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COVID-19: Armageddon before light?

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MANUSCRIPT

COVID-19, five letters, two numbers, five months, three billions. Three billion people confined in a box (1,2). But who turned the light off? Humanity did not expect this. The Doctors did not expect this. Bill Gates did… (3). Unthinkable, but excruciatingly real. The impact of the wave seems indelible. And yet, it's 2020 and everything is possible, AI is more than a promise, it exists. Anybody can become somebody, and any start-up company can become bigger than a State. But who turned the light off?

COVID-19, invisible but potentially on everyone's hands. Nowhere, but already everywhere, even there. In a continuous and disproportionate flow of information, data, the incredulous faces of men, women or children, caregivers and patients pass on our screens, at every moment. The distress of families, the distress of caregivers freezes us. Presidents, ministers, hospital directors in search of hope and solutions and… tests. "Test, test, test"…

Our lives, we walked in crowded streets, we used crowded public transport to get to our laboratories, and then nothing. Locked down. Thousands of patients seen per day and thousands of tubes analyzed every day, and then almost nothing. We just test. Locked down.

On arrival, the repercussions of this pandemic are incommensurate with what we have already experienced. Human and health consequences, hundreds of thousands of sick people and a high death rate. Intensive hospital and care capacities under extreme tension. A crying need for artificial respirators. Caregivers subjected to severe ordeal and extreme cases, confined, stressed populations, scenes of panic in supermarkets, closed schools. Locked down.

But it's a tsunami, a wave train. If the first wave is about health, the next are about economy, social issues... And they will be bigger… If all world stock exchanges are known for dizzying falls, the economic repercussions even for health care players are also gigantic and the losses for laboratories and the *in vitro* diagnostic industry will be colossal with several months of very limited activity. And yet, "test, test, test".

The global response to the darkest unicorn ever that perfectly surfs on the wave of globalization is protectionism. It spreads, we lock and then we lack. The defective globalization, lack of supply of masks, impact on the pre-analytical phase with a lack of swab and on the analytical phase with lack of reagents. We should answer together, but we broker alone and compete. Business first. Our laboratory activity has changed from traditional assays to the thousands of COVID-19 PCR tests and a lack of assay capacity defective by this pandemic requiring thousands of molecular biology tests. We are ready. But we lack.

We lack coordination and that is the most glaring lack, a simple consequence of our withdrawal behavior. The picture is very dark and close to Armageddon. Could we have anticipated it? (3). What if nature wanted to reclaim its rights in the face of an increasingly aggressive human footprint? (4).

However, even on the darkest days, glimmers of hope appear.

First, because we are facing through unprecedented mobilization. The medical and scientific world faces, supported by a citizen impulse in all countries. Solidarity is organized and takes cross-border orientations, medical capacities are shared (5). This solidarity is also cross-business and cross-sector. Willingness to coordinate European logistics, manage logistics and manage population testing. Several countries in the Africa and in the Caribbean are mobilizing resources to implement testing strategies.

Digital platforms are fostering international cooperation, remote work, and mass communication, faster than ever.

Technology also comes in support. Epidemiological and big-data monitoring tools allow real-time monitoring of the dispersion of the pandemic with clinical outcomes monitored in real time.

We hope, too, as through darkness comes light. If Labs have been confined for years in the basement of the temple of Medicine, or second role players, they are now in first line. Clinical biology and the in vitro diagnostic industry are ready. Through screening tests, through their technological capacity to develop molecular biology capacities in real time, they will also respond to the global call. They will test, they will help. But they will need help too. Light gives powers but also responsibilities. Labs lack.

A capacity for adaptation and technological rebound thanks to rapid tests, serological tests. A capacity to respond to the need for critical hospital units but also to triage in emergency situations through point of care testing. But we will need to be accompanied, to coordinate and to validate solutions and pathways altogether.

If our discipline is brought to light as never before, pushing away a lack of recognition, it still suffers from internal divisions that do not allow exploiting its full potential. Let's break them down, a lab is a lab.

Through biological profiles, the disease is better phenotyped, better understood and severity profiles that can be influenced by ethnicity (2,6). We understand that the response to the disease may vary according to individuals, ethnicities and that personalized approaches could be considered (7,8). Science and laboratory medicine are progressing. We also understand that we will be actors in future prevention strategies (9).

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Artificial Intelligence-powered search tools and resources in the fight against COVID-19

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ABSTRACT

Emerging technologies are set to play an important role in our response to the COVID-19 pandemic. This paper explores three prominent initiatives: COVID-19 focused datasets (e.g., CORD-19); Artificial intelligence-powered search tools (e.g., WellAI, SciSight); and contact tracing based on mobile communication technology. We believe that increasing awareness of these tools will be important in future research into the disease, COVID-19, and the virus, SARS-CoV-2.

INTRODUCTION

The COVID-19 pandemic has created unprecedented challenges for the medical and clinical diagnostic community. The fight against COVID-19 is being supported by a number of databases and artificial intelligence (AI)-based initiatives aimed at assessing dissemination of the disease [1], aiding in detection and diagnosis, minimizing the spread of the disease, and facilitating and accelerating research globally [2-7].

Prominent among these initiatives are: the COVID-19 Open Research Dataset (CORD-19) [8-10], and databases curated by the CDC [11,12], NLM [13], and the WHO [14]; AI-powered tools such as those from WellAI [15,16] and the Allen Institute for AI (SciSight) [17-19]; and contact tracing based on mobile communication technology [20,21].

CORD-19

The CORD-19 Dataset has resulted from a partnership between the Semantic Scholar team at the Allen Institute for AI and leading research groups (Chan Zuckerberg Initiative, Georgetown University's Center for Security and Emerging Technology, Microsoft Research, the Kaggle AI platform (Google), and the National Library of Medicine–National Institutes of Health) in coordination with the White House Office of Science and Technology Policy.

