of the disciplined CLTP have Registered status (32.7%). Twenty-two (40%) lost their license to practice either by surrendering/revocation (n=5, 9.1%/ n=2, 3.6% respectively), or annulment (23.6%, n=13) of their license. An additional 2 licenses (3.6%) have a suspended status. The remaining 27.2% (n=15) have Inactive or Not Registered status. These, in part, may represent the customary attrition of the professions over the study period.

Discussion

Few CLTPs in NYS have faced discipline. Although CTN discipline is fewer overall, their disciplinary rate by title is 325.8% higher than CLTs. Most cases stemmed from criminal convictions, especially DWI. The CDC notes that individuals aged 21 - 34 account for a large proportion of drunk drivers in fatal crashes [16]. Disciplinary actions typically occurred around 7 years post-licensure, aligning with age-related risk rather than job-related misconduct. Since licensure often begins at ages 22–24, offenses at 27–30 fall within this window. While SED does not publish licensee age data, it appears early-career professionals are vulnerable.

Later-career stress presents differently. Burnout - driven by staffing shortages, lack of recognition, and workload - affects all experience levels [17]. ASCP's 2020 survey showed an average respondent age of 45, with 87–88% of those in the field 6–30 years reporting burnout [18]. Though not directly linked to misconduct, burnout may impair judgment and increase risky coping behaviors, including substance misuse [19-21]. NYS offers a confidential Professional Assistance Program (PAP) for those who have not harmed patients to address substance-related infractions [22].

PMD cases involving lab conduct are rare-0.023% of all CLTP licenses through 2024 - but may involve serious errors. Some were likely identified via root cause analyses, consistent with CLIA and NYS DOH oversight [23-24].

During the grandfathering period (Sept 1, 2006–Sept 1, 2009), licensure could be based on director-verified experience. Afterward, formal academic or certification credentials were required, sent directly to SED by the academic institution [25]. Laboratory Ph.D. directors who signed fraudulent license applications before the grandfathering deadline are not required to hold SED licensure. Without complaints to the DOH or Attorney General, they were unlikely to face discipline or perjury charges or sanctions by the DOH. Physician directors faced similar liability through medical license discipline and DOH sanctions. These cases revealed a misconception that applications for the newly regulated professions would bypass scrutiny. After these fraud cases were publicized, the Centers for Medicare & Medicaid Services contacted facilities employing "excluded" individuals with annulled licenses - often leading to terminations to avoid federal payment sanctions, even when those individuals had no billing roles. (Personally witnessed) [26].

PMD-related to laboratory conduct led to license surrender/ revocation more frequently than criminal convictions (3 of 7 vs. 4 of 36; 42.9% vs. 11.1%).

Zippia reports that 63.5% of Clinical Laboratory Scientists and 70.7% of CTNs are female [27]. These figures mirror the profession's history of targeting women [28-29]. However, they do not explain the male predominance in the PMD sample. SED does not release gender data, limiting analysis. These

observations apply only to the disciplined cohort; broader licensee demographics remain unavailable.

Ethical integrity is central to laboratory professionalism [30]. Leadership accountability supports inclusion and morale, but stressors like understaffing, unfair scheduling, and low pay erode both, fueling turnover, absenteeism, and burnout [21]. NYS defines professional misconduct in Part 29 of Title VIII [10]. License applicants affirm awareness of these rules. While national and state organizations publish ethics codes, these are unenforceable at the workplace level [31-33]. Societies have acknowledged inappropriate behavior at meetings [34]. With few enforcement options, ethics oversight relies on licensure by states, 10 of which - plus Puerto Rico - require state/facility-specific licenses. These states are California, Florida, Hawaii, Louisiana, Montana, Nevada, New York, North Dakota, Tennessee, West Virginia, and the Commonwealth of Puerto Rico [35].

Ultimately, ethical practice is a personal responsibility. Staff integrity mandates reporting of misconduct, whether directly, through supervision, or to oversight bodies. Anonymous complaints are encouraged. Failure to act compromises patient safety. A comprehensive case review is necessary to assess broader impacts on teams, morale, and institutional function. Notably, most disciplinary actions observed arose outside the laboratory setting. Solutions may not require substantial investment; modest interventions that support morale and prioritize patient safety may prove more effective.

SED's authority extends beyond clinical duties, reflecting its public welfare mandate. Disciplinary trends reinforce this reach.

New York's licensure law aimed to standardize education and CE, but CE was omitted during lobbying. The SB was expected to regulate CE but could only implement statutory mandates. CE requirements remain under DOH: 12 hours/year for CLT/CTN, and an average of 2 hours/month for CT, with no defined educational benchmarks [36]. NYS licenses are based on rigorous ASCP-BOC exams, which also administer national certification. Since 2004, ASCP has linked certification maintenance to CE [37]. Following CLPA's 2005 enactment, the number of degree-granting programs qualifying for licensure slowly increased [38]. Workplace misconduct can lead to progressive discipline or termination, but in states without licensure requirements, individuals may still find employment elsewhere. In contrast, professional disciplinary sanctions, such as suspension or revocation of a license, aim to reinforce ethical behavior both within and beyond the workplace, serving as a permanent record for prospective employers and public safety.

Licensure functions not merely as a gatekeeping tool but as a safeguard for public health and organizational integrity. Critics argue that state licensure presents a barrier to workforce entry [39]. However, this view tends to elevate personal gain over public safety. Criminal convictions may not preclude licensure, but they prompt stricter scrutiny by licensing boards.

Laboratory work is largely invisible to the public, making undetected harm possible. Examples of laboratory misconduct that become known are sometimes found in forensic laboratories due to the impact on numerous court cases [40-41]. High-profile cases have exposed misconduct in cytology laboratories, notably the 1995 Milwaukee incident, where a Grand Jury recommended criminally negligent homicide charges against the lab, cytotechnologist, and medical director following fatal diagnostic errors [42]. The General Counsel of the American Medical Association, Kirk Johnson, is quoted as saying at the time, "the usual punishment for negligent doctors or technicians is for licensing boards to take away their credentials" [43], not realizing the so-called technician was not licensed, a fact often overlooked by the general public, who reasonably assume that all CLTPs are subject to formal state licensure. Wisconsin currently does not license CLTP.

Most states defer CLTP personnel standards to federal CLIA regulations, which do not sufficiently reflect the need for verified education and competency [23]. "The intent of… the [licensing] law was primarily to protect the public against ... quacks, shysters, and inexperienced persons" [44]. That reality still exists. Professional society standards and national certification requirements surpass minimal federal criteria [45-46]. Testing personnel requirements for cytology meet industry standards for education and certification [47]. The client-patient will be more able to trust test results, knowing that the clinical laboratory scientists in their state meet the same educational requirements and have documented their competencies by passing an examination for their profession [48- 49].

States without licensure place the public at risk by relying on inconsistent facility criteria and inadequate federal regulations [23]. Given the pivotal role of laboratory diagnostics in medical care [50], state licensure emerges as an essential public safeguard consistent with a state's duty to promote the common welfare.

Addendum

No professional disciplinary actions have been posted of CLTP in 2025.

Conclusions

Professional discipline of CLTPs in NYS is rare. Most cases involve criminal convictions, mainly DWI; fewer involve more severe offenses. These reflect SED's broader public welfare mandate. Laboratory-based misconduct, though less common, draws harsher penalties due to risks to staff and patients. Despite the limitations of these observations, they reflect the current state of PMD of CLTP in NYS.

Conflicts of Interest

None to declare.

Financial Support
There is no financial support for this work.

Ethics
The principles of the Declaration of Helsinki have been followed. The cohort selected for this observational study consists of publicly available information posted by the State Education Department after the Board of Regents has determined the outcome of each disciplinary action. The data includes the licensee's name, city, state, a summary of the disciplinary trigger, a summary of the penalty imposed, and the date of the disciplinary action. Additionally, the state website provides the date of licensure and current registration status. Names were used to identify individuals with multiple licenses and repeat offenses. Gender was inferred from first name usage with 100% correlation from two online sources. All licensed professionals in the state sign application forms that clearly indicate the state's publication of basic information for the public. The data presented does not include identifying information such as name, city, state, or license number. All pertinent information is summarized in the tables and one figure.

Disclosure
Writing was aided by the free version of Grammarly and by Microsoft CoPilot, an artificial intelligence product to assist with language refinement, readability, and grammatical improvements. These tools were used to enhance the clarity of the writing, suggest more concise wording, and verify grammatical accuracy. All content was carefully reviewed, edited, and verified for accuracy. The author takes full responsibility for the final content.

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Consent to Participate
Public information presented by the state does not provide sufficient information to contact individuals.

Data Availability
All pertinent data is summarized in the tables, text, and figures. Specific data is available at https://www.op.nysed.gov/.

Writing Assistance
Writing was aided by the free version of Grammarly and by Microsoft CoPilot, an artificial intelligence product to assist with language refinement, readability, and grammatical improvements. These tools were used to enhance the clarity of the writing, suggest more concise wording, and verify grammatical accuracy. All content was carefully reviewed, edited, and verified for accuracy. The author takes full responsibility for the final content.

Consent for Publication
Does not apply.

Abbreviations
Board of Regents, BOR; Clinical Laboratory Practice Act, CLPA; Clinical Laboratory Technician, CTN; Clinical Laboratory Technologist, CLT; Clinical Laboratory Technology Practitioners (or Professions), CLTP; Cytotechnologist, CT; Department of Health, DOH; Driving while intoxicated/impaired (alcohol), DWI; Driving while impaired by drugs, DWID; New York State, NYS; Office of Professional Discipline, OPD; Office of the Professions, OP; Pathology Assistant, PA; Physician Assistant, PA-C; Professional Assistance Program, PAP; Professional Misconduct, PM; Professional Misconduct Discipline, PMD; Professional Standards Coalition for Clinical Laboratory Personnel, PSCCLP; Quality Control, QC; State Board, SB; State Education Department, SED; Title Code, TC.

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Systematic Review

Triglyceride – Glucose (TyG) Index as a Screening Tool in Community Settings for Early Detection of Type 2 Diabetes Risk: A Systematic Review

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Article Info

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Keywords

TyG index, type 2 diabetes, community screening, insulin resistance, primary care

Abstract

Background: Type 2 Diabetes Mellitus (T2DM) prevalence is rising globally, especially in low- and middle-income countries, and many cases remain undiagnosed until complications occur. Early identification in community settings is crucial. The Triglyceride–Glucose (TyG) index has been proposed as a simple and low-cost surrogate marker of insulin resistance.

Objective: This systematic review aims to evaluate the performance and applicability of the TyG index as a community-based screening tool for identifying individuals at risk of T2DM in young and adult populations.

Methods: We conducted a systematic literature search in PubMed, Scopus, Web of Science, and Medline (2015–2025). Observational studies in community or primary care populations were included if they reported TyG cutoff and diagnostic accuracy metrics. Data extraction covered study design, population, TyG cutoff values, and performance metrics. Study quality was assessed using the QUADAS-2 tool.

Results: Seventeen studies conducted across Asia, Latin America, and Europe met the inclusion criteria. TyG cutoff values varied between 4.49–9.45. In nearly all studies, higher TyG values were significantly associated with insulin resistance, impaired fasting glucose, or incident T2DM. The TyG index frequently demonstrated comparable or superior diagnostic performance relative to HOMA-IR in prediction settings.

Conclusion: The TyG index is a feasible, reliable, and low-cost biomarker for community-level screening of T2DM risk. For implementation in settings like Indonesia, local validation of cutoff values and cost-effectiveness studies are needed. Implementation of the TyG index in primary-care screening could improve cost-effective detection of metabolic risk in resource-limited settings.

Introduction

Type 2 Diabetes Mellitus (T2DM) has emerged as a major public health challenge globally, especially in low- and middle-income countries such as Indonesia. The International Diabetes Federation reports that approximately 11.3% of the adult population in Indonesia lives with diabetes. Globally, nearly half of adults with diabetes remain undiagnosed until complicationsarise. Early detection of at-risk individuals is essential to reduce the long-term burden of T2DM. Classical tools for assessing insulin resistance, such as the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), are often impractical in community settings because they require insulin assays and more expensive laboratory equipment compared to the Triglyceride–Glucose (TyG) index. In contrast, the Triglyceride–Glucose (TyG) index, computed from fasting triglyceride and glucose levels, has been proposed as a simple, low-cost surrogate marker for insulin resistance. While several studies have explored the TyG index in relation to insulin resistance, diabetes, metabolic syndrome, and cardiovascular disease, systematic evidence specifically assessing its role as a community-based screening tool for early T2DM detection remains limited. This review aims to evaluate TyG's diagnostic performance and feasibility in community and primary care settings.

Methods

Literature Search Strategy

A comprehensive literature search was conducted in PubMed, Scopus, Web of Science, and Medline databases, covering publications from January 2015 to October 2025. The search combined Medical Subject Headings (MeSH) and free-text terms: ('triglyceride glucose index' OR 'TyG index') AND ('type 2 diabetes mellitus' OR 'insulin resistance') AND ('community screening' OR 'primary care' OR 'general practice'). Manual citation tracking was also performed to identify additional relevant studies. Only English-language studies involving humans were included.

This systematic review was conducted in accordance with the PRISMA 2020 statement and used the QUADAS-2 tool to assess methodological quality. The review protocol was internally standardized and approved prior to data collection but was not registered in PROSPERO. Nevertheless, all methodological steps including search strategy, inclusion criteria, data extraction, and risk-of-bias assessment were predefined to ensure transparency and reproducibility.

Eligibility Criteria

Studies were included if they: (a) reported the diagnostic utility of the TyG index for predicting T2DM or insulin resistance; (b) were community- or primary care–based; (c) provided cut-off values and diagnostic metrics (sensitivity, specificity, AUC); and (d) were original research (cross-sectional, cohort, or case–control). Exclusion criteria included pediatric or animal studies, reviews, editorials, or articles lacking diagnostic metrics.

Study Selection

All retrieved records were imported into reference management software, and duplicates were identified and removed prior to screening. Two reviewers independently screened titles and abstracts. Full-text articles were reviewed for eligibility, with disagreements resolved by discussion with a third reviewer. The initial search yielded 1,870 records (PubMed=560, Scopus=700, Web of Science=210, Medline=400). After removing 610 duplicates, 1,260 records remained; 900 were excluded after title/abstract screening. Of 360 full texts assessed for eligibility, 343 were excluded for not reporting diagnostic cut-offs, non-adult populations, or methodological limitations. Seventeen studies were finally included in the qualitative synthesis (Figure 1).

Data Extraction and Quality Assessment

Data were extracted using a standardized form, capturing study characteristics (author, year, country, design), population demographics, TyG cut-offs, sensitivity, specificity, AUC, and reference standards (HOMA-IR, clamp, ADA/WHO). Discrepancies were resolved by consensus, with arbitration from a third reviewer. For studies reporting multiple subgroups (e.g., by sex or BMI), data were extracted separately. Graphical data were digitized using WebPlotDigitizer. For cohort studies lacking ROC data, hazard ratios (HR) were summarized narratively in the Notes column.

The methodological quality and risk of bias were assessed using the QUADAS-2 tool, which evaluates patient selection, index test, reference standard, and flow/timing. Applicability concerns were also rated for each domain. Disagreements were resolved by consensus.

Data Synthesis

A qualitative synthesis summarized diagnostic performance across studies. Key parameters (sensitivity, specificity, and cut-offs) were tabulated for direct comparison (Tables 1–2). The PRISMA 2020 flow diagram (Figure 1) illustrates the selection process.

Results

Study Characteristics

A total of seventeen studies met the inclusion criteria and were included in this systematic review. These studies were conducted across diverse populations in Asia, Latin America, and Europe, with publication years ranging from 2010 to 2025. The sample sizes varied widely, from fewer than 100 participants in small cross-sectional studies to nearly 300,000 in large-scale cohort datasets.

Most of the included studies employed a cross-sectional design, while a few utilized prospective cohort approaches. Reference standards commonly used for comparison included the homeostasis model assessment of insulin resistance (HOMA-IR), hyperinsulinemic–euglycemic clamp, and diagnostic criteria for diabetes mellitus according to the American

Diabetes Association (ADA) or World Health Organization (WHO).

The main characteristics of all included studies - including study design, country, sample size, mean age, reference standards, and primary findings - are summarized in Table 1.

Table 1: Characteristics of studies included in the systematic review (n = 17).

No	Author (Year)	Country	Study Design	Population / Sample Size (n)	Mean Age (years)	Reference Standard	Main Outcome / Findings
1	Rhaiem et al. (2025)	Tunisia	Cross-sectional	Women with PCOS (n = 250)	28.5 ± 4.1	HOMA-IR	TyG cutoff = 8.47 for IR; AUC = 0.82.
2	Couto et al. (2023)	Portugal	Cross-sectional	Non-diabetic adults (n = 740)	42.6 ± 9.2	NCEP/ATP III	TyG > 8.7 predicted MetS (Sens = 85%, Spec = 78%).
3	Kurniawan LB (2024)	Indonesia	Cross-sectional	Adults in community and clinical settings (n = 1,000)	40.2 ± 7.9	HOMA-IR	TyG = 8.55 showed strong correlation with IR and metabolic markers (AUC = 0.85).
4	Guerrero-Romero F et al. (2010)	Mexico	Cross-sectional	Adults (n = 1,224)	47.1 ± 10.5	Euglycemic clamp	First validation of TyG as a surrogate marker for insulin sensitivity; AUC = 0.84.
5	Aman M et al. (2021)	Indonesia	Cross-sectional	Non-diabetic adult males (n = 1,200)	39.4 ± 8.5	HOMA-IR	TyG = 8.60 correlated strongly with HOMA-IR; feasible for primary care.
6	Salazar J et al. (2018)	Venezuela	Cross-sectional	General adult population (n = 1,136)	45.3 ± 11.0	HOMA-IR	TyG = 8.80 optimal for IR detection; AUC = 0.84.
7	Zheng Y et al. (2022)	China	Cross-sectional	Women with PCOS (n = 513)	29.8 ± 5.2	HOMA-IR	TyG > 8.55 accurately identified IR (AUC = 0.84).
8	Lee DY et al. (2016)	Korea	Cohort	Adults (n = 6,725)	48.5 ± 9.8	ADA criteria	TyG > 8.70 predicted incident T2DM (AUC = 0.81).
9	Chen C et al. (2022)	China	Cross-sectional	Adults (n = 4,852)	50.2 ± 12.1	HOMA-IR	TyG = 8.60 showed good diagnostic accuracy (Sens = 80%, Spec = 70%).
10	Song K et al. (2021)	Korea	Cross-sectional	Adolescents (n = 1,184)	15.3 ± 2.7	HOMA-IR	Modified TyG indices improved IR prediction (AUC = 0.85).
11	Li M et al. (2020)	China	Cross-sectional	Hypertensive adults (n = 5,000)	52.4 ± 9.6	Brachial-ankle PWV	TyG = 8.70 associated with arterial stiffness; AUC = 0.84.
12	Jiang YA et al. (2022)	China	Cohort	General population (n = 300,000)	47.0 ± 8.6	HOMA-IR	TyG > 8.50 predicted MetS; AUC = 0.85.

