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In this issue

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eJIFCC

Foreword from the new editor-in-chief

János Kappelmayer, MD, PhD

Dear Readers,

The eJIFCC has become an increasingly recognized journal in the past years greatly due to the dedicated efforts of the former Editor-in-Chief Professor Gábor L. Kovács who managed to recruit numerous outstanding guest editors, hence releasing high quality thematic issues. Furthermore, thanks to his continued perseverance the journal is currently indexed on PubMed Central. In addition - as we have all experienced - the image of the eJIFCC has been considerably refurbished resulting in an outstanding and attractive outfit in recent years. For further promoting our visibility, we are currently in the process of uploading journal issues prior to 2008 onto PubMed Central.

My intention is also to carry on with thematic issues, but at the same time there is an increasing need to publish research articles and free communications. Most likely because of the enhanced visibility of the eJIFCC, we are experiencing a definite increase in the number of submitted manuscripts. As anticipated, this has also resulted in rejection of manuscripts that do not meet the scientific requirements as judged by the peer-review process.

Articles in this maiden issue of 2018 will definitely be of interest to all practicing laboratory specialists. The topics range from molecular testing by next generation sequencing via the investigation of leptin polymorphism in rheumathoid arthritis and studying hematological parameters in HIV infected patients to a concise document of the clinical chemistry curriculum for residents in laboratory medicine. The eJIFCC also publishes Short Communication, Letters, and Book Reviews - this is also well illustrated in this present issue.

These days the unprecedented rise in the number of scientific journals makes it very difficult to dig out what really deserves attention. All of us obtain on a daily basis dozens of 'greetings' from many predator journals sometime bearing names very similar to already existing established scientific journals, thus, for a senior scientist just to manage the e-mails requires a part time job. I am grateful to authors who contributed with their manuscripts, as well as to those colleagues who expedited the peer-review process, typically within a short turnaround time.

As the director of a large clinical laboratory at the University of Debrecen, Hungary (labmed.hu) for many years, I find the eJIFCC a useful periodical that presently reaches a large audience and is accessible to all IFCC member societies. I sincerely hope that it will be followed by a global readership and we also encourage our readers to submit scientific work for potential publication.

I am looking forward to this challenging task and to serve the laboratory medicine community as the newly appointed editor of the eJIFCC and as a member of the Communications and Publications Division of the IFCC.

Debrecen, Hungary - March 15, 2018
János Kappelmayer

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

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Next-generation sequencing approach for the diagnosis of human diseases: open challenges and new opportunities

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ABSTRACT

The rapid evolution and widespread use of next generation sequencing (NGS) in clinical laboratories has allowed an incredible progress in the genetic diagnostics of several inherited disorders. However, the new technologies have brought new challenges. In this review we consider the important issue of NGS data analysis, as well as the interpretation of unknown genetic variants and the management of the incidental findings. Moreover, we focus the attention on the new professional figure of bioinformatics and the new role of medical geneticists in clinical management of patients. Furthermore, we consider some of the main clinical applications of NGS, taking into consideration that there will be a growing progress in this field in the forthcoming future.

INTRODUCTION

The next-generation sequencing (NGS) has been introduced in genomic laboratories about 10 years ago. Its impact on technological revolution has important implications in human biology and medicine [1]. After improvements in accuracy, robustness and handling, it became a widely used and an alternative approach to the direct Sanger sequencing [2,3].

The progress of NGS is leading to the increase of discovery of number of genes associated to human inherited disorders and to the elucidation of molecular basis of complex disease [4]. Moreover, since on NGS platforms it is possible to perform a parallel sequencing of different target regions, NGS is widely used in diagnostics. Recently, the use of NGS in clinical laboratories has became increasingly widespread, used in diagnostics of infectious diseases, immune disorders, human hereditary disorders and in non-invasive prenatal diagnosis, and, more recently, in the therapeutic decision making for somatic cancers [5–12].

A great advantage of NGS approach is based on its ability to deliver clinical diagnosis in a short time [3].

Currently, there are several NGS platforms available for routine diagnostic applications. These sequencers allow performing an high-throughput analysis within few days, considerably decreasing costs [13]. These new technologies are different from Sanger sequencing because they are based on a massively parallel analysis and high throughput. Today two different NGS technologies are mainly used in clinical laboratories: Ion Torrent and Illumina systems [14]. The Ion Torrent Personal Genome Machine (PGM) was launched in 2011, while the widely used Illumina benchtops for diagnostic purpose are MiSeq, marketed in 2011, MiniSeq, launched in 2016, or iSeq100, debuting in the end of 2017. The Ion Torrent exploited the emulsion PCR using native dNTP chemistry that releases hydrogen ions during base incorporation by DNA polymerase and a modified silicon chip detecting the pH modification [15], while Illumina technology is based on the existing Solexa *sequencing by synthesis* chemistry with the use of very small flow-cells, reduced imaging time and fast sequencing process [14].

NGS APPROACH IN CLINICAL LABORATORIES

The increase in number of causative genes associated with human inherited disorders is directly associated with the implementation of NGS.

Until now Sanger sequencing has been the gold standard in clinical laboratories for single-gene tests and it serves as the standard methods by which NGS data should be compared and validated [16]. However, Sanger sequencing achieves the diagnostic goal when there is a clear phenotypic indication of a classical Mendelian disorder and the single-gene test approach is preferred. It eliminates the problem of incidental findings, that we will discuss later, but it may push the patients into a “diagnostic odyssey”, where they could be evaluated by multiple providers, sometimes for years, without a genetic diagnosis [13].

Today there is a different scenario, in which genomic technologies can be very useful to detect genetic variations in patients with a high accuracy and an important reduction of costs, thanks to the first-generation sequencing approach. In particular, next-generation sequencing will increasingly be used for clinically heterogeneous inherited disorders, resulting in an increase in number of reported disease-causing genes [6]. Indeed, in the majority of human inherited diseases not merely one gene but a number of genes may interact leading to overlapping pathological phenotypes [2]. NGS approach is tempting when there is a genetic contribution in heterogeneous and complex diseases, such as

in cardiomyopathies, in cardiac arrhythmias, in connective tissue disorders, in mental retardation or autism, where a large number of genes are involved in a large phenotypic spectrum [10,11,17]. In these cases, NGS approaches allows to test a large number of genes simultaneously in a cost-effective manner [13]. An important issue is to decide which kind of NGS testing strategy is best suited for each clinical case. Two options are currently available: targeted gene panels or whole-exome sequencing (WES) [13].

Targeted sequencing of selected genes offers a good coverage (mean 300X, depending on platforms and number of analyzed samples) for the entire analyzed panel and specific regions refractory to NGS can be sequenced by Sanger sequencing, in order to cover the gap and to validate the NGS data [18,19]. So far, targeted resequencing has been adopted to develop tests for genetic disorders, such as non-syndromic deafness [20,21], common and heterogeneous diseases, such as hypertension and diabetes [22], or in traditional cytogenetic and Mendelian disorder diagnosis [23,24]. The main limitation of targeted sequencing is the rigidity of testing only a selected number of genes. Since the genetic field is rapidly evolving, new genes may be associated with a clinical phenotype and as such redesigning and revalidation of the panel is needed [13,16]. On the contrary a clear advantage of the use of targeted panel is the reduction of number of incidental findings and/or the number of variants of unknown significance, that will be discuss later in this review.

On the other hand, the benefit of WES is testing a greater number of genes, even if, in practice, complete coverage of all coding exons is infeasible. The WES application may be useful, for example, in negative cases in targeted sequencing or in a rare disease, especially in exploiting trios approach. Indeed, it allowed the identification of genes responsible for the dominant Freeman-Sheldon syndrome, the recessive Miller Syndrome and the dominant Schinzel-Giedion Syndrome [25]. However it is important to keep in mind that about 10% of targeted bases sequenced in WES do not get the 20 read depth [26], required for clinical confidence and interpretation, and approximately only 85% of genes associated to human diseases into the principle database (OMIM) receive the adequate coverage [27]. Poor coverage in WES can due to several factors: probes that are not tiled for particular genes probably not included during assay development or because repetitive sequences prevented inclusion or poorly performing probes owing to GC-richness and low mapping quality [6].

However it is important to consider that both of these approaches can significantly reduce costs and turn-around time for a genetic test [13].

THE MAIN ISSUE OF NGS: THE INTERPRETATION OF GENETIC DATA FOR A CLINICAL UTILITY

In the NGS process one limiting step is without doubt the complexity of genetic variation interpretation in whole exome, due to the presence of thousands of rare single nucleotide variations without pathogenic effect. Moreover, in the majority of human diseases the pathological phenotype may be caused by a pathogenic rare mutation with a strong effect or it may be caused by a co-presence of multiple genetic variations [28][29].

Reliable interpretation of the multiple and *de novo* variants identified through NGS will require additional experience and validation before it reaches the clinical stage on a large scale, particularly for diagnosis of complex traits [30]. In the recent past, genetic data did not drive diagnosis but had a primarily confirmatory role. Today the major challenge is to convert pathogenic genetic data into a primary diagnostic tool that can shape clinical decisions and patients management [31].

Actually, the interpretation of genetic variants is based on criteria published by the American college of medical genetics and genomics (ACMG). The ACMG recommends that the variants be allocated to one of the categories reported below [32]:

a. disease causing (class V): the sequence variation is previously reported and recognized as causative of the disorder;
b. likely disease causing (class IV): the sequence variation is not previously reported as expected to cause the disorder, frequently in a known disease gene;
c. variant of unknown clinical significance (VUS; class III): the sequence variation is unknown or expected to be causative of disease and is found to be connected with a clinical presentation;
d. likely not disease causing (class II): the sequence variation is not previously reported and it is probably not causative of the pathology;
e. not disease causing (class I): the sequence variation is already reported and documented as neutral variant.

Moreover, most of these classes of variants are subject to supplementary interpretation focusing on literature reported, population frequencies, clinical findings, mutation databases and possibly case-specific research data [31]. The principal human variant databases are useful to annotate both common and pathogenic variants, such as dbSNP, gnomAD or ExAC database (Exome Aggregation Consortium) [33], and to classify variants previously associated with human disorders, such as Human Gene Mutation Database (HGMD) [34] and ClinVar.

The variants of unknown significance (VUS) represent a problem for the interpretative process. Indeed it is known that hundreds of loss of function variants with unknown clinical significance are present in each individual's genome and today their prioritization remains a primary challenge [35].

In some cases, the interpretation of VUS can be useful in commencing the segregation analysis in large families including affected members or the identification of the occurrence of *de novo* variation in the affected patient. Unfortunately, in many cases the interpretation of VUS remains unresolved and its identification cannot be used for the clinical management of patients and families [29,36].

Until now few clear guidelines are published for the VUS interpretation [36]. Today, in order to try to assign a pathological score to VUS, it is important to consider, for example, its allelic frequency in a control population (1000 Genomes or exome sequencing project consortium [ExAC]), the amino acidic conservation, the predicted effect on protein function and the results of published functional assay [37,38].

Up to now *in silico* prediction algorithms, such as Polyphen, Sift, Mutation Taster or UMD predictor, have been developed and they are widely used for the missense variants interpretation [37]. However, they present some intrinsic caveat and limitations, affecting their specificity and sensitivity, that can lead to possible false-positive and false-negative interpretations [39]. Another existing problem involves the allelic frequency, that is mainly estimated from the 1000 Genome project and ExAC, that represents only a fraction of the worldwide population, so the declared allelic frequency available is not stratified according to the real population groups [29].

Since the problem of the management of VUSs is not yet resolved, it would be fundamental to collect and share VUSs and available clinical data, allowing a progressive and definitive classification of these variants, as deleterious (class V) or neutral ones (class I) [29,30].

Another important challenge of the use of NGS approach in clinical diagnostic is the management of the amount of data generated [40]. Indeed generation, analysis and also storage of NGS data require sophisticated bioinformatics infrastructure [41].

A skilled bioinformatics staff is needed to manage and analyze NGS data, and so both computing infrastructure and manpower impact on costs of NGS applications in clinical diagnostics. Bioinformaticians are to be mandatory in the organization chart of clinical laboratories in the NGS era, where they have to closely collaborate with clinicians and laboratory staff to optimize the panel testing and the NGS data analyses [42].

Bioinformatics has been recently defined as the discipline that develops and applies advanced computational tools to manage and analyze the NGS data. Bioinformatics pipeline developed for NGS are aimed to convert the raw sequencing signals to data, data to information, and information to knowledge [43].

This process can be developed in three different steps - primary, secondary, and tertiary analyses [44]:

- The primary analysis is the process of raw data produced by NGS instruments,
- the secondary analysis is the alignment to a reference sequence and the calling variants and, finally,
- the tertiary analysis is the confirmation or validation of detected variants, providing evidence to facilitate interpretation [41].

All clinical bioinformatics systems require these three steps that should be properly validated and documented. In particular, it requires determination of variant calling sensitivity, specificity, accuracy and precision for all variants reported in the clinical assay [44]. The quality criteria of the performed sequencing test have to be described on the report for clinicians and patients. In particular, it is needed to declare the sensitivity and specificity of the techniques used considering both technical and bioinformatics parameters. It is important to report which target region was not sequenced, the number of reads obtained, the quality of the sequence, the limitations of the chosen sequencing method and of the settings of used bioinformatics pipeline [16,45].

ETHICAL CONSIDERATIONS AND MANAGEMENT OF INCIDENTAL FINDINGS

The development and the widespread use of NGS in clinical laboratories are paired with debate on the ethics for reporting incidental findings [46,47]. In 2013 the ACMG has highlighted the question of the incidental findings (IF), defining them as *"genetic variations identified by genomic sequencing but not related to the disease being investigated"* [48].

According to the European Society of Human Genetics (ESHG) guidelines, the targeted diagnostic testing should be performed minimizing the likelihood of detecting incidental findings, focusing only on genes clinically actionable [49]. It means that genetic testing should aim to analyze the causative genes associated to the primary clinical questions, even if a broader panel of genes or the whole exome sequencing has been performed [49]. It is the role of responsible clinicians requesting the test to disclose an incidental finding to a patient, not the role of the clinical laboratory.

The impact of the IF determines how the genetic finding should be disclosed or not to a patient, also to avoid unwarranted psychological stress. In particular, if it can bring minor consequences or if a clinical intervention is possible, then the variant should be reported.

On the contrary, if the variant is associated to a late onset disorder or has major consequences,

counselling and consent will determine if and when the variant can and should be reported to the patient [36]. This implies that genetic tests should be ordered by medical professionals who are capable of performing appropriate counselling [50]. For that reason, the counselling and the informed consent are critical steps.

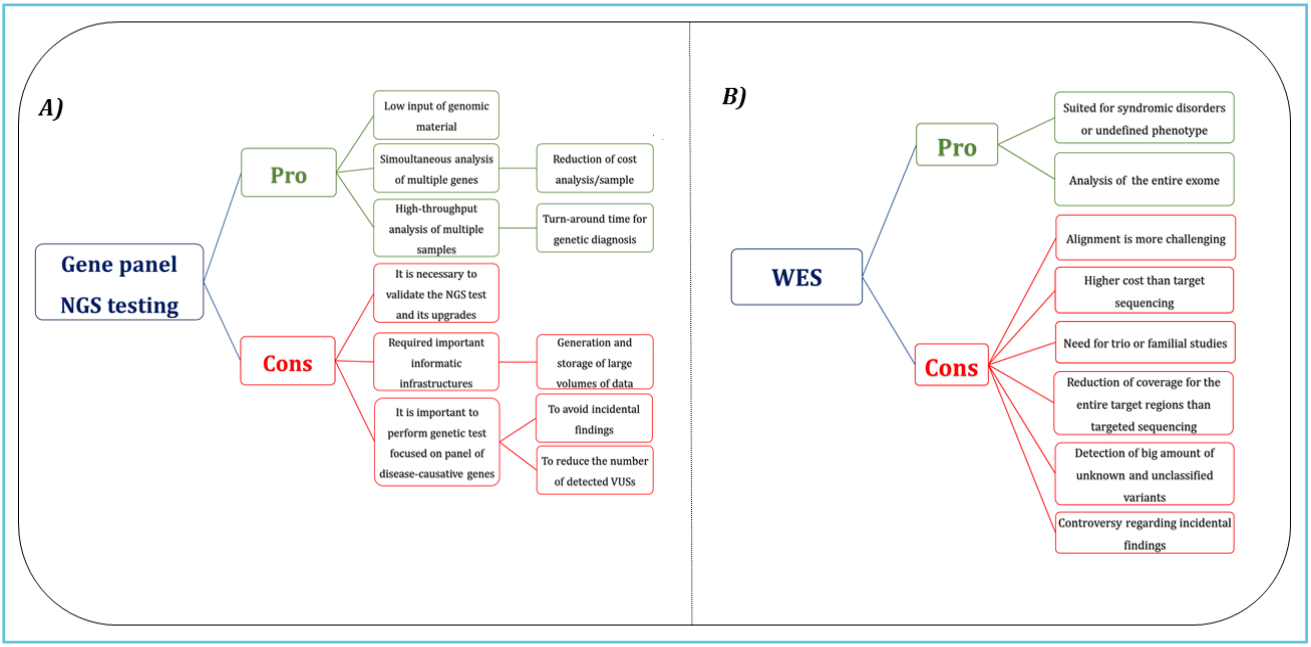
There is a difference between recording and reporting a variant, as well as between who receives this information, clinicians or patients, and when. When a variant is reported to a clinician, it does not mean that it will be revealed to patient. Indeed, the clinician should evaluate the impossible clinical implication of this information, based on the clinical history of patient. For example, the impact of an IF in a case without a known family history for a specific disorder is different from the case in which the patient is already aware of a preexisting familial condition.

Another interesting example is the acute neonatal care, in which immediate reporting of all IFs to patients' families may not be appropriate and the genetic information may be reconsider later in baby's life. Similarly, the report of IFs may be postponed in cases where parents or patients are given a diagnosis linked to poor prognosis or in case of post-mortem genetic testing.

Additional contexts in which the reporting of incidental findings may have an influence on the patients management are carrier testing, prenatal diagnosis, pharmacogenetics testing and additional non-diagnostic testing such as medical research (dependent on the study design), forensic testing, parental and genealogical testing. In conclusion, the issue of IFs requires an appropriate pre and post counselling to correctly inform the patient [16].

The widespread implementation of NGS approach in diagnosis of human pathologies raises the problem of management of IFs and VUSs and it is needed to have clear guidelines for the handling of NGS data in the diagnostics approach (Figure 1).

Figure 1 Advantages and challenges of the use of gene panel NGS testing and WES



So far the application of WES in clinical diagnostics presents more open challenges (B) than targeted sequencing (A).

CONCLUSIONS

Until now Sanger sequencing has been the gold standard in molecular diagnostics and it has been used in clinical testing method for Mendelian disorders, in which most of causative variants are identified in the principal causative genes. Since the rapid and incremental improvements in instrumentations, methodologies and throughput and the significant reduction of costs, the NGS technologies are being integrated into patient care and clinical management. NGS allows sequencing of all genes relevant to a given phenotype starting from a small amount of total DNA. In that way, the limitation factors are no longer the size of the gene or its causative contribution but the actual knowledge of the genetic basis of patient's disease [6].

In the past, clinicians considered genetic tests with a marginal diagnostic value, only if a definitive diagnosis was not yielded or if it had implications on future family planning. Often the positive genetic test results did not influence clinical management of the patient.

However today, with the potentiality of NGS, the parallel sequencing of large multi-genes panel, that may describe a broader range of phenotypes, the clinicians are changing their point of view on the role of the genetics in patients care. Indeed, nowadays the genetic testing may be useful for the evaluation of a clinical case and, if the result were to be positive, it may save time and money in identifying the etiology.

Today physicians often begin their clinical evaluations with the genetic tests. For example, the evaluation of patients with left ventricular hypertrophy begins with genetic testing, given that the genetic diagnosis is achieved in about 80% of hypertrophic cardiomyopathy cases [51].

The results of most targeted genetic tests may be available for clinicians in 2-8 weeks, which is an impressive improvement compared to the time taken for direct Sanger sequencing and the odyssey lived by some patients before to understand the cause of their rare disorder [6].

This strategy of approaching the clinical evaluation has also economically beneficial in patients without diagnosis [52].

The euphoria of the widespread use of the NGS applications to the clinical diagnosis is combined with the awareness of emerged challenges, such as the validation of large number of genetic variations detected, that can be IF or VUSs, the use of standardization processes in clinical diagnostics, the management of terabytes of data and variants interpretation.

In the NGS approach, the analysis of data requires the development of a standard pipeline to process sequencing data. The flow chart analysis includes mapping, variant calling and annotation. Today there are various public database, such as dnSNP [53], the 1000 Genome Project [54], ExAC, as well as several internal control databases.

Targeted panel sequencing or clinical exome sequencing identifies several variations in each person, but as far there are no clear guidelines to filter variants and to delineate their possible pathological meanings. For this reason, the pathogenic validation may be the limiting step. Because of these considerations, it is important to apply the NGS approach in clinical diagnostics for that disorders of which the main causative genes have been identified. Indeed, in this case the genetic tests can successfully reveal a useful result.

Moreover, another consideration involves the fundamental change of the figure of medical geneticist in the NGS era. Indeed, the NGS applications into diagnostic field can lead to useful results for patient's care with genetic disorders. As such, the geneticists will become a pivotal part of the collaborative team of clinicians and their role will be fundamental for the clinical interpretation of NGS data to guide patient care [25].

Consequently, clinical medical geneticists have to complement their skills with expertise in the clinical interpretation of NGS data.

Moreover we have to keep in mind that the medical geneticist has an important and crucial role also in the pre-test counseling, to deliver reliable information to patients [29]. Indeed it is important to clearly explain to the patient and his family the medical implications of the identification of a genetic alteration, regarding the degree of risk for a disease and also the significance of a possible negative results, both in pretest and in the post test counseling [29].

In meanwhile, the NGS approach becomes a cornerstone for the genetic diagnosis, a more efficient and powerful third-generation technologies are expected to further revolutionize genome sequencing [55]. The three commercially available third-generation DNA sequencing technologies are Pacific Biosciences (Pac Bio), Single Molecule Real Time (SMRT) sequencing, the Illumina Tru-seq Synthetic Long-Read technology, and the Oxford Nanopore Technologies sequencing platform.

Third-generation sequencing was made feasible in part by increasing capacity of existing technologies and improvements in chemistry and it allows to sequence a single nucleic acid molecule, eliminating the DNA amplification step, with a longer and easier mapping of sequencing reads with lower costs [55].

Moreover, the use of longer reads than the second-generation allow to overcome the important limitation of NGS in copy number variation analysis (CNV) [56], even if these single-molecule sequencing approaches have to become even more robust for a wider use.

Lastly, few years ago a new technique called Spatial Transcriptomics was developed and gave rise to fourth generation sequencing, also known as single-cell sequencing [55,57]. In this new technology, NGS chemistry is applied to the sequencing of nucleic acid composition directly in fixed cells and tissues providing a throughput analysis, opening great opportunity mainly for the analysis of tumor cells variability *in situ* [58]. In forthcoming future, it holds exciting prospective for research and new insights regarding genomic diagnostics.

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Integration of multigene panels for the diagnosis of hereditary retinal disorders using Next Generation Sequencing and bioinformatics approaches

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ABSTRACT

In recent years, Next-Generation Sequencing (NGS) opened a new way for the study of pathogenic mechanisms and for molecular diagnosis of inherited disorders. In the present work, we focused our attention on the inherited retinal dystrophies (IRDs), a group of specific disorders of the retina, displaying a very high clinical and genetic heterogeneity, whose genetic diagnosis is not easily feasible. It represents a paradigmatic example for the integration of clinical and molecular examination toward precision medicine.