Publications in the collection are sourced from PubMed Central, the bioRxiv and medRxiv preprint servers, and the WHO COVID-19 Database. CORD-19 is freely available, downloadable and it is updated weekly. The collection currently contains over 128,000 publications (with over 59,000 full text as of 26 May 2020) on the disease, COVID-19, and the virus, SARS-CoV-2, and related coronaviruses. It is part of a call to action to the AI community to develop AI techniques in order to generate new insights to assist in the fight against COVID-19 [9]. This call to action has been informed by a series of tasks described in the form of a series of questions that are listed in Table 1 [22].

Table 1 COVID-19 Open Research Dataset Challenge (CORD-19) – tasks

What is known about transmission, incubation, and environmental stability?
What do we know about COVID-19 risk factors?
What do we know about virus genetics, origin, and evolution?
What do we know about vaccines and therapeutics?
What has been published about medical care?
What do we know about non-pharmaceutical interventions?
What do we know about diagnostics and surveillance?
What has been published about ethical and social science considerations?
What has been published about information sharing and inter-sectoral collaboration?

AI-POWERED SEARCH TOOLS

Analysis of the vast amount of COVID-19 data that has already accumulated (e.g., CORD-19 Dataset, COVID-19 cases data, Hospital Data and case statistics) [23] is a daunting challenge, however, this *big data* type of problem is amenable to AI-based search tools [24] such as those from WellAI and the Allen Institute for AI (SciSight). There are several advantages of AI-powered tools that exploit natural language processing (NLP) compared to a conventional search engine, e.g., unlocking buried information [25-27], and these are summarized in Table 2.

WellAI

WellAI has developed a Machine Learning (ML) search and analytics tool, based on four neural networks and incorporating the complete list of NIH medical categories [Unified Medical Language System (UMLS)] semantic types, for interrogation of the CORD-19 Dataset and this is available at https://wellai.health/covid/ [16]. It is now widely agreed that ML has significant applications in the physical and biological sciences [28]. In the WellAI COVID-19 application, a subset of ML -- *i.e*. neural networks – is being used. Neural networks facilitate discovery of highly complex and nonlinear relationships between sets of variables without having to search for a closed form mathematical solution. Neural networks can contain tens of thousands to millions of variables, and this is the basis of their power. The complexity of relationships neural networks can uncover is difficult to fathom but is enabled by an ever-increasing computing power. Somewhat surprisingly, one of the biggest trends of the past 10 years is the increasing scientific role of neural network models of a language. At first glance it seems counterintuitive that something so qualitative and subjective as language, plays a role in learning about physical or biological sciences, which by their nature strive for precision. However, NLP is set to play a major role in scientific learning over the coming decades, because arguably the biggest 'problem' for scientists today is an ever-growing body of data, which defies any traditional tools of comprehension [29]. For example, the CORD-19 dataset already contains >128,000 articles. Digesting such a vast amount of information quickly can only be done by the NLP methods and can extend beyond capturing "known knowledge" and reveal new information and hidden connections [27].

The WellAI COVID-19 application uses NLP neural networks to 'learn' from the CORD-19 dataset in order to summarize existing knowledge. It can also be used to make discoveries in an unsupervised manner. This application is based on unsupervised learning [19, 20], but its main goal is to enable a researcher to generate ideas for the next set of concepts that are relevant to the discovery. The UMLS concepts are used as variables in the model and these concepts provide a vast terminology. Crucially, they deal with synonymy, and by including all of the synonyms, the number of UMLS concepts increased to 4,224,512! Only 60,892 concepts are used in the WellAI COVID-19 model, grouped into 69 categories (or UMLS semantic types). Broader WellAI models are based on >25 million medical articles and use millions of concepts.

These concepts are a helpful starting point. However, they had to be altered for WellAI models because they are somewhat outdated, specifically when it comes to the terminology surrounding the novel coronavirus. The altered concepts were applied to the CORD-19 dataset. This whole process was not trivial because application of concepts requires context. Different words can mean different things in different contexts. Complex ML models sensitive to the context of an article needed to be developed. A series of WellAI neural network models have been utilized to learn relationships between medical concepts. Relationships of any single concept to a

Table 2 Comparison of machine learning tools based on NLP and a conventional search engine

	AI-powered search tool based on NLP (e.g., WellAI)	A publication search engine (e.g., PubMed)
General objective	Neural networks summarize, generalize and predict relationships	Searches for key words and phrases in an article. Cannot make conclusions about relationships.
Synonyms (correlated concepts)	Understands synonyms and correlated concepts. For example, understands that "hypertension" is a synonym for "high blood pressure" and "elevated blood pressure". This knowledge helps build more accurate relationships between concepts.	The results produced match the search words or phrases, without knowledge of synonyms and related concepts.
Result aggregated and summarized?	Yes. Every single concept suggestion is based on a large number of articles.	No. The result is a list of articles that contain the key words or phrases.
Output & next step	A structured list of concepts with ranked probabilities. This narrows the scope of work and results in greater efficiency. Focus on concepts of interest and exploration of relationships - not only between concepts (*e.g.,* COVID-19 and Diagnostics Radiology), but between clusters of concepts (*e.g.,* COVID-19 + Diagnosis, Clinical + Diagnostic Tests and Diagnostics Radiology)	A list of every single occurrence (*i.e.*, every article) of a word or a phrase. Read the articles (time consuming), summarize, and make generalizations.
Example	Starting with "COVID-19" as the pre-selected concept, selecting "READ ARTICLES" corresponding to "Diagnosis, Clinical" produces a list of articles in which the machine learning models have determined there is a relationship between COVID-19 and clinical diagnosis, and not just the whole list of articles that mentions both COVID-19 and clinical diagnosis. In addition, the models know there is a difference between clinical diagnosis and diagnosis.	The result for search terms "COVID-19" and "clinical diagnosis", is a list of all articles that mention "COVID-19" and "clinical diagnosis" irrespective of whether there is a relationship between the two phrases mentioned in the article. For example, hypothetically speaking, the article may not be about clinical diagnosis at all, the phrase "Clinical diagnosis" may be just mentioned in the References section.

set of concepts along with probabilities (strength of the relationship) is routine. However, it is more difficult to work with a group of concepts as inputs, especially if the number of variables is not constant. A researcher may use any number of concepts as a starting point of their research and a model was developed that can accept any number of concepts as inputs and update predicted related concepts, along with actionable probabilities.