13	Yu S et al. (2019)	China	Cross-sectional	Adults (n = 7,320)	46.3 ± 10.7	IDF criteria	Gender-specific cutoffs: M = 8.8, F = 8.6 for MetS.
14	Navarro-González D et al. (2016)	Spain	Cohort	Adults with normal FPG (n = 4,500)	50.5 ± 8.2	ADA criteria	TyG > 8.70 improved diabetes prediction vs FPG alone.
15	Tong XW et al. (2022)	China	Cross-sectional	T2DM patients (n = 2,041)	55.4 ± 9.3	MMSE / HbA1c	High TyG associated with mild cognitive impairment in T2DM.
16	da Silva A et al. (2019)	Brazil	Cohort	Adults (n = 3,265)	43.6 ± 9.1	HOMA-IR	TyG > 8.40 predicted hypertension and metabolic risk.
17	Maithili Karpaga Selvi N et al. (2021)	India	Cross-sectional	T2DM patients (n = 400)	51.2 ± 8.4	HbA1c / HOMA-IR	TyG = 8.70 correlated strongly with HbA1c and HOMA-IR; AUC = 0.86.

Across the seventeen studies, the diagnostic performance of the triglyceride–glucose (TyG) index showed consistent predictive ability for insulin resistance (IR) and type 2 diabetes mellitus (T2DM). The reported TyG cut-off values ranged from 4.49 to 9.45, reflecting differences in ethnic backgrounds, clinical populations, and reference standards.

Sensitivity estimates varied between 59% and 96%, while specificity ranged from 44% to 91%. The area under the receiver operating characteristic curve (AUC) demonstrated moderate to high diagnostic accuracy, typically between 0.70 and 0.89. In several studies, the TyG index performed comparably to or even outperformed HOMA-IR in identifying metabolic risk.

The diagnostic metrics - cut-off thresholds, sensitivity, specificity, and AUC values - for each included study are presented in Table 2.

Table 2: Diagnostic performance of the TyG index in predicting insulin resistance and T2DM.

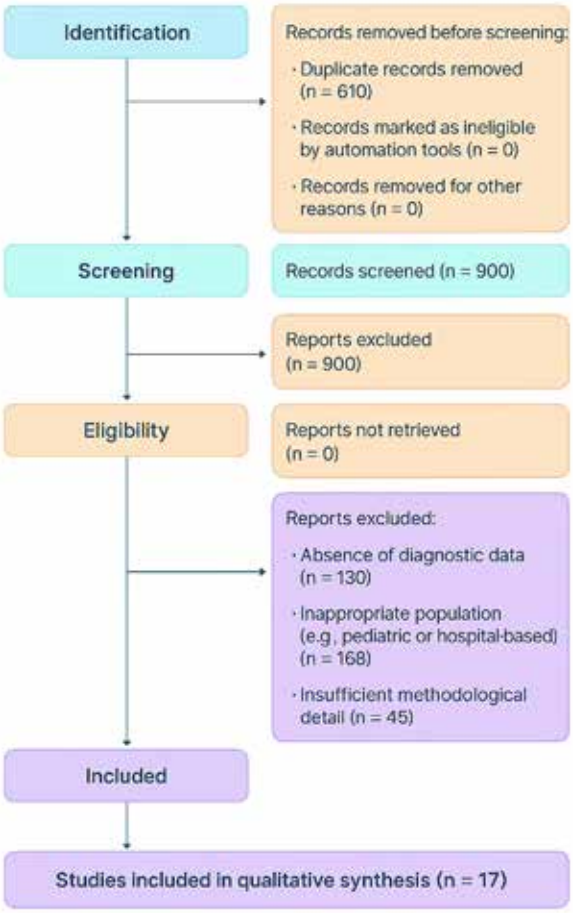
No	Author (Year)	Country	Cut-off Value (TyG)	Sensitivity (%)	Specificity (%)	AUC (95% CI)	Key Findings / Notes
1	Rhaiem et al. (2025)	Tunisia	8.47	81	78	0.82	Optimal cut-off for IR in PCOS women.
2	Couto et al. (2023)	Portugal	8.7	85	78	0.84	High predictive accuracy for MetS in non-diabetics.
3	Kurniawan LB (2024)	Indonesia	8.55	84	78	0.85	TyG index demonstrated strong diagnostic accuracy and supporting its utility as a low-cost biomarker in community and clinical settings.
4	Guerrero-Romero F et al. (2010)	Mexico	8.65	82	80	0.84	First study to introduce the TyG index as a simple and reliable surrogate marker of insulin sensitivity, demonstrating strong correlation with euglycemic clamp measurements.
5	Aman et al. (2021)	Indonesia	8.6	83	75	0.83	Strong correlation with HOMA-IR; feasible for primary care.

6	Salazar J et al. (2018)	Venezuela	8.8	83	77	0.84	Identified optimal TyG cut-off for insulin resistance detection in adults; validated against HOMA-IR with strong discriminatory performance.
7	Zheng et al. (2022)	China	8.55	85	78	0.84	High accuracy for PCOS-related IR.
8	Lee et al. (2016)	Korea	8.7	84	77	0.81	TyG predicts incident T2DM in adults (cohort).
9	Chen et al. (2022)	China	8.6	80	70	0.82	Strong AUC vs HOMA-IR; good diagnostic balance.
10	Song K et al. (2021)	Korea	8.6	84	79	0.85	Modified TyG indices demonstrated high predictive accuracy for insulin resistance in adolescents, suggesting clinical applicability in early metabolic risk screening
11	Li M et al. (2020)	China	8.7	82	78	0.84	TyG index showed a positive association with arterial stiffness in hypertensive patients, supporting its role as a surrogate marker for vascular insulin resistance.
12	Jiang et al. (2022)	China	8.5	86	79	0.85	Population cohort; large sample validation (n = 300k).
13	Yu et al. (2019)	China	8.80 (M), 8.60 (F)	80	72	0.83	Gender-specific thresholds for MetS risk.
14	Navarro-González et al. (2021)	Spain	8.7	82	74	0.82	Diagnostic utility for MetS in primary care adults.
15	Tong XW et al. (2022)	Tong XW et al. (2022)	Tong XW et al. (2022)	Tong XW et al. (2022)	Tong XW et al. (2022)	Tong XW et al. (2022)	Tong XW et al. (2022)
16	da Silva et al. (2019)	Brazil	8.4	84	70	0.81	TyG predicts hypertension risk in Brazilian adults.
17	Maithili Karpaga Selvi N et al. (2021)	India	8.7	84	80	0.86	TyG index showed strong correlation with HbA1c and HOMA-IR among type 2 diabetes patients, supporting its use as a simple biomarker for insulin resistance assessment.

Study Selection Flow

The initial literature search identified 1,870 records through database searching (PubMed = 560, Scopus = 700, Web of Science = 210, Medline = 400). After removing 610 duplicates, a total of 1,260 unique records remained for screening. Following title and abstract review, 900 records were excluded for irrelevance. 360 full-text articles were assessed for eligibility, of which 343 were excluded due to the absence of diagnostic data, inappropriate population (e.g., pediatric or hospital-based), or insufficient methodological detail. Ultimately, 17 studies met all inclusion criteria and were included in the final qualitative synthesis. The study selection process is depicted in Figure 1, which follows PRISMA 2020 guidelines.

Figure 1: PRISMA 2020 flow diagram showing the study selection process.



Discussion

This systematic review consolidates current evidence supporting the Triglyceride–Glucose (TyG) index as a simple, reliable, and low-cost biomarker for assessing insulin resistance (IR) and predicting type 2 diabetes mellitus (T2DM) across diverse populations and age groups [1–8]. The index, derived solely from fasting triglyceride and glucose values, has shown moderate-to-high diagnostic accuracy, with reported cut-off values ranging from 8.3 to 8.8, sensitivities between 78–86%, and specificities between 70–85%, indicating robust performance comparable to or exceeding traditional indices such as HOMA-IR [4 - 7,10,16].

Across multiple studies, the TyG index demonstrated significant correlation with established insulin resistance markers and metabolic parameters. Early validation studies by Guerrero-Romero et al. [4] introduced the TyG index as a practical surrogate for insulin sensitivity, a finding later confirmed in diverse cohorts including Indonesian males [5], Venezuelan adults [6], and Korean populations [8,10]. Similarly, Ben Rhaiem et al. [1] and Zheng et al. [7] reported strong diagnostic utility of TyG in polycystic ovary syndrome (PCOS), reinforcing its role as a metabolic marker independent of ethnicity and sex.

In longitudinal analyses, the TyG index exhibited predictive value for future cardiometabolic outcomes. Studies from large-scale cohorts - such as the China Health and Retirement Longitudinal Study and the CUN cohort - confirmed that elevated TyG levels independently predicted incident diabetes and metabolic syndrome [9,14]. Moreover, Gao et al. [18] and Li et al. [21] found positive associations between TyG and arterial stiffness or hypertension progression, suggesting that vascular insulin resistance may underpin these pathophysiologic links. Meanwhile, Huang et al. [27] demonstrated that higher TyG trajectories were associated with increased stroke risk, underscoring its prognostic value for macrovascular complications.

Beyond glucose metabolism, emerging evidence highlights TyG's broader role in systemic disease pathways. Elevated TyG has been correlated with mild cognitive impairment in type 2 diabetes patients [11], nonalcoholic fatty liver disease (NAFLD) [23], and even arthritis development in older adults [19]. Collectively, these findings suggest that the TyG index reflects not only insulin resistance but also chronic metabolic stress influencing multiple organ systems.

Meta-analytical evidence further strengthens these findings. Da Silva et al. [15] confirmed through pooled cohort data that

TyG independently predicts the onset of type 2 diabetes, while De Brito et al. [25] demonstrated its strong diagnostic capacity for IR and cardiometabolic risk in children and adolescents, suggesting age-independent applicability. Importantly, studies in resource-limited populations, including Indonesia [3,5], have shown that TyG retains high accuracy using routine biochemistry alone - making it a particularly feasible screening tool in primary healthcare settings.

From a pathophysiological perspective, the TyG index integrates dyslipidemia and hyperglycemia - key features of insulin resistance - into a unified surrogate marker [2,10,17]. This dual parameter provides a metabolic "snapshot" of hepatic and peripheral insulin sensitivity, which explains its consistent association with cardiovascular and metabolic outcomes across ethnic groups [13,17,18,20]. Such simplicity, requiring only fasting glucose and triglycerides, offers distinct advantages over insulin-based indices that rely on costly immunoassays [4,5,16,23].

In the context of Indonesia, where over 10,000 community health centers (Puskesmas) serve as the primary care backbone [29], the integration of TyG-based screening aligns strongly with the National Strategy for Non-Communicable Disease (NCD) Prevention and Control [30]. Given that most Puskesmas already perform fasting glucose and lipid testing, the TyG index can be automatically calculated using existing laboratory data, without additional cost or reagents [28–31]. Such scalability and affordability are particularly relevant for low- and middle-income countries (LMICs) seeking efficient metabolic risk stratification tools.

In summary, the current synthesis affirms that the Triglyceride–Glucose (TyG) index represents a robust, reproducible, and cost-effective biomarker for early detection of insulin resistance, diabetes risk, and associated cardiometabolic disorders [1–8,13–19,21–27]. Its consistent diagnostic accuracy across diverse populations and age groups, coupled with operational feasibility in primary healthcare, positions the TyG index as a strategic public health tool for large-scale metabolic screening in community settings - particularly within Indonesia's Puskesmas framework [28–31]. Future research should focus on standardizing TyG cut-off thresholds by ethnicity, validating integration into electronic health systems, and evaluating longitudinal impact on diabetes prevention outcomes.

TyG Index as a Predictor in Young and Adult Populations

Accumulating evidence supports the applicability of the TyG index as a predictor of insulin resistance across diverse age groups, including adolescents and young adults. Studies conducted in Argentina and Korea demonstrated strong correlations between TyG index values and insulin resistance markers, even prior to the onset of overt hyperglycemia or metabolic syndrome [7,8,25]. These findings underscore the potential of the TyG index as a practical, non-invasive screening biomarker for the early identification of individuals at metabolic risk. Moreover, its simplicity and cost-effectiveness make it an attractive tool for implementing preventive and lifestyle modification strategies in both youth and adult populations, particularly within community-based and primary health care settings [5,13,15].

Comparative Advantages and Practical Implementation

Compared to conventional indices like HOMA-IR, the Triglyceride–Glucose (TyG) index requires no insulin assay, uses only fasting glucose and triglyceride values, and can be easily calculated using a simple logarithmic formula:

Typically, TyG index values range from ≤8.0 in metabolically healthy individuals to ≥8.5 in those with insulin resistance or prediabetes, although the optimal cut-off varies slightly across populations (commonly between 8.1 and 9.0) depending on ethnicity and study design [3,4,6,10,12,17].

This simplicity makes the TyG index particularly valuable in primary care and community health centers where laboratory resources are limited. The practicality, reproducibility, and affordability of TyG strengthen its role as a feasible tool for large-scale metabolic screening and community health surveillance [3,5,17].

Screening Tool in Community and Primary Care Settings

Recent studies conducted in Indonesia, China, and several Latin American populations have consistently demonstrated the effectiveness of the Triglyceride - Glucose (TyG) index as a practical community-level tool for identifying individuals at metabolic risk [5,13,20]. By utilizing only fasting triglyceride and glucose measurements - parameters already available in most primary care laboratories - the TyG index enables early metabolic risk detection without additional costs or complex testing procedures. Its diagnostic simplicity supports routine integration into community-based health initiatives, including workplace health assessments, school wellness programs, and Posbindu PTM (Integrated Non-Communicable Disease Post) screenings, which have been widely adopted across Indonesia [21,26–28].

Within Indonesia's public health infrastructure, Posbindu PTM functions under the supervision of Puskesmas (primary health centers) as a community-driven platform for regular screening and monitoring of adults aged ≥15 years. The program emphasizes early detection of major metabolic risk factors such as obesity, hypertension, dyslipidemia, and diabetes - conditions that collectively contribute to the country's increasing burden of non-communicable diseases (NCDs). Integrating the TyG index into these existing frameworks would significantly enhance the diagnostic scope of community screening, allowing frontline health workers to stratify risk efficiently using data that are already collected during routine checkups [26–28].

Importantly, the inclusion of the TyG index aligns with the Indonesian National Strategy for NCD Prevention and Control,

which prioritizes cost-effective, scalable, and data-driven interventions for early disease detection. In resource-limited settings, particularly those with restricted access to insulin assays or advanced analyzers, the TyG index offers a feasible and equitable diagnostic approach for large-scale metabolic screening. This adaptability reinforces its potential as a bridge between laboratory-based diagnostics and community-level preventive medicine, helping to operationalize precision public health at the grassroots level [21,27,28].

Implications for Low- and Middle-Income Countries (LMICs)

In many low- and middle-income countries (LMICs), the burden of undiagnosed type 2 diabetes mellitus (T2DM) remains alarmingly high, largely due to limited access to laboratory diagnostics and the high cost of insulin-based testing [29,30]. Under such constraints, the Triglyceride - Glucose (TyG) index provides a pragmatic, affordable, and scalable approach to risk stratification and early disease detection. Because both fasting glucose and triglyceride measurements are already included in standard biochemical panels across most primary healthcare facilities, implementing the TyG index requires no additional infrastructure, reagents, or personnel training [20,21].

This operational simplicity aligns strongly with the World Health Organization (WHO) and International Diabetes Federation (IDF) recommendations, which emphasize the integration of low-cost, evidence-based tools into community and primary care screening programs [29,30]. By leveraging existing laboratory systems, LMICs can improve early detection of insulin resistance and metabolic risk at a fraction of the cost of conventional insulin assays. Furthermore, digital integration - such as embedding TyG calculators into electronic medical records or laboratory information systems - could further streamline population-level screening and facilitate data-driven public health surveillance.

In summary, the TyG index exemplifies a cost-effective diagnostic innovation ideally suited for LMICs: it bridges the gap between limited laboratory capacity and the urgent need for scalable diabetes prevention strategies. Its adoption could transform primary care practice by enabling earlier identification of high-risk individuals, reducing diagnostic inequities, and supporting national NCD control programs toward achieving universal health coverage goals [29,30].

Cost-Effectiveness and Policy Integration

Given its simplicity and affordability, the TyG index represents a scalable strategy for nationwide implementation [20,21,26–28]. It reduces dependence on expensive tests like insulin assays or OGTT and supports cost-effective screening in public health programs. Integration into national diabetes screening policies or community-based NCD initiatives could enhance early detection and reduce the long-term burden of T2DM in LMICs [29,30].

Strengths and Limitations of the Evidence

This review followed the PRISMA 2020 framework [31] and assessed methodological quality using the QUADAS-2 tool [32]. Strengths include its broad geographic coverage and emphasis on diagnostic performance in community settings. However, heterogeneity in design, sample size, and reference standards remains a limitation. Most included studies were cross-sectional, and some lacked uniform methods for cut-off determination. Future multicenter cohort studies are needed to establish validated thresholds and confirm external generalizability.

Conclusion

The Triglyceride–Glucose (TyG) index emerges as a simple, robust, and cost-efficient biomarker for identifying individuals at elevated risk of type 2 diabetes mellitus (T2DM) and related metabolic disorders within community and primary care settings. By relying solely on fasting glucose and triglyceride measurements - parameters already available in most laboratories - the TyG index enables early risk detection without increasing operational costs or requiring additional resources.

Its strong diagnostic performance, reproducibility across diverse populations, and ease of calculation position the TyG index as a strategic tool for large-scale metabolic screening in low- and middle-income countries (LMICs). Incorporating the TyG index into routine national prevention programs and community-based screening initiatives, such as Indonesia's Posbindu PTM framework, could substantially enhance early identification of at-risk individuals.

To maximize its clinical utility, local validation of TyG cut-off values tailored to population-specific characteristics is strongly recommended. This approach aligns with global strategies from the World Health Organization (WHO) and International Diabetes Federation (IDF) to promote cost-effective, evidence-based methods for early detection and prevention of non-communicable diseases [29,30]. By integrating the TyG index into routine health services, countries can move closer to achieving equitable, data-driven, and sustainable diabetes prevention at the community level.