In this paper, we discuss the use of targeted NGS re-sequencing of selected gene panels in a cohort of patients affected by IRDs. We tested the hypothesis to apply a selective approach based on a careful clinical examination. By this approach we reached a 66% overall detection rate for pathogenic variants, with a 52% diagnostic yield. Reduction of the efforts for

validation and classification of variants is a clear advantage for the management of genetic testing in a clinical setting.

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INTRODUCTION

Inherited retinal dystrophies (IRDs) are a group of rare diseases due to a progressive degeneration of retinal photoreceptors, that can lead to vision loss [1,2]. IRDs comprise several different disorders characterized by clinical and genetic heterogeneity, often displaying a phenotypic overlap [3]. Many IRDs are characterized by progressive degeneration of both cone and rod photoreceptors, making the clinical differential diagnosis difficult, especially in the advanced stages [4].

Additionally, there are also syndromic forms in which retina is not the only affected tissue and additional organs can be involved, such as the Usher Syndrome (USH) and the Bardet-Biedl Syndrome (BBS). Furthermore, clinical symptoms can be progressive with variable onset and intra-familial variability, due to an incomplete penetrance and variable expressivity, making the clinical picture more complex [1]. All these factors often complicate or delay a precise diagnosis [1,2,5].

By a genetic point of view, IRDs displays locus and allelic heterogeneity [6], with more than 200 causative genes, that make the genetic characterization very complex. The advent of next-generation sequencing (NGS) has opened new frontiers in genetic diagnostics of IRDs, exploiting the high-throughput parallel sequencing and the simultaneous analysis of several samples. Indeed, the overall mutation detection rate for IRDs is variable [6], ranging from 36% to 60%, leaving many cases still genetically unsolved. More than 4000 pathogenic variants have been identified in causative genes, that can converge to the same phenotype [6] or can show different symptoms [1], complicating the molecular diagnosis. Lastly, since some IRDs causative genes are associated to specific inheritance traits (AR, AD, X-linked), a targeted genetic analysis could be more effective, although sometimes establishing the inheritance mode in an affected family is difficult [1].

Considering all the above, it is often complex to determine *a priori* which genes are to be analyzed and a "non-hypothesis-driven" approach has been applied in large NGS studies [7-9].

In the diagnostic laboratory, such an approach increases the risk to identify variants of uncertain significance, complicating the interpretation and implying a big effort in classification.

In this paper, we describe the strategy adopted by our multidisciplinary team to optimize the integration of clinical data and NGS targeted resequencing for the diagnostics of the different forms of IRDs. Our approach for the molecular diagnosis of IRDs, including genes that fit with the phenotype, allowed us to obtain a 66% overall mutation detection rate, consistent with the best rates obtained with the "non-hypothesis-driven" approach.

MATERIALS AND METHODS

Clinical diagnosis and sample collection

This investigation conformed to principles outlined in the Declaration of Helsinki.

We collected 35 unrelated affected patients with different forms of IRDs.

All patients underwent an ophthalmic evaluation at the Department of Ophthalmology of San Raffaele Hospital (Milan, Italy), including best corrected visual acuity by means of Early Treatment Diabetic Retinopathy (ETDRS) standard charts, biomicroscopy, color fundus photography, fundus autofluorescence, electrophysiological tests, and spectral-domain optical coherence tomography.

Clinical and family history details were collected during genetic counseling interview. Written informed consent for genetic analysis was obtained from all subjects. Genetic analysis was performed at Laboratory of Clinical Molecular Biology of San Raffaele Hospital (Milan, Italy).

Genomic DNA (gDNA) was extracted from peripheral blood using the automated extractor Maxwell16 (Promega, Milano, Italy); the concentration and gDNA quality were determined using Qubit® Fluorometer (Thermo Fisher Scientific).

Library enrichment and sequencing

Sample enrichment and paired-end libraries preparation were performed using the commercial kit TruSight One (Illumina, San Diego, CA, USA), starting from 50ng gDNA, following the manufacturer's instructions (Document #1000000006694 v00).

TruSight One Sequencing panel includes 4,813 genes associated with known clinical phenotypes, according to the Human Gene Mutation Database_HGMD (http://www.hgmd.cf.ac.uk/ac/index.php), Online Mendelian Inheritance in Man, OMIM (www.omim.org), and GeneTests (www.genetests.org). The entire gene list is published on www.illumina.com (Pub. No. 0676-2013-016 current as of 04 January 2016). Sequencing was performed on NextSeq500 instrument (Illumina, San Diego, CA, USA) with a flow cell high output, 300 cycles PE (150 x 2).

NGS data analysis

The read alignment and variant calling were performed with BaseSpace Onsite Sequence Hub. For each case, the analysis of variants was focused on one or more gene panels based on the different clinical phenotypes. The variants were then annotated using Illumina VariantStudio data analysis software. For the identification of possible causative variants, filters were applied taking into account: 1) the quality parameter, 2) the MAF (Minor Allele Frequency) >2% in the 1000Genomes and ExAC database, 3) the localization of the variants, considering only the exonic and intronic regions at ± 20 bp from the coding regions, to identify possible splice-site variations.

In order to optimize the data analysis process and to focus on the identification of causative variants, we created panels of disease genes associated to the different forms of IRDs, as reported in Table 1. Particularly, we set panels for non-syndromic forms (Achromatopsia (ACHM); Best vitelliform macular dystrophy; Congenital stationary night blindness (CSNB); Choroideremia; Stargardt disease; Retinitis pigmentosa) and for syndromic forms (Bardet-Biedl S., Refsum disorder, Cohen S., Stickler S., Usher S.). We chose causative genes for each disease panel based on public databases, such as OMIM (http://www.ncbi.nlm.nih.gov/omim) or RetNet™ (https://sph.uth.edu/retnet/) and from the literature [4,6,10-12]. After primary analysis, the search for causative variants started by considering the panel of genes associated to the clinical suspicion. If the suspicion was less focused, more than one panel is analyzed.

Interpretation of putative variants was performed using Alamut® Visual (Interactive biosoftware), that integrate data from several databases, such as NCBI, UCSC, ClinVar, HGMD Professional, and *in silico* tools prediction, such as Polyphen, Sift, Mutation Taster. Candidate variants were classified according to the ACMG criteria in 5 categories:

- class 1: benign,
- class 2: likely benign,
- class 3: uncertain significance (VUS),
- class 4: likely pathogenic,
- class 5: pathogenic [13,14].

Analysis flow chart is reported in Figure 1.

Table 1 Different panels of disease genes associated to the different forms of IRDs

Inherited Retinal Dystrophies											
	Non-Syndromic Forms						Syndromic forms				
	Achromatopsia	Best macular dystrophy	Congenital Stationary Night Blindness	Choroideremia	Stargardt disease-cone-rod dystrophy	Retinitis pigmentosa-rod-cone dystrophy	Bardet-Biedl Syndrome	Cohen Syndrome	Stickler Syndrome	Usher Syndrome	Refsum disease
Orpha	49382	1243	215	180	827	791	110	193	828	886	773
ICD-10	H53.5	H35.5	H53.6	H31.2	H35.5	H35.5	Q87.8	Q87.8	Q87.0	H35.5	G60.1
Incidence	1-9 / 100 000	1-9 / 100 000	Unknown	1-9 / 100 000	1-5 / 10 000	1-5 / 10 000	1-9 / 1 000 000	Unknown	1-9 / 100 000	1-9 / 100 000	1-9 / 1 000 000
Onset	Infancy, Neonatal	Childhood, Adolescent	Neonatal	Childhood, Adolescence, Adulthood	Childhood, Adolescence, Adulthood	Childhood, Adolescent, Adult	Prenatal, Neonatal, Childhood	Neonatal, Childhood	Childhood	Neonatal, Childhood	Infancy, Childhood, Adolescence, Adulthood
Inheritance mode	AR	AD	AD; AR; X-linked	X-linked	AD; AR	AD; AR; X-linked; Mitochondrial inheritance	AR	AR	AR; AD	AR	AR
Prevalence of mutations	75-90%	96%* (familial forms) 70%* (non familial forms)	95%*	95%*	65-70%	75%	90%*	70%*	100%*	80-85%*	100%*
N. of genes of panel	7	3	14	1	43	63	18	1	5	11	2
Genes	ATF6	BEST1	CABP4	CHM	ABCA4	ABCA4	ARL6	VPS13B	COL11A1	ADGRV1	PEX7
	CNGA3	IMPG2	CACNA1F		ADAM9	BBS1	BBS1		COL11A2	CDH23	PHYH
	CNGB3	PRPH2	CACNA2D4		AIPL1	BBS2	BBS10		COL2A1	CIB2	
	GNAT2		GNAT1		C2orf71	C2orf71	BBS12		COL9A1	CLRN1	
	PDE6C		GNB3		C8orf37	C8orf37	BBS2		COL9A2	HARS	
	PDE6H		GPR179		CABP4	BEST1	BBS4			MYO7A	
	RPGR		GRK1		CACNA1F	CA4	BBS5			PCDH15	
			GRM6		CACNA2D4	CDHR1	BBS7			PDZD7	
			NYX		CDH3	CERKL	BBS9			USH1C	
			PDE6B		CDHR1	CLRN1	CEP290			USH1G	
			RHO		CEP290	CNGA1	LZTFL1			USH2A	
			SAG		CERKL	CNGB1	MKKS				
			SLC24A1		CLN3	CRB1	MKS1				
			TRPM1		CNGA3	CRX	NPHP1				

					C1QTNF	CYP4V2	SDCCAG8				
					CNGB3	DHDDS	TRIM32				
					CNNM4	EYS	TTC8				
					CRB1	FAM161A	WDPCP				
					CRX	FLVCR1					
					CYP4V2	FSCN2					
					ELOVL4	GUCA1B					
					FSCN2	HGSNAT					
					GNAT2	IDH3B					
					GUCA1A	IMPDH1					
					GUCY2D	IMPG2					
					KCNV2	KLHL7					
					PDE6C	LRAT					
					PDE6H	MAK					
					PITPNM3	MERTK					
					PROM1	NR2E3					
					PRPH2	NRL					
					RAB28	PDE6A					
					RAX2	PDE6B					
					RDH12	PDE6G					
					RDH5	PRCD					
					RGS9	PROM1					
					RGS9BP	PRPF3					
					RIMS1	PRPF31					
					RP1L1	PRPF6					
					RPGR	PRPF8					
					RPGRIP1	PRPH2					
					SEMA4A	RBP3					
					TIMP3	RBP4					
						RDH12					
						RGR					
						RHO					
						RLBP1					
						ROM1					
						RP1					

						RP1L1					
						RP2					
						RP9					
						RPE65					
						RPGR					
						SAG					
						SEMA4A					
						SNRNP200					
						SPATA7					
						TOPORS					
						TTC8					
						TULP1					
						USH2A					
						ZNF513					

Data available on Orphanet (http://www.orpha.net - Last update: August 2017) and Genereviews (https://www.ncbi.nlm.nih.gov/books/NBK1116).

** Data reported on Genereview.*

Identified variants were validated using Sanger Sequencing on AB3730 sequencer (Applied Biosystem), according to the manufacturers' protocols. (Primer and PCR conditions available on request). Moreover, in order to avoid undetected variants in regions with a low number of reads, all target regions of causative genes with a coverage <10X were analyzed by Sanger sequencing.

RESULTS AND DISCUSSION

Parameters of NGS raw data

All the 35 patients have been sequenced for 4813 genes, included in TruSight One panel (Illumina) using Illumina NextSeq500.

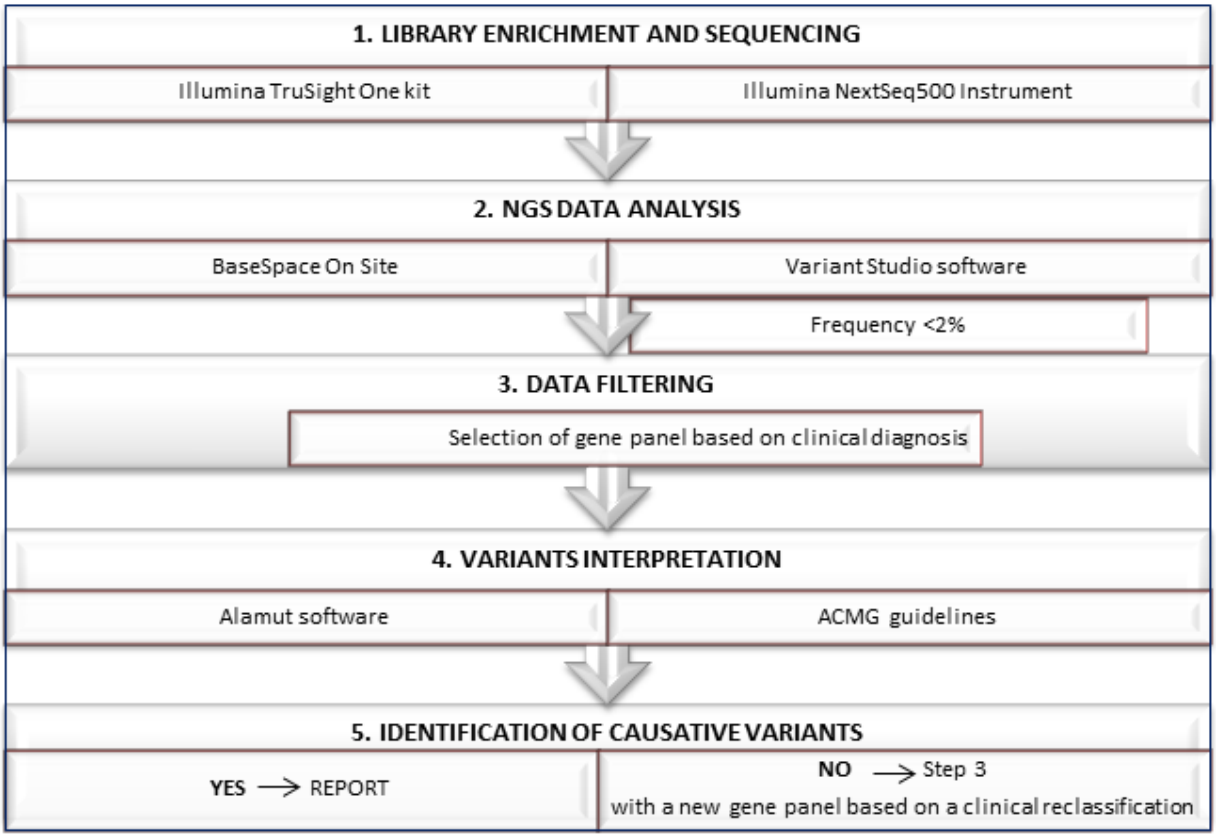
Runs had a mean cluster density equal to 217 k/mm2. We obtained a mean read enrichment of 59% and target aligned read of 99%. The mean coverage for the analyzed genes associated to the different forms of IRDs was 300X.

Analysis and classification of detected variants

In our cohort, excluding common variants, we detected a total of 57 variants in 29 genes; 30 were novel and 27 were already reported in dbSNP as rare variants. In three patients no variants were found (9%), while the others (91%) presented with different variants with the exception of two pathogenic variants in *ABCA4* (NM_000350.2: c.5882G>A; NM_000350.2: c.5018+2T>C), identified in four different unrelated patients.

Considering all the detected variants, 66.7% (38/57) were missense, 10.5% (6/57) were stop-gain, 7% (4/57) were frameshift changes, 8.8% (5/57) may alter splice sites, 1 variant was a start-loss (1.8%), 1 was an in-frame insertion (1.8%), 1 was an in-frame deletion (1.8%) and 1 was a deletion of two whole exons (1.8%) (Figure 2). All the 57 variants were confirmed by Sanger sequencing or MLPA.

Figure 1 **Workflow of NGS analysis**



The flow chart illustrates the main steps from the sequencing to the clinical report.

Among the 29 genes, the majority (22/29) present a single variant while seven genes are multivariated (Figure 3).

According with the ACMG guidelines [13], 11 variants were classified as pathogenic (class 5), 19 as likely pathogenic (class 4) and the remaining 27 as variants of unknown significance (VUS, class 3).

Evaluation of the diagnostic yield and genotype-phenotype correlation

We found pathogenic or likely pathogenic variants in 23/35 (66%) patients and consistent with the subject clinical presentation. Among these, we were able to reach the genetic diagnosis in 18/35 (52%) patients while in 5/35 (14%) patients we obtained only a partial diagnosis because of the detection of only one causative recessive variant. In 9/35 (26%) patients we identified heterozygous variants with unknown significance (VUS) in disease-genes but in 5 of them the genotype did not fit to the disease inheritance manner and the genetic diagnosis remained incomplete. Finally, 3 patients were wild-type in analyzed causative genes. In these cases, a multidisciplinary re-discussion would be suggested in an attempt to define further testing or the potential for a research approach.

The majority of patients not reaching the genetic diagnosis had non-syndromic phenotypes, in particular two of the patients with no variants had a

Table 2 The percentance of complete, partial and total diagnostic yield obtained using our multigene panel approach for each disease

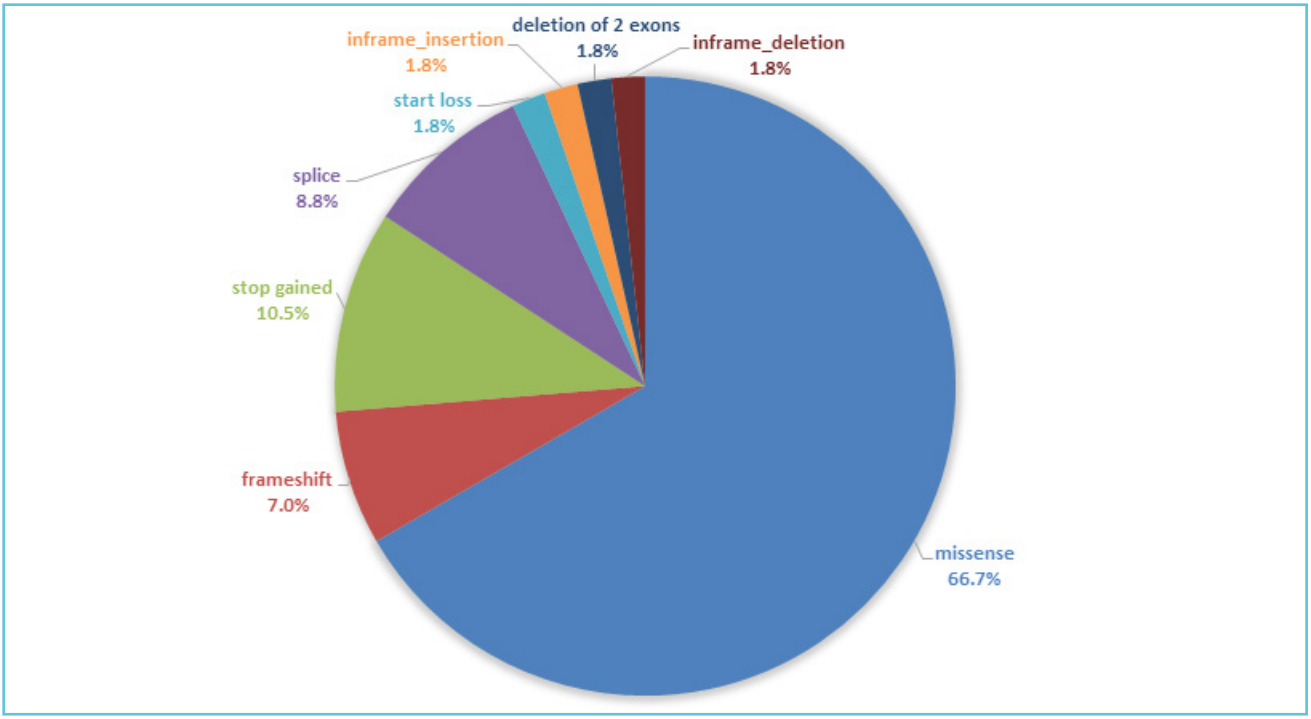
Total patients = 35 Overall diagnostic yield (%) = 51	Clinical phenotype	Patients (n)	Complete diagnostic yield % (n)	Partial diagnostic yield % (n)	Total diagnostic yield %
Disease	Pattern dystrophy	1	100 (1)		100
	Bardet-Biedl S.	1	100 (1)		100
	Best Disease	5	60 (3)		60
	Complex phenotype; retinal dystrophy (rod-cone or cone-rod)	11	36 (4)	18 (2)	54
	Retinitis Pigmentosa	4	75 (3)		75
	Stargardt disease	11	36 (4)	27 (3)	63
	Stickler S.	1	100 (1)		100
	Usher S.	1	100 (1)		100

clinical diagnosis of Best, while in the case of retinal dystrophy, retinitis pigmentosa and Stargardt, a high proportion of patients had a partial or inconclusive diagnosis due to the presence of only one pathogenic variant or to the presence of VUS. In particular, for 5 patients with a partial diagnostic yield we can suspect the presence of a second pathogenic variant in a deep intronic region, as is the case for *ABCA4* or the presence of a structural variant not identified by sequencing. In Table 2, we reported the obtained diagnostic yield for each disease.

In Table 3 are listed all the genes with variants identified in the present work in association with different diseases. It is possible to appreciate that the larger genetic overlap is between the retinal dystrophies and RP phenotypes (Table 3, the shaded lines).

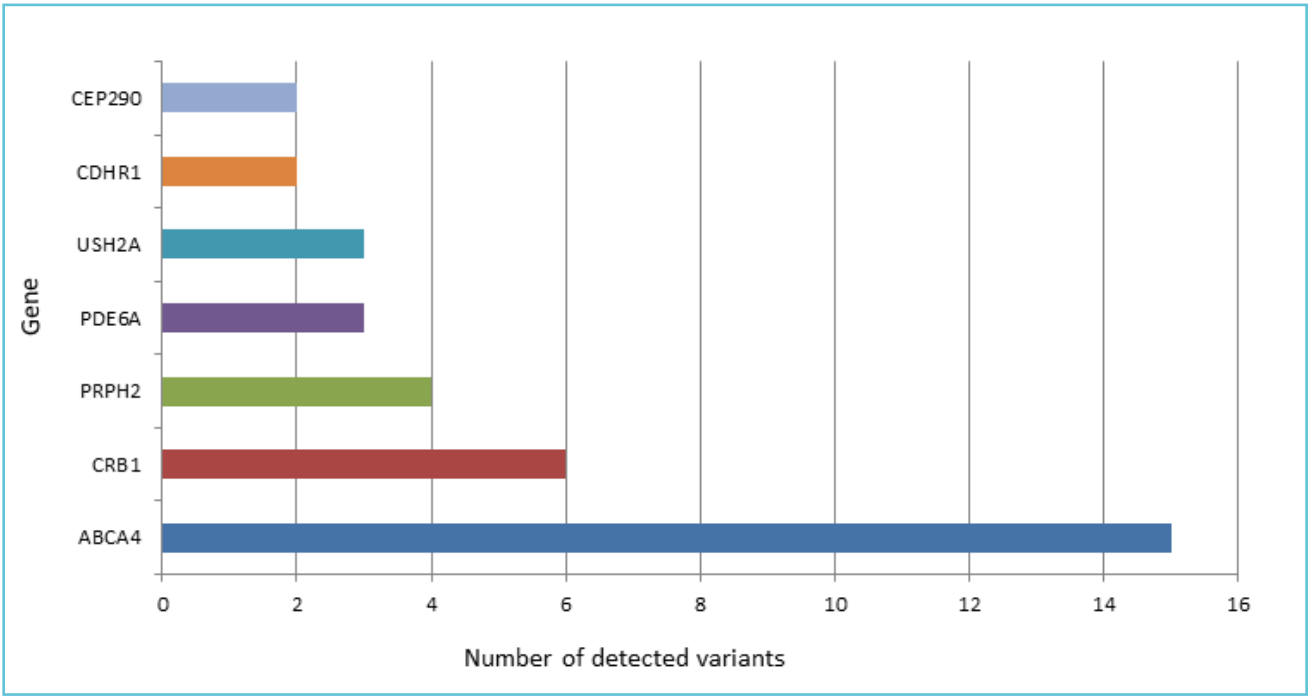
In the present work, we applied a targeted NGS resequencing for genetic testing of IRDs; selection of gene panels was done based on the clinical suspicion (Table 1) allowing us to reduce the number of genes tested. We reached a diagnosis in a proportion of patients that was consistent with the results from other studies, where wider panels were used. Based on these findings, this approach, reducing the efforts needed for classification and validation of variants, seems to be more suited in the diagnostic field.