At a practical level, searching the CORD-19 Dataset using the WellAI tool begins with the results of the initial analysis, based on COVID-19 and SARS Coronavirus as the preloaded concepts, and this produces a list of 69 concept categories. Each concept category has an associated list of concepts, ranked according to their significance in relation to COVID-19 based on log probability (or negative log likelihood loss) [30] of the strength of the concept relationship to COVID-19, according to the WellAI neural networks.

For clinical diagnostics there are several relevant major concept categories in the list, including: "Diagnostic Procedure"; "Laboratory Procedure"; "Laboratory or Test Result".

Associated with each major concept category is a list of related concepts, each linked to relevant publications ("READ ARTICLES"). The search can be refined by adding any of the concepts to the "Selected Concepts" list. A rerun of the search ("Find by selected concepts" option) produces the new lists of concepts that are most related to the new list of "Selected Concepts" (Figure. 1).

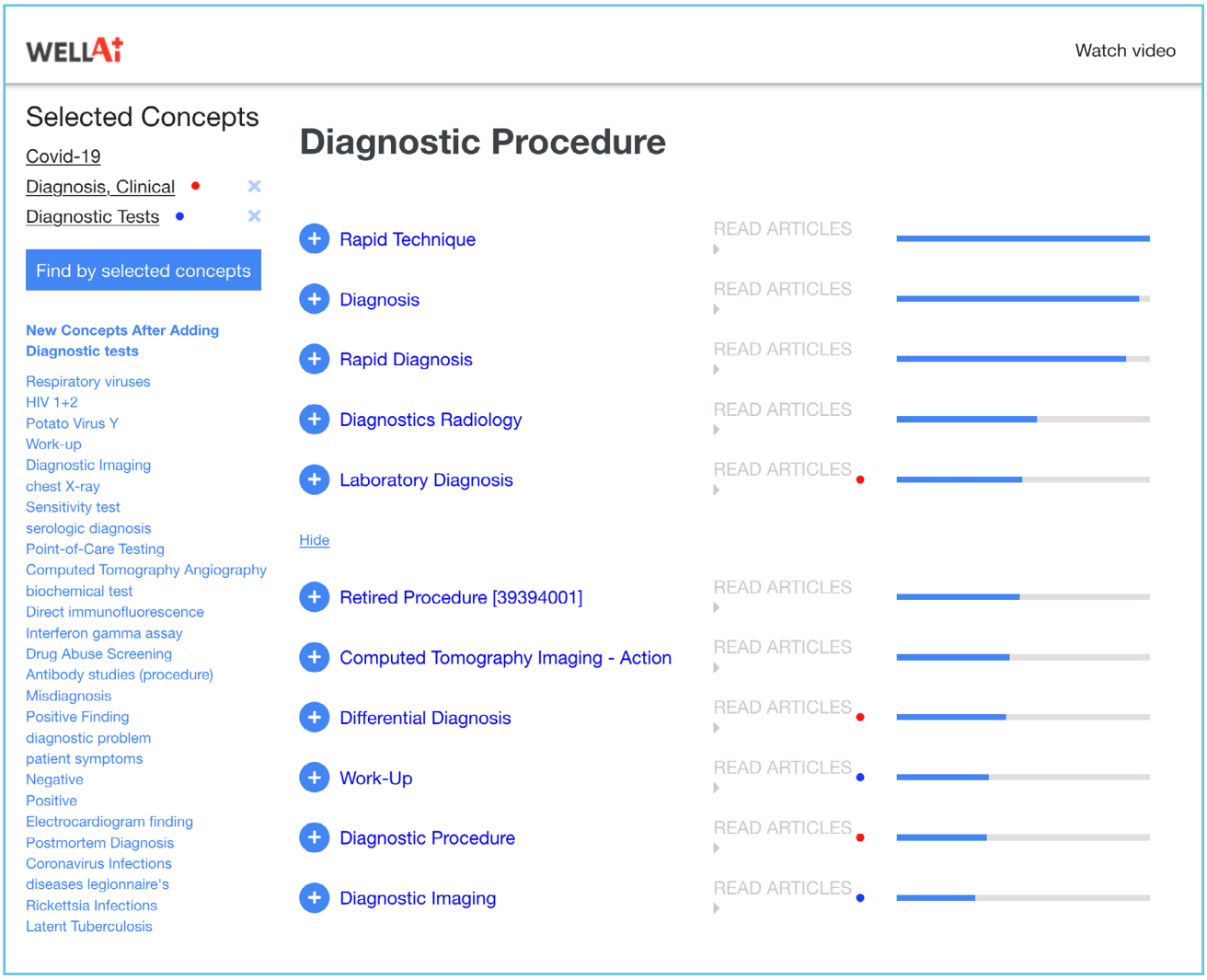
Underlying this AI-powered tool is a network of servers that make the searching quick and seemingly effortless. Significantly, most of the questions in Table 1 could be answered by the WellAI COVID-19 tool by entering a concept (e.g., transmission mode) or looking at the relevant concept category (e.g., "Gene or Genome" for virus genetics and virus origin question).

SciSight

SciSight is an AI-powered visualization tool for exploring associations between concepts appearing in the CORD-19 Dataset and visualizing the emerging literature network around COVID-19 [17-19,31]. It is available at: https://SciSight.apps.allenai.org/ [17]. SciSight is based on SciBERT, a pretrained language model, trained on a large corpus of scientific publications, to provide improved performance in natural language processing [32]. Initially, SciSight provides four different search options, namely, two scientific concepts that are important to the study of the virus, "Proteins/genes/cells" and "Diseases/chemicals", and a "Network of Science" search and a "Faceted search".