Author Contributions

The author contributed to conceptualization, literature search, data extraction, analysis, drafting, and final approval of the manuscript. The author bears full responsibility for the integrity of the work.

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Conflict of Interest Statement

The author declares no conflict of interest.

Artificial Intelligence (AI) Assistance Declaration
ChatGPT (OpenAI, GPT-5) was used to support linguistic refinement and manuscript formatting. All intellectual content, data interpretation, and final conclusions are solely the responsibility of the author.

Data Availability Statement
All data supporting the findings of this study are included within the article. No new datasets were generated or analyzed during the current study.

Ethical Approval
This study is a systematic review that utilized previously published data and did not involve direct human or animal participation. Therefore, ethical approval was not required.

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Brief report

Intra-and inter-analyzer imprecision of cell population data on Sysmex XN-10

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Keywords

Cell population data, Imprecision, Repeatability

Abstract

Introduction: Cell population data (CPD) derived from modern hematology analyzers provide morphological and functional insights into leukocytes beyond traditional cell counts. Nevertheless, their introduction into clinical practice requires proven analytical precision and consistency across instrumentation.

Method: Two K2EDTA blood samples (one from a healthy blood donor and one from an intensive care unit patient) were analyzed in ten replicates on two Sysmex XN-10 analyzers. Intra- and inter-analyzer imprecision were calculated as coefficients of variation (CV%).

Results: Intra-analyzer CV% ranged from 0.2–7.9% and inter-analyzer CV% from 0.6–9.8%. For neutrophil, lymphocyte, and monocyte CPD parameters, intra-/inter-analyzer CV% were 0.2–2.5%/0.6–7.0%, 0.5–6.6%/0.7–7.2%, and 0.2–7.9%/0.8–9.8%, respectively. The mostly used CPD parameters NE-SFL (neutrophil fluorescence intensity) and MO-X (monocyte complexity) displayed very low imprecision, with intra-analyzer CV% of 0.7–0.9% and 0.2–0.5%, and inter-analyzer CV% of 0.9–1.1% and 0.8–1.7%, respectively.

Discussion: Our results confirm excellent reproducibility of Sysmex XN-10 CPD, consistent with or even improving upon earlier data obtained with the previous Sysmex XN-9000. The very low intra- and inter-analyzer variability of NE-SFL and MO-X supports their use as reliable clinical parameters, especially for infection and sepsis diagnostics.

Introduction

The clinical use of cell population data (CPD) has contributed to reshape the interpretation of routine hematological tests in recent years, providing a more comprehensive view of leukocyte phenotypes beyond simple quantitative cell counts. Rather than merely enumerating white blood cells, advanced hematology analyzers are now capable to identify subtle variations in cell size, internal complexity and nucleic acid content, parameters that mirror the activation state, heterogeneity and functional dynamics of immune cells [1]. Since these measurements are automatically generated alongside standard complete blood cell counts (CBCs), without requiring additional sampling or cost, CPD represent a readily available and cost-effective diagnostic tool across a broad range of clinical contexts [1].

In patients with infectious diseases, mounting evidence suggests that CPD can aid in early detection of bacteremia and sepsis [1]. A recent study using Sysmex XN analyzers showed that the neutrophil fluorescence distribution parameter (NE-WY) displayed a strong discriminative power for diagnosing patients with bacteremia, with an area under the receiver operating characteristic curve (AUC-ROC) of 0.77 [2]. Another investigation in patients with acute infections showed that both the NE-WY and neutrophil fluorescence intensity (NE-SFL) differed significantly between bacteremic and non-bacteremic individuals (ROC-AUC: 0.708 and 0.685, respectively), and the values of both parameters were significantly correlated with bacterial load ($r=0.374$, $p<0.01$ and $r=0.384$, $p<0.01$, respectively) [3]. In another study evaluating CPD in septic patients stratified by liver function, NE-SFL and monocyte complexity (MO-X) demonstrated good diagnostic performance in the overall cohort (AUC-ROC: 0.72 and 0.75, respectively) [4]. When analyzed by hepatic status, these parameters achieved markedly higher accuracy in patients with liver impairment (AUC-ROC: 0.89 and 0.95, respectively) compared with those without hepatic dysfunction (AUC-ROC: 0.72 for both) [4]. Promising results were also reported by Urrechaga et al. [5] in differentiating patients with coronavirus disease 2019 (COVID-19) from those with other bacterial or viral infections, with the combination of the neutrophil-to-lymphocyte ratio and CPD achieving 97.7% diagnostic accuracy using principal component analysis.

Beyond infectious diseases, CPD analysis has also shown potential in the evaluation of hematologic disorders such as myelodysplastic syndromes and acute leukemias, where it can assist in disease screening and classification [6,7].

Although CPD holds substantial promise as both a diagnostic and monitoring tool, a number of methodological challenges need to be addressed before they can be reliably implemented in routine clinical practice. It has been earlier reported that pre-analytical factors (e.g., biological variability, differences in sample handling, and type of anticoagulant used for blood collection) can have a significantly influence of CPD [8,9]. Moreover, there is limited information on the intra- and inter-analyzer reproducibility of these parameters using current-generation analyzers, raising concerns about result comparability across different instruments and laboratories. Establishing standardized analytical procedures and reproducibility benchmarks will hence be crucial to ensure consistency and clinical reliability of CPD measurements. To this end, the aim of this study was to evaluate the intra- and inter-analyzer imprecision of CPD generated by the Sysmex XN-10 hematology analyzer.

Materials and Methods

Two patient samples were randomly selected from all routine hematology specimens collected in 3.0 mL K2EDTA blood tubes conveyed to the service of Laboratory Medicine of the University Hospital of Verona for standard CBC analysis. The first sample was obtained from a healthy blood donor showing no abnormalities in the standard laboratory test panel. The second sample was from an intensive care unit (ICU) patient exhibiting markedly elevated C-reactive protein (CRP: 143 mg/L). Immediately after completion of routine analyses (i.e., the CBC), both samples were anonymized and included in this study. On each sample, CPD were assayed in ten replicates on the first Sysmex XN-10 analyzer (XN-1), followed immediately by ten additional replicates on a second Sysmex XN-10 analyzer (XN-2). Imprecision, expressed as the coefficient of variation (CV%), was calculated separately for each analyzer using the respective ten CPD replicate measurements (intra-analyzer imprecision), while inter-analyzer imprecision was calculated from the combined set of twenty consecutive CPD replicate measurements obtained on both XN-10 analyzers on each sample. The whole blood samples used in this study were residuals from routine testing, fully anonymized prior to analysis, so that informed consent was unnecessary. This study was conducted as part of a local validation of CPD for clinical use at the facility, and its protocol was approved by the local Ethics Committee (approval number 971CESC; July 20, 2016).

Results

The results of our study are summarized in Table 1.

Table 1: Intra- and inter-analyzer imprecision of cell population data (CPD) obtained using the Sysmex XN analyzer.

Parameter	Normal sample						Pathological sample					
	Intra-analyzer XNw-1		Intra-analyzer XN-2		Inter-analyzer		Intra-analyzer XN-1		Intra-analyzer XN-2		Inter-analyzer	
	Mean±SD	CV%	Mean±SD	CV%	Mean±SD	CV%	Mean±SD	CV%	Mean±SD	CV%	Mean±SD	CV%
NE-SSC	149.3±0.5	0.30%	151.2±0.4	0.30%	150.2±1.0	0.70%	160.3±0.3	0.20%	162.4±0.3	0.20%	161.4±1.1	0.70%
NE-WX	312.2±4.8	1.50%	303.1±5.2	1.70%	307.7±6.7	2.20%	331.8±4.6	1.40%	324.4±.0	1.50%	328.1±6.1	1.80%
NE-SFL	45.4±0.4	0.90%	46.0±0.3	0.70%	45.7±0.5	1.10%	56.6±0.5	0.90%	56.9±0.4	0.80%	56.7±0.5	0.90%
NE-WY	652.8±13.8	2.10%	605.5±14.4	2.40%	629.2±27.5	4.40%	758.1±6.7	2.20%	733.3±8.9	1.20%	745.7±18.3	2.40%
NE-FSC	87.3±0.4	0.50%	86.4±0.2	0.30%	86.9±0.5	0.60%	97.8±0.3	0.30%	95.8±0.2	0.20%	96.8±1.0	1.10%
NE-WZ	664.2±16.9	2.50%	582.0±12.4	2.10%	623.1±43.7	7.00%	651.1±12.6	1.90%	584.5±2.4	2.10%	617.8±35.6	5.80%
LY-X	78.5±0.4	0.50%	84.4±0.5	0.60%	81.4±3.0	3.70%	81.3±0.6	0.80%	87.1±0.5	0.50%	84.2±2.9	3.50%
LY-WX	550.6±19.0	3.40%	503.8±28.7	5.70%	527.2±33.8	6.40%	463.6±27.7	6.00%	424.5±15.4	3.60%	444.1±29.8	6.70%
LY-Y	67.8±0.5	0.70%	68.6±0.6	0.90%	68.2±0.7	1.00%	78.5±1.0	1.30%	73.2±3.4	4.60%	75.9±3.6	4.80%
LY-WY	849.6±25.1	3.00%	835.3±55.2	6.60%	842.5±43.4	5.20%	867.9±46.4	5.30%	875.5±45.9	5.20%	871.7±46.3	5.30%
LY-Z	60.9±0.3	0.50%	60.4±0.3	0.50%	60.6±0.4	0.70%	61.4±0.4	0.70%	61.1±0.6	1.00%	61.2±0.5	0.90%
LY-WZ	507.7±20.2	4.00%	450.4±17.9	4.00%	479.1±34.4	7.20%	554.2±29.4	5.30%	520.3±29.4	5.60%	537.3±33.9	6.30%
MO-X	117.9±0.4	0.30%	121.7±0.5	0.40%	119.8±2.0	1.70%	124.7±0.6	0.50%	126.6±0.3	0.20%	125.6±1.0	0.80%
MO-WX	256.3±16.9	6.60%	233.2±18.4	7.90%	244.8±21.1	8.60%	258.9±4.0	1.50%	249.3±9.5	3.80%	254.1±8.7	3.40%
MO-Y	107.9±2.0	1.90%	109.0±2.5	2.30%	108.4±2.4	2.20%	94.3±1.0	1.00%	95.4±1.1	1.10%	94.8±1.2	1.20%
MO-WY	710.3±41.0	5.80%	707.7±45.7	6.50%	709.0±43.5	6.10%	790.4±23.5	3.00%	762.4±34.8	4.60%	776.4±32.8	4.20%
MO-Z	70.2±0.9	1.30%	71.4±0.5	0.70%	70.8±1.0	1.30%	66.2±0.4	0.50%	67.3±0.5	0.70%	66.8±0.7	1.10%
MO-WZ	524.0±30.5	5.80%	445.4±22.8	5.10%	484.7±47.6	9.80%	622.5±22.5	3.60%	537.3±20.3	3.80%	579.9±47.7	8.20%

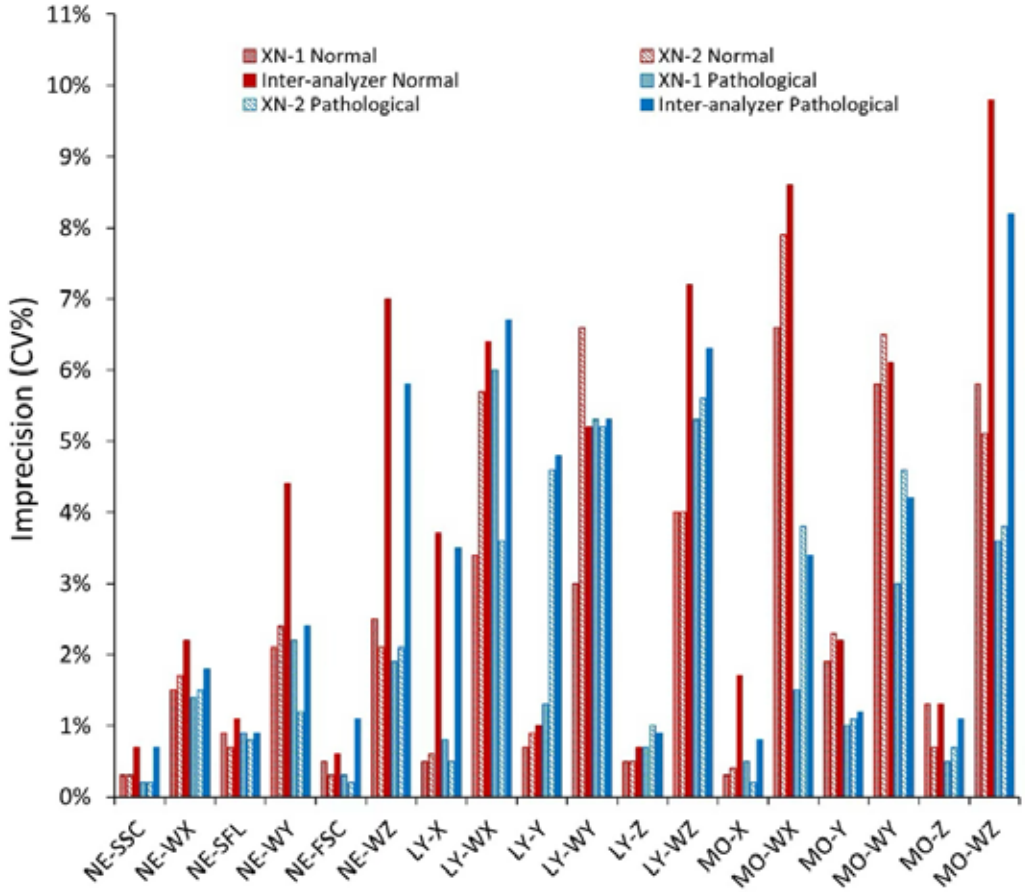
CV%, coefficient of variation; SD, standard deviation

Abbreviations and detailed descriptions of individual parameters are provided in Supplementary Table 1.

The intra-analyzer CV% of CPD on XN-10 ranged between 0.2-7.9% (0.3-7.9% in the normal patient sample and 0.2-6.0% in the pathological patient sample), while the inter-analyzer CV% ranged between 0.6-9.8% (0.6-9.8% in the normal patient sample and 0.7-8.2% in the pathological patient sample).

As concern the specific cell populations, the intra- and inter-analyzer CV% ranged between 0.2-2.5% and 0.6-7.0% for the neutrophil CPD parameters, between 0.5-6.6% and 0.7-7.2% for the lymphocyte CPD parameters, and between 0.2-7.9% and 0.8-9.8% for the monocyte CPD parameters, respectively. Overall, the intra- and inter-analyzer CV% of the mostly used in clinical practice CPD parameters ranged between 0.7-0.9% and 0.9-1.1% for NE-SFL and between 0.2-0.5% and 0.8-1.7% for MO-X, respectively (Figure 1).

Figure 1: Intra- and inter-analyzer imprecision of cell population data (CPD) obtained using the Sysmex XN analyzer.



CV%, coefficient of variation

Abbreviations and detailed descriptions of individual parameters are provided in Supplementary Table 1.

Discussion

Growing evidence supports the use of CPD as valuable tools in evaluation of infectious diseases and other life-threatening conditions, including leukemia and pre-neoplastic syndromes [10]. Nevertheless, before CPD can be routinely implemented in clinical practice, it is essential to verify the consistency and repeatability of measurements both within the same hematology analyzer and across different instruments. This aspect is particularly relevant because modern clinical laboratories often employ multiple, interconnected hematology analyzers operating on the same line [11]. Under such configurations, samples from the same patient, especially those needing frequent retesting, such as in sepsis, may be analyzed by different instruments, making analytical comparability a critical prerequisite for clinical reliability.

The purpose of this study was to evaluate the intra- and inter-analyzer imprecision of CPD parameters generated by the Sysmex XN-10 hematology analyzer, with the objective of assessing their analytical robustness and suitability for clinical application. Overall, intra-analyzer CV% ranged from 0.2 to 7.9%, while inter-analyzer CV% varied between 0.6 and 9.8%, confirming excellent repeatability and reproducibility across instruments. These findings are consistent with, and in most cases superior to, previous data obtained on the earlier Sysmex XN-9000 platform, where within-run CV% were reported between 0.4 and 14.1% [12]. Specifically, neutrophil-related CPD parameters exhibited imprecision between 0.4–5.5%, lymphocyte CPD parameters between 0.7–8.7%, and monocyte CPD parameters between 0.8–14.1%, with good agreement among five different XN-9000 modules.

Among the various CPD parameters, NE-SFL (neutrophil side fluorescence light intensity) and MO-X (monocyte complexity) showed remarkably low intra- and inter-analyzer imprecision, with CV% values consistently below 1.1% and 1.7%, respectively. This degree of analytical precision is excellent for optical and fluorescence-based hematology parameters, strongly supporting their reliability for routine diagnostic use. Such high reproducibility, in fact, provides a solid analytical foundation for clinical implementation, especially in critical care settings where rapid and reliable indicators of immune activation are needed.

The excellent imprecision performance further demonstrates that CPD measurements obtained with the Sysmex XN-10 analyzer are highly robust to inter-analyzer variability, enabling

reliable comparability of results across instruments of the same model and manufacturer. Such reproducibility is essential for standardization within integrated laboratory networks and for broader incorporation of CPD into diagnostic and monitoring algorithms. Although some distribution-width CPD parameters displayed higher CV% values, these remained within acceptable analytical limits for morphological indices and did not compromise interpretive accuracy.

The findings of this study have some important implications for laboratory practice and future research. The low intra- and inter-analyzer imprecision observed supports the robustness of Sysmex XN-10 CPD and strengthens their potential integration into laboratory quality assurance (QA) programs. However, to reach wider clinical implementation, harmonization across different analyzer models and brands will be essential. Variability in optical systems, signal processing, or calibration algorithms may influence CPD comparability, emphasizing the need for multicenter assessments and manufacturer-independent standardization efforts. The excellent precision observed in this study may also have direct implications for laboratory quality control and clinical interpretation of CPD metrics. In particular, parameters such as NE-SFL and MO-X, which demonstrated very low imprecision, could serve as indicators of internal stability within routine hematology quality control programs. The high analytical consistency also supports the use of CPD in longitudinal patient monitoring and automated interpretation algorithms, where minimizing analytical noise is crucial for distinguishing biological changes from technical variations.