Figure 2 Different types of identified variants



We identified 57 variants in 29 genes in our cohort and in the pie chart the percentage of each type of detected variant is reported.

Figure 3 Seven genes are multivariated in our cohort



Graph represents the number of detected variants (x-axis) for each gene (y-axis).

Table 3 The genetic overlapping between retinal dystrophies and RP phenotypes

Pattern dystrophy	Bardet-Biedl Syndrome	Best Disease	Complex Phenotype; retinal dystrophy (rod-cone or cone-rod)	Retinitis Pigmentosa	Stargardt disease	Stickler Syndrome	Usher Syndrome
PRPH2	BBS4	BEST1	ABCA4	ABCA4	ABCA4	COL2A1	USH2A
		IMPG2	CDH23	C2ORF71	ATF6		
		PRPH2	CDHR1	CDHR1	CEP290		
			CEP290	CRB1	CRX		
			CNGA3	USH2A	GNAT2		
			CRB1		GPR98		
			FSCN2		PCDH15		
			IMPDH1		TOPORS		
			KCNV2				
			PDE6A				
			PDE6B				
			PITPNM3				
			PRPH2				
			RIMS1				
			RP1				
			RP1L1				
			RPGRIP1				

The coloured cells indicate the genes mutated in different clinical phenotypes.

CONCLUSION AND GENERAL REMARKS

Thanks to NGS, genetic testing costs are reducing rapidly with the potential for a broader access in the frame of health care systems. As NGS allows parallel analysis, it currently realizes a real improvement for personalized medicine, shortening the time needed to reach a diagnosis, nevertheless we still have to face a number of criticisms [15]. This report, showing an overall mutation detection rate for IRDs of approximately 60%, addresses the challenges ahead,

which include: a better understanding of the clinical significance of variants in disease genes; improvement of variant calling, especially for deep-intronic regions, regulatory sequences, promoters and structural variants (i.e.: extension of captured regions and improvement of tools for CNV detection); improvement of genotype–phenotype correlations and comprehension of more complex or not yet understood genetic mechanisms of diseases.

Correspondingly, the simultaneous sequencing of a large number of genes has resulted in increased detection of variants of unknown significance, which require interpretation for clinical purposes. The development of databases such as ClinVar and WES (Whole Exome Sequencing) variant allele frequency by ExAC Browser are gradually improving variant interpretation.

Similarly, programs such as SIFT, PolyPhen-2, and NNSPLICE are now widely used to predict the influence of a variant on protein localization, structure, and/or function. However, *in silico* predictions are not always consistent with functional studies and, despite recent advances, pathogenicity assessment remains challenging, particularly for hypomorphic, synonymous and non-coding variants. Ultimately, better tools are required, as well as improved knowledge of the genome and genome function.

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Influence of serum leptin levels and Q223R leptin receptor polymorphism on clinical characteristic of patients with rheumatoid arthritis from Western Mexico

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ABSTRACT

Objective

The aim of the present study was to evaluate the possible association between the Q223R Leptin receptor (*LEPR*) polymorphism (A>G; rs1137101) and leptin levels in patients with rheumatoid arthritis (RA) from Western Mexico.

Methods

A cross-sectional study was performed with 70 RA patients and 74 controls subject (CS). Disease activity was evaluated using DAS28 score, the Q223R *LEPR* polymorphism was determined by the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and serum leptin levels, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and rheumatoid factor (RF) were quantified.

Results

RA patients had significant high serum leptin levels compared with CS; leptin levels correlated strongly with body composition measures, but not with inflammatory markers, disease evolution, and activity. The genotype and allele frequencies of the Q223R *LEPR* polymorphism were not associated with RA. Similarly, leptin levels did not differ between Q223R *LEPR* genotypes.

Conclusion

The *LEPR* Q223R polymorphism was not associated with RA risk in patients from Mexican population, even though high levels of serum leptin were present and these could explain the low weight observed in RA patients when they were compared to control subjects. However, the serum leptin levels did not correlate with inflammatory markers, severity and disease evolution.

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INTRODUCTION

Rheumatoid arthritis (RA) is one of the most common inflammatory rheumatic disease in which joints of the hand and feet are mainly affected (1). One of the main features in the pathophysiology of this disease is the production of inflammatory cytokines in the synovial membrane and the subsequent joint damage.

Leptin is a cytokine that mediates both immune cell recruitment and complex intracellular signaling control mechanisms that characterize inflammation, thus it may have a potential role in RA. Several studies have reported a two to three times elevation in serum leptin levels in women as compared to men (2,3), furthermore, serum leptin levels are high in RA patients as compared to controls (4-11). Leptin levels also correlate with disease activity (5-7,9) and pro-inflammatory activity (12).

In 1994, it was discovered that leptin is produced by adipocytes and is crucial for the control of appetite (13). This hormone exhibits a structural similarity with the Granulocyte Colony-Stimulating Factor (G-CSF) and with a number of cytokines including Interleukin 6 (IL-6), Ciliary Neurotrophic Factor (CNTF) and Leukemia Inhibitory Factor (LIF) (14,15).

In humans, blood leptin levels are proportional to the amount of body fat (16). At the level of the Central Nervous System (CNS) leptin controls energy expenditure (17), whereas in the periphery it regulates endocrinal function, reproduction, and immunity (18).

Leptin acts by binding to specific receptors present on cell membranes. This receptor is expressed in many tissues in multiple isoforms, resulting from a "splicing" of its alternate mRNA.

The different isoforms of the receptor can be classified as long (OB-Rb), short (OB-Ra, OB-Rc, and OB-Rd) and soluble (OB-Re) (19). Considering the multiple isoforms, it is possible to hypothesize the possibility that leptin exerts multiple effects on tissues.

Several single nucleotide polymorphisms and mutations of leptin receptor (*LEPR*) gene have been linked to diseases accompanying obesity and/or obesity-related diseases in different populations (20-24), nonetheless there are very few studies evaluating their association with RA.

As far, three missense variants Q223R, K109R, K656N in the *LEPR* gene have been described, and their associations with adiposity examined. In most cases, the *LEPR* Q223R polymorphism has been associated with high serum leptin levels; this variant is a substitution of G>A at 668 position, a putative leptin binding region (20,21).

The aim of the present study was to evaluate the possible association between the Q223R *LEPR* polymorphism and leptin levels in patients with RA from Western Mexico.

MATERIALS AND METHODS

A cross-sectional study was performed with a Mexican mestizo population of 70 RA patients and 74 Control subjects (CS) from Guadalajara, State of Jalisco, Mexico. The diagnosis of RA was based on the American College of Rheumatology criteria and the patients were selected from an outpatient rheumatology clinic from the public healthcare system. The CS were randomly selected from an open population sample. The study was approved by the Investigation, Ethics and Biosecurity Committee of the Health Sciences University Center of the University of Guadalajara. All participants gave informed consent prior to inclusion in the study; the study was carried out in accordance with the principles of the World Medical Association's Declaration of Helsinki.

Following blood sampling, serum leptin levels were determined, DNA was extracted and *LEPR* genotypes were identified. Additionally, a detailed clinical history was collected from each participant.

Leptin levels were quantified using a solid phase enzyme-linked immunosorbent assay (ELISA) (DRG International, Inc., USA). The erythrocyte sedimentation rate (ESR) was determined using the Westergren method. The C-reactive protein (CRP) and rheumatoid factor (RF) were quantified using Immage™ Immunochemistry system (Beckman Coulter System).

The Miller method was used to extract DNA. The DNA pellet was resuspended in 200 µL of sterile water, and concentration and integrity were calculated by spectrophotometry. The Q223R variants of the *LEPR* gene were determined by PCR-RFLP technique, as described previously (25).

Descriptive statistics (mean and standard deviation or median and percentile 5-95) were used to describe participant characteristics, additional statistical analysis was performed following data normality check by the Kolmogorov-Smirnov test.

Chi-square test was used to evaluate the statistical association of the polymorphism. Odds Ratio (OR) was calculated for the evaluation of polymorphism as a risk factor associated with rheumatoid arthritis.

The Student *t* or Mann-Whitney test was used to evaluate mean or median differences between the two study groups. One-way ANOVA with Bonferroni *post-hoc* tests were used to evaluate the mean differences between the genotypes of the *LEPR* gene.

Pearson or Spearman correlations were used to evaluate the correlation between leptin levels with age, weight, height, Body Mass Index (BMI), CRP, RF, DAS28 and disease duration. Statistical significance was reported when p values were below 0.05. Data analysis was carried out with the SPSS version 19.0 statistical package (SPSS, Inc., Chicago, IL).

RESULTS

The clinical characteristics of the study subjects are shown in Table 1. A total of 70 RA patients were enrolled, 4 were males and 66 females, with an age mean of 48±13 years and disease duration of 7.5 years, the clinical activity according to the DAS28 score was 5.1±1.6. A total of 74 CS were selected, 24 were male and 50 females, with a mean age of 37±12 years. Upon comparing the two groups, there was no statistically significant difference in height and BMI (p>0.05, data not shown), and there was a significant difference in age, weight, inflammation markers (CRP and ESR) and RF (p<0.05, data not shown).

Table 1 Clinical characteristics of the study groups

Variables	RA (n= 70)	CS (n=74)
Age (years) [c]	48 ± 13	37 ± 12
Sex (male/female) [b]	4 / 66	24/50
Weight (kg) [a]	60.2 (41.1 - 90.3)	67.35 (51-94)
Height (m) [c]	1.55 ± 0.1	1.64 ± 0.1
BMI (kg/m^2) [c]	25.8 ± 5.1	25.9 ± 4.5
Disease status		
Duration of disease (years) [a]	7.5 (0.5-30)	
DAS28 [c]	5.1 ± 1.6	
Clinical assessment		
Leukocytes (X10^9/L) [c]	6.2 ± 1.8	6.4 ± 1.6
CRP (mg/dL) [a]	2.3 (0.3-40.8)	0.2 (0.1-0.9)
ESR (mm/h) [c]	37 ± 14	18.9 ± 11.7
Rheumatoid factor (UI/mL) [a]	258.5 (20-3426)	20 (20-21.4)

[a] *Data provided in median (p5–p95)*
[b] *Data provided in n*
[c] *Data provided in mean ± SD*
RA: rheumatoid arthritis; CS: control subject; ESR: erythrocyte sedimentation Rate; CRP: C reactive protein; DAS-28: disease activity score 28. Normal distribution was determined using Kolmogorov-Smirnov test.

Genotypes and allele frequencies of the *LEPR* Q223R polymorphism are shown in Table 2 and they were in Hardy-Weinberg equilibrium (χ^2 0.08, p=0.77). When we evaluated the genotypes, no causal or statistical association was found between the RA patients and the control subjects.

Serum leptin levels were elevated in patients with RA as compared to CS (12.4 (1.7-48.5) ng/mL *vs* 9.4 (1.4-29.9) ng/mL, p=0.03) (Figure 1).

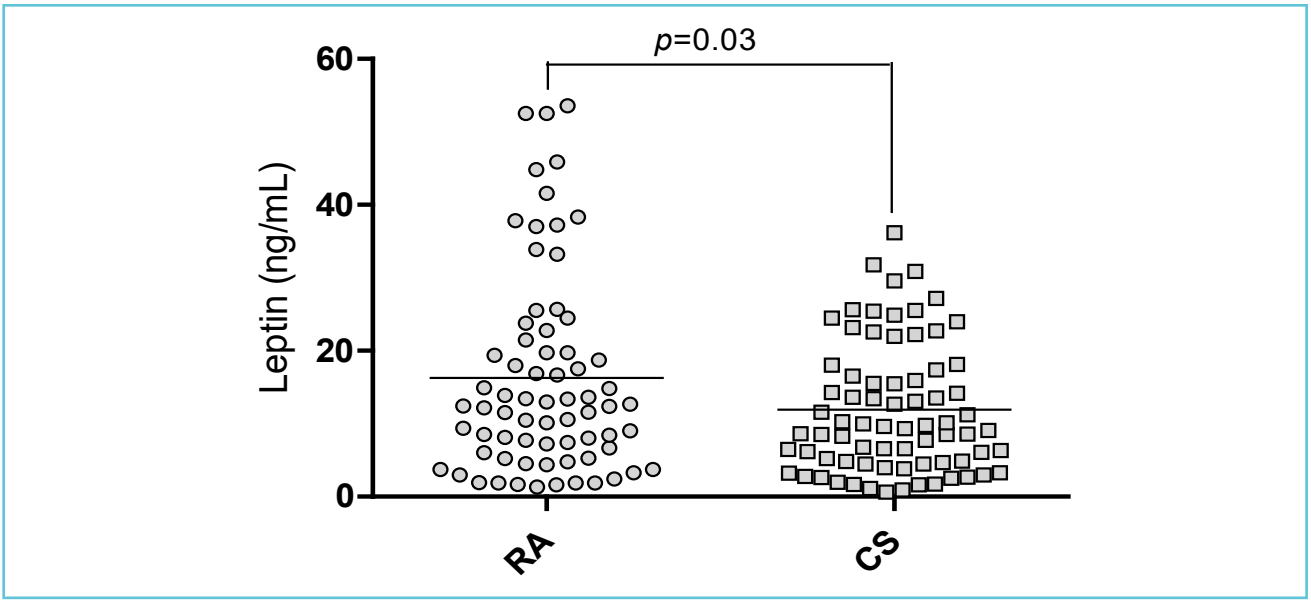
When the serum leptin levels were stratified by genotype in RA patients group, the carriers of the AA genotype had a median of 13.4 (1.8-45.6) ng/mL, while carriers of the AG and GG genotypes had a median of 11.8 (1.6-52.5) ng/mL and 9.9 (2.4-52.5) ng/mL, respectively (Figure 2A). In the CS group, carriers of the AA genotype had a leptin median of 6.34 (0.7-23.5) ng/mL, while the carriers of the AG and GG genotypes had a median of 10.1 (1.5-31) ng/mL and 11.7 (1.6-36.1) ng/mL, respectively (Figure 2B).

Table 2 Genotypic distribution and allelic frequencies of Q223R polymorphism

SNP	Genotype	RA (n=70) % (n)	CS (n = 74) % (n)	*p value*[a]	OR (95% CI); *p*[§]
668 A>G (Q223R)					
Codominant	AA[b]	34.3 (24)	33.8 (25)	0.43	1.0
	AG	54.3 (38)	47.3 (35)		1.1 (0.54-2.33); 0.73
	GG	11.4 (8)	18.9 (14)		0.6 (0.21-1.67); 0.32
Dominant	AA[b]	34.3 (24)	33.8 (25)	0.94	1.0
	AG+GG	65.7 (46)	66.2 (49)		0.9 (0.49-1.94); 0.94
Recessive	AA+AG[b]	88.6 (62)	81.1(60)	0.21	1.0
	GG	11.4 (8)	18.9 (14)		0.5 (0.21-1.41); 0.21
Allele	A[b]	61.4 (86)	57.4 (85)	0.49	1
	G	38.6 (54)	42.6 (63)		0.8 (0.52-1.35); 0.49

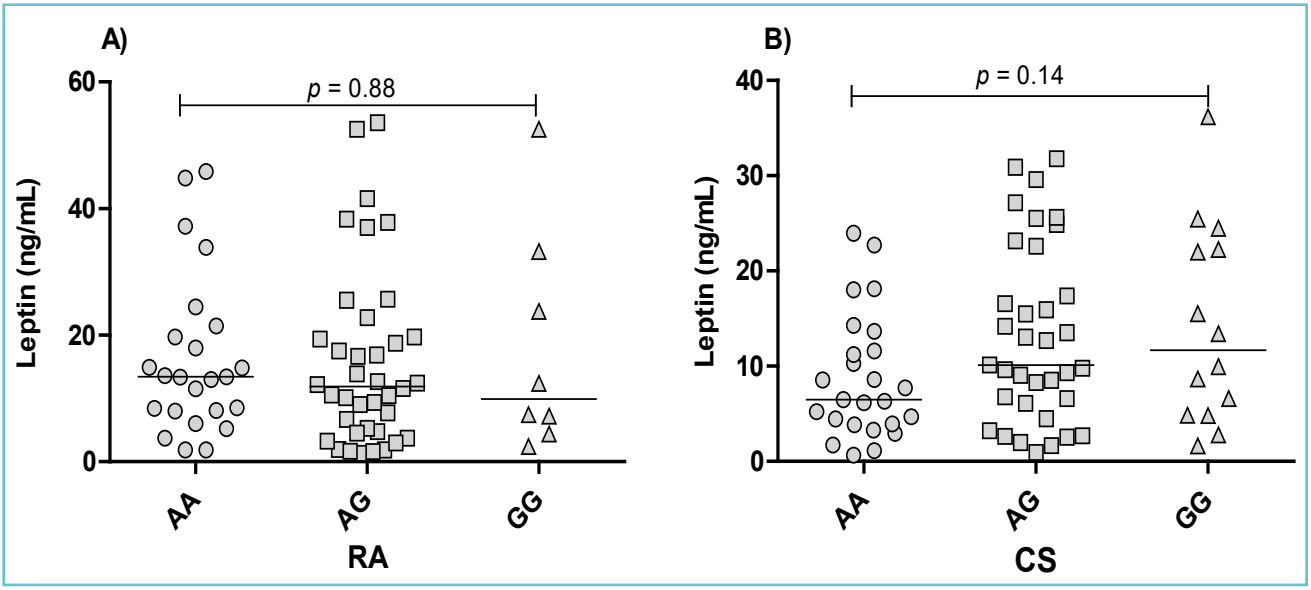
Percentages were obtained by count direct.
RA: rheumatoid arthritis; CS: control subject; OR: odds ratio; 95% CI, 95% confidence interval;
[a]p value was calculated by χ^2 test; [b] Reference category; [§]p value was calculated by logistic regression.

Figure 1 Serum leptin levels in RA patients and CS



Statistical analysis was performed using Mann–Whitney U test.

Figure 2 Comparison of circulating serum leptin levels according to *LEPR* Q223R genotype in RA patients (A) and CS (B)



Statistical analysis was performed using Mann–Whitney U test.

Table 3 Correlation between serum leptin levels and clinical characteristics of AR patients

	Leptin levels	
	r	*P*
Age (years)*	0.172	0.154
Height (kg)*	0.534	<0.001
Weight (m)*	-0.236	0.050
BMI (Kg/m^2)*	0.702	<0.001
ESR (mm/h)**	0.072	0.154
CRP (mg/dL)**	0.121	0.317
RF (UI/mL) **	0.018	0.880
DAS28*	-0.135	0.266
Disease evolution (years)*	-0.018	0.881

Evaluated with Pearson or Spearman** correlation.*
RF: rheumatoid factor; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

In both groups, the mean differences were not statistically significant.

In RA patients, serum leptin levels correlated strongly with weight, height, and BMI. No correlation was found between leptin levels and RF, CRP, ESR, disease duration and DAS28 index (Table 3).

DISCUSSION

Several studies suggest the importance of the adipose tissue as a hormone producer. These hormones are called "adipokines" which play an important role in immunity and inflammation. One of these hormones is leptin and emerging evidence indicates that this hormone acts as a proinflammatory cytokine in immune responses such as systemic lupus erythematosus, multiple sclerosis, psoriasis and RA (26). Thus, the present study attempted to find an association between the Q223R leptin receptor gene polymorphism and leptin levels in patients with rheumatoid arthritis from Western Mexico. We found that RA patients had lower weight as compared to CS. These data are interesting because Chen Y et al., reported that in males with RA, the height is inversely associated with the severity of disease and disability, and this association is independent of other factors. In the case of females, it appears that there is an association of sex and a lower height with severe disease activity and disability (27). The significantly lower weight in the RA patients is also consistent with a previous report, where weight, height, and BMI loss was significant in females with RA (27, 28). However, our results should be interpreted with caution, as most RA patients were women with very short stature as compared to the CS group. Additionally, it is notable that BMI value of patients and controls are the same.

Goldring SR et al., have argued that the loss in weight and height observed in RA patients could be explained by a generalized involvement of the bone tissue, that causes osteopenia and bone erosion (29). Several studies propose that various factors such as proinflammatory cytokines, low mobility, and a poor nutrition can promote osteopenia (30-32). This may be due to increased appetite-regulating adipokines such as leptin, as reported in certain inflammatory conditions, such as obesity or in this case in RA.

We found significantly elevated serum leptin levels in patients with RA as compared to the control subjects. These results are consistent with previous reports (4-11). Although, the values that we report in this study are higher than the healthy subjects, they are considered low as compared to other populations (16, 33), this observation may be due to differences in age, sex and ethnical background (2, 3, 33, 34). Similarly to patients with obesity and insulin resistance, the observed increase in leptin levels may be explained in part by macrophage infiltration into the adipose tissue (35-37) and not due to presence of the studied polymorphism. Further investigations are needed.

The frequencies of the allele G of the polymorphism Q223R *LEPR* gene reported in our study (0.42) are similar to those reported for Caucasians (0.41 to 0.58), and lower than that in Asians (0.81) (38). Additionally, it needs to be noted that there is a marked difference in the frequency of this allele within the Mexican population ranging between 0.33-0.39 and 0.49 for Central Mexico (39,40) and Western Mexico (25), respectively, probably explained by the increased inter-racial mixing within the population (38, 41).

In the present study, we did not find an association between LEPR Q223R polymorphisms and serum leptin levels. These results are similar to those reported by others, where no significant differences were found in leptin levels

with genotypes in healthy subjects and subjects with metabolic abnormalities (20, 21, 33). Even *in vitro* experiments have shown that the presence of this polymorphism does not affect the signaling pathway of its receptor (42).

In both groups, serum leptin levels had a strong correlation with BMI and weight, and are consistent with previous reports, which reported positive correlation between leptin levels and body composition variables in CS (43-45) and RA patients (46-48). On the other hand, in RA patients, no correlation was found between leptin levels and ESR, RF, CRP, disease duration and disease activity (DAS28). Some studies (10, 49-52) support our findings, while others have reported positive correlation between serum leptin with inflammatory markers (5-7, 9). This discrepancy could be explained by the differences in sample size, study design, and ethnic background.