The user can explore associations between either of two preselected scientific concepts – "Proteins/genes/cells" or "Diseases/chemicals" in the CORD-19 Dataset as follows. Selection of one of the preselected concepts displays the "Try:" list below the search box, and this lists salient keywords with high relevance to SARS-CoV-2. There is also a graphical display of the network of associations between the preselected scientific concept and the top related terms mined from the Dataset. The thickness of the edges signifies that terms are co-mentioned more often in close proximity to each other in publications in the database. Clicking on an edge reveals the list of linked full text papers and hovering over a term reveals co-mentioned terms. This is illustrated in Figure 2 for the associations between the preselected concept "diseases/chemicals" and the key words "virus infection" selected from the "Try:" list. Alternatively, one of two preselected scientific concepts can be chosen, and a search term entered. This generates and displays a list of autocompleted search suggestions. Selecting one of these suggestions again displays the network of top associations in the dataset.

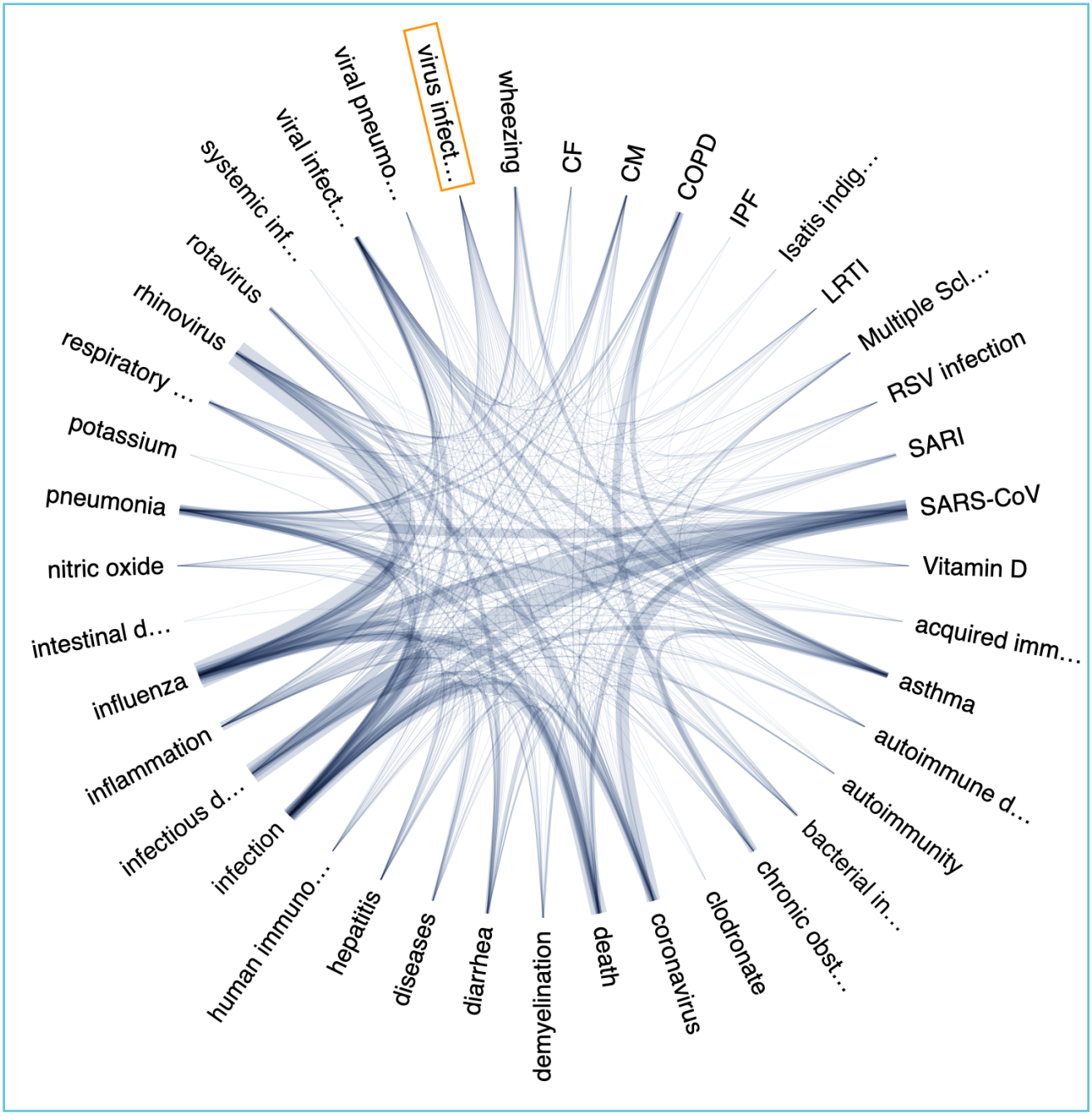
Figure 1 **Example of WellAI search results for the combination of three concepts - "Covid-19" and "Diagnosis, Clinical" and "Diagnostic tests"**



The column to the left shows the list of new concepts resulting from the inclusion of the last item in the list of "Selected Concepts". A part of the results is shown to the right and shows one of the categories ("Diagnostic Procedure") and the related concepts. Each related concept has a link to the relevant articles in the CORD-19 Dataset, and each is ranked by relevance to COVID-19 (depicted by blue bars on the right), where relevance is represented by log probability (or negative log likelihood loss) of the strength of the concept relationship to COVID-19, according to the WellAI neural networks. (Reproduced with permission from WellAI).

A "Network of science" search option allows the user to visualize research groups and their ties in the context of COVID-19. Searches can be by "Topics", "Affiliation" or "Authors" or by the seven preloaded topics in the "Try:" list. Multiple combinations of "Topics", "Affiliation", or "Authors" can be selected. Results are shown as a network of boxes that are color coded from high to low relevance. Each box shows top authors, top affiliations and top topics in a group, and the color-coded links between boxes reveal shared authors or topics. Selection of a box provides a list of publications relating to the contents of that particular box. Also, results are ranked within each topic category (e.g., "Author") by means of a shaded bar.

Figure 2 **Example of SciSight search results for combination of "diseases/chemicals" associated with "virus infection"**



Top related terms are indicated along the edges of the network. Lines denote the associations of the two concepts in the network. (Reproduced with permission from the Allen Institute for AI).