Nonetheless, some limitations must be acknowledged. First, the sample size was limited to only two specimens (one from a healthy donor and one from a patient with a marked inflammatory response), selected to represent a biologically relevant range. Although this design allowed an initial assessment of intra- and inter-analyzer imprecision across contrasting physiological conditions, it inevitably underrepresents the biological variability encountered in routine practice. Studies including broader and different sample cohorts, encompassing multiple pathological conditions and demographic categories, would provide a more comprehensive evaluation of CPD variability. Second, even if this study robustly controlled technical sources of variation (e.g., through replicate measurements and standardized analyzer operation), pre-analytical factors, especially sample collection and transportation, could not be experimentally controlled. Finally, the use of residual patient material did not permit inclusion of standardized quality control materials because they currently lack assigned target values for CPD parameters. It is also important to note that potential sources of analytical bias or confounding that may affect CPD repeatability include differences in reagent lots, instrument maintenance status, calibration drift, and environmental conditions such as temperature and humidity. Both Sysmex XN-10 analyzers used in this study were maintained according to the same internal quality standards, operated under identical laboratory conditions, and utilized reagents from the same manufacturing lot, thereby excluding major sources of variability due to these factors.

In conclusion, this study confirms that CPD parameters generated by the Sysmex XN-10 analyzer exhibit satisfactory intra- and inter-analyzer imprecision, with NE-SFL and MO-X emerging as stable and reproducible. These results reinforce the potential of CPD for integration into clinical workflows, particularly for infection and sepsis assessment, where their analytical robustness and previously demonstrated diagnostic value make them powerful tools for precision laboratory medicine.

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Author contributions

Marco Tosi: Development, Investigation. Laura Pighi: Development, Investigation. Mariateresa Rizza: Development, Investigation. Gian Luca Salvagno: Development, Investigation. Giuseppe Lippi: Conceptualization, Data Analysis, Write-up.

Conflict of Interests

None to declare.

Ethics approval

This study was conducted as part of a local validation of CPD for clinical use at the facility, and its protocol was approved by the local Ethics Committee (approval number 971CESC; July 20, 2016).

Consent for Publication

Consent to submit has been received explicitly from all coauthors, as well as from the responsible authorities. Authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

Data availability

The data included in this study is available upon request to the corresponding author.

Acknowledgement

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Supplementary

Supplementary Table 1: Description and clinical significance of leukocytes cell population data (CPD). Adapted from [4].

Parameter	Description	Clinical significance
NE-SSC	Neutrophil cell complexity	Increases when neutrophils contain more granules (e.g., toxic granules), vacuoles, or other cytoplasmic inclusions; decreases with reduced granularity or hypogranulation.
NE-WX	Neutrophil complexity – distribution width	Higher values indicate greater heterogeneity in neutrophil internal structure, relative to NE-SSC.
NE-SFL	Neutrophil fluorescence intensity	Reflects nucleic acid content; higher values suggest increased cellular RNA and DNA, as observed in immature or activated neutrophils.
NE-WY	Neutrophil fluorescence intensity – distribution width	Represents variability in nucleic acid content within the neutrophil population, compared with NE-SFL.
NE-FSC	Neutrophil cell size	Indicates average neutrophil volume; may change in the presence of abnormally large or small cells.
NE-WZ	Neutrophil cell size – distribution width	Higher values indicate a broader size variation within the neutrophil population, compared with NE-FSC.
LY-X	Lymphocyte cell complexity	Increases with the presence of cytoplasmic granules or vacuoles (e.g., large granular lymphocytes).
LY-WX	Lymphocyte complexity – distribution width	Elevated values reflect higher heterogeneity in lymphocyte internal structure, relative to LY-X.
LY-Y	Lymphocyte fluorescence intensity	Correlates with nucleic acid content; elevated in activated, abnormal, or blast-like lymphocytes.
LY-WY	Lymphocyte fluorescence intensity – distribution width	Represents the degree of variability in nucleic acid content among lymphocytes, compared with LY-Y.
LY-Z	Lymphocyte cell size	Indicates average lymphocyte volume; may increase in activated cells or decrease in apoptotic (pyknotic) forms.
LY-WZ	Lymphocyte cell size – distribution width	Higher values indicate greater variation in lymphocyte size, relative to LY-Z.
MO-X	Monocyte cell complexity	Increases with more granules, vacuoles, or inclusions; decreases when monocytes exhibit simpler internal morphology.
MO-WX	Monocyte complexity – distribution width	Higher values denote greater heterogeneity in monocyte complexity, relative to MO-X.
MO-Y	Monocyte fluorescence intensity	Reflects cellular RNA and DNA content; elevated values are typical of activated monocytes or monoblasts.
MO-WY	Monocyte fluorescence intensity – distribution width	Represents the degree of heterogeneity in nucleic acid content across the monocyte population, compared with MO-Y.
MO-Z	Monocyte cell size	Indicates average monocyte volume; changes may reflect abnormal cell enlargement or shrinkage.
MO-WZ	Monocyte cell size – distribution width	Higher values correspond to greater variation in monocyte size, relative to MO-Z.

Brief report

Urgent Call for Action: Bridging Gaps in Asia-Pacific Laboratories' Transition to ISO 15189:2022

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Abstract

ISO 15189:2022 introduces key updates to medical laboratory standards, emphasizing risk management, ethics, and technical competence. With the December 2025 deadline for ISO 15189:2012 to 15189:2022 transition nearing, a cross-sectional survey was conducted during the Asia-Pacific Federation of Clinical Biochemistry and Laboratory Medicine webinar on February 21, 2025, to assess readiness. On 303 total responses, awareness was high, with 85% familiar with the revised standard and 92% recognizing its stronger focus on risk management. Most (78%) viewed the transition as highly important, and 82% expected improvements in quality and patient care. Major barriers included financial constraints (65%), insufficient training (72%), and resistance to change (45%). Preparation efforts reported were gap analyses (68%), training programs (75%), and policy updates (70%). While optimism is strong, resource limitations and skills gaps threaten timely adoption. The findings highlight the urgent need for structured training, financial support, and expert guidance to help laboratories, particularly in resource-limited settings, meet the new requirements. Collaboration among laboratories, professional bodies, and regulatory authorities will be crucial to ensure a smooth and effective transition to ISO 15189:2022, enabling more accurate, reliable, and patient-centered diagnostics.

Brief Report

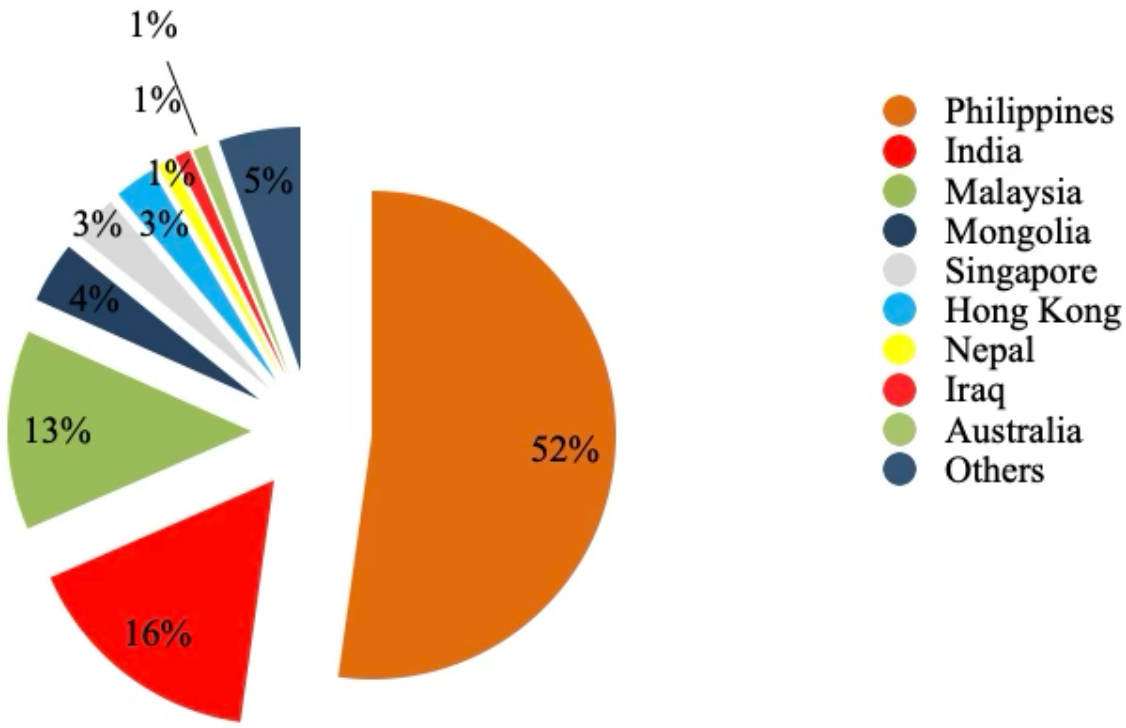
The impending December 2025 deadline for ISO 15189:2022 accreditation presents both an opportunity and a challenge for medical laboratories worldwide [1]. The December 2025 deadline refers to the end of the three-year transition period set by the International Laboratory Accreditation Cooperation (ILAC) for laboratories to move from ISO 15189:2012 to the 2022 version [1]. Our recent survey of 303 laboratory professionals across the Asia-Pacific region reveals critical gaps between awareness and implementation that threaten to leave many laboratories behind, particularly in resource-constrained settings. With 85% of respondents aware of the updated standard but only 20% in low-income countries having begun implementation, these findings demand immediate attention from accreditation bodies, professional organizations, and policymakers.

The transition to ISO 15189:2022 represents a significant evolution in quality management for medical laboratories. Building upon the 2012 version, the new standard emphasizes risk-based thinking, integrates point-of-care testing requirements previously covered under ISO 22870, and aligns more closely with ISO 17025 [2]. These changes aim to create a more robust, patient-centered approach to laboratory medicine. However, as our survey conducted during a February 2025 APFCB webinar demonstrates, many laboratories are struggling to translate these requirements into practice.

A cross-sectional, web-based survey was developed by the APFCB Communication and Publications Committee to assess regional readiness for the ISO 15189:2022 transition and its implementation. The questionnaire, validated for content by a multi-national panel of laboratory experts with no conflict of interest. It captured data across three core domains: awareness of the standard's changes, perceived implementation challenges, and current transition activities (Table 1). It was administered via the online One24 platform on February 21, 2025 following a webinar conducted for laboratory professionals and decision makers involved in accreditation [3]. Participants were instructed that only one response per institution from the primary decision-maker for accreditation (e.g., Quality Manager or Lab Director) should be submitted. From the 1,673 webinar attendees, this yielded 303 complete responses, each representing a unique institution, from professionals across 12 Asia-Pacific countries, with strong representation from India (n=48), Malaysia (n=39), Mongolia (n=12), and the Philippines (n=154) (Figure 1). Participants were allowed to select more than one response for relevant questions. Data were analyzed using descriptive statistics.

What emerged was a picture of uneven progress that correlates strongly with national economic resources. While high-income countries like Australia and Japan report 100% transition completion, laboratories in developing nations face multiple barriers.

Figure 1: Geographic Distribution of Participants across the Asia-Pacific.



Others: Pakistan, Indonesia, Macau, Japan, Thailand, Vietnam, UAE, China

Table 1: Summary of Survey Results on Knowledge, Perspective, and Practices regarding ISO 15189:2022 Transition.

Category	Key Findings	Percentage (number)
Awareness of ISO 15189:2022	Yes	**85% (258)**
	Somewhat aware	**10% (30)**
	No awareness	**5% (15)**
Key Changes Recognized	Enhanced focus on risk management	**92% (279)**
	New requirements for POCT	**78% (236)**
	Revised technical requirements	**65% (197)**
Sources of Information	Training sessions/workshops	**55% (167)**
	Professional associations	**25% (76)**
	Online resources	**20% (61)**
Perceived Importance	Very important	**78% (236)**
	Important	**18% (55)**
	Neutral/Not sure	**4% (12)**
Overall Attitude	Positive (will improve quality and patient care)	**82% (248)**
	Neutral (unsure of impact)	**15% (45)**
	Negative (unnecessary challenges)	**3% (9)**
Major Challenges	Financial constraints	**65% (197)**
	Lack of staff training	**72% (218)**
	Resistance to change	**45% (136)**
	Difficulty in updating procedures	**60% (182)**
	Limited access to expert consultation	**50% (152)**
Transition Steps Taken	Conducted gap analysis	**68% (206)**
	Provided staff training	**75% (227)**
	Updated policies and procedures	**70% (212)**
Tools Used for Transition	Internal quality teams	**50% (152)**
	Online training modules	**30% (91)**
	Consulting services	**20% (61)**
Additional Support Needed	More training for staff	**60% (182)**
	Increased budget allocation	**40% (121)**
	Access to expert consultants	**35% (106)**
	Clearer guidance documents	**30% (91)**

Financial constraints emerged as the most significant obstacle, cited by 65% of respondents. The costs associated with updating quality management systems, purchasing new equipment for compliance, and funding accreditation processes pose particular challenges for public laboratories in low-resource settings [4].

Even more concerning is the training gap, identified by 72% of participants as a major implementation barrier. The survey revealed that many laboratory professionals, while aware of the standard's existence, lack detailed understanding of specific requirements. A common misconception observed during post-webinar discussions was that risk management requires expensive software, whereas the standard intends a practical, patient-focused approach [5]. This misunderstanding highlights the need for clearer guidance and education about the standard's actual requirements.

Resistance to change within organizations presented another significant hurdle, mentioned by 45% of participants. This resistance is compounded by the fact that 60% of respondents reported difficulties in updating their procedures to meet new requirements, often due to limited access to expert consultation. The survey also examined current transition activities, revealing that most laboratories are relying on internal quality teams (50-70% across countries) rather than external consultants. While this approach may reduce costs, it potentially limits exposure to best practices. Common implementation steps included gap analyses (68%), staff training (75%), and policy updates (70%), though the depth and quality of these activities varied widely. Online training modules, which could offer scalable solutions, were underutilized (only 30-50% adoption), suggesting either lack of awareness or concerns about effectiveness.

Perhaps most responses were the regional disparities in

implementation progress. While 100% of participating Australian and Japanese laboratories had completed their transition, rates in other countries told a different story [Table 2]. The Philippines reported 70% of laboratories in planning stages, India 65%, Malaysia 60%, and Mongolia 80%. These numbers correlate closely with both national laboratory accreditation histories and healthcare funding levels, underscoring the economic dimensions of standards implementation [6-8].

Table 2: Country-Specific Challenges, Transition Progress, Tools Utilized, and Support Needs.

Country	Top Challenges	Transition Progress	Tools Used for Transition	Requested Support
Philippines (n=154)	Lack of staff training (75%, n=116), Budget constraints (70%, n=108)	Ongoing/Planning (70%, n=108)	Internal Quality Teams (50%, n=77), Online Training (40%, n=62), Consulting Services (30%, n=46)	Staff Training (80%, n=123), Budget Increase (60%, n=92)
India (n=48)	Staff training (65%, n=31), Resistance to change (50%, n=24)	Ongoing/Planning (65%, n=31), Completed (20%, n=10)	Internal Quality Teams (60%, n=29), Online Training (50%, n=24)	Staff Training (70%, n=34), Clearer Guidelines (40%, n=19)
Malaysia (n=39)	Limited understanding (60%, n=23), Budget (50%, n=20)	Ongoing/Planning (60%, n=23)	Internal Quality Teams (70%, n=27)	Budget Increase (50%, n=20)
Mongolia (n=12)	Budget constraints (80%, n=10), Staff training (60%, n=7)	Ongoing/Planning (80%, n=10)	Internal Quality Teams (70%, n=8), Online Training (60%, n=7)	Staff Training (90%, n=11)

The consequences of uneven implementation could be significant. Laboratories that fail to meet the 2025 deadline risk losing accreditation, potentially compromising patient care and international recognition of test results. This is particularly concerning for countries where laboratory medicine is still developing, as accreditation serves as a crucial quality benchmark. Moreover, the disparities may widen existing gaps in healthcare quality between high-income and developing nations in the region.

Our findings suggest several urgent interventions. First, targeted training programs must address both technical requirements and change management strategies. Second, accreditation bodies should consider developing tiered implementation pathways for resource-constrained settings. Third, regional professional organizations like the APFCB could establish mentoring programs pairing advanced laboratories with those earlier in their transition journey. Finally, clearer, simplified guidance documents with practical examples could help dispel common misconceptions about the standard's requirements.

This study has limitations inherent to its design. The use of convenience sampling from a webinar audience may introduce self-selection bias and limit the generalizability of findings, despite the event being targeted at senior laboratory professionals who are the decision makers for accreditation. Furthermore, the sample size varied significantly between countries, and the lack of specific demographic data on professional roles prevents analysis of how perspectives may differ by responsibility. Nonetheless, as an APFCB initiative, this brief report provides a crucial first assessment of the regional transition landscape, identifying key challenges to enable rapid dissemination and prompt further, more extensive research. The time for action is now. With few months remaining until the December 2025 deadline, laboratories across the Asia-Pacific need coordinated support to ensure no institution is left behind. This transition represents more than compliance, it's an opportunity to elevate the quality of laboratory medicine across the region. By addressing the identified gaps in training, resources, and guidance, we can turn this challenge into a catalyst for improved patient care and strengthened health systems throughout the Asia-Pacific.

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Ethical Approval

Participation in the survey was voluntary, and informed consent was obtained from all respondents before they proceeded with the questionnaire. Data confidentiality and anonymity were maintained throughout this survey study. This survey study was

conducted in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki.

Author Contributions

VP, PKD: Conceptualization. VP: Formal analysis, Writing original draft. DP, PKD, MU: Data curation, Writing-Review and Editing. RO, MLS: Writing-Review and Editing.