CONCLUSION

Our findings suggest that in patients with RA, there is no association between the *LEPR* Q223R polymorphism and RA, despite high serum leptin levels. The elevated leptin levels may explain the low weight observed in RA patients as compared to the control subjects. Furthermore, in our study the serum leptin levels did not correlate with the studied inflammatory markers, and disease severity and duration.

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Anemia and thrombocytopenia in the cohort of HIV-infected adults in northwest Ethiopia: a facility-based cross-sectional study

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anemia, prevalence, HAART,
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ABSTRACT

Background

Anemia and thrombocytopenia are frequent hematological abnormalities in patients with human immunodeficiency virus (HIV) infection and have been associated with increased morbidity and mortality. However, there is a paucity of data on the prevalence and correlates of these hematological abnormalities among HIV infected adults in Ethiopia. The aim of this study was to determine the prevalence and correlates of anemia and thrombocytopenia in a cohort of HIV-1 infected adults in northwest Ethiopia.

Methods

A total of 320 HIV-infected adults were enrolled into the study, from March 2016 to July 2016. Sociodemographic and clinical characteristics of the study participants were recorded. Blood samples were collected from each patient to determine hematological and immunological parameters. A binary logistic regression model was fitted to identify factors associated with each hematological abnormality. The odds ratio with a 95% confidence interval was

calculated. A p-value <0.05 was considered statistically significant.

Results

Out of 320 HIV-1 positive participants, 203 (63.4%) were female. Overall, anemia was found in 25% (95% CI: 20.23 - 29.8%) of the study participants, of whom 2.5% (n=2) had severe and 21.2% (n=17) had moderate anemia. About 83.8% (67/80) anemic patients were on highly active antiretroviral therapy (HAART) for a minimum of six months, and 31 of them were receiving Zidovudine (AZT)-based HAART regimen. Multivariable logistic regression analysis showed that being HAART-naïve (AOR= 5.5, 95% CI: 1.5-19.9) and having CD4 count below 200 cells/µl (AOR= 2.4, 95% CI: 1.3-4.9) were independent and significant predictors of anemia. Thrombocytopenia was noted in 6.3% (95% CI: 3.58-8.9%) of the study participants. Sixty percent of thrombocytopenic (n=12) subjects were over the age of 40 years.

Conclusion

We found an overall high prevalence of anemia in the cohort of HIV-infected adults in northwest Ethiopia. HAART naïve subjects and those with CD4 count less than 200 cells/µl were found to be at higher risk for developing anemia. This data has an important implication for management of hematological abnormalities in HIV patients and highlights the need for early initiation of HAART to reduce the burden of anemia.

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BACKGROUND

Human immunodeficiency virus (HIV) is a retrovirus which can be transmitted via sexual intercourse, shared intravenous drug paraphernalia, and mother-to-child transmission (1). As to UNAIDS estimation, in 2013 an estimated 35 million people were living with HIV worldwide. Sub-Saharan Africa accounted for 71% of the global burden of HIV infection. According to this estimate, ten countries that include South Africa (25%), Nigeria (13%), Mozambique (6%), Uganda (6%), Tanzania (6%), Zambia (4%), Zimbabwe (6%), Kenya (6%), Malawi (4%) and Ethiopia (3%) accounted for almost 80% of all people living with HIV in Sub-Saharan Africa (2, 3).

Human immunodeficiency virus (HIV) infection is associated with profound hematological abnormalities. Anemia is one of the major hematological problems, frequently observed in patients with HIV infection. It has been estimated to vary from 30% to 95%, with the greatest burden in patients with advanced disease (4-6). The etiology of anemia in HIV patients may be related to factors such as opportunistic infections, HIV-associated neoplastic diseases, HIV medications, and the virus itself (7-10). HIV has shown to induce anemia either by direct infection of hematopoietic progenitor cells or by inducing autoantibody against erythropoietin, thus, blunting the physiological response to this cytokine (11).

Anemia has been associated with diverse consequences that compromise the quality of life and survival of HIV patients that leads to fatigue, congestive cardiac failure, and an increased risk of HIV-associated dementia (12, 13). Furthermore, anemia has been correlated with accelerated disease progression, deteriorated clinical outcomes, and increased mortality (14). Studies in large cohorts of HIV patients showed that anemia associated with a high risk of mortality irrespective of the first CD4 count and opportunistic infections. On the other hand, recovery from anemia has been demonstrated to correlate with improved survival (15-16). Therefore, it is vital to monitor the magnitude and associated factors of anemia in this vulnerable group, particularly in poor settings to improve therapeutic options and disease management.

Thrombocytopenia is another hematological complication that occurs in HIV patients. The prevalence of thrombocytopenia ranges from 4-40% in different study settings, and it was found to associate with all stages of the disease (17-19). It has also been linked to an increased morbidity and mortality of HIV patients, due to its association with risks of bleeding in different tissues. Mechanisms of thrombocytopenia development in the context of HIV-infection include immune-mediated destructions of platelets, toxic effects of HIV medications, and impaired hematopoiesis (20, 21). A decline in platelet count has been associated with increased viral load and predicted a rapid decline of CD4 cells count (22). Several studies have reported that highly active antiretroviral therapy (HAART) has reduced the prevalence of thrombocytopenia (23, 24). However, there are also considerable numbers of reports that showed an ongoing occurrence of this hematological abnormality even in patients receiving HAART (25).

Although hematological abnormalities of different blood cell lineages in HIV-infected adults have been widely reported, there is a paucity of data on the prevalence and correlates of anemia and thrombocytopenia from Ethiopia. We hypothesize that the risk factors for anemia and thrombocytopenia in our setting could be different from those in developed countries due to the prevailing high rates of parasitic infections and nutritional factors. Therefore, this study sought to investigate the prevalence and correlates of anemia and thrombocytopenia in a cohort of HIV-1 infected adults in northwest Ethiopia.

MATERIALS AND METHODS

Study design and settings

This cross-sectional study was conducted from March 2016 to July 2016 in an out-patient setting, in the antiretroviral treatment (ART) clinic of the University of Gondar Hospital. The hospital provides medical services, including HIV care and treatment, for about five million people in northwest Ethiopia. The ART for HIV has been available in the hospital since 2005 and the regimen consisted of a combination of nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTI and NNRTI). As of 2014, there were about 10,000 HIV-positive patients with ongoing follow-up at the University of Gondar hospital ART clinic, of which 6000 were on combination ART. Gondar is located in North of Lake Tana and Southwest of the Siemen Mountains. It has a latitude and longitude of 12°36'N 37°28'E with an elevation of 2133 meters above sea level.

Study population and sampling

The sample size was determined based on a single population formula, considering the previously reported 20.7% prevalence of anemia (19), with 95% confidence interval (CI) and 5% margin of error. A total of 320 HIV-infected adults were selected by a systematic random sampling technique in an outpatient setting and provided the necessary information and samples. On a daily basis, an average of 15 adult HIV-children patients were getting service at ART Clinic of University of Gondar Referral Hospital.

A total of 1200 study participants were estimated to visit the ART clinic during the study period. The study participants were chosen at regular intervals from their sequence of ART clinic visit at approximate sampling interval of four (1,200/320). The first sample order to be included in the study was selected by lottery from the first four order of adult HIV-infected who were visiting ART clinic. Thereafter, at every 4th interval study participants were included in the study until the total sample was achieved.

The study populations were all HIV-infected adults who were on follow-up at the ART clinic of the University of Gondar Hospital during the study period. HIV-infected adults with chronic renal failure, pregnancy, cancer, and a history of blood transfusion (within three months) and radiotherapy/cytotoxic chemotherapy (within two months) prior to data collection were excluded.

Information regarding socio-demographics of the study participants was collected using pretested questionnaires as pretesting is the means to test the consistency, validity and reliability of the survey questions before the commencement of data collection. Variables were chosen for inclusion based on their clinical relevance, and included age, sex, marital status, residence, occupation, and education levels. Clinical data were extracted from patients' charts.

Laboratory procedures

About 4 mL of venous blood was collected in EDTA vacutainer tubes from each participant in the morning, from 8:00 am to 11:00 am, and processed within three hours of collection. Hematological parameters were determined by a Cell-Dyn® 1800 (Abbott Laboratories, USA) which was standardized against a 4C® Plus blood control. Immunological parameters were analyzed using the BD FACSCount™ system (Becton Dickinson, San Jose, CA, USA). Performances of the instruments were controlled by running control samples prior to the test samples.

Definitions of study outcomes

The laboratory limits for hematological abnormalities were defined based on WHO criteria as follows (26): anemia in men was defined as an adjusted hemoglobin (Hb) concentration <13 g/dL (11.0–12.9 g/dL=mild; 8.0– 10.9 g/ dL= moderate, and <8.0 g/dL=severe), whereas anemia in non-pregnant women was defined as an adjusted Hb <12.0 g/dL (11.0–11.9 g/ dL=mild, 8.0–10.9 g/dL=moderate, and <8.0 g/dL=severe). Anemia was further defined based on mean corpuscular volume (MCV); as microcytic for MCV value <80 fL, normocytic if it was between 80-100 fL and macrocytic if MCV was >100 fL. Furthermore, it was defined as hypochromic if mean corpuscular hemoglobin (MCH) was <27 pg. Thrombocytopenia was defined as platelets count (PLT) < 150,000 cells/µL (19).

Data analysis

Data analysis was performed using SPSS version 20 statistical package software (SPSS Inc., Chicago, IL). Descriptive statistics as percentages, mean, median, IQR and standard deviation were applied as appropriate. Bivariate and multivariable logistic regression analyses were performed to determine the association between variables. Odds ratio and its 95% confidence intervals were calculated to determine the strength of association between hematological abnormalities and relevant socio-demographic and clinical factors.

Variables having p-value less than or equals to 0.2 in univariable binary logistic regression analysis were fitted to the final multivariable logistic regression model. P-values less than 0.05 in multivariable logistic regression analysis were considered statistically significant.

Ethical approval

The study was ethically approved by the Institutional Review Board of the University of Gondar. All subjects provided written informed consent for their participation. To ensure the confidentiality of participants and their information, no patient identifiers were included in the dataset. Laboratory findings of study participants were communicated with the responsible clinicians at the ART Clinic. This study was performed according to the declaration of Helsinki.

RESULTS

Socio-demographic and clinical characteristics of study participants

A total of 320 HIV positive adults were recruited into this study, of which 203 (63.4%) were female with a female-to-male ratio of 1.7. The Demographic and clinical characteristics of the study participants are shown in Table 1.

The median age was 38 years (IQR: 27-49 years), and the median CD4+ T lymphocytes count was 412 cells/mm3 (IQR: 91-733). Most of the participants (94.4%) included in this study were on HAART containing combinations of three drugs; two NRTIs and one NNRTIs.

Zidovudine (AZT) containing drugs were found to be the most frequently used ART regimen in

Table 1 Sociodemographic and clinical characteristics of study subjects at ART clinic of the University of Gondar Hospital, northwest Ethiopia

Characteristics		Number (%)
Sex	Male	117 (36.6)
	Female	203 (63.4)
Age, years	18- 30	50 (15.6)
	31-40	157 (49.1)
	>40	113 (35.3)
	Median (IQR)	38.0 (27-49)
Marital status	Married	194 (60.6)
	Single/divorced	126 (39.4)
Residence	Urban	280 (87.5)
	Rural	40 (12.5)
Educational status	Unable to read and write	134 (41.9)
	Primary education	65 (20.3)
	Secondary education	82 (25.6)
	Tertiary education	39 (12.2)
HAART status	On HAART	302 (94.4)
	HAART-Naïve	18 (5.6)

HAART duration, years (n=302)	Median (IQR)	6.0 (4.0)
HAART regimen (n=302)	Stavudine (d4T) based	49 (16.2)
	Zidovudine (AZT) based	157 (52.0)
	Tenofovir (TDF) based	96 (31.8)
CD4 count (cell/µl)	Mean (SD)	431.1 (243.9)
	Median (IQR)	410 (91-733)
WHO clinical stage	I	296 (92.5)
	II-IV	24 (7.5)

CD4: Cluster of differentiation 4; HAART: highly active antiretroviral therapy; IQR: Interquartile Range; SD: standard Deviation; WHO: World Health Organization

our setting (52%), followed by a Tenofovir (TDF) based regimen (31.8%).

Prevalence and correlates of anemia

Overall, anemia was found in 25% (95% CI: 20.23 - 29.8%) of the study participants, of which 2.5% (n=2) had severe and 21.3% (n=17) moderate anemia. In terms of RBCs morphologic classification of anemia, 7.5% (n=6) were microcytic-normochromic, 47.5% (n=38) normocytic-normochromic and 45% macrocytic-normochromic (Table 2).

Out of the 80 anemic patients, 67 were on HAART for a minimum of six months (Table 3), and 31 of them were receiving AZT-based HAART regimen.

Bivariate and multivariable binary logistic regression analyses were performed to identify risk factors for anemia in HIV-infected adults. Variables like age, sex, residence, occupation, current CD4+ T lymphocytes count, co-morbidities with other infections, alcohol abuse, history of surgery, WHO stage, HAART status, HAART regimen, and HAART duration were included in the analysis. After controlling confounders, we found that being HAART-naïve (AOR= 5.5, 95% CI: 1.5-19.9) and having CD4 count less than 200 cells/µl (AOR= 2.4, 95% CI: 1.3-4.9) were independent and significant predictors of anemia (Table 3).

Prevalence and correlates of thrombocytopenia

Thrombocytopenia was detected in 6.3% (95% CI: 3.58-8.9%) of the study participants (Table 2). All of the thrombocytopenic subjects were on HAART, and 60% (n=12) were in the age group of above 40 years (Table 4).

We analyzed factors associated with the thrombocytopenia among HIV-infected patients in a logistic regression model. As depicted in table 4, none of the variables were significantly associated with thrombocytopenia.

DISCUSSION

Anemia and thrombocytopenia are frequent hematological abnormalities in patients with HIV, and have been associated with increased morbidity and mortality.

These abnormalities can be reversed by proper care and treatment. Results from the present study revealed that having low CD4+ T cells

Table 2 Prevalence of anemia and thrombocytopenia in cohort of HIV-infected adults in Gondar, northwest Ethiopia

Characteristics		Number (%)
Overall Anemia, N=320		80 (25)
Severity of Anemia, n=80	Mild	61 (76.2)
	Moderate	17 (21.3)
	Severe	2 (2.5)
MCV-MCH, n=80	Microcytic-normochromic	6 (7.5)
	Normocytic-normochromic	38 (47.5)
	Macrocytic-normochromic	36 (45.0)
Thrombocytopenia, N=320		20 (6.3)

MCV: mean cell volume; MCH: mean cell hemoglobin.

count (<200cells/mm3) and being HAART-naïve were significantly associated with an increased risk of developing anemia. Although it was not statistically significant, thrombocytopenia was more frequent in subjects over the age of 40 years.

The overall prevalence of anemia in this study was 25%, with the majority of subjects having mild to moderate anemia. This prevalence rate was within similar range with those in previously published reports from Jimma (23.1%), Debre Tabor (23%), and Gondar (20.7%), Ethiopia (19, 27, 28).

However, it was considerably lower when compared to the 37% prevalence rates in West Africa cohort, 65.5% in India, and 60.6% in Nigeria (29-31). The difference between our finding and others may be explained by the fact that in our study, the proportion of subjects with an advanced stage of HIV was very small.

Therefore, the observed dissimilarities in the prevalence of anemia could be ascribed to variations in the study population with respect to their HIV clinical stages, and other sociodemographic factors.

Furthermore, majorities of the subjects in our study had received HAART (94%) for a minimum of six months. Thus, the relatively lower prevalence rate of anemia in this study could be attributed to the effect of HAART suppression of viral load, which might have improved erythropoiesis and/or reduced destruction of hematopoietic cells (24).

In addition, immune restoration by HAART could also play a role by decreasing the incidence of opportunistic infections. Taken together, in spite of the relatively low prevalence rate, the result of this study showed that anemia is still a public health concern in the cohort of HIV-infected adults in the study area.

Table 3 Bivariate and multivariable binary logistic regression analysis of associated factors for anemia in cohort of HIV-infected adults in Gondar, northwest Ethiopia

Variables	Anemia		COR (95% CI)	P-value	AOR (95% CI)	P-value
	Yes (%)	No (%)				
HAART						
Yes	67 (22.2)	235 (77.8)	1.0	-	1.0	-
No	13 (72.2)	5 (27.8)	9.1 (3.1-26.5)	<0.001	5.5 (1.5-19.9)	0.01
WHO clinical stage						
I	68 (23.0)	228 (77.0)	1.0	-	1.0	-
II-IV	12 (50.0)	12 (50.0)	3.4 (1.4-7.8)	0.005	1.3 (0.4-4.1)	0.68
CD4 count						
<200	24 (47.1)	27 (52.9)	3.4 (1.8-6.3)	<0.001	2.4 (1.3-4.9)	0.008
>200	56 (20.9)	212 (79.1)	1.0	-	1.0	-

CI: confidence interval; COR: crude odds ratio; AOR: adjusted odds ratio; HAART: highly active antiretroviral therapy; WHO: World Health Organization.

In this study, normocytic-normochromic anemia was predominant. This was comparable with previous studies (19, 32). Furthermore, our result showed that macrocytic-normocytic anemia was found to be the second common type of anemia, 45 % (n=36). This could be due to the well-established effect of AZT on the MCV, as the majority of anemic cases in this study were receiving AZT based regimens (35/67) (33).

This study revealed that HIV-infected adults with CD4 count less than 200 cells/µl were at higher risk of developing anemia (AOR= 2.4, 95% CI: 1.3-4.9). This could be due to erythropoietic dysfunction resulting from the increased viral load as immunity deteriorates. However, we could not verify the effect of viral load on the risk of developing anemia as data on this parameter is lacking.

Similarly, being HAART-naïve was significantly associated with a risk of developing anemia as previously described (14, 15, 24). It is of note that all but 5 non-anemic study participants were on HAART. This may substantiate the role of HAART in reducing the risk of developing anemia. Collectively, our data suggesting that there is a need for routine monitoring of HIV patients for anemia, particularly HAART-naïve subjects and those with CD4 count <200 cells/µl.

The overall prevalence of thrombocytopenia in this study was 6.3%, which was similar to the previous report from Ethiopia (19).

Table 4 Bivariate and multivariable binary logistic regression analysis of sociodemographic and clinical risk factors for thrombocytopenia in cohort of HIV-infected adults in Gondar, northwest Ethiopia

Variables	Thrombocytopenia		COR(95%CI)	P value	AOR (95%CI)	P-Value
	Yes (%)	No (%)				
Sex						
Male	11(9.4)	106(90.6)	2.2(0.9-5.6)	0.08	1.7(0.7-4.5)	0.27
Female	9(4.4)	194(95.6)	1.00	-	1.00	-
Age category						
<30 years	2(4.0)	48(96.0)	1.00	-	1.00	-
30-40 years	6(3.8)	151(96.2)	1.0(0.2-4.9)	0.90	0.8(0.2-4.3)	0.81
>40 years	12(10.6)	101(89.4)	2.9(0.6-13.2)	0.1	2.6(0.5-13.1)	0.25
CD4 count						
<200	5(9.8)	46(90.2)	1.8(0.6-5.3)	0.2	0.4(0.2-1.4)	0.16
>200	15(5.6)	253(94.4)	1.00	-	1.00	-

CI: confidence interval; CD4: cluster of differentiation 4; COR: crude odds ratio; AOR: adjusted odds ratio.

However, it is lower when compared with 26% rate in Australia (22), 17.4% in Uganda (34), and 20% in Iran (35). This is probably due to higher proportions of subjects with previously established risk factors for thrombocytopenia such as advanced HIV disease, injection drug users or low compliance to HAART regimen in the aforementioned studies. Furthermore, most of our study subjects were on HAART with over half of the participants receiving AZT containing combination antiretroviral drug.

Therefore, the low prevalence of thrombocytopenia in this study could also be partially attributed to the role of HAART in restoring platelets count (36). Similar to other reports, we found a high prevalence of thrombocytopenia in subjects over the age of 40 years (19, 37). This could be related to increased cases of myelodysplasia in older patients (22, 37).

This study has some limitations in that we lack data on the prevalence of helminth infections like hookworm, one of the risk factors for anemia in sub-Sahara Africa.

Besides, inconsistencies of cut-of value to define anemia and thrombocytopenia between different studies have imposed some limitation in comparing our estimate with other studies. However, given that very few anemic patients

(12/80) were from a rural area (i.e., high-risk area for helminths infection), the contribution of this parameter to anemia could be minimal. In addition, our study lacks data on economic status and lifestyle of the study participants which might affect their hematologic profiles.

CONCLUSION

In conclusion, we found an overall high burden of anemia in the cohort of HIV-infected adults in northwest Ethiopia. HAART naïve subjects and those with CD4 counts less than 200 cells/µl were found to be at higher risk for developing anemia. Therefore, routine monitoring of at risk groups for anemia and early initiation of HAART could be beneficial to reduce the burden of these hematological abnormalities.

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Abbreviations

AOR: *Adjusted odds ratio*
CD4: *Cluster of differentiation 4*
CI: *Confidence interval*
COR: *Crude odds ratio*
HAART: *Highly active antiretroviral therapy*
HIV: *Human Immunodeficiency Virus*
MCH: *mean cell hemoglobin*
MCV: *mean cell volume*
WHO: *World Health Organization*

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Authors' Contributions

Conceived and designed the experiments: TD, DD; performed the experiments: TD, DD, MW, MG, MM; Analyzed the data: TD, DD, MM; interpreted results: TD, DD, MW, MG, MM. All authors contributed to the writing and editing of the manuscript and approved the final version.

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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Competing interests

The authors have declared that no competing interests exist.

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Reference intervals for a complete blood count on an automated haematology analyser Sysmex XN in healthy adults from the southern metropolitan area of Barcelona

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ABSTRACT

Background

The examination of peripheral blood is routinely used as a basic test in daily medical practice. Reliable reference intervals are necessary to avoid misdiagnoses, and the establishment of those intervals is an important task for clinical laboratories.

The aim of the present study was to establish the reference intervals for complete blood count (CBC) on a Sysmex XN haematology analyser in healthy adults from the southern metropolitan area of Barcelona (Spain).

Methods

A total of 213 apparently healthy adults who received a general health examination at Hospital Universitari de Bellvitge were recruited to this study. Blood samples collected in K_3EDTA tubes were analysed on a Sysmex XN. Statistically relevant gender based partition was assessed, outliers removed, and the reference intervals calculated in concordance with Clinical

and Laboratory Standards Institute (CLSI) EP28-A3C guidelines.

Results

The CBC reference intervals were established in 191 adults (64 men and 127 women) who fulfilled all of the inclusion criteria. Significant gender-dependent differences in red blood cells, haematocrit, haemoglobin and platelets were found. The rest of the CBC reference intervals were obtained from the overall data.

Conclusions

We report CBC reference intervals established on a Sysmex XN analyser, a widely used automated analyser for which reference intervals were previously lacking in the literature. However, these reference intervals we recommend should be validated by individual laboratories for the local population as recommended by CLSI.

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INTRODUCTION

One of the main aims of clinical laboratories is to provide accurate results as well as appropriate reference intervals for a better interpretation of a patient's results. The reference intervals of a biological parameter depend on the origin of the population and its measurement procedure. Metrological variability is the main reason why different measurement procedures provide different results.