Another search option is "Faceted search". This reveals how authors and topics interact over time in the context of COVID-19. Searches can be made by selecting combinations of Author, Co-author, Characteristic, Intervention, Outcome, Journal, License or Source, and/or by selecting one of seven preloaded topics in the "Try:" list. Multiple combinations of Topics, Affiliation, or Authors

can be selected. Results are ranked within each topic category (e.g., Author) by means of a shaded bar and a list of relevant publications and a graphic shows the number of papers per year.

DIGITAL CONTACT TRACING

Population-wide datasets are now emerging that show the response of society to COVID-19. The data includes commonly used terms in internet search engines, satellite mapping data of human activity and the emerging interactive data from digital contact tracing. Contact tracing is an essential monitoring process for combating the spread of an infectious disease [19-21]. It comprises three basic steps: 1) Contact identification; 2) Contact listing; and, 3) Contact follow-up - and it forms one part of the "Test, Trace and Quarantine" mantra. Conventionally, contact tracing is a manual process relying on finding individuals who have tested positive, and then interviewing those individuals to identify all individuals who need to be quarantined. The widespread availability of mobile communication technology (*e.g.,* smartphones) is providing new ways of enabling contact tracing by using Bluetooth to track nearby phones, keep logs of those contacts, and to warn people about others with whom they have been in contact. In the digital age, contact tracing can be passively achieved and integrated with diagnostic testing results. On an individual level, the actions can be bi-directional. An individual can test positive and then initiate a cascade of notifications of all recent contacts. Alternatively, an individual can be notified that they were in Bluetooth proximity to an anonymous person who has tested positive. Public health authorities empowered with digital tracing can quickly identify positive contacts with a minimal workforce.

In the US, Apple and Google are collaborating on tracking technology for iOS and Android smartphones [33]. Elsewhere in the world, an example of a contact tracing app is TraceTogether which has been deployed in Singapore [34,35]. If a person is found to be positive for COVID-19, then the app uses a smartphone's Bluetooth network to notify every participating TraceTogether user that person was within 2 meters of for more than 30 minutes.

In China, the Alipay Health Code on the Alipay app dictates freedom of travel based on three categories: green for unrestricted travel, yellow for a seven-day quarantine, and red for a two-week quarantine [36]. In South Korea, people receive location-based emergency text messages from the government to inform them if they have been in the vicinity of a confirmed case of COVID-19 [37]. In Italy the app "Immuni" [38,39] combines a personal clinical diary and contact tracing. Anonymous identification codes are generated by the user's app rather than a central server in order to improve privacy. By placing identification on the individual user's device, the contact tracing information is separate from identification. The App complies with the European model outlined by the PEPP-PT (Pan European Privacy-Preserving Proximity Tracing) consortium [40]. It is delivered for free and on a voluntary basis. There has been resistance to app-based monitoring [39], but the Italian government expect 60-70% of people will download the app. In the UK, a contact tracing app (NHS COVID-19) is currently being trialed in a limited geographical area with a population of ~140,000 [41]. This app registers duration and distance between devices and the data is fed into a centralized system where a risk algorithm estimates infection risk and triggers notifications.

Other examples of pandemic data infrastructures include the Google tool, COVID Near You, to identify patterns and hot spots by location (zip-code) [42]; COVID Trace [43] that warns of exposure to COVID-19 by comparing your locations over the previous 3 weeks against the time and locations of reported exposures; CoronApp, which provides localized, real-time data about

COVID-19 based on the geographic location of their smartphones [44]; and a hashtag tracking tool for the evolution of COVID hashtags on Twitter (>628 million tweets about COVID-19) [45]. Twitter is also being used to understand the impact of COVID-19 (e.g., psychological impact) [46]. One significant concern over digital contact tracing has been ethical issues (e.g., privacy) and the consequent impact on the rate of adoption of the apps [47,48]. Some technology developers are focused on developing tracing apps that ensure privacy protection [49].

Currently, in response to COVID-19, clinical laboratories and the IVD industry are grappling with test development, test validation, fast-track clearance (e.g., Emergency Use Authorization) [50], availability of analyzers, tests and related supplies, and testing capacity for both molecular tests for SARS-CoV-2 and tests for IgM/IgG antibodies against this virus [51,52]. Once these issues have been resolved, the next major hurdle will be contact tracing to reduce the risk of future outbreaks. AI-powered tools will be valuable to identify trends and associations between digital contact tracing, tests and outbreaks of disease.

CONCLUSIONS

Easily accessible AI-powered tools and databases are valuable in all types of research, but especially so, in the context of the urgent diagnostic and therapeutic challenges presented by the COVID-19 pandemic. It is hoped that the new AI-powered search tools will accelerate research and development in COVID-19 as the world strives to develop efficient and timely testing and effective therapies to combat this disastrous pandemic. Another important part of our fight against COVID-19 will be efficient digital contact tracing enabled by mobile communication technology linked with massively scaled-up testing as outlined in the recent "Roadmap to Pandemic Resilience" [53].

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Reference value for serum zinc level of adult population in Bangladesh

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ABSTRACT

Background

Zinc is an essential trace element that has an enormous role in regulation of physiological processes whose deviant value leads to malfunction in the body. So, establishing a country specific reference value is needed to serve as a standard for the interpretation of laboratory results during clinical decision making.

Objective

The objective of this study was to determine the reference value of serum zinc level of adult population in Bangladesh.

Materials and methods

The overnight fasting blood was collected from 154 apparently healthy individuals aged 18 to 65 years,

from a rural community after considering several criteria. Graphite furnace atomic absorption spectrophotometry (GF-AAS) method was used for serum zinc analysis. The 2.5th and 97.5th percentiles of zinc level were calculated for the reference value according to the recommendations of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

Results

The estimated reference range of serum zinc level in sample population was 60-120 µg/dL, where the range was 59-125 µg/dL for male and 50–103 µg/dL for female. Significant differences of serum zinc level between male and female ($p<0.001$) was observed. However, there was no significant correlation between age of the respondents and serum zinc level ($r=0.110$, $p>0.05$).