Conflict of interest

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Case Report

A variant in the TSH β gene resulted in discordant TSH levels in an Indian patient

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Keywords

TSH immunoassay interference, thyroid-stimulating hormone, TSHβ gene variant, epitope alteration, assay harmonization, Indian Patient

Abstract

A clinically euthyroid patient of Indian origin was identified with persistently undetectable TSH concentrations using our laboratory's third-generation Ultra TSH assay, raising concerns of assay interference. The discordant results, flagged by the treating physician, prompted an in-depth investigation to determine the cause of undetectable TSH values despite the patient's euthyroid clinical status. Over an eight-month period, three consecutive serum samples consistently showed TSH levels below 0.008 µIU/mL on our routine platform. To rule out analytical artifacts such as the high-dose hook (prozone) effect, heterophilic antibody interference, and other pre-analytical or analytical errors, the samples were re-evaluated under various dilution protocols and assay conditions. Reanalysis using two alternate FDA-approved TSH immunoassays (CLIA and ECLIA platforms) revealed a TSH concentration of 6.81 µIU/mL, consistent with the clinical picture and in stark contrast to our initial results. Given the persistence of this discrepancy and the suspected interference with antibody recognition, genetic analysis of the TSHB gene was performed. Sanger sequencing of the entire coding region revealed a homozygous A-to-G substitution (c.223A>G; AGA>GGA) in exon 3, resulting in an arginine-to-glycine amino acid change at codon 75 (R75G) in the mature TSH β-subunit (RefSeq: NP_000540.2). This variant was absent in a control subject of similar ethnic background with normal TSH levels on the same assay, supporting its role in the observed interference. The mutation likely alters the epitope conformation of the TSH molecule, reducing its recognition by monoclonal antibodies used in specific immunoassays without impairing its biological activity. This case underscores the importance of correlating laboratory results with clinical findings and highlights the need for cross-platform verification when discordant TSH values are encountered. Genetic variants affecting TSH structure can lead to misinterpretation of thyroid function, and efforts toward assay standardization and harmonization are essential to mitigate such diagnostic pitfalls.

Introduction

Thyroid-stimulating hormone (TSH) is a primary biomarker for evaluating thyroid function and is routinely measured using third-generation immunoassays due to their high sensitivity and analytical reliability. These assays, including chemiluminescent immunoassay (CLIA) and electrochemiluminescent immunoassay (ECLIA), typically employ monoclonal antibodies targeting epitopes on the TSH β-subunit. However, despite their robustness, these immunoassays are susceptible to analytical interference that can compromise accuracy. Such interference may arise from endogenous factors like heterophilic antibodies, high-dose hook effects, or more rarely, genetic variants that structurally modify the TSH molecule [1]. The clinical implications of platform diversity in immunoassay systems are multifaceted and significant for patient care. Immunoassay platforms utilize diverse antibodies, both polyclonal and monoclonal, designed by different manufacturers, with IVD validation typically occurring on specific populations, leading to generalized results that may not adequately account for global population diversity. This variability has profound patient impact, as genetic variants present significant clinical implications where patients may receive unnecessary treatment modifications despite having functionally normal TSH levels within established reference ranges, thereby emphasizing the critical importance of maintaining robust clinical-laboratory correlation in endocrine diagnostics. The key message from our findings is that such genetic variants, while relatively rare in occurrence, can lead to substantial diagnostic confusion and inappropriate clinical management if not properly recognized and addressed through comprehensive evaluation protocols. Immunoassay technology faces inherent limitations that challenge modern laboratory medicine, highlighting the urgent need for standardization and harmonization across analytical platforms. Until more harmonized assays or advanced techniques like mass spectrometry-offering greater specificity and less interference-are widely adopted, enhanced clinical-laboratory communication remains crucial. Our case study emphasizes the importance of vigilance among laboratory professionals and clinicians when results are discordant without clinical correlation, as such discrepancies may reflect analytical interferences rather than true pathology. Recognizing these interferences requires a systematic review of manufacturer instructions, considering factors like Human Anti-Mouse Antibodies (HAMA), Human Anti-Animal Antibodies (HAAA), prozone effects, biotin interference, and genetic variants such as the TSH-β R75G mutation that affect epitope recognition and cause platform-specific errors. This complex interference landscape underscores the need for clinical suspicion, cross-platform verification, and close collaboration between laboratory and clinical teams to ensure accurate diagnosis and optimal patient care. Variants that alter key amino acid residues within epitope regions can disrupt antigen-antibody binding, resulting in underestimation or non-detection of TSH levels. This form of interference is particularly concerning, as it may lead to misclassification of thyroid status, including the false diagnosis of hyperthyroidism. In some cases, TSH remains biologically active but is not immunoreactive in certain assay systems due to altered epitope conformation. These discrepancies emphasize the importance of assay design, particularly epitope selection, and the need for cross-platform verification when TSH results are incongruent with clinical findings. Furthermore, molecular genetic analysis can serve as a valuable tool to identify sequence variants in the TSHB gene that may affect assay performance. Awareness of these potential limitations and the implementation of harmonization strategies across assay platforms are critical for improving the diagnostic accuracy of TSH measurements in routine clinical practice [2], [3].

Case

A 41-year-old male resident of West Bengal, with a known history of carcinoma tongue status post-surgical resection and adjuvant radiotherapy completed two years prior, presented for routine follow-up. During longitudinal monitoring, a persistent and significant discrepancy in thyroid-stimulating hormone (TSH) levels was noted between our laboratory and an external reference laboratory, with both tests conducted within a one-week interval. Our laboratory consistently reported undetectable TSH levels (<0.008 µIU/mL) across three serum samples over an 8-month period using a third-generation ultra-sensitive immunoassay. In contrast, reanalysis of the same serum sample using an alternate FDA-approved assay platform revealed a TSH concentration of 6.81 µIU/mL, which was clinically concordant. Extensive analytical validation, including serial dilutions, interference checks, and assessment for prozone effect and heterophilic antibodies, failed to identify technical anomalies. Cross-platform evaluation using CLIA and ECLIA-based assays confirmed the discordance as assay-specific. To further investigate potential causes, the patient underwent thorough clinical re-evaluation by the treating physician, including a detailed review of medication history, which revealed no use of exogenous thyroid hormones or drugs known to interfere with TSH assays. Genomic DNA was subsequently extracted, and the full coding region of the TSHβ subunit gene was sequenced. Bioinformatic analysis using NCBI reference databases did not identify any pathogenic variants, suggesting the possibility of an assay-specific epitope alteration or a structurally atypical TSH isoform contributing to the immunoassay interference [4].

TSH estimation was performed using the Siemens Atellica IM module (Siemens Healthineers, USA) with Siemens reagents at our lab. For comparative analysis, the Alinity i analyzer (Abbott, USA) and Roche Cobas, Switzerland was also used with corresponding reagents. A significant discrepancy was observed in TSH values below 0.008 µIU/mL on the Siemens Atellica platform, which showed poor clinical correlation and lacked comparability with the Alinity i results.

CLIA (Siemens Atellica IM TSH3-UL): Third-generation chemiluminescent immunoassay employing:
•Anti-FITC monoclonal antibody covalently bound to paramagnetic particles
•FITC-labeled anti-TSH capture mouse monoclonal antibody
• Tracer with proprietary acridinium ester and anti-TSH mouse monoclonal antibody conjugated to BSA
• Direct relationship between TSH concentration and relative light units (RLUs)

ECLIA (Roche Cobas): Electrochemiluminescent sandwich immunoassay (18-minute duration):
• 1st incubation: 50 µL sample with biotinylated monoclonal TSH-specific antibody and ruthenium complex-labeled antibody
• 2nd incubation: Streptavidin-coated microparticles for solid-phase binding
• Detection via chemiluminescent emission after voltage application

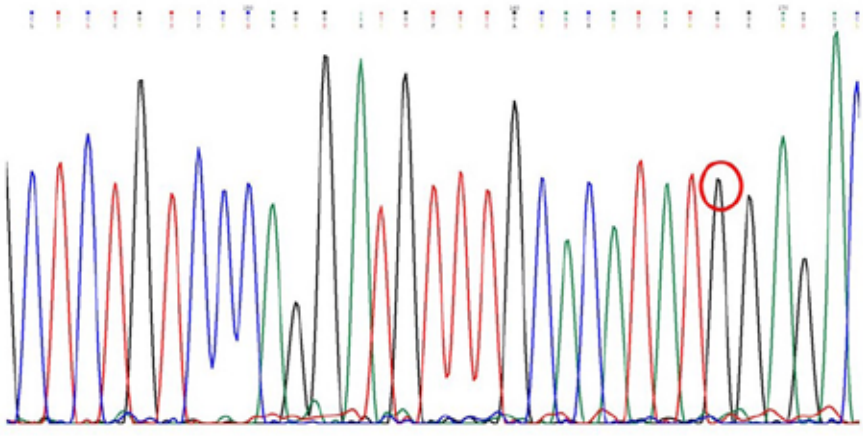
Table 1: Consistent pattern demonstrates reproducible platform-specific interference affecting CLIA methodology while ECLIA maintains detection capability for the variant TSH, confirming the genetic variant's differential impact on antibody recognition systems.

Testing Instances	Siemens Atellica (CLIA)	Roche Cobas (ECLIA)
1st Testing	<0.008 µIU/mL	6.81 µIU/mL
2nd Testing	<0.008 µIU/mL	10.3 µIU/mL
3rd Testing	<0.008 µIU/mL	4.1 µIU/mL

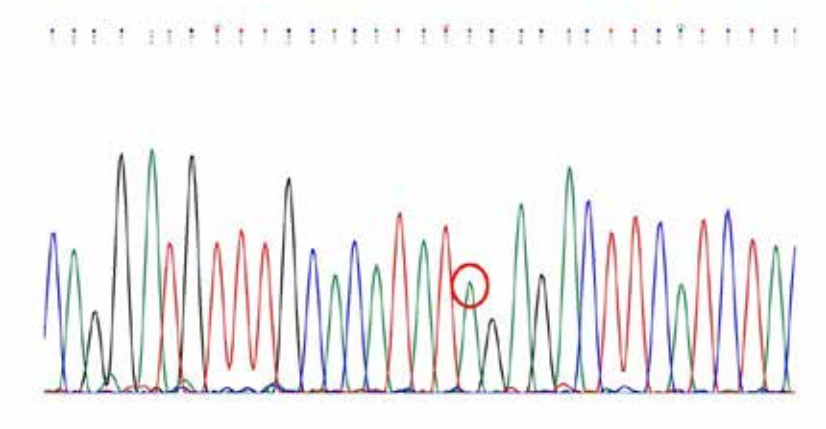
To investigate the cause of this discrepancy, Sanger sequencing of the entire coding region of the TSHB gene was performed. For variant detection, DNA was isolated using the QIAamp DNA Blood Mini Kit (QIAGEN, Germany) with automated extraction performed on the QIACUBE system (QIAGEN, Germany). Primers targeting exon 2 and exon 3 of the TSHβ gene were designed using the NCBI Primer Designing Tool and synthesized by Eurofins Genomics India Pvt. Ltd. (Bangalore, India). PCR amplification was conducted using the Bio-Rad C1000 Touch Thermal Cycler (California, USA), with the reaction mixture prepared using Takara PCR Master Mix (Clontech / DSS-Takara, New Delhi, India). The PCR products were purified using Exo-SAP IT (Thermo Fisher Scientific, USA). Sequencing was carried out using Invitrogen Sequencing Master Mix (India) on an ABI 3500 Genetic Analyzer (USA). PCR conditions were as follows: initial denaturation at 95°C for 5 minutes; followed by 35 cycles of denaturation at 95°C for 45 seconds, annealing at 56.3°C for exon 2 and 60°C for exon 3 for 45 seconds, and extension at 72°C for 45 seconds; with a final extension at 72°C for 5 minutes. The PCR reaction mix (total volume 25 µL) consisted of: 14.3 µL distilled water, 2.5 µL of 10× PCR buffer, 1.0 µL of 20.5 mM dNTPs, 1.0 µL each of 10 pmol/µL forward and reverse primers, 5.0 µL of DNA template (20 ng/µL), and 0.2 µL of Taq polymerase (5 U/µL).

This revealed a homozygous A-to-G nucleotide substitution in exon 3, resulting in an arginine-to-glycine amino acid change at codon 75 (R75G) fig. (A) and (B) in the mature TSH β-subunit protein (RefSeq: NP_000540.2). No such variant was detected in a control individual with normal TSH levels, supporting the hypothesis that the R75G mutation interferes with antibody recognition in our assay, rather than reflecting a true deficiency of TSH.

Figure 1: Sanger sequencing chromatograms of the TSHB gene. (A) Patient sample showing a homozygous c.223A>G (R75G) variant in exon 3 (red circle). (B) Control sample with wild-type sequence at the same position.



Case(A)



Control(B)

The alignment shows a homozygous A>G substitution at nucleotide position c.223, resulting in an arginine-to-glycine substitution at codon 75 (R75G), indicated by a red box.

Figure 2: Alignment of the patient's TSHB gene sequence with the reference sequence (NP_000540.2).

Range 3: 505 to 624 Graphics ▼ Next Match ▲ Previous Match ▲ First Match

Score	Expect	Identities	Gaps	Strand
217 bits(117)	2e-60	119/120(99%)	0/120(0%)	Plus/Plus

```
Query  150  CAGGATGTTTGCACATATGGAGACTTCATCTACAGGACTGTAGAAATACCAGGATGCCCA  209
            ||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  505  CAGGATGTTTGCACATATAGAGACTTCATCTACAGGACTGTAGAAATACCAGGATGCCCA  564

Query  210  CTCCATGTTGCTCCCTATTTTTCCTATCCTGTTGCTTTAAGCTGTAAGTGTGGCAAGTGC  269
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  565  CTCCATGTTGCTCCCTATTTTTCCTATCCTGTTGCTTTAAGCTGTAAGTGTGGCAAGTGC  624
```

Range 4: 402 to 505 Graphics ▼ Next Match ▲ Previous Match ▲ First Match

Score	Expect	Identities	Gaps	Strand
187 bits(101)	1e-51	103/104(99%)	0/104(0%)	Plus/Plus

```
Query  107  GGATATCAATGGCAAACTGTTTCTTCCCAAAATATGCTCTGTCCCAGGATGTTTGCACATA  166
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  402  GGATATCAATGGCAAACTGTTTCTTCCCAAAATATGCTCTGTCCCAGGATGTTTGCACATA  461

Query  167  TGGAGACTTCATCTACAGGACTGTAGAAATACCAGGATGCCCAC  210
            | ||||||||||||||||||||||||||||||||||||||||||
Sbjct  462  TAGAGACTTCATCTACAGGACTGTAGAAATACCAGGATGCCCAC  505
```

Range 5: 342 to 384 Graphics ▼ Next Match ▲ Previous Match ▲ First Match

Score	Expect	Identities	Gaps	Strand
80.5 bits(43)	2e-19	43/43(100%)	0/43(0%)	Plus/Plus

```
Query  107  GGATATCAATGGCAAACTGTTTCTTCCCAAAATATGCTCTGTCC  149
            |||||||||||||||||||||||||||||||||||||||||||
Sbjct  342  GGATATCAATGGCAAACTGTTTCTTCCCAAAATATGCTCTGTCC  384
```

Discussion

Immunoassay-based measurement of thyroid-stimulating hormone (TSH) is a cornerstone of thyroid function testing. However, in rare cases, discordant TSH results may arise due to interference caused by structural variants of the TSH molecule. We report a case in which an undetectable TSH concentration was observed using a routine immunoassay platform, while clinical evaluation and free thyroid hormone levels indicated a euthyroid state. Subsequent genetic analysis identified a homozygous missense mutation in exon 3 of the TSHB gene, resulting in an arginine-to-glycine substitution at codon 75 (R75G) of the mature TSH β-subunit protein. This mutation likely alters the conformation of the TSH molecule in a manner that impairs recognition by specific antibodies used in certain immunoassays.

The identified R75G mutation has been previously described by D Shaki et al. in individuals of Ashkenazi Jewish ancestry, where it was similarly associated with anomalously low or undetectable TSH concentrations despite preserved thyroid function. This mutation is postulated to affect a key epitope targeted by monoclonal antibodies in specific TSH immunoassays. Importantly, the affected patients in those studies also displayed normal levels of free thyroxine (FT4) and triiodothyronine (FT3), as in our case, supporting the notion that the mutation compromises immunoreactivity rather than biological activity of the hormone [5].

To validate the hypothesis of assay interference, we analyzed the same patient sample using two additional TSH immunoassay platforms. Both alternative assays yielded measurable TSH concentrations, in stark contrast to the undetectable result on our primary system. This inter-platform discrepancy reinforces the role of the R75G variant in altering the immunoreactive profile of TSH, likely through conformational changes that mask or disrupt epitope-antibody interactions specific to certain assay designs.

While this particular mutation was consistent with the findings of Shaki et al., similar phenomena have been described in the literature involving other TSH variants. For example, J Drees et al. reported a distinct TSH β-subunit variant causing falsely low TSH values due to immunoassay interference. Although the molecular basis differed, the clinical presentation and diagnostic challenge were similar, highlighting a broader issue of variant-dependent immunoassay limitations [6].

Our findings underscore a critical limitation of various current TSH immunoassays: their vulnerability to genetic variation in the hormone structure, particularly when such variants occur within or near epitopes used for antibody binding. These cases demonstrate the necessity of considering genetic causes when TSH levels are unexpectedly low and discordant with the clinical picture. In such situations, confirmation with alternative immunoassay platforms or functional bioassays may be warranted.

Furthermore, this case exemplifies the importance of assay transparency and epitope mapping in test development, especially in diverse populations where rare variants such as R75G may be under recognized. Broader awareness of such mutations and their analytical consequences will improve diagnostic accuracy and prevent misclassification of thyroid status, especially in asymptomatic individuals or during routine screening. Literature reports a higher prevalence of certain genetic variants in Asian populations, including Indian Jews, with our index case from eastern India aligning with this geographic pattern. Current evidence suggests that ethnic-specific genetic architectures contribute to the greater prevalence observed in Asian populations compared to European and North American groups. Clinically, this underscores the need for stringent monitoring when TSH values are discrepant without clear clinical correlation, alongside the use of alternative testing platforms as the best current practice. Patient education about genetic variations and genetic counseling are also essential to support informed healthcare decisions. Moving forward, prospective studies are necessary to better define the prevalence of these variants within different ethnic groups and to develop diagnostic strategies tailored to specific populations [5, 6].

Conclusion

This case highlights the clinical and analytical implications of a homozygous R75G mutation in the TSHB gene, which leads to falsely low or undetectable TSH levels on specific immunoassay platforms due to epitope interference. The mutation does not impair TSH biological activity but disrupts antibody recognition, resulting in assay-specific underestimation. The patient could have modification like glycosylation in TSH due to the variant in TSHβ subunit gene, may have altered TSH epitope expression which induced variable antibody recognition which may be the cause for discordant TSH levels in our laboratory reports. Recognition of such variant-induced assay interference is essential to avoid misdiagnosis and inappropriate clinical management. Comparative testing across multiple assay platforms and genetic analysis should be considered when TSH results are discordant with clinical findings. Standardization and Harmonization of TSH assay may limit such discrepancies in the results.