Some international organisations, such as the International Federation of Clinical Chemistry (IFCC), recommend that all clinical laboratories should develop their own biological reference values (1). Moreover, the standard ISO 15189:2012 requires that biological reference intervals be reviewed periodically and whenever there is any change in laboratory technology (2). However, few laboratories currently follow these recommendations because of its difficulty, large time consumption and high costs.

A complete blood count (CBC) including differential leukocyte count is widely used in clinical practice. Recently developed automated haematology cell counters incorporate technologic improvements including, new parameters and the ability to determining the CBC with better accuracy.

The information from manufacturers about the reference intervals for the CBC is often very limited which highlights the need for laboratories to establish references intervals, that are based on the use of their own equipment and routines. The establishment of local reference intervals for a CBC provides useful data to a laboratory because it reflects the population for which the tests are targeted.

The Sysmex XN-series system (Sysmex, Kobe, Japan) is a recently launched automated haematology analyser with new methods of measurement. Sysmex XN aims to improve the quality of the results of the CBC.

The impedance method is the basis for red blood cell and platelet counts. Some channels count red blood cells and platelets using the sheath flow direct current detection method. The cell signals are sensitively captured because of innovations in the unique digital waveform processing technology.

The haemoglobin concentration is measured using sodium lauryl sulfate (SLS). This reagent haemolysis the red blood cell membrane and SLS-haemoglobin shows absorption at a wavelength of 555 nm.

Using a semiconductor laser, flow cytometry counts and classifies cells by irradiating them with a 633 nm laser beam and analysing their forward scattered light (FSC), side scattered light (SSC) and side fluorescent light (SFL). The intensity of the two types of scattered light (FSC

and SSC) reflects cell surface structure, particle shape, nucleus form, refractive index and reflectivity of the cells. In general, the FSC signal is stronger for larger cells, and the SSC signal becomes stronger as the intracellular structures become more complex. The intensity of the SFL mainly reflects the type and amount of nucleic acids and cell organelles. These three signals are used to differentiate and count white blood cells, nucleated red blood cells, reticulocytes, and platelets, and to detect abnormal cells and immature cells with the help of unique digital technology and algorithms (3, 4).

The aim of the present study was to establish reference intervals for the following haematology parameters using the Sysmex XN-series analyser in an local adult population: red blood cells (RBC), haematocrit, haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), erythrocyte distribution width, white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosinophil and basophil counts and differentials, platelets, mean platelet volume (MPV), reticulocyte counts and differentials and erythroblast counts.

MATERIALS AND METHODS

Subjects and samples

Our study protocol followed the recommendations of the Clinical and Laboratory Standards Institute (CLSI: former National Council of Clinical Laboratory Services) (5-7) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (1). The study was performed in accordance with the ethical standards laid down in the 1964 Helsinki Declaration.

The Hospital Universitari de Bellvitge (HUB) is a 700-bed teaching hospital that specialises in adult patient care. It provides assistance to a population of 300,000 inhabitants, and it is also a reference centre for more than 2 million people from the southern metropolitan area of Barcelona. The Clinical Laboratory of the HUB is accredited according to the ISO standard 15189.

To produce the reference values for the haematological parameters, blood samples were obtained from apparently healthy adults who received a general health examination at the HUB. In total, 213 subjects (70 men and 143 women), ranging from 18 to 70 years of age, were enrolled in this study. The different age groups were represented equally. To exclude unhealthy individuals, blood and serum biomarkers from liver and renal function, inflammation and iron status and CBC were evaluated. The following parameters were assessed: CBC, differential leukocyte count and the fraction number of reticulocytes, catalytic concentration of alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase and alkaline phosphatase: substance concentration of urea and creatinine and glomerular filtration rate (CKD-EPI) and erythrocyte sedimentation rate (ESR) and mass concentration of ferritin.

Individuals were excluded if they had a result outside of the reference interval for any parameter used in our clinical laboratory. Blood was drawn by venipuncture with the Vacuette® blood collection system (Vacuette Greiner Bio-One GmbH, Frickenhausen, Germany) and BD Vacutainer® system (Beckton Dickinson, San Jose, California, USA). For CBC and ESR, tri-potassium ethylenediaminotetraacetate (K3-EDTA) was used as an anticoagulant, and serum was collected in serum gel tubes for all biochemical parameters.

The CBC and differential leukocyte count was initially performed in the current laboratory haematology analyser, a Pentra DF120 analyser (HORIBA® ABX SAS, Montpellier, France). The

erythrocyte sedimentation rate (ESR) was measured using a microagglutination method on TEST 1 (Alifax, Padova, Italy). Biochemical parameters were measured on a Cobas c 711 analyser (Roche Diagnostics®). The measurement method for all parameters was spectrometry molecular absorption, except for the mass concentration of ferritin, which was determined by immunoturbidimetry.

All samples were then processed in the Sysmex XN-2000 analyser within six hours of venipuncture, following International Council for Standardization in Haematology (ICSH) guidelines (8-9).

Quality control

Three-level commercial quality controls (XN-Check Control) were processed daily on the Sysmex XN-2000 system during the study in order to evaluate bias and the between-day analytical imprecision, which fulfilled the metrological requirements.

Statistical analysis

All calculations to determine reference intervals were based on the CLSI guidelines (former National Council of Clinical Laboratory Services) document EP28-A3C (5).

Differences between genders were evaluated using the Harris and Boyd criteria as suggested by CLSI (10). Outliers were detected and excluded from the study by Tukey's boxplot.

Overall and gender stratified data were evaluated for a normal distribution using the Anderson-Darling test. The reference ranges for each parameter were calculated according to the CLSI guidelines document EP28-A3C (5).

All statistical analyses were carried out using Analyse-It software (Analyse-It software Ltd., Leeds, UK) for Microsoft Excel. *P* values of 0.05 or lower were considered to be statistically significant.

RESULTS

Of the 213 subjects who were recruited, 191 (64 men and 127 women) fulfilled all of the inclusion criteria and were selected for the reference interval calculation.

The mean age of all participants at study entry was 42 (±15) years Based on gender, the mean age was 42 (±14) and 41 (±15) years for women and men, respectively.

Table 1 shows selected haematology reference intervals for our population. Reference intervals for: RBC, haematocrit, haemoglobin and platelets were defined according to gender as dictated by the Harris and Boyd's Test for partitioning the reference values.

DISCUSSION

Sysmex XN modular system is a new automated haematology analyser that is often used in clinical laboratories in our country. The lack of information from manufacturers about the reference ranges for the CBC parameters highlights the need for laboratories to establish reference intervals for the CBC, using their own equipment and routines. The importance of this study relies on the usefulness of these results for other laboratories with European populations that use this analyser system.

There are previous studies on reference intervals using the Sysmex analysers (XE-Class analysers) (11, 12); however, for the first time, we report reference intervals for parameters on the new Sysmex XN-Class analysers on Spanish population.

We studied blood samples from 191 healthy adults using the Sysmex-XN-2000 analyser, and we calculated reference intervals for the CBC, including leukocyte differential and reticulocytes. We confirmed that there are relevant gender differences for RBC, haematocrit and platelet counts. However, none of the other parameters

Table 1 References intervals for all haematology parameters stratified by gender when it was mandatory

Parameters	Units	n (men/women)	Men	Women
Red blood cells (RBC)	$x10^{12}$/L	64/126	[4.3-5.6]	[3.9-5.1]
Haematocrit	%	59/124	[40-50]	[36-45]
Haemoglobin	g/L	59/126	[137.4-164.7]	[120.0-146.8]
MCV	fL	188	[83.6-97.0]	
MCH	pg	188	[27-32]	
MCHC	g/L	185	[314-319]	
Erythrocyte distribution width	%	189	[11.6-14.3]	
Leukocytes	10^9/L	188	[3.9-9.5]	
Neutrophils	10^9/L	188	[1.5-5.7]	
Neutrophils	%	189	[37.1-68.4]	
Lymphocytes	10^9/L	187	[1.3-3.4]	
Lymphocytes	%	190	[21-50]	
Monocytes	10^9/L	191	[0.31-0.92]	
Monocytes	%	188	[5.1-11.2]	
Eosinophils	10^9/L	181	[0.03-0.39]	
Eosinophils	%	188	[0.4-6.6]	
Basophils	10^9/L	190	[0.01-0.09]	
Basophils	%	189	[0.2-1.3]	
Platelets	10^9/L	63/124	[149-303]	[153-368]
MPV	fL	187	[9.7-13.2]	
Reticulocytes	10^9/L	113	[34-102]	
Reticulocytes	%	113	[0.68-1.86]	
Erythroblasts	/100 Leukocytes	190	0-0.01	

showed significant variations by gender, which was in accordance with most of the published data (13-15).

We calculated the reference values for haemoglobin, but we recommend the use of the cut-off value for anaemia defined by the World Health Organization (WHO) (10, 11). The reference interval concept describes the ranges found in studied populations, and WHO definition of anaemia represents a value based on a medical decision. The lower reference value must always be higher than the cut-off for anaemia because it is calculated with healthy subjects that are not anaemics.

The finding of higher values for haemoglobin, haematocrit and erythrocytes in males compared to females may be partly due to the influence of androgens on erythropoiesis, menstrual loss and gravidity. As found in other similar studies, platelet counts in women were higher than men. It seems to reflect different hormonal profiles or a compensatory mechanism associated with menstrual blood loss (15, 16). In the present study, we found a great gender difference in the upper reference limit (303 x 10^9/L for men vs 368 x 10^9/L for women). This was in concordance with the study directed by Pekelharing et al. (11), which was carried out with the Sysmex XE-5000 analyser; more marked differences were observed in the upper reference limit (308 x 10^9/L and 390 x 10^9/L for men and women, respectively).

As expected, the presented reference intervals are similar to previous published data using Sysmex XE-5000 (11) on a population in the Netherlands. Those findings seem logical because the analysers were similar and in both studies the population was European. However, it is mandatory to establish references intervals when there is a change on routine analyser. It is worth mentioning that the reference range for platelets using the Sysmex technology seems to be lower than most textbook data. Pfaeffli et al. (17) showed that reference limits for Sysmex XE-2100 were 130–330 x 10^9/L, which is lower than 150–400 x 10^9/L from the published literature.

Based on the use of the impedance method with the Sysmex technology, the results for platelets do not seem to be interchangeable with the results from other measuring systems that also use the impedance method (18).

At the moment, there are few publications that establish references ranges for CBC in adult population using the Sysmex-XN analyser and that also exhibit significant differences by gender for haemoglobin, RBC, haematocrit and platelet count (19). One of these studies was carried out using samples from an Italian population, and the results were comparable. The results of a second study have been obtained in a Korean population (19). There is enough literature describing relevant differences when comparing references values in different populations, which emphasises the need for population specific reference intervals. Factors like the environment and nutritional status may play an important role (12).

One limitation of our study was the small number of blood samples analysed. However, the method was robust enough to establish reference intervals following the CLSI guidelines when the number of samples was fewer than 120. Also, it has to be considered that age-related differences were not assessed for the establishment of reference intervals due to the limited sample size.

In summary, we established reference intervals for CBC in apparently healthy adults from the southern metropolitan area of Barcelona using the Sysmex-XN analyser. Establishing such intervals is recommended by international guidelines whenever there is a change in any analyser in a clinical laboratory.

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The IFCC Curriculum - phase 1

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IFCC Committee for Distance Learning – for the IFCC eAcademy project

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IFCC CURRICULUM

THE IFCC CURRICULUM - PHASE 1

The IFCC Curriculum was developed as part of the IFCC eAcademy project by the Committee for Distance Learning.

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INTRODUCTION

The IFCC curriculum is being developed as a guide for its member societies in their development of syllabuses for postgraduate trainees in laboratory medicine, appropriate for use in their own countries. The curriculum should be viewed as a framework into which requirements specific to different regional or national practice can be incorporated.

It is also intended to provide a resource for trainees in planning their private study in preparation for academic and professional qualifications which lead to formal recognition of expertise and status as experts and leaders in the field of laboratory medicine.

The curriculum has been developed in response to a request from some National Societies, by the Committee on Distance Learning (C-DL) and will inform the development of the IFCC e-Academy. The C-DL is grateful to the following National Societies who responded to the call in 2013 and submitted their own curricula for us to consult in considering format and content:

- Australia and New Zealand
- Canada
- Croatia
- Netherlands
- Romania
- Slovak Republic
- South Korea
- Sweden
- Switzerland

A laboratory medicine expert is expected to have a comprehensive knowledge of the science and medicine on which the specialty is based and to use this knowledge to develop and provide a safe, effective, efficient and high quality service to its users. The curriculum is designed to provide a framework of learning both practical and theoretical components through which this expertise can be achieved.

Herein, is phase 1 of the IFCC curriculum to support ongoing education in laboratory medicine. It is not intended to include references in this curriculum. Instead, the learning objectives are designed to link back to the online learning material of the IFCC eAcademy where relevant additional reading will be provided within these presentations, Figure 1.

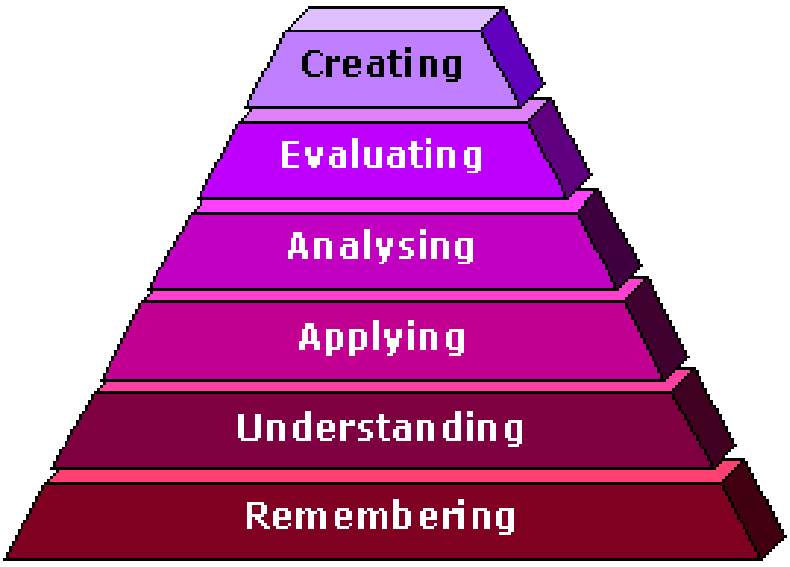
Learning Skills

IFCC recommends a stepwise approach to the acquisition and application of knowledge by trainees. Bloom's taxonomy defines six categories and cognitive processes, refined in 2001[1]. Each category is shown with appropriate verbs that may be used to construct learning objectives or assessment questions.

- **Remembering** - retrieving, recognizing, and recalling relevant knowledge
  Define, memorise, list, name

- **Understanding** - constructing meaning through interpreting, exemplifying, classifying, summarizing, inferring, comparing, and explaining
  Restate, discuss, describe, identify, report, explain, review, recognise
- **Applying** - carrying out or using a procedure through executing, or implementing
  Translate, interpret, apply, illustrate, demonstrate, use
- **Analysing** - breaking information into parts to explore understanding and relationships through differentiating, organizing, and attributing
  Distinguish, differentiate, appraise, analyse, calculate, compare, contrast
- **Evaluating** - making judgements based on criteria and standards through checking and critiquing
  Appraise, evaluate, revise, score, estimate, choose, assess
- **Creating** - putting elements together to form a coherent or functional whole; generating new ideas, products or ways of viewing things
  Compose, plan, propose, design, prepare, organise

Figure I **In diagrammatic form, the learning process, creating and evaluating are key functions for those in the most senior professional roles.**



[1] Bloom B S (ed.) (1956) Taxonomy of Educational Objectives, the classification of educational goals – Handbook I: Cognitive Domain New York: McKay)

Program Learning Objectives

Many components contribute to safe and effective laboratory investigation of patients and their diseases. These can be summarised in Figure II.

Figure II **Program Learning Objectives - Summarised**

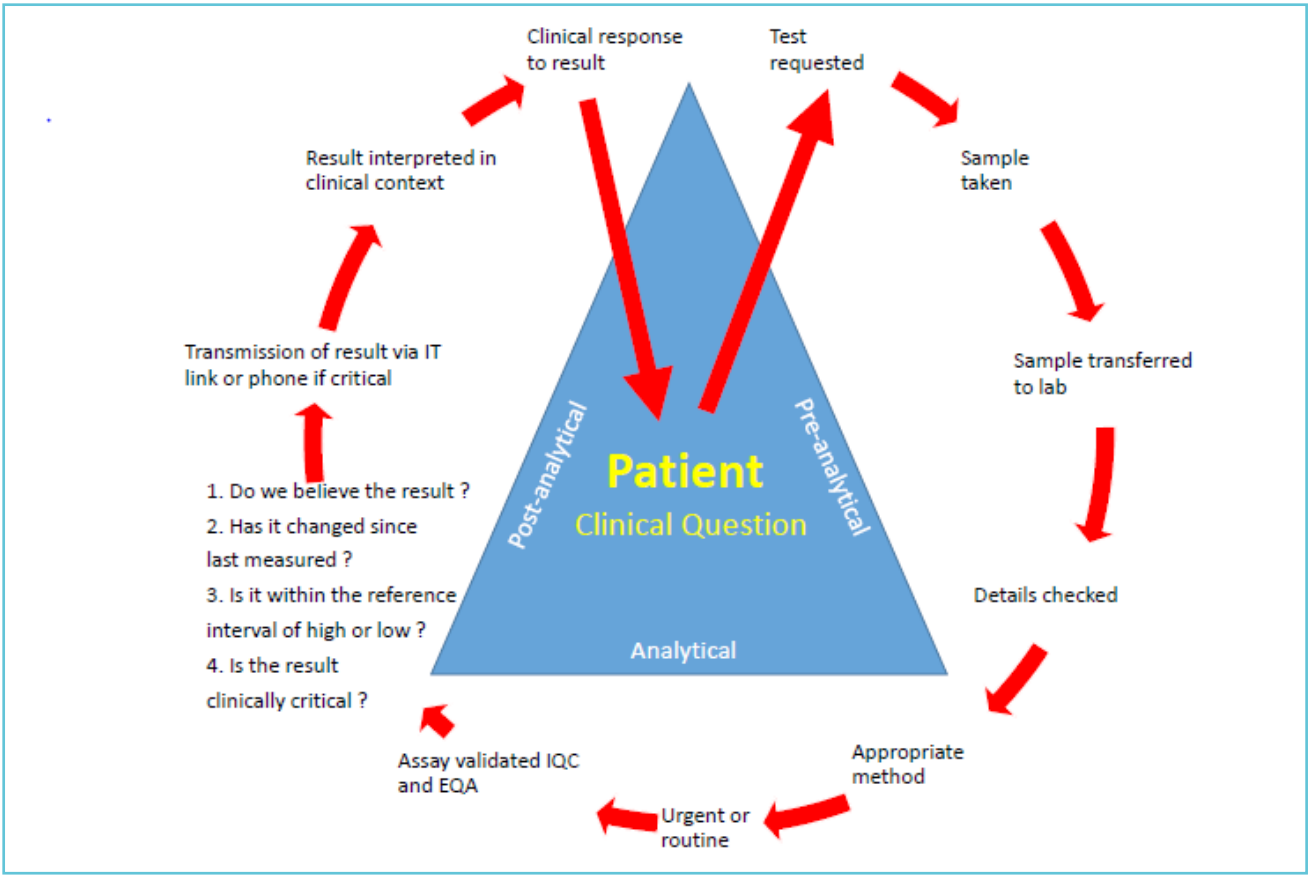


Figure II. The specialist functions of a clinical scientist are:

- *To advise on testing protocols and the appropriateness of testing*
- *To provide meaningful analytical results that can be used with confidence in the diagnosis and management of patients with disease.*
- *To provide interpretation of laboratory results in the light of clinical findings in the individual patient.*
- *To reach this level of expertise the clinical scientist must have detailed knowledge of:*
  - ◊ *Human physiology, biochemistry and organ function*
  - ◊ *The causes, clinical signs and symptoms of disorders of organs, systems, metabolic disease and genetic disease*
  - ◊ *Laboratory analyses and testing protocols for investigation of such disorders*
  - ◊ *The options for and principles of measurement of specific analytes*
  - ◊ *The pre-analytical, analytical and post analytical factors that may influence testing*
  - ◊ *The limitations of analyses, including specificity, sensitivity, bias and imprecision*
  - ◊ *Biological variation, reference intervals and critical test results*

Clinical Interpretation

Clinical interpretation of laboratory results is complex and it is important to adopt a systematic approach. There must be procedures in place in the laboratory, to ensure that specimen quality can be guaranteed and that the analytical quality of the result is fit for purpose. The result can then be interpreted with respect to reference intervals, clinically critical limits or significant changes in value and the causes of any abnormal or unexpected laboratory results be considered. It is very useful to have a structured approach to help interpretation of results, particularly those that are unexpected or unfamiliar. Considering the possible underlying pathological processes is an excellent starting point and using an acronym such as the one shown below, provides structure to the thought processes and minimises the risk of missing an important element.

A useful memory prompt is "A Vitamin C Def" (Table 1).

Table 1 A VITAMIN C DEF acronym to use as a memory prompt

A	acquired
V	vascular
I	infective/inflammatory
T	traumatic
A	autoimmune
M	metabolic
I	iatrogenic/idiopathic
N	neoplastic
C	congenital
D	degenerative/developmental
E	endocrine/environmental
F	functional

Curriculum Structure

The curriculum is designed to represent the three specialties of laboratory medicine; Blood Sciences, Microbiology and Molecular Genetics. Within each of the specialities, clinical and analytical aspects are considered separately and there is further breakdown of the clinical section into sub-specialties. These are then subdivided into disease groupings, based on organ system, metabolic disease and genetic disease. Within each of these groupings, individual clinical conditions are specified. Analytical aspects are broken down primarily into technology groupings then further into specific techniques. The whole structure is therefore presented in a hierarchical way. Laboratory Organisation and Management, common to all specialties, is considered separately.

In this version (i.e. part one) of the curriculum, concepts related to Laboratory Organisation and Management and Blood Sciences (Clinical Chemistry and Immunology) have been developed. Future additions to the curriculum will include curricula related to Haematology, Microbiology, and Molecular Genetics, Figure 3.