Conclusion

The estimated reference range for serum zinc level in adult population of Bangladesh can serve as a useful indicator for clinical decision making.

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INTRODUCTION

Zinc, an inorganic micronutrient, has significant effects on the metabolism of proteins, lipids and carbohydrates through enzymatic activity. Moreover, it has effects on growth regulation, sexual maturation and function, taste acuity, psychogenetic function, maintaining epithelial integrity, wound healing, and immunity (1, 2).

Both deficiency and excessive accumulation of zinc might result in multiple dysfunctions in the body (3). In this context, a reference value helps physicians for screening the serum zinc status and making a clinical decision (4). Age, gender, ethnicity, geo-chemical factors and altitude of the population have influence in fixing the reference value (4, 5, 6). The reference values used in most of the laboratories of the developing countries are obtained either from the values of scientific literature or manufacturers' manuals (7), which is also applicable for Bangladesh. The International Federation for Clinical Chemistry and Laboratory Medicine (IFCC), and the Clinical and Laboratory Standards Institute (CLSI) recommended that every laboratory should have fixed reference values for bio-analytes (8). Thus, the study aimed to determine the reference value of serum zinc levels in Bangladeshi adult population of a selected rural area.

MATERIALS AND METHODS

Study area

This study was conducted in Sirajdikhan, a subdistrict of Munshiganj district of Bangladesh, located approximately 29 km southeast of the capital city Dhaka. This agro-based area is about 180 km^2 with the geographical extension of 23°30' and 23°41' latitude, and 90°14' and 90°27' longitude. The inhabitants of this region have limited food diversity and mostly rely on rice and vegetables with supplemented fish or meat (9).

Study participants and data collection

Around 200 healthy controls from another project on tuberculosis were considered as participants of this study (Author's manuscript, unpublished). These healthy controls were selected from a community during November to December 2012 considering several selection criteria. Participants with a known history of malabsorption syndrome, nephrotic syndrome, tuberculosis, chronic liver disease, diabetes mellitus, neoplasm and chronic renal failure were excluded. In addition, participants having a history of blood transfusion within the last three months, hospitalized in the past month, participants on zinc medication, pregnant and lactating women, and women taking oral contraceptive pill were also excluded. A semi-structured

questionnaire was administered to collect data which included age, sex, socioeconomic status, educational level and food habit of the participants. Informed written consent was obtained from each participant before the interview. After overnight fasting, 6 ml blood was collected aseptically via anti-cubital venepuncture to estimate serum zinc, serum albumin, serum creatinine and blood glucose level. Serum creatinine, serum albumin and fasting blood sugar were done in order to exclude respondents having chronic renal disease, chronic liver disease and diabetes mellitus, respectively. Among all the participants, seven participants had elevated serum creatinine value (>1.4 mg/dL), five had low serum albumin (<3.5 gm/dL) and another five had elevated blood glucose levels (≥7.0 mmol/L) (10). Chest radiography and Mantoux test (MT) were done for the exclusion of tuberculosis. Moreover, 22 participants were excluded due to haemolyzed blood samples through visual inspection. Finally, data from 161 participants were considered for analysis.

Determination of serum zinc level

Prior to sample collection, all plastic wares were made free from metallic contamination. The screw capped plastic tubes were opened and immersed in detergent water for at least half an hour, washed thoroughly in running tap water and then air-dried. These tubes and caps were later immersed sequentially in diluted nitric acid (20% v/v) for 24 hours, rinsed thoroughly and washed thrice in de-ionized water. Afterwards, the tubes and caps were again air-dried and capped. Then the screw capped plastic tubes were stored in capped plastic container and used only once. About 2 ml blood was collected and transferred immediately in clean deionized screw capped plastic tubes. The samples were transported from the study area to the laboratory on the same day. A cool ice box with liquid nitrogen containing ice pack was used for transporting the tubes.

Serum was obtained from blood after centrifugation at 1300-1350 g force for 5 minutes. The serum was kept in clean metal free polypropylene tubes and stored at -20^{0}C until analysis. It was subjected to a single freeze-thaw cycle at the time of analysis. The tests were done at the Department of Biochemistry, Bangabandhu Sheikh Mujib Medical University (BSMMU) by graphite furnace atomic absorption spectrometer (GFAAS) (Shimadzu model 6650; 1996, Japan) (11) equipped with deuterium (D_2) lamp background correction system (12). A zinc hollow cathode lamp was operated at an 8-mA (milliampere) intensity with a spectral width of 0.5 nm and the selected analytical wavelength for zinc was 213.9 nm. All samples were diluted (1:50) using diluent (matrix modifier- $MgNO_3$ 10ml: Deionized water 90 ml) and analysed in triplicate.

The linearity of the calibration curve was in strong positive correlation (r=0.9993). The lowest limit of detection of serum zinc in the current method was 250 ppb (parts per billion) (25.0 µg/dL). The inter-assay CVs of serum zinc were within 5% for normal range and within 10% for pathological range. The level of serum zinc was expressed in µg/dL from ppb value.

Ethical consideration

The study protocol was approved by the Institutional Review Board of BSMMU, Dhaka, Bangladesh (2012/12497) in accordance with the Declaration of Helsinki.

Statistical methods

The normality assumption of serum zinc level was done by Shapiro-Wilk test (W value=0.881, p<0.000) and found not in Gaussian distribution. Tukey's method was then employed to remove outliers which reduced the sample size to 154 from 161 (13). A graphical presentation of the data distribution on serum zinc level before and after outlier removal is depicted in Figure 1.

Recommended statistical approach of IFCC–CLSI was used to estimate the reference value of serum zinc (8). Reference values of serum zinc level for male and female were estimated by Robust method due to inadequate sample size (8, 14).

Descriptive analysis was done on respondents' socio-demographic variables. Frequency and percentages were estimated as summary measures for the categorical variables and arithmetic mean, standard deviation, median and percentile were used to describe the continuous variables. The measure of association for the categorical variables was calculated by chi-square test, while Mann-Whitney U test and ANOVA for continuous variables. Spearmen correlation was used for correlation of the continuous variables.