Future Research Looking ahead, we aim to collaborate with genetic research teams to initiate a longitudinal, multicentric prospective study, contingent on funding and ethical approvals. This research will focus on characterizing ethnic-specific variant prevalence and analyzing platform-specific interference patterns. The ultimate goal is to develop harmonized detection strategies that enhance diagnostic accuracy across diverse populations.

Conflict of interest

Authors do not have any conflict of interest.

Artificial intelligence statement

The authors used Grammarly and ChatGPT to help make the writing clearer.

Disclosure

The authors have no relevant financial or non-financial interests to disclose.

Acknowledgment: During the preparation of this manuscript, the authors Grammarly, OpenAI's language(ChatGPT) model to improve clarity and coherence. The authors reviewed and edited the final content and take full responsibility for its accuracy.

Ethical Approval

This study is in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki. The study is approved from Institute Ethical Committee TMC-IEC III, Tata Memorial centre Advanced Centre for Treatment Research & Education in Cancer, Kharghar, Navi Mumbai -410210, India vide approval letter Rr. No.524 dated 09 July 2025.

Informed consent statement

Informed written consent was obtained from the patient.

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Availability of data and materials
Data will be provided on request.

Consent for publication
All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Author Contributions
Avinash Pagdhune contributed to conceptualization, original draft preparation, formal analysis, review and editing, and project administration. Poonam Gera was involved in Conceptualization, visualization, methodology, validation, and formal analysis. Preeti Chavan contributed to resources, methodology, and supervision. Sujeet Kamtalwar participated in resources, investigation, and data curation. Ashwini More provided resources. Varsha Jadhav contributed to methodology, software, Visualization, and validation. Prafulla Parikh was involved in resources and supervision. Sharda Haralkar contributed to methodology. Rajni Mohite and Madhura Patil were involved in methodology. Rushikesh Samal contributed to data curation and resources. Rajiv Sarin was responsible for supervision and resources.

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Case Report

The ethical aspects of AI in scientific publishing

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Article Info

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Keywords

artificial intelligence, ethics, medical publishing

Abstract

Introduction: Printing allowed the scientific revolution. Scientific journals established peer review. AI is driving the next wave of scientific progress. Ethical aspects of AI in publishing are an emerging area of concern.

Key issues: AI tools are used in generating papers. This raises questions about authorship and accountability: who is responsible? If AI contributes, should they be credited as authors? Are researchers accountable for AI-generated content? If AI is involved in writing, this should be disclosed to maintain transparency. Otherwise, there could be concerns about misrepresentation or lack of rigor.

Another consequence is intellectual property: if AI generates portions of a paper, who owns the rights to that work? Frameworks for intellectual property were designed for human creators, so these might be rethought. Many journals require a written statement regarding AI use. AI use in publishing could exacerbate inequality in research access, leading to a divide between well-funded and less-funded institutions. Global inequality in science sharpens: AI might skew research toward countries with more technological resources.

AI can be used to assist peer review. This challenges peer review integrity: relying on AI could undermine the integrity of human oversight. AI does not replace but complements reviewers' expertise. AI-driven tools might lack the nuanced human understanding. Over-reliance on AI could compromise publishing quality.

Conclusion: AI offers possibilities to speed up and to improve scientific publishing, but it is essential to judge and to address the ethical implications. This requires guidelines and rules warranting an honest, transparent and integer approach of publishing.

Introduction

Book printing allowed for the mass distribution of scientific ideas, kicking off the scientific revolution. Scientific journals established formal peer review and standardized scientific communication, accelerating the exchange of knowledge. The internet exponentially increased access to research and fostered global collaboration, making scientific progress faster and more inclusive.

When OpenAI launched ChatGPT - a natural language processing chatbot driven by generative Artificial Intelligence (GenAI) - in November 2022 [1], it became the fastest-growing consumer application in history, reaching 100 million active users in just two months after its launch [2]. AI is now driving the next wave of scientific progress, enabling faster analysis, discovery, and innovation, and changing how research is conducted and applied. There are definitely potential benefits and challenges associated with the use of AI in scientific writing [3]. As data generation increases, AI is employed to handle the complex analysis required. AI enables the analysis of enormous datasets from large scale experiments in ways that were previously impossible. AI has been helping accelerate discoveries by processing vast amounts of data at speeds far beyond human capabilities. This is especially visible in rapidly progressing fields like drug discovery and genomics e.g. AI-driven tools like AlphaFold (predicting protein structures) have revolutionized medicine, solving a problem that had stumped scientists for decades. AI became a significant force in scientific progress in the 2000s with the advent of machine learning and deep learning technologies. AI has brought profound changes to how research is conducted, from data analysis to hypothesis generation.

AI and research

AI tools are increasingly automating many aspects of research (e.g., in areas like hypothesis generation, experimental design, and simulation). In medical research, AI is used not only for analyzing patient data and predicting disease outcomes but also for creating personalized treatment plans. AI can identify potential drug candidates more quickly than traditional methods. The COVID-19 pandemic spurred AI applications for vaccine development, epidemiological modeling, and diagnostics.

Researchers are leveraging AI to predict outcomes and guide experiments, reducing the time needed for testing and discovery.

AI - based tools are being used to assist in scientific writing, generating reports, and proposing new research directions. Integration of AI in science is driven by significant investment. Research organizations/universities are building AI-focused units to foster this growth. Venture capital is flowing into AI-powered startups in science, aiming at tackling complex problems and bringing innovations to market faster. In recent years, the ethical aspects of AI in scientific publishing are an important and emerging area of concern [4, 5, 6]. A balanced approach to integrating AI into the writing process is advocated [3]. There are several key issues that need to be considered:

AI and authorship

First of all, authorship and accountability require attention: AI tools are increasingly being used to assist in writing, data analysis, and even generating papers [6]. This raises questions about authorship and accountability: Who is responsible for the research? If AI tools contribute significantly to the work, should they be credited as authors? Should the human researchers be held accountable for AI-generated content? Also, transparency is of importance: if AI is involved in the analysis or writing of a paper, this should be clearly disclosed to maintain transparency. Otherwise, there could be concerns about misrepresentation or lack of rigor. There is potential for AI-generated text to resemble existing research too closely, leading to accusations of plagiarism. It could also be difficult to detect AI-generated work unless proper tools and safeguards are in place. Most scientific journals still rely on the fact that good manuscript reviewers can (still) detect stylistic differences between paragraphs or text parts.

Intellectual property

Another consequence of the use of AI-generated content are the complex intellectual property questions: A first aspect is the ownership: if an AI system generates significant portions of a paper or analysis, there are questions about who owns the rights to that work - the developer of the AI, the user who directed it, or others?

AI has an impact on traditional copyright systems: The existing frameworks for intellectual property were designed with human creators in mind, so these might need to be adapted or rethought in light of AI's role. Many journals require in 2025 a written statement of authors and reviewers regarding the use of AI [7].

In a recent survey [8] 78% of 78 medical journals provided guidance on use of AI in peer review. Of these provided guidance, 59% explicitly prohibit using AI, while 32 allow its use if confidentiality is maintained and authorship rights were respected. Internationally based medical journals are more likely to permit limited use than journals' editorial located in the US or Europe, and mixed publishers had the highest proportion of prohibition on AI use. Among the journals that provided guidance, 91% prohibited uploading manuscript-related content to AI, and 32% permitted restricted use of AI that mandated reviewers disclose in review reports. Regarding the mention of AI tools, 47% cite chatbots, and 27% mention large language models; 32 journals (41%) link to the publisher's website, which had preferences in AI use. Seventeen journals (22%) also provide links to statements from the International Committee of Medical Journal Editors or World Association of Medical Editors that permit limited use of AI, although 5 journals' guidance contradict these statements. The main reason for prohibiting or limited use of AI

is confidentiality concerns (96%).

Funding of AI research

Furthermore, the use of AI in scientific publishing could exacerbate inequality in access to research. Not all researchers, particularly those from underfunded or underrepresented backgrounds, may have access to the cutting - edge AI tools in publishing. This could lead to a divide between well-funded and less-funded research institutions. The global inequality in science is sharpening AI's influence on the publishing process might skew research toward countries with more technological resources, leaving other regions behind. Open AI models (e.g., GPT-NeoX, BLOOM) could narrow the gap by allowing researchers in developing regions to experiment with state-of-the-art tools without major infrastructure investments. AI can help with coding, data analysis, literature reviews, and even experiment design -amplifying individual researchers' productivity.

Effect on publishing practices

In some cases, AI could be abused to manipulate data or present false conclusions, potentially leading to unethical publishing practices. The proliferation of AI-generated papers has also recently led to an increase in predatory publishing practices. Researchers, particularly those from underfunded institutions, may be exploited by publishers that accept low-quality AI-generated manuscripts for a fee. This not only dilutes the quality of scientific literature but also exacerbates inequalities by providing a platform for substandard research [9].

AI and peer review

AI can be used to assist in the peer review process by automating certain tasks like checking for plagiarism, errors, or suggesting improvements. However, this also brings challenges, for example peer review integrity: Relying on AI for parts of the peer review process could undermine the integrity of human oversight. It is essential that AI does not replace but rather complements the expertise and judgment of human manuscript reviewers. AI - driven tools may be fast, but they might lack the nuanced understanding that human reviewers bring to the process. Over - reliance on AI could compromise the quality of modern scientific publishing.

Conclusion

In conclusion, although AI definitely offers many possibilities to speed up and to improve scientific publishing, it becomes essential to judge and to address the ethical implications. This requires a collaboration between scientists, publishers, and ethicists to develop appropriate guidelines and rules which warrant an honest, transparent and integer approach of scientific publishing [3, 4, 8, 9, 10]. According to the International Association of Scientific, Technical and Medical Publishers (STM), the use of AI tools can be allowed for basic author support such as refining, correcting, editing, and formatting text and documents without disclosure, but disclosure becomes necessary when the AI use goes beyond basic author support [11]. The ETHICAL framework, a set of principles has been designed to guide the responsible use of generative AI in scientific research. It emphasizes the importance of ethical considerations such as transparency, accountability, and the mitigation of bias in AI - generated content. [12]. There is an urgent need for scientists to upskill in AI and modern data science to fully utilize the potential of these new tools.

Declaration of Conflict of interests

The author of this article declares that there is no conflict of interest with regard to the content of this manuscript.

Submission declaration

The work described has not been published previously in this form. The article is not under consideration for publication elsewhere. The article's publication is approved. No funding of any kind has supported writing this manuscript.

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Case Report

Case-Based Analysis of Pre-analytical and Analytical Non-Conformities in Urinary Protein Testing

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Abstract

Accurate quantification of urinary protein is fundamental for the diagnosis, monitoring, and management of kidney disease. Despite technological advances, both pre-analytical and analytical non-conformities in laboratory testing remain significant sources of misinterpretation that can adversely affect clinical decision-making. This report analyzes two such cases to highlight common but critical pitfalls in proteinuria assessment. We present two illustrative cases: the first involves a pediatric patient, where a pre-analytical non-conformity led to a significant overestimation of proteinuria severity. The second case describes an elderly diabetic patient where an analytical non-conformity resulted in a profound underestimation of albuminuria. In both instances, discrepancies between semi-quantitative and quantitative results were the critical clues that prompted investigation. These cases underscore that urinary protein results, while quantitative, are not infallible. Vigilant attention to pre-analytical procedures, strict adherence to analytical limits, and the integration of semi-quantitative results as a plausibility check are essential to prevent diagnostic non-conformities. Effective communication between clinicians and laboratory professionals is paramount to ensure that laboratory results accurately inform, rather than misdirect, clinical decision-making.

Introduction

Proteinuria is a key laboratory finding that reflects a wide spectrum of renal and systemic diseases. Accurate measurement of urinary protein is essential for diagnosis, monitoring, and prognosis in kidney disease. However, despite standardized definitions and improved assay technologies, measurement reliability still depends critically on both pre-analytical and analytical factors [1].

Although terminology and classification schemes may vary across clinical and laboratory settings, the core framework generally includes the anatomical source of protein loss, temporal pattern, quantity of protein excretion, and predominant protein type. Recognizing these categories is essential for accurate diagnosis, interpretation of laboratory results, and appropriate clinical management, as each pattern reflects distinct underlying mechanisms and disease implications (Table 1) [2-4].

Among these, microalbuminuria, defined as urinary albumin excretion between 30 and 300 mg/day or its equivalent in spot urine samples, represents an early and sensitive marker of glomerular injury, particularly in diabetic kidney disease and other conditions associated with increased glomerular permeability. Detecting microalbuminuria allows for timely diagnosis and intervention before the onset of overt proteinuria and irreversible renal damage [5].

Laboratory methods for proteinuria assessment encompass a broad range of qualitative, semi-quantitative, and quantitative techniques. These include dipstick analysis, precipitation assays, and more precise turbidimetric, colorimetric, or immunoturbidimetric methods. While each approach offers advantages in terms of sensitivity, specificity, expediency, and cost-effectiveness, they also exhibit limitations and susceptibility to analytical and pre-analytical interferences. Awareness of these factors is essential for accurate clinical interpretation [6].

Despite continuous advancements in assay technologies, both pre-analytical and analytical sources of non-conformities remain significant challenges in accurately evaluating proteinuria. Pre-analytical issues, such as incomplete or improperly timed urine collections, sample mislabeling and contamination, variability in patient adherence to collection protocols, and improper storage or transport conditions, can result in misleading or inconsistent measurements [6]. Analytical challenges can be equally impactful. A critical issue is failure to recognize assay linearity; protein concentrations exceeding the reportable range without proper dilution result in significant underestimation of proteinuria [7]. At the other end, the detection limit of certain methods, such as the sulfosalicylic acid (SSA) precipitation test, may fail to identify mild microalbuminuria, potentially resulting in false-negative findings during early stages of kidney disease [8]. Additionally, common interfering factors such as highly alkaline urine, radiographic contrast agents, elevated concentrations of certain drugs, and substances can adversely affect the accuracy of turbidimetric and colorimetric assays [9]. Moreover, poor harmonization between different analytical methods can yield discordant results, complicating the longitudinal monitoring of a patient's proteinuria [10]. To address these challenges, strict adherence to method harmonization, quality control, and correlation with clinical findings is essential to ensure reliable and clinically meaningful urine protein measurements.

In this report, we present two illustrative cases that highlight critical laboratory challenges in the evaluation of proteinuria. The first case involves a pediatric patient whose proteinuria appeared more severe due to inadvertent use of a 24-hour urine sample instead of a random sample, emphasizing the impact of pre-analytical handling non-conformities. The second case describes an elderly diabetic patient in whom failure to dilute a urine sample exceeding the microalbumin assay's linear range resulted in significant underestimation of albuminuria, illustrating an analytical oversight. These cases illustrate how subtle pre-analytical and analytical non-conformities can distort proteinuria results and demonstrate the value of integrating qualitative and quantitative methods for error detection. We also provide a brief literature review of urine protein measurement methodologies to contextualize these cases within current best practices.

Table 1: Overview of proteinuria categories and their characteristics.

Classification	Categories	Representative Causes and Notes
Source	Pre-renal (Overflow)	Multiple myeloma (light chains), hemolysis (hemoglobinuria), rhabdomyolysis (myoglobinuria)
	Renal – Glomerular	Nephrotic syndrome, glomerulonephritis
	Renal – Tubular	Tubulointerstitial nephritis, acute tubular injury
	Post-renal	Urinary tract infections, prostatic secretions
Temporal Pattern	Transient (Functional)	Exercise, fever, dehydration, congestive heart failure, cold exposure
	Orthostatic (Postural)	Adults (benign)
	Constant	Chronic kidney disease
Quantity	Microalbuminuria	30–300 mg/day albumin; early diabetic nephropathy
	Overt Proteinuria	>300 mg/day total protein
	Nephrotic-range Proteinuria	>3500 mg/day total protein
Composition	Selective Glomerular	Predominantly albumin (e.g., minimal change disease)
	Non-selective Glomerular	Albumin and larger proteins (advanced glomerulopathies)
	Low-Molecular-Weight Proteins	Tubular dysfunction (β2-microglobulin, light chains)

Classification integrates information from KDIGO 2012 Clinical Practice Guidelines [2], Tietz Textbook of Clinical Chemistry and Molecular Diagnostics (8th edition) [3], and Brunzel's Fundamentals of Urine and Body Fluid Analysis (5th edition) [4].

Case Description

Case 1

A 13-year-old girl with a history of persistent proteinuria was referred for reevaluation of renal function and urinary protein excretion. The initial assessment in January 2025 showed a markedly increased random urine protein concentration of 348 mg/dL and a protein-to-creatinine ratio (PCR) of 2.07. The urine protein measurement was performed on a Mindray BS-480 biochemistry analyzer (Mindray, Shenzhen, China) using a pyrogallol red colorimetric method (Ziest Chem Diagnostic, Iran, Lot: 10-545). The manufacturer's reportable range for this assay was 4–120 mg/dL. The initial analysis triggered a ">LIN" (above linearity) flag, indicating the concentration exceeded this range. The 348 mg/dL result was obtained after a manual dilution was performed according to the kit manufacturer's instructions, consistent with overt proteinuria. At follow-up in early March 2025, a 24-hour urine collection demonstrated total protein excretion of 1848 mg/24 h, confirming significant proteinuria. Urinalysis at that time revealed 2+ protein by the SSA precipitation test (Table 2). Later that month, during routine follow-up, quantitative analysis of what was recorded as a random urine sample produced unexpectedly high protein (997 mg/dL) and creatinine (407 mg/dL) concentrations, values comparable to those of the previous 24-hour specimen. In contrast, the SSA result remained unchanged at 2+, raising concern about the result's plausibility. A repeat, freshly voided random sample collected on the same day revealed markedly lower protein (182 mg/dL) and creatinine (55 mg/dL) concentrations, confirming persistent but less severe proteinuria.

An internal review determined that the specimen labeled as random urine had been inadvertently drawn from the patient's 24-hour collection container rather than from a new voided sample. The mix-up led to a major overestimation of proteinuria severity and unnecessary clinical concern.

This case underscores the potential for pre-analytical non-conformities to profoundly affect the interpretation of proteinuria severity and highlights the importance of integrating clinical assessment, method comparison, and systematic review of laboratory processes in evaluating unexpected results.

The discrepancy was identified during internal verification, and the corrected result was issued before clinical reporting. Therefore, the error did not influence patient management or clinical decision-making.

Review of internal quality-control (IQC) records for total urine protein assays before and after the incident showed all results within acceptable control limits, and external quality-assessment (EQA) participation confirmed satisfactory performance during the same period.