Figure III **Summary of Curriculum Design**

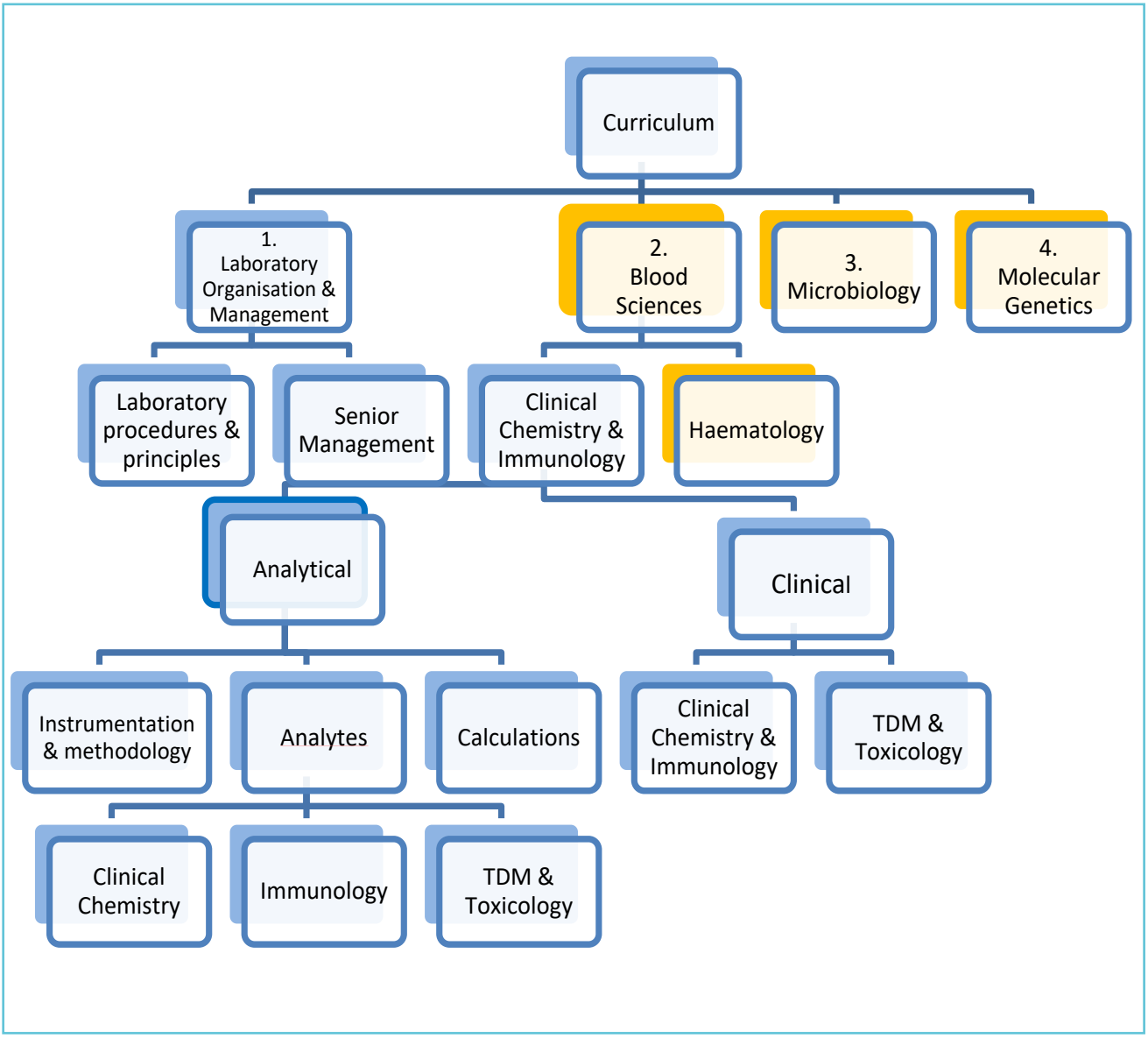


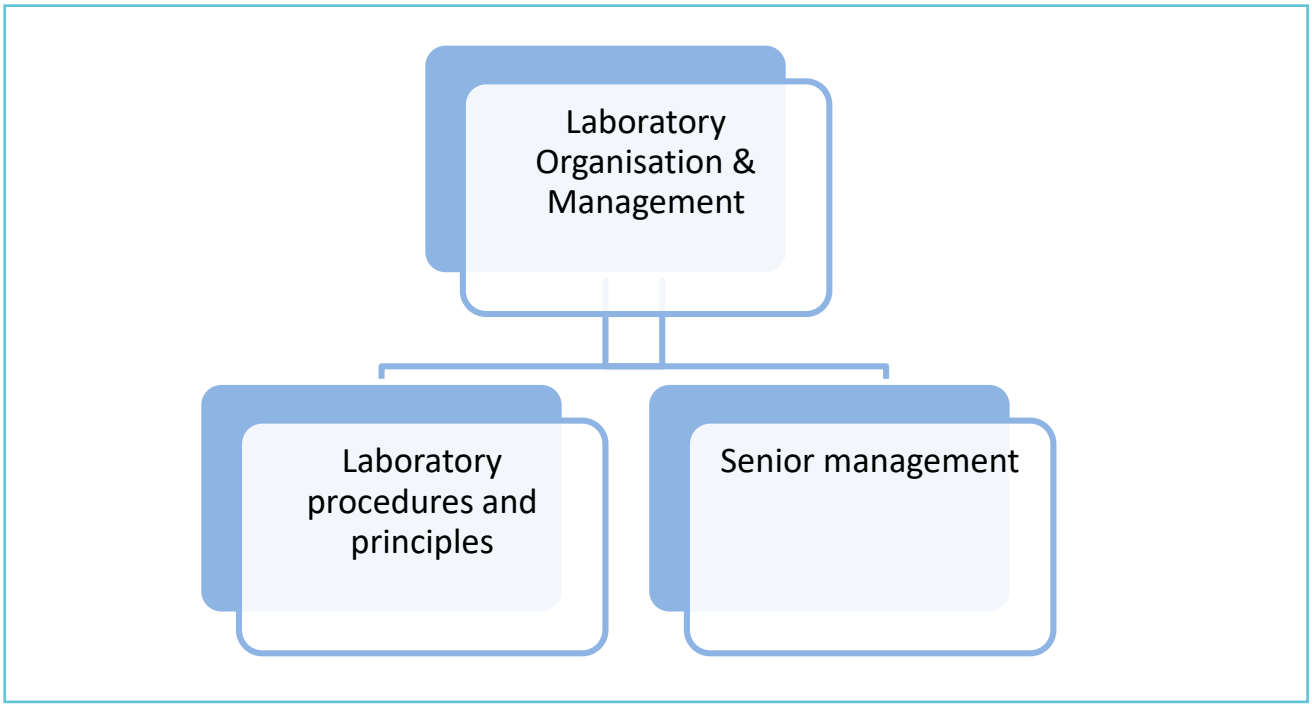
Figure III:

- *The sections covered in version 1 of the curriculum are shown in blue.*
- *The yellow cells indicate areas of the curriculum to be developed further as part of future versions.*

SECTION A: LABORATORY ORGANISATION AND MANAGEMENT

Laboratory Organisation and Management skills and concepts are considered in two categories, those which are relevant to all laboratory staff and those which are required by those in senior laboratory roles who have responsibilities for leadership in all aspects of the service.

Figure A1 Hierarchical structure of Laboratory Organisation and Management section of the curriculum



Section A1. Laboratory Principles and Procedures: Learning Objectives

Be able to discuss and demonstrate specific skills and competences for laboratory organisation and management

- Basic information communication technology (ICT) skills
- Basic instrument and equipment maintenance
- Basic research skills
- Basic statistical techniques
- Biological variation
- Document control
- External quality assessment (EQA)
- Health and safety issues
- Internal quality control (IQC)
- Laboratory safety
- Method evaluation
- Patient safety
- Pre-analytical variables
- Reference intervals (RI)
- Specimen collection
- Specimen preparation
- Standard curves
- Standard operating procedures
- Temperature monitoring

- Waste management
- Water purity monitoring

Section A2. Senior Laboratory Management: Learning Objectives

Be able to discuss and demonstrate specific skills and competences for laboratory organisation and management at a senior or advanced level

- Accreditation and ISO15189
- Advanced communication skills
- Advanced research skills
- Budget management
- Clinical and professional liaison
- Evidence-based laboratory medicine
- Facilitating training and continuing professional development (CPD) for all department staff
- Initiation of and collaboration in research activities
- Instrumentation and method selection
- Laboratory information systems (LIS), hospital information systems (HIS) and interfaces
- Medico-legal requirement and legislation
- Personnel management
- Point of care testing policies and management
- Professional ethics
- Requesting and reporting policies
- System design
- Total quality management and quality systems
- Traceability

Section A3. Evidence Based Laboratory Medicine (EBLM): Learning Objectives

Be able to discuss the relevance of evidence based laboratory medicine as applied to your organisation at an advanced level

Core topics

- Economic Evaluation of Diagnostic Tests
- Evaluating the literature: Appraisal instruments; e.g. STEP
- Evidence Based Laboratory Medicine (EBLM): definition, how it differs from Evidence Based Medicine (EBM) and why we need it
- The EBLM process: Ask, Acquire, Appraise; Act, Audit
- Formulation of the Question (PICO; CAPO and other formats)
- Guideline development, levels of evidence and appraisal. AGREE instrument
- Laboratory Medicine and Clinical Outcomes; Applying the Evidence
- Meta-analysis
- The Role of Clinical Audit
- Searching the Evidence; PubMed; EMBASE; Cochrane Library
- Sources of bias in studies of diagnostic accuracy, STARD criteria
- Study design: cross-sectional, cohort and randomized controlled trials (RCTs)
- Systematic Reviews versus Narrative Reviews
- Sources of Guidelines. Cochrane Collaboration

EBLM: measures of diagnostic performance

- Sensitivity, Specificity, Predictive Value, Effect of Prevalence

- Likelihood Ratios (and their utility), Odds Ratios – Fagan's nomogram
- Receiver Operating Characteristic (ROC) Curve analysis

Evidence Based Laboratory Medicine and Point of Care Testing (POCT)

- Outcomes of relevance for laboratory tests, including POCT
- Examples of evidence base for POCT including HbA1c, the Emergency Department, self-monitoring of blood glucose and INR testing
- POCT troponin testing and rural health; examples from Australia
- POCT in the Primary Care environment

Section A4. Conducting Research in Laboratory Medicine: Learning Objectives

Be able to discuss the key aspects related to the conduct of research in laboratory medicine

Why is research in laboratory medicine important?

- To describe medical research
- To discuss the types of medical research that are undertaken
- To define laboratory medicine in the context of the research guide
- To explain why research in laboratory medicine is important to stakeholder groups

Choice of suitable project

- To describe factors that stimulate research projects
- To discuss how the importance of research may be assessed
- To consider the practicability of a research project
- To explain research supervision and mentoring

Conduct a literature search

- To describe literature study
- To explain the questions to be asked when reading a research publication
- To recognise the importance of critical appraisal
- To discuss how critical appraisal of research publications is undertaken

Formulating a research plan

- To discuss the research question
- To explain the importance of hypothesis based research
- To describe the construction of aims and objectives
- To consider the research design

Submitting a research proposal for external approval and funding or formulating a research plan

- To recognise the importance of preparation for a research proposal
- To discuss ten steps on the road to success
- To describe the structure of a research proposal
- To evaluate feedback from a submitted research proposal

Conducting a research investigation and analysing findings

- To explain the importance of planning for research implementation
- To describe conducting and recording research investigations
- To discuss the analysis of results and the use of statistics

- To describe the drawing of conclusions from results

Writing research papers to publish

- To recognise the objectives to publish research results
- To recognise the initial preparation in writing research paper for publication
- To recognise the importance of all contents in a manuscript
- To recognise ethical considerations in medical research

Delivering research findings as oral presentations

- To recognise the importance of presenting research findings
- To provide guidance on preparing to present research findings
- To describe good practice in oral presentations
- To describe good practice in poster presentations

Auditing Research and planning for the future

- To describe audit
- To explain the process of auditing research
- To describe how audit findings should be recorded and analysed
- To discuss planning for the future

SECTION B: ANALYTICAL SECTION

The Analytical section is sub-divided into 'Instrumentation and Methodology' and 'Analytes' represented as follows for Clinical Chemistry and Immunology. From this section you should aim to be able to discuss the method principle and potential advantages and limitations of instruments and analytical techniques commonly used in Core and Specialist Clinical Laboratories. It is expected that practical experience and competence in the use of generic laboratory equipment, basic techniques and laboratory equipment will be acquired in the workplace as appropriate.

See Figure B1 for Hierarchical structure of Blood Sciences as related to Clinical Chemistry and Immunology section specifically related to the analytical component of the curriculum.

The clinical component is shown in yellow to demonstrate the relationship between the analytical and clinical sections; which is presented separately in section C.

Section B1. Generic Laboratory Equipment: Learning Objectives

Be able to discuss the basic principle of use of generic laboratory equipment:

- Balances
- Centrifuges
- Microscopes
- pH meters
- Quantities and Units
- Waterbaths

Section B2. Basic Techniques: Learning Objectives

Be able to discuss the basic techniques as applied in laboratory medicine

- Buffer preparation
- Calculations
  - ◊ Basic statistics of central tendency
  - ◊ Basic statistics of dispersion
  - ◊ Addition dilution
  - ◊ Serial dilution
- Centrifugation
- Dialysis

- Extraction techniques
  - ◊ Liquid-liquid
  - ◊ Solid Phase
  - ◊ Supported liquid
- Extractions
- Filtration
- Freeze drying
- Pipetting
- Ultrafiltration
- Volumetric measurement

Figure B1 Hierarchical structure of Blood Sciences as related to Clinical Chemistry and Immunology *(related to the analytical component)*

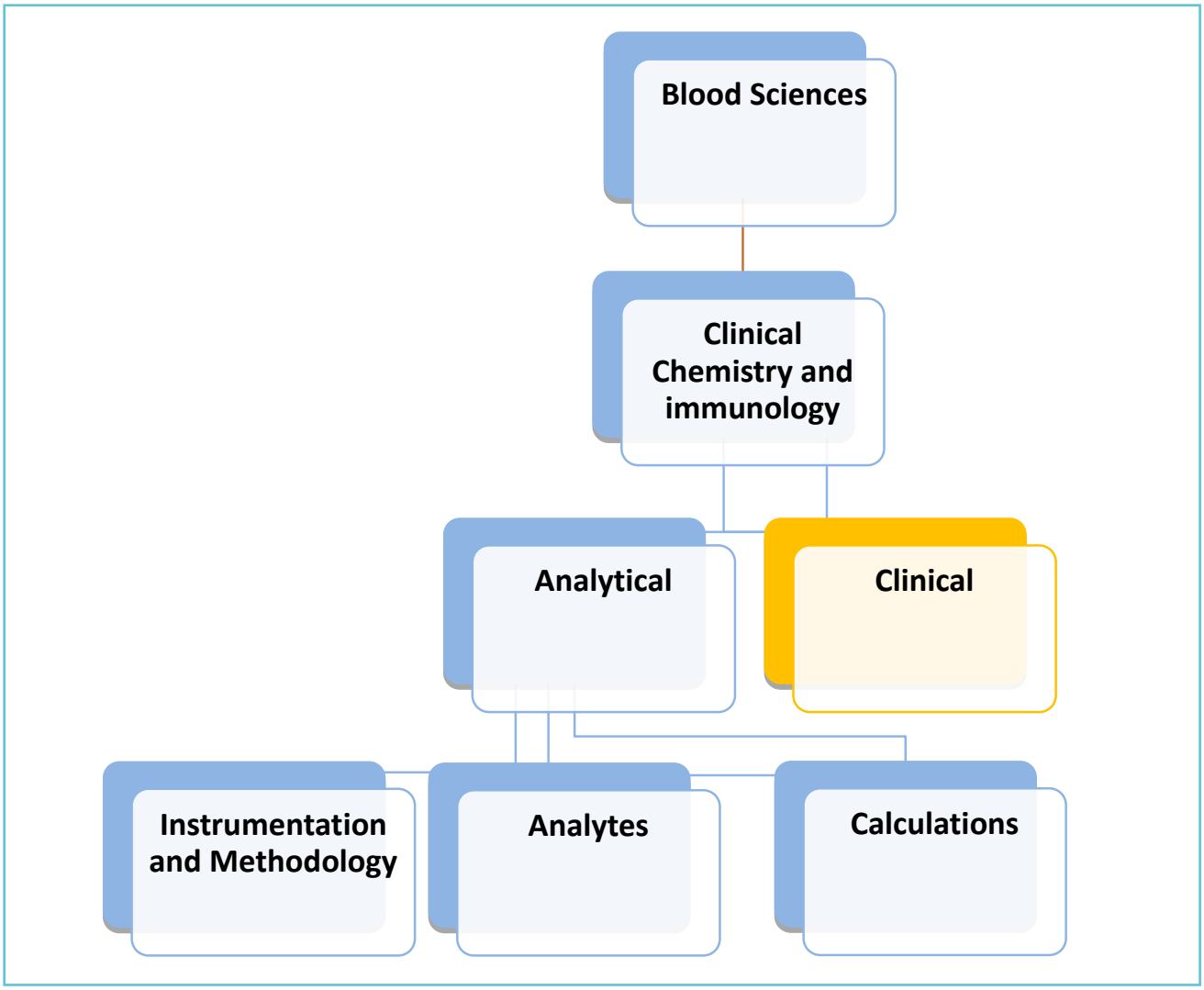


Figure B1:

- *Hierarchical structure of Blood Sciences as related to Clinical Chemistry and Immunology section specifically related to the analytical component of the curriculum.*
- *The clinical component is shown in yellow to demonstrate the relationship between the analytical and clinical sections; which is presented separately in section C.*

- Weighing

Section B3. Laboratory Instruments – Routine: Learning Objectives

Be able to discuss the method principle and potential advantages and limitations of instruments and analytical techniques commonly used in a Core Laboratory

- Absorption, nephelometry and turbidimetry
- Acid-base measurement
- Immunoassay and detection systems
- Ion selective electrodes
- Main analyser platforms and automation.
- Osmometry

Section B4. Laboratory Instruments – Spectroscopy: Learning Objectives

Be able to discuss the method principle and potential advantages and limitations of instruments and analytical techniques commonly used in Specialist Clinical Laboratories

- Luminescence and fluorescence
- Spectrophotometry
- Spectrophotometry and ICP-MS
  - ◊ Atomic absorption
  - ◊ Flame emission photometry

Section B5. Laboratory Instruments – Electrophoresis: Learning Objectives

Be able to discuss the method principle and potential advantages and limitations of instruments and analytical techniques commonly used in Specialist Clinical Laboratories

- Electrophoresis
  - ◊ 2-dimensional
  - ◊ Agarose
  - ◊ Capillary zone
  - ◊ Cellulose acetate
  - ◊ Isoelectric focusing (IEF)
  - ◊ Immunofixation
  - ◊ Isotachophoresis
  - ◊ Polyacrylamide

Section B6. Laboratory Instruments – Chromatography: Learning Objectives

Be able to discuss the method principle and potential advantages and limitations of instruments and analytical techniques commonly used in Specialist Clinical Laboratories

- Chromatography
  - ◊ Affinity
  - ◊ Column
  - ◊ Direct and reverse phase liquid chromatography
  - ◊ Gas chromatography
  - ◊ Gas vs liquid chromatography
  - ◊ High-performance liquid chromatography
  - ◊ Ion-exchange
  - ◊ High pressure liquid chromatography (HPLC)
  - ◊ Partition techniques
  - ◊ Planar
  - ◊ Principles of chromatography
  - ◊ Size exclusion techniques

Section B7. Laboratory Instruments – X-Mass Spectrometry: Learning Objectives

MS - Basic Concepts

Describe the basic principles of mass spectrometry (MS)

Explain the mass to charge ratio and mass spectra

Outline the components of a mass spectrometer, including ionisation sources (electron impact, chemical ionisation, electrospray ionisation), mass filters (quadruple, magnetic), detectors

- Mass spectrometry
  - ◊ Chromatographic separation (see above)
  - ◊ Gas Chromatography-MS (GC-MS)
  - ◊ Inductively Coupled Plasma MS (ICP-MS)
  - ◊ Liquid Chromatography MS (LC-MS)
  - ◊ LC-MS-MS
  - ◊ Matrix Assisted Laser Desorption/ Ionization – Time of Flight (MALDI-TOF)

Discuss the past, current and potential future clinical applications of mass spectrometers, including full scan, selective ion monitoring, multiple reaction monitoring, TOF

Instrumentation

Critically discuss and compare mass spectrometry instruments in terms of their place in clinical testing along with advantage and limitations

- GC- with single or tandem quadrupoles
- High- resolution MS
- ICP-MS
- LC-MS/MS with single or tandem quadrupoles
- MALDI-TOF
- Orbitrap
- Quadrupole – TOF (Q-TOF)

Principles of Analysis X-MS

Sample Preparation

Critically discuss the approaches to sample preparation in clinical testing along with advantage and limitations of each

- Derivatisation
- Dilute and shoot
- Liquid-liquid extraction (LLE)
- Protein precipitation
- Supported liquid extraction (SLE)
- Solid phase extraction (SPE)

Analytical – chromatography

Discuss the processes of chromatographic separation including the selection of columns and mobile phases

Define the terms isomer and epimer and explain the importance of chromatographic separation

Analytical - quality

Discuss the important considerations to support analytical quality

- Calibrators including traceability and commutability
- External Quality Assurance (EQA)
- Interferences
- Internal quality control (IQC)
- Internal standard selection, including non-isotopic, deuterated and carbon 13
- Post implementation quality assessment
- Qualitative scans
- Quantifiers and qualifier selection
- Quantitation – calibration curves

Post analytical

Discuss the implementation of mass spectrometry reference intervals

Outline the criteria for accepting an analytical run

Outline the requirements of reporting a result

Setting up a LC-MS/MS assay

Critically discuss the pre implementation quality assessment i.e. method validation required.

Critically discuss the role and selection of mass spectrometry instruments in terms of automation and processes.