Principle component analysis was adopted to measure the wealth index based on the household characteristics: type of wall, roof, floor and toilet; utilities: cooking fuel, source of light and water; ownership of the house; and crowding index (15).

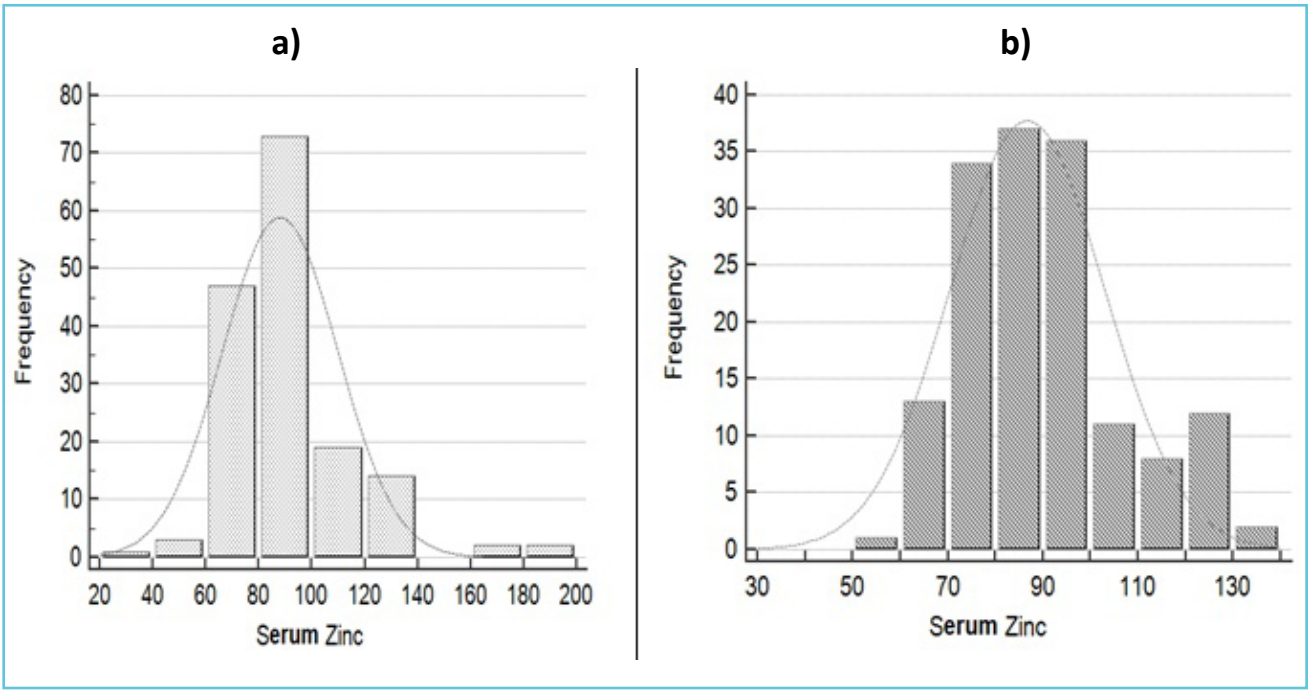
The socio-economic status (SES) was categorized as lower, middle and upper based on the wealth index (16). Descriptive analysis was done by SPSS version 24 (Statistical Package, Chicago, USA), and "reference Intervals" of R Programming language (R Software version: 3.51) (17) was used to determine outlier and reference interval. A p value of less than 5 percent was considered as significant.

RESULTS

Socio-demographic profile and zinc value

Among the 154 healthy participants, ages 18 to 65 [mean (SD) age 33.2 (11.9) years], 53 percent were male, and 47 percent were female. Approximately 50 percent of the respondents were normal in weight [BMI mean (SD): 22.2(1.9) kg/m^2], while 40 percent respondents were overweight [BMI mean (SD): 26.9 (1.4) kg/m^2]. However, no significant weight difference was found between male and female respondents.

Figure 1 Normal distribution curve on a) original data of serum zinc values (µg/dL) (n=161) and b) that of after outlier treatment (n=154)



Around three fourth of the respondents consumed meat 1-3 times, 60 percent consumed fish 4-6 times and half of the respondents consumed egg 1-3 times on a regular week, while the food consumption behaviour of male and female was almost identical (p value>0.05). Among all respondents, 16.9 percent belonged to lower SES, 42.2 percent to middle SES and 40.9 percent to upper SES.

The mean (SD) value of serum zinc level was 85.5 (16.0) µg/dL. This value showed significant difference between male [mean (SD): 92.0 (15.3) µg/dL] and female [mean (SD): 78.0 (13.8) µg/dL, p value<0.05]. However, serum zinc level had no significant correlations with age (spearman correlation, r=0.110; p>0.05) and BMI (r=-0.052; p>0.05).

The reference value of serum zinc level among the adult participants of this study was 60-120 µg/dL. The estimated reference intervals of serum zinc level for both genders are summarized in Table 1.

Comparison with published studies

A comparative picture of serum zinc level of different studies conducted in various countries is shown in Table 2.

DISCUSSION

A number of factors needs to be considered for determining the reference interval of bio-analytes. In this regard, the study participants were enrolled through accounting several selection criteria, and also controlling intra-individual variation by specimen collection timing, fasting duration and exercise restriction. Quality control was also ensured in sample collection, storage and analytic procedures. Finally, an appropriate statistical method, with outlier treatment, was followed for the measurement of reference interval of the bio-analytes (18).

The notable finding of the present study, considering the reference range of serum zinc in the normal adult population, was found as 60-120 µg/dL, after taking the necessary precautions in order to avoid the potential pre-analytical contamination while collecting samples, storing them and preparing for instrumental analysis. The scarcity of data on the standard reference range of serum zinc in Bangladesh makes the finding incomparable in the local context. In comparison to the finding of the current study, several other studies revealed almost similar reference ranges of serum zinc level (19–22) while few studies also reported inconsistent findings

Table 1 Measured reference intervals of serum zinc value (µg/dL)

Population categories	Median	2.5th – 97.5th percentile	90% CI of lower limit (2.5th percentile)	90% CI of upper limit (97.5th percentile)
Total (n=154)	85.0	60-120	55-65	120-135
Male (n=83)	90.0	59-125	54-63	118-131
Female (n=71)	75.0	50-103	43-54	97-110

P-value for male vs. female: <0.0001 (Mann-Whitney U test);
Reference interval calculated using Robust algorithm and confidence intervals (CI) calculated by bootstrapping, R = 5000.