Following the identification of the specimen mix-up, an internal root-cause analysis (RCA) was conducted. The investigation traced the non-conformity to a lapse in specimen segregation during processing of concurrent random and 24-hour urine samples. The event was documented in the laboratory information system (LIS) as a pre-analytical non-conformity. Corrective actions included staff retraining on specimen labeling and verification, implementation of color-coded collection containers for different urine sample types, and mandatory double-checking of sample identifiers before analysis. These measures were incorporated into the laboratory's quality management plan to prevent recurrence.

Table 2: Serial laboratory and urinary findings in a 13-year-old girl with persistent proteinuria.

Parameter	15 Jan 2025 (Random)	1 Mar 2025 (24-hour)	21 Mar 2025 (Mislabeled†)	21 Mar 2025 (Repeat) (Repeated Random Sample)	Reference Interval (Unit as indicated)
Serum Urea	-	23	26	-	10 – 40 mg/dL
Serum Creatinine	-	0.8	0.7	-	0.5 – 1.1 mg/dL
Random Urine Protein	348	319	997	182	< 15 mg/dL
Random Urine Creatinine	168	46	407	55	20 – 275 mg/dL*
Protein/Creatinine Ratio	2.07	6.93	2.44	3.3	< 0.2 mg/mg
24-hr Urine Protein	-	1848	1054	-	< 150 mg/24h
Urine analysis: Protein (SSA)††	-	2+	2+	-	Negative
Urine analysis: RBCs	-	10–15	10–15	-	< 3/HPF
Urine analysis: WBCs	-	3–4	2–3	-	< 5/HPF
Urine analysis: Granular Casts	-	3–4	4–5	-	Rare or absent/LPF

* Urine creatinine varies by hydration and muscle mass; the approximate expected range is shown.

† This sample was inadvertently collected from the 24-hour collection container rather than a fresh void.

†† SSA (sulfosalicylic acid) grading scale: Negative (<6 mg/dL), Trace (6-30 mg/dL), 1+ (30-100 mg/dL), 2+ (100-200 mg/dL), 3+ (200-400 mg/dL), 4+ (>400 mg/dL).

Dash (-) indicates test not performed.

Abbreviations: SSA, sulfosalicylic acid; HPF, high-power field; LPF, low-power field; RBC, red blood cell; WBC, white blood cell.

Case 2

A 76-year-old woman with a long-standing history of diabetes mellitus was referred for laboratory evaluation due to possible renal impairment. Initial testing revealed fasting hyperglycemia (170 mg/dL), elevated serum urea (56 mg/dL), and increased creatinine (2.5 mg/dL), consistent with reduced renal function. Urinalysis demonstrated 2+ protein by the SSA precipitation test and 1+ glucosuria on dipstick.

Quantitative measurement of urinary microalbumin was performed on a Mindray BS-480 biochemistry analyzer (Mindray, Shenzhen, China) using an immunoturbidimetric assay (AUDIT Diagnostics, Iran; Lot No. 242537). According to the manufacturer's specifications, the reportable range for this method is 2–200 mg/L. The analyzer produced an initial microalbumin result of 249 mg/L, accompanied by a ">LIN" (above linearity) flag, indicating that the concentration exceeded the assay's validated upper limit. However, the flagged result was inadvertently reported without dilution or verification. The unexpectedly modest albumin concentration appeared discordant with the SSA finding of 2+ proteinuria, prompting supervisory review. Upon re-examination, the same specimen was reanalyzed after a 1:20 dilution in accordance with the manufacturer's instructions. The corrected result was 2227 mg/L, confirming severe albuminuria and aligning the quantitative result with the SSA test (Table 3).

This case exemplifies a classic analytical non-conformity in urinary protein measurement, where failure to recognize and correct for assay linearity led to a tenfold underestimation of albuminuria severity. It highlights the critical importance of maintaining awareness of reportable ranges, performing appropriate dilutions, and integrating semi-quantitative results as plausibility checks.

The non-conformity was detected and resolved during supervisory review before result validation, and the corrected report was released to the clinician. Consequently, the incident had no impact on patient care or management decisions.

The analytical non-conformity was documented in the laboratory's non-conformity log as an analytical oversight. A focused RCA revealed that the ">LIN" flag was overlooked during routine result verification. Review of IQC and EQA records confirmed no prior issues with assay linearity or dilution procedures. Corrective measures included refresher training for staff on instrument flag interpretation, reinforcement of mandatory flag acknowledgment in the LIS before result release, and revision of the standard operating procedure (SOP) for handling flagged results.

These interventions were implemented to strengthen analytical vigilance and prevent recurrence.

Table 3: Laboratory and urinary findings in a 76-year-old woman with diabetes.

Parameter	Result	Reference Interval (Unit as indicated)
Blood sugar	170	<140 mg/dL
Serum Urea	56	21-45 mg/dL
Serum Creatinine	2.5	0.6-1.3 mg/dL
Serum Uric Acid	7.6	2.5-6.8 mg/dL
Serum Sodium	144	135–145 mmol/L
Serum Potassium	4	3.5–5.0 mmol/L
Urine analysis: Protein (SSA)†	2+	Negative
Urine analysis: RBCs	10-15	< 3/HPF
Urine dipstick: Glucose	1+	Negative
Urine random microalbumin*	249	< 20 mg/L
Urine random microalbumin**	2227	< 20 mg/L

† SSA (sulfosalicylic acid) grading scale: Negative (<6 mg/dL), Trace (6-30 mg/dL), 1+ (30-100 mg/dL), 2+ (100-200 mg/dL), 3+ (200-400 mg/dL), 4+ (>400 mg/dL).

* Initial result without dilution; exceeded assay upper linearity limit of 200 mg/L.

** Result obtained after 1:20 dilution per manufacturer protocol.

Abbreviations: SSA, sulfosalicylic acid; RBC, red blood cell; HPF, high-power field

Discussion

Accurate urine protein measurement critically depends on the type of specimen collected and meticulous specimen handling during the preanalytical phase. The preanalytical phase encompasses urine sampling, storage, transport, and preparation prior to analysis, involving both patient-dependent and laboratory-controlled steps that significantly impact test reliability and clinical interpretation [6].

Among specimen types, 24-hour urine collection has traditionally been regarded as the gold standard for quantifying proteinuria because it integrates protein excretion over an entire day, accounting for physiological fluctuations [11]. However, drawbacks include patient inconvenience, potential incomplete collection, and handling and storage non-conformities, which can introduce significant inaccuracies [6].

Recognizing these challenges, spot urine samples, particularly the PCR or albumin-to-creatinine ratio (ACR) in first-morning voids or random midstream specimens, have gained wide acceptance in clinical practice as practical and reliable alternatives. Several studies show strong correlations between spot urine PCR/ACR and 24-hour urine protein, supporting their use for screening, diagnosis, and monitoring of renal pathology such as nephrotic syndrome and preeclampsia. Spot samples improve patient compliance and reduce collection non-conformities but require normalization against creatinine to account for urine concentration variability. First-morning void samples are generally preferred to minimize diurnal variation and orthostatic effects, enhancing reproducibility [12,13].

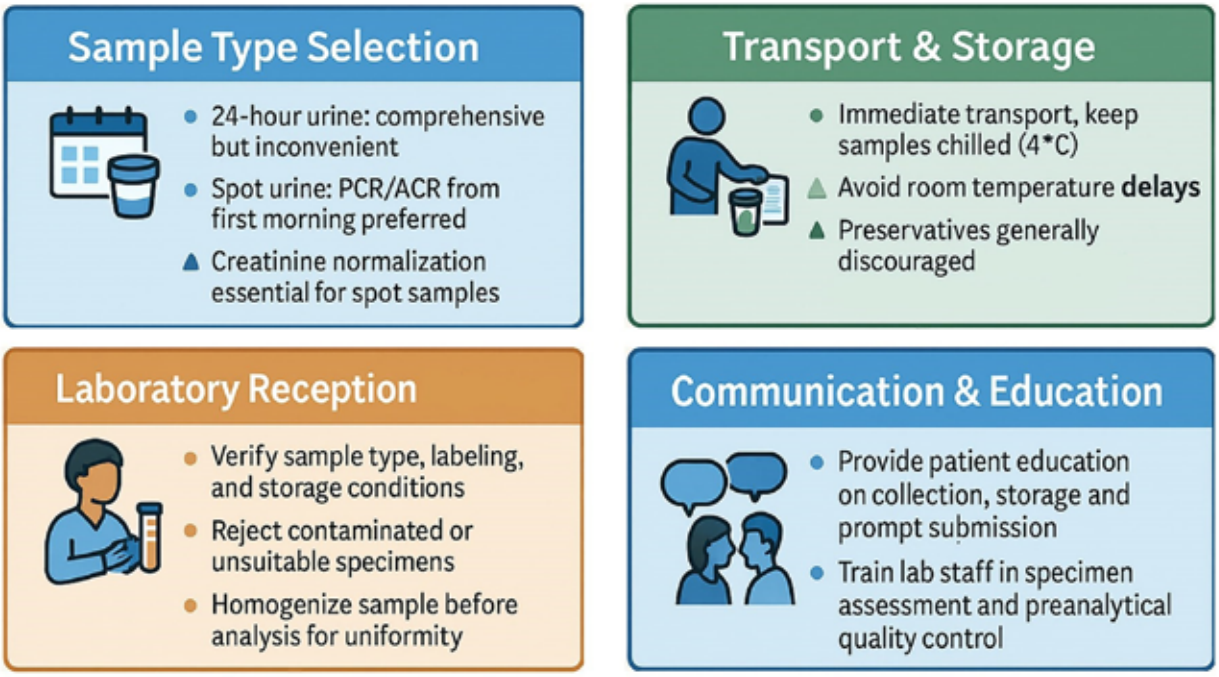
Beyond collection type, specimen handling critically influences the reliability of urine protein measurements. Urine sampling is patient-dependent and susceptible to non-conformities such as contamination with vaginal secretions, menstrual blood, or other extraneous materials, which can cause false elevations or spurious results. Proper midstream clean-catch techniques help minimize contamination [6].

Once collected, urine specimens are biochemically and microbiologically unstable if stored or transported improperly. Proteins can degrade or precipitate, especially when specimens are kept at room temperature for prolonged times. Refrigeration at 4 °C is strongly recommended if immediate processing is unavailable, as it preserves protein integrity and prevents bacterial overgrowth. When transport is required, samples should be kept chilled and delivered to the laboratory promptly to minimize degradation. Studies assessing various preanalytical variables confirm that the stability of urinary proteins significantly declines after extended delays or inappropriate temperature exposure. Furthermore, sample homogenization prior to aliquoting ensures representative protein distribution, critical for accurate measurement [14].

The routine use of chemical preservatives for urine protein is generally discouraged, as they may interfere with specific analytical methods, especially immunoassays. If refrigeration is not feasible, acidification (e.g., with hydrochloric acid) or the addition of preservatives like boric acid may be considered with caution and only when compatible with the intended analytical procedure. Ensuring appropriate storage, labeling, and timely transport remains critical to minimizing pre-analytical variability [15].

The success of accurate urine protein measurement hinges not only on adhering to technical protocols but also on effective communication between laboratory professionals and patients. Proper instruction on urine collection procedures is essential to prevent common pitfalls such as missed voids, sample contamination, or mislabeling. Patients should be clearly educated on the importance of midstream collection, specimen refrigeration, and timely submission. Likewise, laboratory staff must diligently verify specimen type, assess storage conditions, and ensure thorough sample homogenization prior to analysis. Standardized training programs and clear, written instructions are vital to minimizing preanalytical non-conformities and enhancing test reliability across various healthcare settings [6]. A summary of key preanalytical considerations for accurate urine protein measurement is illustrated in Figure 1.

Figure 1: Key preanalytical considerations in urine protein measurement.



This figure summarizes the principal pre-analytical variables that influence the accuracy of urinary protein testing. Critical factors include patient instruction on proper collection technique (midstream, clean-catch, or 24-hour collection), sample labeling and chain of custody, timely transport under refrigeration (4 °C), and avoidance of prolonged storage or chemical preservatives that may interfere with protein stability. Each step can introduce non-conformities if not standardized. Implementing clear protocols and patient–laboratory communication minimizes these errors and ensures valid proteinuria assessment.

Accurate interpretation of proteinuria depends not only on correct specimen collection but also on the selection and performance of the laboratory method used. A wide range of techniques is available for urinary protein measurement, each with distinct analytical characteristics and clinical applications. Understanding these differences is essential, particularly when discrepancies in results occur, as illustrated in our cases.

Initial screening for proteinuria in clinical practice often begins with qualitative or semi-quantitative methods, such as urine dipstick testing or the SSA precipitation test. Dipstick analysis is rapid, inexpensive, and widely available, using a colorimetric reaction to detect primarily albumin via pH-dependent interaction with tetrabromophenol blue. However, it does not detect other protein types such as globulins or Bence-Jones proteins. In addition, its accuracy may be compromised by extreme urine pH, specific gravity, or the presence of interfering substances. While valuable as an initial tool, dipstick testing is best interpreted as part of a broader diagnostic framework, not in isolation [16].

To improve sensitivity to early renal damage, albumin-specific dipsticks or immunochromatographic strips have been developed. These methods target albumin explicitly and demonstrate improved sensitivity for microalbuminuria detection, often with thresholds as low as 2 mg/dL. However, these tests remain semi-quantitative and are also influenced by urine concentration and interfering substances, necessitating cautious interpretation [17].

The SSA test offers a broader protein detection range by precipitating total proteins in urine. This method can detect albumin, globulins, and other high- and low-molecular-weight proteins, and is often used as a semi-quantitative backup to dipstick results. It is particularly helpful when dipstick findings are equivocal or when non-albumin proteins are suspected. However, SSA lacks standardization, and its interpretation is subjective and susceptible to inter-observer

variability. It can also produce false-positive results in the presence of contrast media, penicillin, or highly concentrated urine [18]. In our first case, the SSA result provided a critical qualitative flag that was inconsistent with unexpectedly high quantitative measurements, prompting further investigation into sample integrity and specimen handling.

For definitive assessment of proteinuria, quantitative laboratory methods are required. These include turbidimetric and colorimetric assays, which are routinely used in clinical laboratories. Turbidimetric assays, such as those employing benzethonium chloride or trichloroacetic acid, work by precipitating proteins and measuring light scatter. They are cost-effective and suitable for total protein measurement, but are non-specific and vulnerable to interferences. Their linearity range is typically limited to 300–500 mg/dL, and failure to dilute highly concentrated specimens can result in significant underestimation [19].

Colorimetric assays measure urinary protein concentration based on the intensity of color formed when the protein reacts with specific dyes, such as the pyrogallol red–molybdate complex, which offers improved specificity and is widely adapted to automated platforms. These assays are more resistant to chemical interference and have a broader dynamic range, making them well-suited for routine clinical applications. However, like turbidimetric methods, they are primarily suited for total protein measurement and do not distinguish between albumin and other protein types [20].

In patients at risk of glomerular injury, such as those with diabetes or hypertension, measurement of albuminuria using immunochemical methods is essential. Techniques like immunoturbidimetry and immunonephelometry utilize antibodies specific to human albumin and offer high sensitivity, with detection limits as low as 1–2 mg/L. These assays enable early diagnosis of microalbuminuria (30–300 mg/day) and facilitate timely therapeutic intervention before overt nephropathy develops. Nevertheless, their accuracy is highly dependent on proper calibration, reagent integrity, and recognition of their reportable range [21]. As our second case revealed, exceeding this range without proper dilution can lead to gross underestimation, falsely reassuring clinicians and delaying necessary management.

Beyond routine quantitative methods, advanced techniques are employed to provide a more detailed characterization of proteinuria in specific clinical contexts. Urine protein electrophoresis (UPEP) and immunofixation electrophoresis (IFE) allow for the detection and characterization of specific protein fractions, particularly monoclonal immunoglobulin light chains, and are essential tools in the evaluation of suspected paraproteinemias, multiple myeloma, or amyloidosis. These methods, while not quantitative, can distinguish glomerular, tubular, and overflow proteinuria patterns, offering diagnostic clarity when standard assays are inconclusive or discordant [22]. In the research and advanced diagnostic context, mass spectrometry–based proteomic techniques, such as MALDI-TOF or LC-MS/MS, enable the detailed identification and quantification of urinary proteins and peptides. These high-resolution methods support biomarker discovery, disease phenotyping, and insights into renal pathophysiology. However, due to their high cost, technical complexity, and lack of standardization, they are not currently suited for routine clinical use [23].

While advanced techniques provide diagnostic clarity in complex cases, their utility must be weighed against practicality and clinical need. For most patients, a combination of standardized quantitative assays and complementary semi-quantitative methods offers a reliable framework for proteinuria evaluation. As illustrated by our cases, awareness of each method's analytical limitations is crucial for avoiding misinterpretation and ensuring accurate clinical decisions. Despite advances in assay technologies, inconsistencies between qualitative and quantitative methods and failures to adhere to analytical protocols remain significant sources of non-conformities, as illustrated by our cases. Effective harmonization requires understanding the analytical limitations of each method, implementing standardized procedures, and fostering correlation strategies that integrate multiple data sources for clinical decision-making.

Screening methods such as SSA precipitation and dipstick analysis provide rapid, inexpensive detection of proteinuria, but they are inherently semi-quantitative and subject to variable interpretation. SSA has an approximate detection limit of 5-10 mg/dL, enabling detection of moderate proteinuria but with limited sensitivity for very low-level albumin excretion. For example, a urinary albumin concentration of 35 mg/L (equivalent to 3.5 mg/dL) would typically fall below the SSA detection threshold, resulting in a negative finding despite clinically relevant microalbuminuria. The SSA test provides a semi-quantitative grading that correlates with total urinary protein concentration. According to standard urinalysis references, expected turbidity grades correspond to the following approximate protein concentrations: negative (< 6 mg/dL), trace (6–30 mg/dL), 1+ (30–100 mg/dL), 2+ (100–200 mg/dL), 3+ (200–400 mg/dL), and 4+ (> 400 mg/dL). These ranges can serve as practical plausibility thresholds for verifying the consistency of semi-quantitative and quantitative results [24]. For instance, if a specimen graded as 1+ by SSA corresponds to an expected range of 30–100 mg/dL, a substantially higher or lower quantitative result should prompt verification for potential non-conformities. Incorporating such plausibility thresholds into routine review protocols can help laboratories identify errors early and ensure internal consistency between methods.