- Fully automated solutions
- Kit-user setting
- Lab developed tests
- Near patient testing
- Identification, optimisation, qualitative assessment, sample preparation to full quantitation

Applications

Discuss the clinical applications of mass spectrometry

- Creatinine
- Drug of abuse testing
- Genomics
- Glycated haemoglobin
- Inborn errors of metabolism
- Metabolomics - Translation from discovery phase to targeted assays and clinical practice
- Microbiology
- Non-peptide hormones
- Peptide hormones
- Proteomics
- Therapeutic drug monitoring
- Toxicology including General unknowns screen – targeted MS/MS or high-resolutions MS
- Vitamins

Discuss the application of mass spectrometry in a variety of clinical matrices

- Blood including serum and plasma
- Dried blood spots
- Misc e.g. dried urine, dried saliva spots, hair, vitreous humour
- Saliva
- Urine

Section B8. Laboratory Instruments – Not otherwise classified: Learning Objectives

Be able to discuss the method principle and potential advantages and limitations of instruments and analytical techniques commonly used in Specialist Clinical Laboratories

- Cell counters
- Emerging technologies,
  - ◊ Multiplex analysis
  - ◊ Nuclear magnetic resonance (NMR) spectroscopy
  - ◊ Sensors
- Enzymology
- Flow cytometry
- Isotopic techniques

Section B9. Analytes: Learning Objectives

Be able to identify the clinical relevance of each of these analytes (as applied in Section C)

Be able to discussion the basic methods, including their advantages and limitations, that could be used for the analysis of common analytics measured in laboratory medicine

Be able to discuss the matrix or matrices (including their advantages and limitations) used for measuring a particular analyte

Lung

- Base Excess
- Bicarbonate
- pCO_2
- pH
- pO_2

Renal

- Aluminium
- Cystatin C
- Creatinine
- Inulin
- Potassium
- Sodium
- Urea
- Uric acid
- Urine albumin
- Urine creatinine
- Urine protein

Liver

- Albumin
- Alkaine phopstase (ALP)
- Alanine Aminotransferase (ALT)
- Aspartate Aminotransferase (AST)
- Bile acids
- Bilirubin
  - ◊ Total
  - ◊ Conjugated or Direct
  - ◊ Unconjugated or Indirect
  - ◊ Fractions
- Gamma-Glutamyl Transferase (GGT)
- Insulin like growth factor (IGF)-1
- Lactate dehydrogenase (LDH)
- Total protein

Metabolism

- Ammonia
- Glucose
- Lactate
- HbA1c

Heart - muscle

- B-type natriuretic peptide (BNP) and Pro-BNP
- Cholesterol
- Creatine kinase (CK)
- CK-MB
- High density lipoprotein (HDL)
- Low density lipoprotein (LDL)
- pCO_2
- Triglycerides
- Troponin I
- Troponin T

Bone

- 1,25 di hydroxy vitamin D
- Calcium
- Magnesium
- Phosphate
- Parathyroid hormone (PTH)
- Vitamin D (25 hydroxy vitamin D)

Endocrinology - Pituitary

- Adrenocorticotropic hormone (ACTH)
- Growth hormone (GH)
- Follicle stimulating hormone (FSH)
- Luteinizing hormone (LH)
- Thyroid stimulating hormone (TSH)

Endocrinology - Peripheral

- 17 hydroxyprogesterone
- Aldosterone
- Anti-mullerian hormone (AMH)
- Cortisol
- Dehydroepiandrosterone (DHEA)
- DHEA sulfate (DHEA-S)
- Estradiol

- free triiodothyronine (fT3)
- free thyroxine (fT4)
- Insulin like growth factor I (IGF-I)
- IGF binding protein 3 (BP3)
- Inhibin
- Progesterone
- Steroid hormone profiles
- Sex hormone binding globulin (SHBG)
- Testosterone

Dynamic Function Tests

- Dexamethasone suppression
- Glucagon stimulation Test
- Growth hormone releasing hormone (GHRH)-Arginine Test
- Insulin Tolerance Test
- Metyrapone Suppression Test
- Oral Glucose Tolerance Test (OGTT)
- OGTT for growth hormone (GH) suppression
- Synacthen stimulation
- Water deprivation

Gastrointestinal - nutrition

- Amylase
- Ceruloplasmin
- Copper
- Faecal calprotectin
- Faecal elastase
- Ferritin
- Insulin
- Intestinal permeability
- Iodine
- Iron
- Lead
- Lipase
- Phorphyrin screen
- Selenium
- Vasoactive intestinal peptide (VIP)
- Vitamin A - retinol
- Vitamin B1
- Vitamin B2
- Vitamin B3
- Vitamin B6
- Vitamin B9 – folate
- Vitamin B12
- Vitamin C
- Vitamin D
- Vitamin E
- Vitamin K
- Transferrin
- Zinc

Biomarkers of Cancer

- Alpha foeto protein
- 5-hydroxyindoleacetic acid (5HIAA)
- Carbohydrate antigen (CA) 19-9
- CA125
- Calcitonin
- Catecholamines
- Carcinoembryonic antigen (CEA)
- Chromogranin A
- Homovanillic acid (HVA)
- Prostate specific antigen (PSA)
- Thyroglobulin

Therapeutic Drug Monitoring and Toxicology

- Acetominophen (paracetamol)
- Carbamazepine
- Carbon monoxide

- Cyclosporine
- Digoxin
- Ethanol
- Ethylene glycol
- Lithium
- Mycophenolic acid
- Methanol
- Methotrexate
- Phenytoin
- Salicylate
- Saliva drug screen
- Sirolimus
- Tacrolimus
- Theophylline
- Urine drugs of abuse screening

Immunology

- Albumin
- Alpha-1-antitrypsin quantification, phenotyping and genotyping
- Beta -2 microglobulin
- C1 esterase inhibitor
- Total complement activity (CH50)
- Complement C3 and C4
- C-reactive protein (CRP)
- Cryoglobulins
- Full blood count
- Haptoglobin
- Hepatitis A, B and C serology
- HIV serology
- IgE and specific IgE
- Immunofixation electrophoresis (IFE)
- Immunoglobulins (IgA, IgG, IgM)
- IgD
- IgG subclasses
- Immunosubtraction/immunotyping
- Lymphocyte subsets
- Monoclonal protein quantification
- Oligoclonal banding (isoelectric focusing)
- Prealbumin (transthyretin)
- Rheumatoid factor
- Serum and urine protein electrophoresis
- Serum free light chains
- Total complement activity (CH50)
- Transthyretin (prealbumin)
- Tryptase

Autoantibodies

- Acetyl choline receptor
- Adrenal
- Antibodies associated with neurological diseases (paraneoplastic antibodies)
- Centromere
- Cyclic citrullinated peptide
- Double stranded DNA
- Endomysium
- Extractable nuclear antigens
  - ◊ SSA (Ro)
  - ◊ SSB (La)
  - ◊ Ribo nuclear protein (RNP)
  - ◊ Sm
  - ◊ Jo1
  - ◊ SCL70
  - ◊ Scl Sm
- Gliadin
- Glomerular basement membrane
- Intrinsic factor
- Liver

- ◊ Liver kidney microsomal
- ◊ Mitochondrial
- ◊ Smooth muscle

- Neutrophil cytoplasm (ANCA)
  - ◊ Myeloperoxidase
  - ◊ Proteinase 3
- Nuclear components (ANA)
- Ovary
- Pancreas
  - ◊ Glutamic acid decarboxylase
  - ◊ Insulin
  - ◊ Insulinoma antigen 2 (IA-2)
  - ◊ Zinc Transporter 8 (ZnT8)
- Skin
  - ◊ Basement membrane
  - ◊ Intercellular cement
- Thyroid
  - ◊ Microsomal
  - ◊ Peroxidase/microsomal
  - ◊ Thyroglobulin
  - ◊ TSH receptor
- Tissue transglutaminase

Section B10. Laboratory Calculations: Learning Objectives

Be able to discuss the process for calculating common equations relevant to laboratory medicine

- Addition dilution
- Anion Gap
- Bicarbonate
- Converting between mass and molar units
- Corrected calcium
- Corrected sodium
- Estimated glomerular filtration rate (eGFR)
- Globulins
- INR
- LDL
- Osmol Gap (including calculated osmolarity)

Be able to perform basic statical calculations used in the laboratory.

Be able to distinguish the appropriate statistical tests to employ based on the distribution of the data.

- Bias
- Biological variation
- Evaluation of differences between populations
- Measures of central tendency
- Measures of dispersion
- Multiple of Median (MOM)
- Positive and negative predictive value
- Reference interval
- Recovery
- Sigma
- Uncertainty of measurement

Section B11. Standardization, Traceability and Harmonization Learning Objectives

Core topics

Understand why different methods for the same analyte give different results

Explain why reducing between-method variability is important

Describe traceability in laboratory medicine

List the stakeholders involved in achieving traceability in laboratory medicine together with their respective roles

Advanced

Standardization in general vs standardization in the metrology of chemistry

Understanding the concept of "amount of substance" and its unit the mole

Understand the concept "measurand"

The meaning of comparisons in measurement

Traceability as a property of the measurement result

Standardisation in the analytical phase and in the pre-and post-analytical phases of the total testing chain

Harmonisation and the importance of commutability

Standardisation as a top-down regulatory process which is stable in time and space

Harmonisation as a bottom up consensus process based on commutable patient samples and with less stability than standardisation in time and space

Examples of harmonisation projects

SECTION C: CLINICAL SECTION

In laboratory medicine it is essential for professionals to have a sound understanding of both analytical tools to achieve the best clinical outcomes. It is therefore essential that laboratory professionals have a sound working knowledge of physiology and pathophysiology related to their testing area. This section of the curriculum details the clinical areas related to clinical chemistry and immunology as detailed in Figure C1.

Section C1. Fluid and Electrolyte Disorders: Learning Objectives

Basic Concepts

To understand the principles and control of fluid and electrolyte balance:

- Clinical assessment of ECF volume
- Extracellular and intracellular fluid volumes
- Hormonal control of fluid and electrolyte balance (renin, angiotensin, aldosterone, ADH)
- Principles of correcting fluid and electrolyte losses

Disorders and disease states

To understand the causes, clinical signs and symptoms of the following features of disorders relating to fluid and electrolyte metabolism.

To describe which laboratory investigations are important in their detection, diagnosis and management

- Dehydration
- Diabetes insipidus
- Extracellular fluid (ECF) volume loss
- Hypernatremia
- Hyperkalemia and pseudohyperkalemia
- Hypokalemia
- Hyponatremia and pseudohyponatraemia
- Shock
- Syndrome of inappropriate antidiuretic hormone (SIADH)

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important to the study of fluid and electrolyte disorders.

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- Aldosterone
- Electrolytes (sodium, potassium, chloride)

Figure C1 Hierarchical structure of Blood Sciences as related to Clinical Chemistry and Immunology *(Clinical component)*

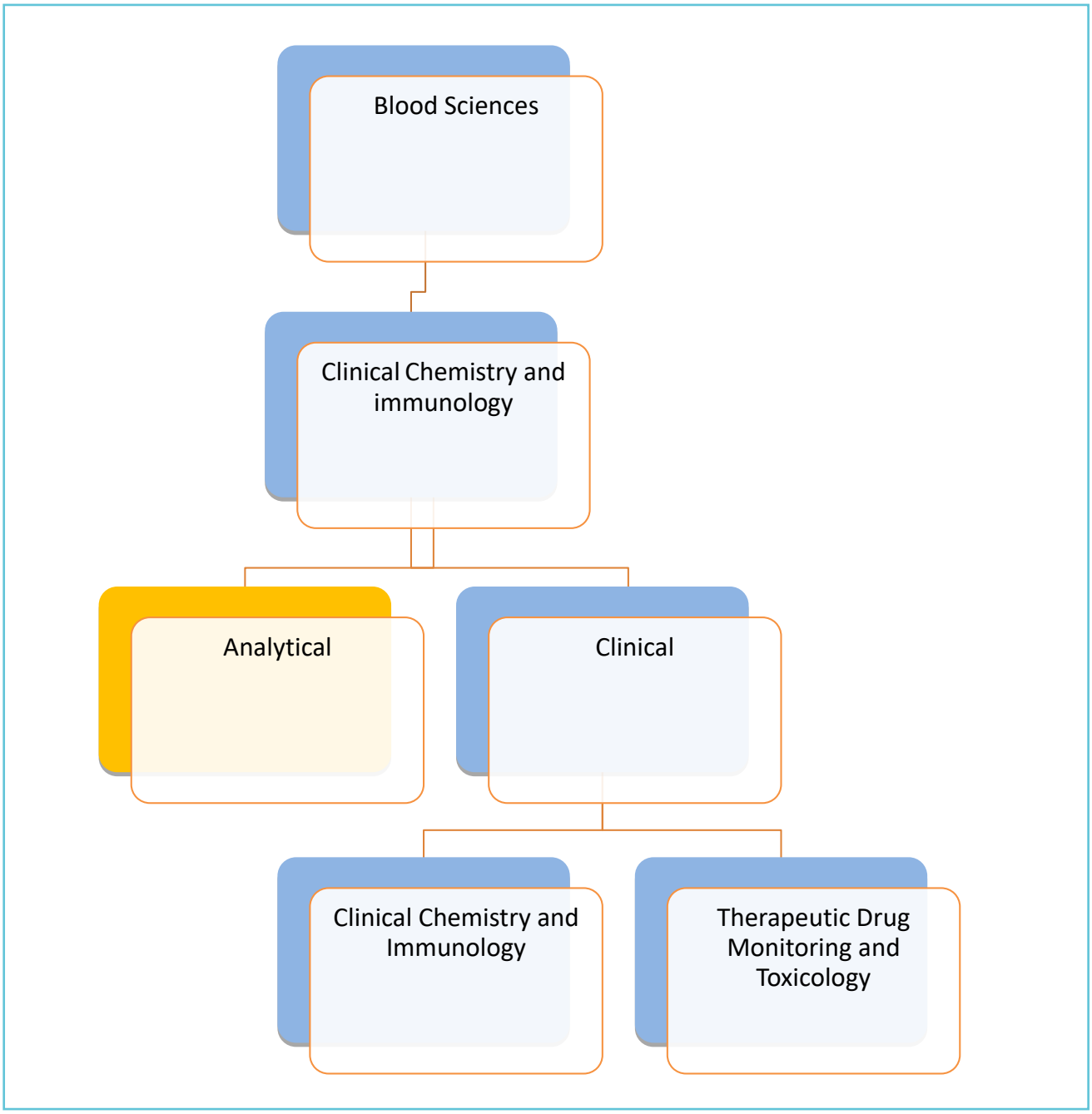


Figure C1:

- *Hierarchical structure of Blood Sciences as related to Clinical Chemistry and Immunology section specifically related to the clinical component of the curriculum.*
- *The analytical component is shown in yellow to demonstrate the relationship between the analytical and clinical sections; which was presented separately in section B.*

- Serum and urine osmolality
- Renin-angiotensin axis (RTA) assessment
- Urine and faecal electrolytes

Section C2. Acid-Base Regulation and Pulmonary Function: Learning Objectives

Basic Concepts

To understand the principles and control of acid – base balance and pulmonary function

- Anion gap
- Compensation for acidosis and alkalosis
- Control of respiration
- Henderson-Hasselbach equation
- Haemoglobin dissociation curves and limitations of calculated oxygen saturation
- Osmol gap
- Systematic approach to investigating acid-base disturbances

Disorders and disease states

To understand the causes, clinical signs and symptoms of the following features of disorders relating to acid-base balance and pulmonary function.

To describe which laboratory investigations are important in their detection, diagnosis and management

- Alpha 1 antitrypsin (A1AT) deficiency *(see also Section C7, Hepatobiliary disease)*
- Carbon monoxide poisoning
- Metabolic acidosis
- Metabolic alkalosis
- Pyloric stenosis
- Renal tubular acidosis *(see also Section 3, Disorders of kidney and urinary tract)*
- Respiratory acidosis
- Respiratory alkalosis

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important to the study of acid-base balance and pulmonary function.

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- Alcohols (ethanol, methanol, ethylene glycol, isopropanol)
- Anion gap
- Blood gas and hydrogen ion measurements
- Co-oximetry (carboxyhaemoglobin and methaemoglobin)
- Calculated blood gas parameters and their limitations
- Ketones (urine and serum)
- Lactate
- Osmolality
- Osmol gap
- Salicylate

Section C3. Disorders of Kidney and Urinary Tract: Learning Objectives

Basic concepts

To understand the principles and control of renal function and the urinary tract, including renal stones and purine and pyrimidine metabolism

- Clearance (creatinine, cystatin C, inulin)
- Creatinine standardisation
- Disorders and disease states

- Estimated glomerular filtration rate (eGFR) calculations
- Endocrine functions of the kidney
- Formation of renal calculi
- Haemodialysis
- Peritoneal dialysis
- Proteinuria; glomerular permeability, tubular proteinuria
- Renal stone formation
- Steady state renal function
- Transplant biochemistry

To understand the causes, clinical signs and symptoms of the following features of disorders relating to renal function, the urinary tract and uric acid metabolism

To describe which laboratory investigations are important in their detection, diagnosis and management

- Acute kidney injury
- Amyloid
- Antineutrophil cytoplasmic antibodies (ANCA) associated vasculitis
- Chronic kidney disease
- Cryoglobulin associated renal damage
- Drug-induced renal damage
- Glomerulonephritis
- Goodpasture's syndrome
- Gout
- Hyperuricaemia
- Myeloma associated renal damage
- Nephritic syndrome
- Nephrotic syndrome
- Renal tubular acidosis *(see also Section 2: Acid-base regulation and pulmonary function)*
- Systemic lupus erythematosus (SLE)
- Urinary tract infection (UTI)
- Types of renal stone
- Uraemia
- Vasculitis (ANCA associated)

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important to the study of renal function, the urinary tract and uric acid metabolism

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- Antinulear antibodies (ANA)
- ANCA
- Beta 2 microglobulin
- Complement C3 and C4
- Creatinine (serum and urine)
- Cryoglobulins
- Cystatin C
- Erythropoietin
- Examination and identification of renal calculi
- Measurement of immunosuppressant drugs (cyclosporine, tacrolimus, sirolimus)
- Phosphate, calcium and magnesium
- Renal calculi analysis
- Serum and urine electrophoresis and immunoglobulins
- Urea and urea kinetics
- Uric acid
- Urine albumin

- Urine dip stick analysis
- Urine microscopic analysis
- Urine oxalate

Section C4. Immunology: Learning Objectives

Basic concepts

To understand the principles, components and control of the immune system

Components of the immune system (Cells, Lymphoid tissue, Soluble components and mediators)

- Acute phase response
- Adaptive Immune system
- Complement
  - ◊ Alternative pathway
  - ◊ Classical pathway
  - ◊ Mannose-binding lectin (MBL) pathway
- Cytokines
  - ◊ Colony stimulating factors and haematopoietic
  - ◊ Interferons
  - ◊ Interleukins
  - ◊ Tumour necrosis factors
- Hypersensitivity reactions
  - ◊ Types I – IV (or V)
- Immunoglobulins
  - ◊ Function
  - ◊ Gene rearrangement
  - ◊ Structure
- Innate Immune system
- Lymphocytes
  - ◊ B lymphocytes
  - ◊ T lymphocytes

Disorders and disease states

To understand the causes, clinical signs and symptoms of the following features of disorders relating to the immune system

To describe which laboratory investigations are important in their detection, diagnosis and management

- Allergy and anaphylaxis
- Autoimmune diseases
  - ◊ Endocrine
  - ◊ Gastro intestinal (GI) tract
  - ◊ Liver
  - ◊ Renal
  - ◊ Rheumatic and articular
  - ◊ Skin
- Lymphoid malignancy
  - ◊ B cell
  - ◊ T cell
- Primary immune deficiency
- Secondary immune deficiency
- Transplantation

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important to the study of disorders of the immune system

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- Autoantibodies to
  - ◊ Skin basement membrane, intercellular cement

- ◊ Nuclear (ANA), smooth muscle, mitochondrial
- ◊ Neutrophil cytoplasmic (ANCA), glomerular basement membrane, ANA
- ◊ Rheumatoid factor, cyclic citrullinated peptides, nuclear, double stranded DNA, extractable nuclear antigens
- ◊ Thyroid, pancreas, adrenal, ovary, (testis)
- ◊ Tissue transglutaminase, endomysial, intrinsic factor

- Full blood count
- HLA typing
- Immunoglobulin quantification and IgG subclasses
- Lymphocyte subsets (CD3, 4, 8, 16/56, 19)
- Monoclonal protein identification and quantification in serum and urine
- Total and specific IgE
- Tryptase

Section C5. Diabetes Mellitus: Learning Objectives

Basic concepts

To understand the pathogenesis of diabetic states and the following aspects of the study of diabetes mellitus

- Aetiology of Type I, Type 2 and gestational diabetes
- Complications of diabetes (microvascular, macrovascular)
- Diabetic ketoacidosis
- HbA1c standardisation
- Guidelines for the screening, diagnosis and monitoring of diabetes
- Non-ketotic hyperosmolar coma
- Self-monitoring of blood glucose

Disorders and disease states

To understand the causes, clinical signs and symptoms of the following aspects of diabetes mellitus

To describe which laboratory investigations are important in their detection, diagnosis and management

- Diabetic ketoacidosis
- Gestational diabetes
- Hypoglycaemia
- Metabolic syndrome
- Non-ketotic hyperosmolar coma
- Type I diabetes
- Type 2 diabetes

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important to the study of diabetes mellitus

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- Anti-glutamic acid decarboxylase (GAD) antibodies
- Anti insulin antibodies
- Blood gas and hydrogen ion measurements
- C-peptide
- Fructosamine and other glycated proteins
- Glucagon
- Glucose
- Glucose tolerance tests
- HbA1c

- Insulin
- Insulinoma antigen 2 (IA-2)
- Ketones, (β-hydroxy butyrate)
- Microalbumin
- Zinc Transporter 8 (ZnT8)

Section C6. Gastrointestinal and Pancreatic Disease: Learning Objectives

Basic concepts

To understand the principles and control of gastrointestinal and pancreatic function

- Acute and chronic pancreatitis
- Autoimmune bowel disease
- Bowel cancer screening
- Causes of gastric ulceration
- Endocrine and exocrine functions of the pancreas
- Inflammatory bowel disease
- Intestinal absorption of proteins, fats and carbohydrates
- Neuroendocrine Tumours
- Vitamin B12 absorption

Disorders and disease states

To understand the causes, clinical signs and symptoms of the following features of disorders relating to gastrointestinal and pancreatic function

To describe which laboratory investigations are important in their detection, diagnosis and management

- Acute and chronic pancreatitis
- Carcinoid syndrome
- Coeliac Disease
- Crohn's Disease
- Food allergy
- H. pylori infection
- Intestinal malabsorption, including vitamins, *(see also Section 5 : trace elements and vitamins)*
- Neuroendocrine tumours
- Pyloric stenosis *(see also Section 2, Acid base regulation and pulmonary function)*
- Pernicious anaemia
- Zollinger-Ellison syndrome

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important to the study of disorders of gastrointestinal and pancreatic function

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- $^{14}CO_2$ urea breath test for H. pylori
- Amylase and macroamylase
- CA 19-9
- Carcinoembryonic antigen (CEA)
- Chromogranin A
- Elastase
- Endomysial autoantibodies
- Faecal calprotectin
- Faecal occult blood
- Hydrogen breath test for lactose intolerance
- IgE and specific IgE
- Intrinsic factor antibodies
- Lipase
- Serotonin and 5 HIAA
- Transglutaminase autoantibodies

- Vitamin B12 and folate
- Xylose absorption test

Section C7. Hepatobiliary Disease: Learning Objectives

Basic concepts

To understand the metabolic functions of the liver and the following aspects of hepatobiliary disease

- Autoimmune disease
- Cholestasis
- Drugs – acute and chronic
- Genetic – e.g. A1AT deficiency *(see also Section 2, Acid-base regulation and pulmonary function)*
- Inflammatory and infective liver disease (hepatitis)
- Liver autoantibodies
- Liver cirrhosis
- Liver function – synthesis, conjugation, detoxification
- Liver transplantation
- Liver tumours – primary or secondary
- Origin, metabolism and transport of bilirubin

Disorders and disease states

To understand the causes, clinical signs and symptoms of the following features of disorders relating to hepatobiliary function

To describe which laboratory investigations are important in their detection, diagnosis and management

- Acute hepatitis
- Alcoholic liver disease
- Allograft rejection
- Biliary damage and dysfunction
- Chronic hepatitis
- Cirrhosis (Including primary biliary cirrhosis)
- Elevated bilirubin levels: conjugated, unconjugated and total
- Elevated neonatal bilirubin levels
- Infectious hepatitis
- Paracetamol (Acetominophen) overdose

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important to the study of disorders of hepatobiliary function

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- Albumin (blood and ascites)
- Alpha-1 antitrypsin concentration and phenotype/genotype
- Alphafoetoprotein (AFP)
- Ammonia
- Bile acids
- Bilirubin – conjugated, unconjugated, total, transcutaneous
- Caeruloplasmin
- Ethanol
- Ferritin
- Hepatitis serology
- Iron
- Liver enzymes: ALT, AST, ALP, GGT
- Measurement of immunosuppressant drugs (cyclosporine, tacrolimus, sirolimus)
- Paracetamol (Acetominophen)
- PT-INR

Section C8. Lipids and Disorders of Lipoprotein Metabolism: Learning Objectives

Basic concepts

To understand the principles and control of lipid metabolism and the following aspects of the study of lipid and lipoprotein disorders

- Apolipoproteins: functions, receptors (e.g. LDL-R)
- Cardiovascular disease risk calculation and evaluation, and cost-effectiveness of lipid screening strategies.
- Fatty acid transport and oxidation
- Lipid absorption, transport and metabolism
- Lipoprotein metabolism: endogenous and exogenous pathways

Disorders and disease states

To understand the causes, clinical signs and symptoms of the following features of disorders relating to lipid and lipoprotein disorders

To describe which laboratory investigations are important in their detection, diagnosis and management

- Atherosclerosis
- Hypercholesterolaemia
- Hyperlipidemia
  - ◊ Inherited disorders
  - ◊ Non-inherited disorders
- Hypolipidemia
- Metabolic syndrome

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important to the study of disorders of lipid and lipoprotein metabolism

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- Apolipoprotein A and B
- Genotyping of ApoE, LDL receptor and LPL
- HDL cholesterol
- High sensitive CRP
- LDL cholesterol – direct and calculated methods
- Lipoprotein electrophoresis
- Lipoprotein ultracentrifugation
- Lp(a)
- Non-HDL cholesterol (calculated)
- Total cholesterol
- Triglycerides

Section C9. Cardiovascular Disorders and Hypertension: Learning Objectives

Basic concepts

To understand the causes and manifestations of the cardiovascular disease the assessment of individual risk

- Acute coronary syndrome
- Cardiac risk assessment
- Myocardial infarction

Disorders and disease states

To understand the causes, clinical signs and symptoms of the following features of cardiovascular disorders

To describe which laboratory investigations are important in their detection, diagnosis and management

- Atherosclerosis
- Cardiac amyloid

- Congestive heart failure
- Hypertension
- Myocardial infarction
- Stable angina
- Unstable angina

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important to the study of cardiovascular disease and hypertension

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- Anti-heart muscle antibodies (Dresslers)
- High-sensitivity CRP
- Natriuretic peptides
- Serum and urine electrophoresis and immunoglobulins
- Troponin and high-sensitivity troponin

Section C10. Calcium, Magnesium, Parathyroid, Bone Disorders: Learning Objectives

Basic concepts

To understand the control of calcium and phosphate homeostasis including the following specific aspects of the process.