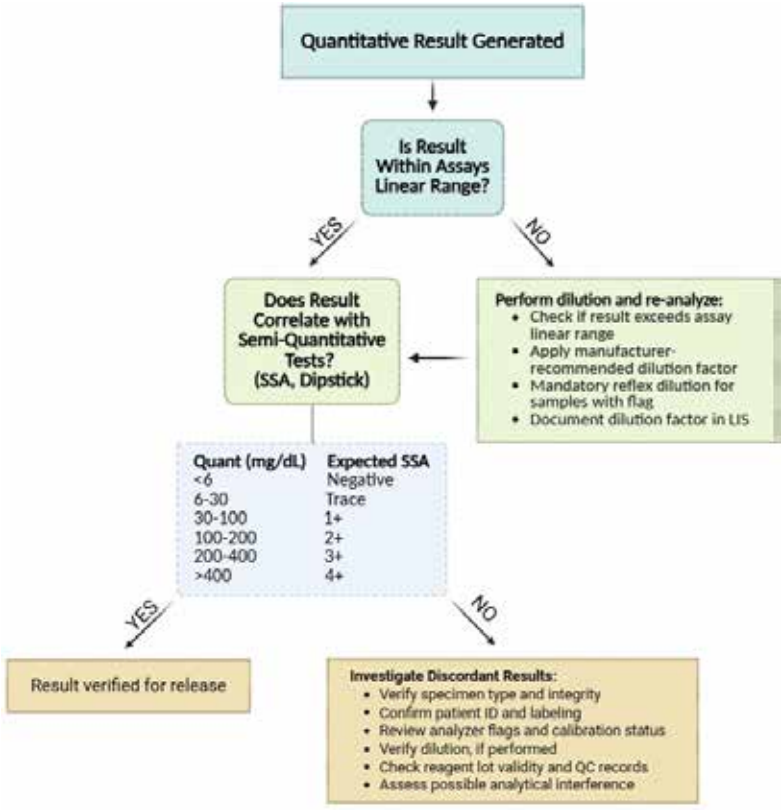
In addition to correlating qualitative and quantitative results, maintaining assay linearity and adhering to proper dilution protocols are critical to ensure accuracy in quantitative urine protein measurements. Quantitative assays have defined reportable ranges within which results are considered reliable. Samples with protein concentrations exceeding the

upper limit of linearity can yield falsely low results if not appropriately diluted, leading to significant underestimation of proteinuria severity [25]. To address this issue, laboratories should implement multiple safeguards, including automated analyzer flags or LIS alerts for results beyond the linear range, and enforce mandatory dilution procedures as part of standard operating protocols. Comprehensive staff training on manufacturer guidelines and dilution techniques is essential to ensure procedural compliance. Incorporation of automated dilution systems, where available, can further minimize non-conformities and enhance consistency. Furthermore, periodic audits and quality control assessments should be conducted to verify the proper execution of dilution protocols and adherence to linearity criteria. Laboratories should also include confirmatory or reflex testing protocols for borderline or discordant results and educate clinicians about assay limitations to ensure accurate interpretation. Collectively, these measures enhance analytical reliability, reduce the risk of clinically significant misinterpretations, and promote accurate patient diagnosis and management.

A major challenge in urinary protein measurement is achieving consistency across different laboratories, as variability arises from differences in analytical methods, calibrators, and reagent formulations. Turbidimetric, colorimetric, and immunochemical assays often lack full standardization, resulting in significant inter-laboratory bias. This variability can lead to clinical misclassification, particularly when patients transition between care settings or results are compared longitudinally across different facilities [26]. To minimize these discrepancies, laboratories should adopt methods traceable to reference measurement procedures and participate in EQA or proficiency testing programs. Such programs provide benchmarks for identifying systematic deviations and enable corrective actions to align with peer laboratories [27]. The use of commutable reference materials and internationally recognized calibrators is essential to reduce method-dependent variability [28].

A multimodal approach that combines standardized assays, rigorous quality control, and proactive clinician-laboratory communication is essential to ensure accurate diagnosis, timely intervention, and improved patient outcomes. A proposed workflow for ensuring accurate urinary protein results is shown in Figure 2.

Figure 2: Algorithmic workflow for verification of urinary protein results.



The workflow outlines key decision steps for validating quantitative urine protein results. It incorporates automated detection of values exceeding the assay's linear range, mandatory reflex dilution and re-analysis with documentation in the laboratory information system (LIS), and plausibility cross-checking against semi-quantitative methods such as the sulfosalicylic acid (SSA) test. Expected SSA ranges serve as reference thresholds for assessing consistency. Discordant findings prompt review of specimen integrity, labeling, and analytical performance before result release.

Lessons Learned and Recommendations for Laboratory Implementation

SOP Modifications

- Color-coded labeling: Use distinct labels for 24-hour vs. random specimens
- Mandatory dilution protocols: Establish automatic dilution for samples with flags
- Concordance verification: Routinely cross-check SSA/dipstick with quantitative results
- Supervisory review: Require approval before releasing flagged or discordant results

LIS Enhancements

- Automated alerts: Flag multiple specimen types from the same patient within 24 hours
- Result release blocks: Prevent reporting when linearity exceeded without dilution
- Dilution documentation: Mandate entry of dilution factor before result authorization
- Specimen tracking: Implement barcode verification at collection and aliquoting

Staff Education and Competency

- Initial training: Comprehensive modules on specimen handling and flag recognition
- Annual refresher courses: Competency testing on dilution techniques and result verification

Quality Assurance Measures

- Monthly audits: Review all results with linearity flags for dilution compliance
- EQA participation: Include high-concentration samples to challenge upper linearity
- Periodic validation: Verify dilution protocols and concordance criteria quarterly
- Clinician feedback: Establish communication channels for unexpected results

Patient Education

- Written instructions: Provide clear collection guidelines for 24-hour specimens
- Visual aids: Use diagrams showing proper collection techniques
- Verbal reinforcement: Have staff review collection procedures with patients

Conclusion

Accurate urinary protein measurement is essential for reliable assessment of renal function, yet remains vulnerable to both pre-analytical and analytical non-conformities. The two cases presented illustrate how specimen misidentification and failure to recognize assay linearity can profoundly distort clinical interpretation. These findings emphasize the necessity of rigorous specimen verification, adherence to assay performance limits, and continuous quality assurance. Integrating semi-quantitative plausibility checks and strengthening clinician–laboratory communication are key strategies to prevent diagnostic error. Ongoing efforts toward harmonization and staff training are critical to ensure that urinary protein results consistently support, rather than compromise, patient care.

Ethic statement

The study protocol, including the presentation of clinical case data and associated laboratory findings, was reviewed and approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (Tehran, Iran). Written informed consent was obtained from the patient and the legal guardian for publication of de-identified clinical information, laboratory data, and tables derived from their results. All identifying details were removed to ensure confidentiality in accordance with institutional and international ethical standards for human subject research.

Data availability

The data supporting the findings of this study are derived from clinical case records and laboratory results of individual patients. To protect patient confidentiality, these data are not publicly available. De-identified excerpts relevant to the cases may be shared by the corresponding author upon reasonable request and with permission from the institutional ethics committee.

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Conflict of interest

The authors declare that there is no conflict of interest concerning this study.

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Case Report

Lipemia-Induced Hemoglobin Overestimation and Correction by Plasma Replacement in a Pediatric Acute Lymphoblastic Leukemia Patient

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Keywords

analytical interference, hemoglobin, lipemia, plasma replacement, acute lymphoblastic leukemia (ALL)

Abstract

Background: Pre-analytical and analytical errors in laboratory testing can lead to clinical misinterpretation. This case highlights a falsely elevated hemoglobin level due to lipemia and the corrective laboratory intervention.

Case: A 3-year-7-month-old girl with acute lymphoblastic leukemia underwent a follow-up complete blood count which reported a hemoglobin level of 16.9 g/dL. The hemoglobin result was inconsistent with previous clinical findings and hematocrit. A simultaneously drawn venous blood gas sample showed a hemoglobin value of 9.2 g/dL. The biochemistry sample showed visible lipemia, with a lipemia index of 3041. The same sample revealed a triglyceride level of 8042 mg/dL (1:50 dilution) and total cholesterol of 492.2 mg/dL. These findings indicated a falsely elevated hemoglobin due to lipemia. The patient was not on parenteral nutrition. Pediatric endocrinology consultation attributed lipemia to L-asparaginase and corticosteroids in the treatment regimen. To eliminate lipemic interference, the EDTA blood sample was centrifuged at 1000 x g for 10 minutes, and the lipemic plasma was replaced with an equal volume of 0.9% NaCl solution. The sample was gently mixed to restore whole blood integrity. After this plasma replacement procedure, hemoglobin was measured as 10.2 g/dL, consistent with the blood gas result and clinical picture.

Conclusion: This case emphasizes the need to correlate laboratory results with clinical and biochemical data. In lipemic samples, plasma replacement may provide a practical correction method for falsely elevated hemoglobin values when resampling is not feasible. Recognition and prompt correction of lipemia-induced errors are crucial to avoid inappropriate clinical decisions.

Introduction

In laboratory medicine, pre-analytical and analytical errors can significantly compromise the accuracy of test results, potentially leading to misdiagnosis, delayed treatment, or inappropriate clinical decisions. The reliability of laboratory findings is particularly critical in guiding the diagnosis, monitoring, and management of patients. Ensuring analytical precision is therefore essential for optimal patient outcomes [1].

Lipemia refers to visible turbidity of serum or plasma caused by an elevated concentration of large lipoprotein particles, especially chylomicrons and very low-density lipoproteins (VLDL). This accumulation causes visible turbidity which interferes with optical measurement techniques commonly used in clinical chemistry and hematology analyzers. Spectrophotometric readings can be affected by light scattering, and automated cell counters may also be influenced by changes in the sample's refractive index, leading to potential errors in test results [1,2]. In the context of complete blood count (CBC) testing, lipemia can cause false elevation of hemoglobin (Hb) values. Since mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) are calculated parameters derived in part from Hb, such interference can lead to inaccurate red blood cell indices as well [3].

Case Presentation

A 3-year-7-month-old girl diagnosed with acute lymphoblastic leukemia (ALL) was undergoing induction chemotherapy according to Protocol 1A, which included dexamethasone, vincristine, daunorubicin, and L-asparaginase. She was also receiving multiple supportive medications, including piperacillin-tazobactam, amikacin sulfate, pantoprazole, azithromycin, fluconazole, calcimax, and vitamin D.

During routine follow-up, a CBC analyzed using the Sysmex XN-1000 showed a Hb value of 16.9 g/dL, which was markedly inconsistent with the clinical picture and with previous hematologic data. Two days earlier, Hb had been reported as 10.7 g/dL. The discrepancy was confirmed during specialist validation approximately two hours after sampling. A simultaneously drawn venous blood gas sample, analyzed on the ABL90 FLEX, showed a Hb value of 9.2 g/dL, further supporting the suspicion of analytical interference.

Biochemistry analysis on the Roche Cobas C702 revealed visible lipemia, with a lipemia index of 3041. Triglycerides were markedly elevated at 8042 mg/dL (measured with a 1:50 dilution), and total cholesterol was 492.2 mg/dL. These findings indicated a falsely elevated Hb due to lipemia.

Subsequent biochemistry, coagulation, and CBC samples also appeared lipemic. The patient was not receiving parenteral nutrition. Pediatric endocrinology was consulted, and it was suggested that the hyperlipidemia might be related to the chemotherapy agents, particularly L-asparaginase and corticosteroids. Initiation of fenofibrate treatment was recommended.

Comparison of hematological parameters with those obtained two days earlier is shown in Table 1. Hb, MCH and MCHC were markedly increased, while other parameters remained relatively stable.

Table 1: Hematologic Parameters: Two Days Prior vs Current.

Parameter	WBC	RBC	PLT	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC(g/dL)
Two Days Prior	0.38	3.09	72	10.7	26.6	86.1	34.6	40.2
Current	0.5	3.01	171	16.9	25.6	85	56.1	66

To correct the lipemia related interference, the EDTA whole blood sample was centrifuged at 1000 x g for 10 minutes. The lipemic plasma was carefully removed and replaced with an equal volume of isotonic NaCl solution, without disturbing the buffy coat layer. The sample was gently mixed to restore whole blood integrity. After this plasma replacement procedure, Hb was measured as 10.2 g/dL, consistent with the blood gas result and clinical picture. Changes in other parameters were also observed, most notably MCH, which returned toward reference ranges following plasma replacement. These changes are summarized in Table 2.

Table 2: Plasma Replacement Effect on CBC Parameters.

Parameter	WBC	RBC	PLT	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Before Replacement	0.5	3.01	171	16.9	25.6	85	56.1	66
After Replacement	0.36	3.13	173	10.2	19.1	61	32.6	53.4

Discussion

Accurate laboratory results are essential for appropriate diagnosis and treatment, especially in hematology and oncology patients. In this case, a grossly lipemic sample led to a falsely elevated Hb value in a pediatric patient with ALL, raising concerns of analytical interference. The discrepancy between the CBC and venous blood gas Hb values, along with the visibly lipemic serum and extremely high lipemia index of 3041, prompted further investigation.

Lipemia is a well-known interferent in photometric analyses. It increases sample turbidity, which causes light scattering and affects absorbance-based measurements. Hematology analyzers, such as the Sysmex XN-1000, estimate Hb via spectrophotometry after chemical lysis of red cells. In contrast, co-oximetry-based blood gas analyzers like the ABL90 FLEX measure Hb at multiple wavelengths and are less affected by lipemia [3-5]. This may account for the more reliable 9.2 g/dL Hb reading obtained from the blood gas analyzer.

The falsely elevated Hb level on CBC also results in spurious increases in calculated indices, such as MCH and MCHC [3-5]. In this case, the falsely elevated Hb (16.9 g/dL) also caused artificial increases in MCH (56.1 pg) and MCHC (66 g/dL). After plasma replacement, MCH decreased to 32.6 pg, returning to the normal reference range, while MCHC dropped to 53.4 g/dL but remained above normal. This persistent elevation in MCHC may be attributed to the influence of hematocrit (Hct) values on its calculation, as MCHC is derived from Hb and Hct. The post-replacement Hct (19.1%) decreased, possibly contributing to continued MCHC overestimation.

The observed reduction in mean corpuscular volume (MCV) and Hct values after plasma replacement may be explained by using 0.9% NaCl solution instead of the analyzer's proprietary diluent, which aligns with one of the implementation methods outlined by Gulati et al. [3].

According to established recommendations, the CBC results obtained after plasma replacement can be considered reliable if white blood cell (WBC), red blood cell (RBC), and platelet (PLT) values remain within the expected between-run reproducibility limits when compared to the initial run. In cases where discrepancies exist between initial and rerun counts, it is acceptable to report the original WBC, RBC, PLT, Hct, MCV, and red cell distribution width (RDW) values, while incorporating the corrected Hb, MCH, and MCHC from the post-replacement measurement [3]. In our case, the close agreement of WBC, RBC, and PLT values before and after replacement provided confidence in the validity of the rerun Hb and MCH result. To clearly report the correction and assist interpretation, we added the following note to the laboratory report, as recommended in the literature: "Lipemic sample was treated with isovolumetric replacement to reduce interference. The affected results were corrected after treatment" [2].

Various methods such as high-speed centrifugation and lipid extraction have been suggested to reduce lipemia-related interference in serum or plasma samples. However, these approaches are not applicable to CBC testing, which requires whole blood [2].

Several practical strategies have been proposed to address Hb interference due to lipemia in CBC analysis. Another approach is to measure lipemic plasma Hb and apply a correction formula to derive accurate Hb, MCH, and MCHC values [3,6]. Additionally, dilution with isotonic diluent followed by correction for dilution factor has been suggested. Point-of-care devices, which are less affected by lipemia, may also serve as an alternative for reliable Hb measurement. If none of these methods can be applied, reporting only the unaffected parameters (WBC, RBC, PLT, Hct, MCV, RDW) with an interpretive comment noting that Hb, MCH, and MCHC values could not be reliably obtained due to lipemia [3].

In our case, pediatric endocrinology consultation was requested due to the absence of parenteral nutrition and the extreme degree of hypertriglyceridemia. The patient was receiving both L-asparaginase and dexamethasone as part of her ALL-induction protocol. These agents, while essential in the treatment of ALL, are known to significantly disrupt lipid homeostasis. L-asparaginase has been shown to elevate serum triglyceride levels by increasing endogenous synthesis of VLDLs [7,8]. Concurrently, glucocorticoids such as dexamethasone can further exacerbate this effect by activating lipoprotein lipase, stimulating hepatic cholesterol synthesis, and enhancing de novo lipogenesis [7,9]. The combined influence of these agents leads to the rapid accumulation of lipoproteins in circulation, with diminished clearance capacity, resulting in transient but marked hyperlipidemia [7]. Although typically self-limiting, this biochemical derangement may significantly interfere with laboratory testing, as observed in our patient's falsely elevated Hb and derived parameters.

In addition to analytical inaccuracy, lipemia and other preanalytical interferences may also incur hidden costs by necessitating repeat testing, delaying clinical decisions, and prompting unnecessary investigations [10]. Therefore, implementing corrective measures and standardized protocols is of dual importance-not only to safeguard patient safety but also to enhance healthcare cost-effectiveness. Despite growing recognition of lipemia-induced errors in CBCs, standardized guidelines for whole blood analysis remain limited, underscoring the need for institutional protocols and further collaborative efforts.

Conclusion

Lipemia must be recognized as a critical source of analytical interference in CBC interpretation, especially due to its potential to cause falsely elevated Hb levels. This case highlights the importance of correlating analytical results with the clinical and biochemical context to avoid misinterpretation. Plasma replacement with isotonic NaCl solution may serve as a simple and effective technique to correct lipemia related errors when immediate resampling is not feasible. Timely identification and correction of such interferences improve

test result accuracy and support appropriate clinical decision-making.

Conflict of Interest Statement
None declared.

Ethical Approval
This case report was conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from the patient. Ethical committee approval was not required according to institutional policy for single anonymized case reports.

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Data Availability
All data generated or analyzed during this study are included in this published article.

Author Contributions
ZŞE conceptualized the study, curated the data, and prepared the initial draft. KTU contributed to validation, visualization, and writing – review and editing. Both authors revised the manuscript for intellectual content, and all authors read and approved the final version of the manuscript.

Abbreviations
ALL: Acute Lymphoblastic Leukemia
CBC: Complete Blood Count
Hb: Hemoglobin
Hct: Hematocrit
MCH: Mean Corpuscular Hemoglobin
MCHC: Mean Corpuscular Hemoglobin Concentration
MCV: Mean Corpuscular Volume
RBC: Red Blood Cell
RDW: Red cell Distribution Width
WBC: White Blood Cell
VLDL: Very Low-Density Lipoproteins

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