- Circulating forms of calcium
- Metabolism of vitamin D
- Markers of bone resorption and bone formation
- Primary versus secondary hyper/hypo calcaemia
- Regulation of calcium and phosphate levels

Disorders and disease states

To understand the causes, clinical signs and symptoms of the following disorders and manifestations of calcium and phosphate metabolism and bone disease

To describe which laboratory investigations are important in their detection, diagnosis and management

- Hypercalcaemia
- Hypermagnesaemia
- Hyperparathyroidism
- Hyperphosphataemia
- Hypocalcaemia
- Hypomagnesaemia
- Hypoparathyroidism
- Hypophosphataemia
- Hypophosphatasia
- Pseudohypoparathyroidism
- Osteogenesis imperfecta
- Osteoporosis
- Paget's disease

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important to the study of calcium and phosphate metabolism asn bone disease

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- 1,25 di-hydroxy vitamin D
- 25 hydroxy vitamin D

- C- and N-telopeptides
- Calcium – total and ionized
- Magnesium
- Osteocalcin
- Phosphate
- PTH
- PTHrp
- Pyridinolines
- Total and bone-specific ALP

Section C11. Iron and Haemoglobin Disorders, including the Porphyria's: Learning Objectives

Basic Concepts

To understand the control and metabolism of iron and haem, the mechanisms which lead to iron overload and deficiency, and the implications of these states.

To understand the enzymatic defects of haem synthesis which lead to the porphyrias

- Haem biosynthesis
- Haem metabolism
- Iron absorption, transport and storage
- Iron deficiency
- Iron overload

Disorders and disease states

To understand the causes, clinical signs and symptoms of the following aspects and manifestations of disorders of iron and haem metabolism

To describe which laboratory investigations are important in their detection, diagnosis and management

- Acute and chronic porphyrias and their differential diagnosis
- Anaemia secondary to malignancy
- Glucose-6-phosphate dehydrogenase (G6PD) deficiency
- Haemochromatosis
- Haemoglobinopathies
- Intravascular haemolysis
- Iron deficient anaemia
- Thalassaemia

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important to the study of iron and haem metabolism and the porphyrias

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- Delta-aminolaevulinic acid (ALA)
- Ferritin
- Full blood count
- G6PD
- Haptoglobin
- Haemoglobin and haemoglobin variants
- Haemopexin
- Iron
- Porphobilinogen (PBG)
- Porphyrins
- Soluble transferrin receptor
- Total iron binding capacity
- Transferrin
- Transferrin saturation

Section C12. Vitamins and Trace Elements: Learning Objectives

Basic Concepts

To understand the importance of trace elements and vitamins to metabolic processes and wellbeing, the mechanisms of their actions and the consequences of deficiency and overload states.

- Essential and non-essential metals
- Fat soluble vitamins; A and carotene, D, E and K
- Folate metabolism and function
- Genetic disorders of copper metabolism
- Genetic disorders of iron metabolism
- Potential vitamin toxicity
- Toxic and non-toxic metals
- Vitamin B12 absorption, metabolism and function
- Water soluble vitamins; B group vitamins and C

Disorders and disease states

To understand the causes, clinical signs and symptoms of the following aspects of disorders of trace element and vitamin metabolism

To describe which laboratory investigations are important in their detection, diagnosis and management

- Cadmium poisoning
- Copper deficiency/excess
- Folate deficiency
- Iron overload
- Lead poisoning
- Mercury poisoning
- Vitamin B12 deficiency

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important to the study of trace elements and vitamins

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- Cadmium in blood and urine
- Caeruloplasmin
- Delta amino laevulinic acid (ALA)
- Ferritin
- Folate
- Homocysteine
- Intrinsic factor antibody
- Laboratory assessment of specific B vitamin status
- Lead in blood and urine
- Mercury in blood and urine
- Methylmalonic Acid (MMA)
- Serum and urinary iron
- Serum and urinary copper
- Transferrin
- Vitamin B12
- Vitamin D

Section C13. Pregnancy and Prenatal Diagnosis: Learning Objectives

Basic Concepts

To understand the following concepts in relation to the provision of a clinical laboratory service to support pregnancy and the health of the mother and foetus.

- Biochemical, haematological and endocrine changes during pregnancy
- Changes in analyte levels throughout pregnancy
  - ◊ Multiples of the median
- Foetal lung maturity
- hCG doubling time
- hCG forms
- Maternal serum screening – purpose, limitations, screen vs. definitive testing
  - ◊ First trimester screening
  - ◊ Integrated screening
  - ◊ Second trimester screening
- Premature rupture of membranes and pre-term labour
- Rh isoimmunisation

Disorders and disease states

To understand the causes, clinical signs and symptoms of the following, complications of pregnancy and foetal development.

To describe which laboratory investigations are important in their detection, diagnosis and management

- Choriocarcinoma
- Ectopic pregnancy
- Gestational diabetes
- Molar pregnancy
- Obstetric cholestasis
- Open neural tube defects
- Pre-eclampsia, HELLP syndrome
- Rh isoimmunisation
- Trisomy 21, 18 and 13
- Trophoblastic disease

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important to the study of pregnancy and foetal development

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- AFP
- hCG
- Inhibin A
- PAPP-A
- Plasme nucleic acids
- Serum bile acids
- Unconjugated estriol

To be able to discuss the clinical rational for tests using amniotic fluid

- Acetylcholinesterase
- AFP
- Bilirubin – absorbance at 450 nm
- Karyotype

Section C14. General Paediatric Clinical Chemistry: Learning Objectives

Basic Concepts

To understand the following special considerations in relation to the provision of a general paediatric clinical laboratory service.

- How to collect heel prick samples
- Issues with capillary specimens
- Paediatric reference intervals – dynamic changes with growth, development and puberty

- Sample volume and collection issues, including sweat collection

Disorders and disease states

To understand the causes, clinical signs and symptoms of the conditions which may present to a general paediatric laboratory.

To describe which laboratory investigations are important in their detection, diagnosis and management

- Congenital thyroid disease
- Cystic fibrosis
- Delayed puberty
- Diabetes mellitus type 1
- Disorders of sex development, including congenital adrenal hyperplasia
- Growth retardation and growth hormone deficiency
- Hypocalcaemia
- Inborn errors of metabolism
- Neonatal hypoglycaemia
- Neonatal jaundice
- Neuroblastoma
- Precocious puberty
- Respiratory distress

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important to the study of disorders presenting in the neonate and in childhood

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

For all analyses, sample size is of key importance in method selection

- Acid base / blood gases
- Calcium
- Glucose
- Hormone measurement *(see Section 16, Endocrinology)*
  - ◊ Anterior hypothalamic hormones
  - ◊ Cortisol
  - ◊ Neuropeptides
  - ◊ Ovarian hormones
  - ◊ Testicular Hormones
  - ◊ Thyroid function
  - ◊ Other adrenal hormones
- HVA, dopamine
- Performance of the sweat test
- Plasma catecholamines
- Total and differential bilirubin

Result interpretation

To recognise that the population-based reference intervals for many of children's chemistries change throughout childhood.

To recognise that these reference intervals change differently for different analytes.

To recognise some of the problems associated with reporting children's chemistries.

To be aware of some of the problems that arise from failing to report correct children's chemistry reference intervals

Section C15. Inborn Errors of Metabolism: Learning Objectives

Basic Concepts

To understand all aspects of screening for disease including the rationale for screening and the process.

- Challenges with newborn screening
- Diseases appropriate for newborn screening – characteristics
- Newborn screening process

Disorders and disease states

To understand the causes, clinical signs and symptoms of the following inherited conditions

To describe which laboratory investigations are important in their detection, diagnosis and management

- Congenital hypothyroidism
- Cystic fibrosis
- Enzyme deficiencies: biotinidase, galactokinase
- Fatty acid oxidation disorders: short, medium, long and very long chain
- Haemoglobinopathies
- Homocystinuria
- Lysosomal disease, glycogen storage diseases: lipidoses, hexosaminidases, Fabry's disease
- Maple syrup urine disease
- Phenylketonuria
- Tyrosinaemia

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important to the study of inherited metabolic diseases

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- Acylcarnitines
- Alpha-1-antitrypsin genotyping
- Amino acids
- Haemoglobin electrophoresis
- Immunoreactive trypsinogen
- Organic acids
- Specific enzyme testing (e.g., biotinidase, galactokinase)
- Sweat chloride and conductivity

Section C16. Endocrinology: Learning Objectives

Basic Concepts

To understand all aspects of hormone action, including feedback inhibition and other controlling mechanisms for hormone release and to acquire a detailed knowledge of the major endocrine organs and systems.

Hypothalamic-pituitary axis

- Anterior pituitary hormones
- Communication between pituitary and hypothalamus – anterior vs. posterior
- Hirsutism and virilisation
- Hypothalamic hormones
- Inhibitory hormones
- Posterior pituitary hormones
- Primary vs. secondary causes
- Renin-angiotensinogen-aldosterone pathway
- Steroid biosynthesis pathway
- Stimulation tests

- Suppression tests

Disorders and disease states

To understand the causes, clinical signs and symptoms of the following endocrine disorders

To describe which laboratory investigations are important in their detection, diagnosis and management

- Acromegaly/gigantism
- Addison's syndrome
- Adrenal insufficiency
- Congenital adrenal hyperplasia
- Cushing's syndrome and Cushing's disease
- Female infertility
- Growth hormone deficiency
- Hyperaldosteronism
- Hyperprolactinemia
- Hyperthyroidism
- Hypothyroidism
- Male infertility
- Phaeochromocytoma
- Polycystic ovarian syndrome
- Premature ovarian failure
- Sheehan's syndrome
- Thyroid cancer

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important to the study of endocrine disorders

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- 17 hydroxyprogesterone
- ACTH
- ADH
- Aldosterone, renin and ratio
- Androstenedione
- Angiotensin converting enzyme (ACE)
- Autoantibodies to the relevant endocrine organ
- Catecholamines (plasma and urine)
- Cortisol – serum, urine, salivary
- Dexamethasone suppression test
- DHEAS
- Estradiol
- FSH
- Growth hormone
- Growth hormone suppression test (OGTT)
- Insulin-like growth factor 1 (IGF-1)
- LH
- Metanephrines (plasma and urine)
- Progesterone
- Prolactin and macroprolactin
- SHBG
- Testosterone – total, free, bioavailable
- Thyroglobulin, anti-thyroglobulin antibodies
- Thyroid function tests: TSH, Free T3, free T4 Total T3, Total T4
- TSH receptor antibodies

Section C17. Neurological and Psychiatric Disorders: Learning Objectives

Basic Concepts

To understand the following key concepts relating to neurological processes and disruption of these

- Blood brain barrier
- Paraneoplastic syndromes

Disorders and disease states

To understand the causes, clinical signs and symptoms of the following disorders, some of which are primary neurological disorders and some of which have neurological manifestations

To describe which laboratory investigations are important in their detection, diagnosis and management

- Acute porphyria's
- Alzheimer's disease
- Meningitis
- Multiple sclerosis
- Myasthenia gravis
- Paraneoplastic syndrome

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important to the study of neurological disorders

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- Aminolaevulinic acid (ALA)
- Anti-acetylcholine receptor antibodies
- Anti-Hu, Anti-Yo antibodies
- CSF beta-2-transferrin in rhinorrhoea and otorrhoea (fistula)
- CSF glucose
- CSF protein
- Examination of synovial fluid
- Oligoclonal banding (isoelectric focusing)
- Paraneoplastic antibodies
- Porphobilinogen (PBG)
- Porphyrins (urine, feces, serum)

Section C18. Biochemical Aspects of Monitoring Malignant Disease: Learning Objectives

Basic Concepts

To understand the following key concepts relating to the choice, use and measurement of biomarkers of cancer

- Characteristics of an ideal biomarkers of cancer
- Uses and limitations of current biomarkers of cancer
- Uses of biomarkers of cancer: prognosis, monitoring, recurrence

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important in the management of malignant diseases

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- AFP
- ALP isoenzymes
- CA 15-3
- CA 19-9
- CA 125
- Calcitonin
- CEA
- HE4

- hCG
- HER2/Neu
- LDH isoenzymes
- Mammary specific antigen
- PSA (total and free)
- PTHrp
- Protein electrophoresis
- Thyroglobulin, anti-thyroglobulin antibodies
- Other emerging biomarks as deemed relevant

Section C19. Musculoskeletal Diseases: Learning Objectives

Basic concepts

To understand muscle function and the use and limitations of autoimmune testing in diagnosis

- Autoimmune testing
- Muscle function

Disorders and disease states

To understand the causes, clinical signs and symptoms of the following musculoskeletal disorders

To describe which laboratory investigations are important in their detection, diagnosis and management

- Duchenne / Becker dystrophy
- Osteoarthritis
- Rhabdomyolisis
- Rheumatoid arthritis
- Systemic lupus erythematosus
- Vasculitis

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important in the management of musculoskeletal diseases

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- Anti-CCP antibodies
- Anti-ds DNA
- Anti-neutrophilic cytoplasmic antibodies (ANCA)
- Anti-nuclear antibodies (ANA and specific antibodies SSA, SSB, Sm, RNP)
- Rheumatoid factor
- Serum creatine kinase

Section C20. Therapeutic Drug Monitoring and Toxicology: Learning Objectives

Basic Concepts

To understand the following key concepts of pharmacology and the key factors relevant to drug action and measurement.

To understand the mode of action and clinical uses of drugs in the categories listed.

Pharmacokinetics (PK)

- Absorption
- Bioavailability
- Compliance
- Distribution
- Excretion
- Metabolism
- Peak vs. trough drug levels
- Steady state

Pharmacodynamics (PD) and Pharmacogenetics (PG)

- Antibiotics
- Antidepressants

- Anti-epileptic drugs
- Anti-psychotic drugs
- Carbon monoxide poisoning
- Cardioactive drugs
- Common drugs of abuse
- Drugs appropriate for TDM
- Drug screens vs. confirmatory tests for drugs of abuse
- Free drugs
- Immunosuppressants
- Lithium
- Methotrexate and rescue
- Thiopurines
- Warfarin

Disorders and disease states

To understand the causes, clinical signs and symptoms of the following toxic conditions and the treatment regimes which may be used,

To describe which laboratory investigations are important in their diagnosis and management

- Acetaminophen toxicity
- Carbon monoxide poisoning
- Ethanol, alcohol toxicity
- Organophosphate poisoning
- Salicylate toxicity

Specific Laboratory Investigations

To gain an in depth knowledge of the following specific laboratory investigations important in for drug monitoring and detection of toxic concentrations of drugs

To be familiar with analytical methods available for their measurement

To be able to select laboratory investigations and interpret the analytical results in the light of the clinical signs and symptoms in the individual patient

- Acetaminophen
- Carbon monoxide – carboxyhaemoglobin
- Cholinesterase and pseudocholinesterase
- Cyclosporine
- Ethanol
- Ethylene glycol
- Isopropanol
- Methanol
- Mycophenolic acid (MPA)
- Osmolality
- Point-of-care drug screens
- Sirolimus
- Salicylates
- Tacrolimus

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Serum ferritin as a biomarker of polycythemia vera?

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LETTER TO THE EDITOR

The measurement of serum ferritin and transferrin saturation are the most commonly used tests for evaluating iron deficiency with low ferritin concentrations reflecting iron depletion (1). One cause of iron deficiency is the over-production of red blood cells, as evident in most patients with the myeloproliferative neoplasm of polycythemia vera (PV) (2), a malignancy in which there is constitutive activation of erythropoietin receptor signalling pathway due the acquisition of the *JAK2* p.V617F mutation.

The current World Health Organization (WHO) criteria for PV diagnosis are gender-specific raised hemoglobin or hematocrit levels, tri-lineage bone marrow hypercellularity, the presence of the *JAK2* p.V61F (acquired in more than 95% of cases) or exon 12 mutations, and a low serum erythropoietin concentration (3).

Additionally, these guidelines also detail the entity of masked PV, in which an iron deficiency due to the enhanced red blood cell proliferation results in an apparently normal presenting hemoglobin concentration, the diagnostic principles of which have subsequently been validated (4).

Despite subnormal serum ferritin not being a diagnostic requirement for PV, a low ferritin level in the absence of other features of PV has become a sporadic trigger for requesting *JAK2* p.V617F molecular analysis. In order to address the clinical value and laboratory impact of such requests, a retrospective audit was performed on all *JAK2* p.V617F requests received at a molecular diagnostics centre for hematological malignancies.

From January 2006 to December 2017 inclusive, 15562 diagnostic requests for *JAK2* p.V617F mutation analysis were received. Of these, 64 requests (0.4%) were received with the only clinical details provided on the request form of a subnormal serum ferritin (normal range 23–393 ng/mL). The median age was 58 years and comprised 42 males and 22 females. Using a standardised allele-specific PCR screening assay (5) capable of detecting a 2% mutant allele burden and unchanged throughout the audit period, the *JAK2* p.V617F mutation was not detected in any of these 64 patients.

Which patients to screen for the myeloproliferative neoplasm associated mutations of *JAK2*, *CALR* and *MPL* requires careful consideration in order to optimise laboratory resources (6,7). While the number of requests for *JAK2* p.V617F mutation in patients with low serum ferritin does not appreciably impact on overall laboratory workload, reflexive screening for the *JAK2* p.V617F mutation in patients with isolated subnormal ferritin levels and no further evidence of PV or masked PV appears inappropriate.

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Book review: "Neonatology and Laboratory Medicine"

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BOOK REVIEWED

"Neonatology and Laboratory Medicine"
by Sarah Heap, Jim Gray, and Andrew Ewer

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RECENSION

The majority of newborns do not require laboratory testing apart from a blood sample for the neonatal screening programme. Nonetheless, this book fills an important niche with its comprehensive coverage of laboratory medicine for a distinct group of patients. Laboratory support is crucial in the management of sick newborns for a broad spectrum of clinical needs, from simple monitoring to the diagnosis of complex inherited disorders.

The opening chapter gives an overview of neonatal care and biochemistry. Approximately 11% of newborns admitted to the neonatal unit (NNU) in the UK are preterm. Besides undertaking actual analysis, the laboratory should be involved in the collection, transport and pre-analytical processing of samples. One of the major concerns of laboratory testing is the collection of samples from neonates, a topic that is adequately covered in this chapter, and in an appendix.

The second chapter deals with the requirements for laboratory services and related issues such as quality considerations. Point-of-care-testing (POCT) and the

factors deciding its use are discussed. Credit should be given to the authors for discussing ethical issues in the laboratory testing of neonates in this, and some other chapters.

Most of the remaining chapters cover the underlying pathophysiology of the clinical problems that are encountered in neonates and the interpretation of related laboratory results. Neonates, particularly premature infants, are vulnerable to disorders of electrolyte, water and acid-base metabolism and these are covered in two chapters. The common minor neonatal problems that require laboratory support are jaundice, hypoglycaemia and infection. The chapter on neonatal jaundice covers its pathophysiology and, *inter alia*, provides an algorithm for the investigation of prolonged jaundice. Blood glucose falls rapidly after birth but normalizes by 12 hours of life. Prolonged severe hypoglycaemia can result in death and brain damage and the causes of hypoglycaemia are extensively discussed.

Neonatal laboratory screening is undertaken in the UK, for nine inherited disorders, to identify infants who may be at high risk, for those who are treatable, before symptoms develop. An initial positive screening test is confirmed with specific second-tier diagnostic tests. The advent of rapid high throughput technologies and DNA analysis offer the potential to screen for numerous disorders but the expansion of newborn screening also raises ethical dilemmas and treatment issues. The chapter on Inherited Metabolic Diseases is the longest with 36 pages. Its coverage is wide-ranging with descriptions of the clinical and biochemical manifestations, and the investigations that need to be undertaken.

Haematology and the neonate are covered in a single chapter. Anaemia is a common disorder in neonates. Its causes and a useful diagnostic algorithm of the condition are provided. The chapter also touches on most common disorders of the haemostatic system.

Infections can be acquired before, during and after birth. Babies in the NNU are at high risk. The chapter describes the common pathogens, epidemiology, clinical features, diagnosis and management of infections in neonates. The high incidence and mortality of neonatal sepsis worldwide require an earlier diagnosis and more accurate monitoring of the disease [1]. The definition of paediatric sepsis is a matter of controversy, and the conventional tests (WBC and differential count, micro-ESR and CRP) have limitations [2]. Newer molecular tests, the application of proteomics and metabolomics for risk stratification and prognosis, and the clinical use of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) for the identification of various bacteria and yeasts, are discussed. The availability of these sophisticated biochemical and molecular tests and of innovative technologies can significantly improve outcomes [1]. A separate chapter discusses the important topic of infection surveillance for prevention and control, and the management of outbreaks of infection.

Babies are not small adults and need their own reference intervals (RI). Although these are provided in this book, the authors have suggested that laboratories should establish their own RI. Since there are numerous challenges in trying to do so for children, it would have been helpful if the authors had addressed the ways this could be done.

The book consists of 15 chapters and 4 appendices. Each chapter begins with a "Summary" and ends with a list of references. Among attractive features of the book are the useful diagnostic algorithms, and tables/figures that conveniently summarize the main body of the text.

There are not many books that comprehensively cover all areas of laboratory medicine. For the

future, the authors have opined that closed, self-contained fully integrated platforms will allow testing to be carried out on demand and nearer to the patient. In microbiology, antibiotic resistance is likely to be one of the main challenges. Though most of the chapters of this book are on clinical biochemistry, there are also chapters on nutrition and drugs besides those on infections (microbiology) and haematology.

This excellent title has been updated from its previous edition to ensure continued relevance. While the authors modestly offer this book as a "basic guide for junior doctors, laboratory scientists and neonatal nurses," its appeal should range much wider. In the preface, they enigmatically thank the publishers "for providing us with the opportunity for this *final* (my italics) edition." One must hope that there will be another update of this remarkable publication at the appropriate time.

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