Table 2 Review of reference values of zinc concentration in various countries

Reference	Country	Sample	Age (year)	Analytic Method	Statistical parameter	Zinc level (µg/dL)#
Present study	Bangladesh	154	18-65	GF-AAS	P2.5-P97.5	60-120
Alimonti et al (19)	Italy	110	20-61	ICP-AES	P5-P95	59.7-102.8
Forrer et al (20)	Switzerland	110	-	ICP-MS	P5-P95	63.7-100.4
Rahil-Khazen et al (21)	Norway	141	21-87	ICP-AES	P2.5-P97.5	71.26-108.53
McMaster et al (22)	Northern Ireland	499111	25-64	AAS	P5-P95	Survey 1: 60.82-96.13 Survey 2: 66.05-111.18
Hussain et al (23)	Pakistan	450	20-29	Flame AAS	P2.5-P97.5	75.01-240.14
Abiaka et al (24)	Kuwait	560	15-80	Flame AAS	Range (mean±2SD)*	59.50-151.03
Rükgauer et al (25)	Germany	68	22-75	AAS	mean±2SD	108.53±40.54
Grandjean et al (26)	Denmark	200	-	Flame-AAS	P2.5-P97.5	53.61-102.65

P = percentile;
 # All zinc levels were calculated in µg/dL by conversion factor. (1 µg/dL = 0.153µmol/L or 1 µg/L= 10 µg/dL);
** Range from frequency distribution plot;*
ICP-AES: Inductively Coupled Plasma Spectrometry with Atomic Emission;
ICP-MS: Inductively Coupled Plasma–Mass Spectrometer;
GF-AAS: graphite furnace atomic absorption spectrometer.

(23–26). Grandjean et al (26) reported lower reference range of serum zinc level than the present study, but higher reference range was also reported by some other studies (23, 25). Different results of serum zinc reference level are subjected to variation of laboratory techniques (23, 25, 26), statistical procedures (20, 25) and eco-environmental factors.

The mean serum zinc concentration was statistically higher in male (about 18%) than female ($p<0.001$). In congruent with this study, male gender was reported to have more zinc concentration than female (19, 24). As an explanation, high concentration of zinc was reported in the prostate gland and semen to maintain normal physiology of sperm (27). This condition makes

adult men 3 times more recommended to zinc consumption than women (28). On the contrary, few studies found no significant difference in respect of gender (24) or opposing higher zinc value in female (29).

A non-significant correlation between zinc values and participants' age was observed in this study (r=0.110; p>0.05). Analogously, the similar non-linear relationship was also detected by McMaster et al (22). However, Li et al found an increasing tendency of serum zinc in different age groups (30). Similarly, Zhang et al also reported increased value of serum zinc in subjects older than 50 years, but decreasing tendency before the age of 50, with any unnoticeable change of zinc value in different age groups (31). Another study even showed descending zinc values with ascending age (21).

This study warrants following limitations: the result of this study is difficult to generalize as the samples were taken from a selective rural area. After gender partitioning, the sample size became smaller. So, the gender specific reference ranges may not be representative for the whole population. However, the study findings recommend the importance of measuring reference values of every biochemical analytes with larger sample size ensuring proper quality. The study also recommends reduction of dependency on literature or test kit reference value for clinical decision making.

CONCLUSION

The result of this study provides important information about the reference value of serum zinc in adult population of Bangladesh.

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Blood lead levels in rag-pickers of Kathmandu and its association with hematological and biochemical parameters

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ABSTRACT

Introduction

Lead poisoning is a common health problem in Nepal and there are a limited number of studies on blood lead levels in various population groups. Rag-pickers are those people who visit from house to house to collect the materials that can be recycled and thus earn their livelihood. The present study was designed to evaluate blood lead level (BLL) and its relationship between hematological and biochemical parameters in rag-pickers working in Kathmandu.

Methods

An observational cross-sectional study among 50 rag-pickers working in the selected area of Kathmandu was done in May 2019 after obtaining ethical approval from the Nepal health research council. Capillary and venous blood was drawn from each participant after written consent to measure the BLL, aspartate aminotransferase, alanine aminotransferase, total

bilirubin, creatinine, glucose and to test for a complete blood count. Whole blood was also screened for the presence of hemoglobin variants in cases with abnormal red blood cell indices. Data was analyzed using SPSS (Version 20.0).

Result

All rag pickers were men with mean age of 32.56 ± 12.51 years. The mean BLL among rag-pickers was 11.6 ± 7.23 µg/dL. High eosinophil count was found (8.27 ± 5.49 %) in 27 cases (54%) having no significant association with BLL. The mean BLL was higher (12.89 µg/dL) in a cohort of workers who pick and recycle electronic waste. Beta-Thalassemia trait was seen in four cases, all of them had high BLL. No significant association of BLL with the number of years worked by rag picker was found. Similarly, no significant association of BLL with hematological and biochemical parameters was found.

Conclusion

Rag-pickers working in Kathmandu are at increased risk of lead toxicity. The use of protective gloves, masks, shoes and clothes along with a regular medical examination of this vulnerable group is recommended.

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INTRODUCTION

Lead poisoning is a common health problem in Nepal. The sources of lead exposure in Nepal include the combustion of petroleum products, occupational exposure from paint industry, cosmetics, ayurvedic medication and environmental pollution. A study done in Nepal found that 76% of the tested paints contained lead at concentrations greater than 90 parts per million [1].

A limited number of studies have been conducted to estimate the BLL in various population groups in Nepal. One such study found the mean blood lead level of 20 µg/dl in school children in an industrial area of Nepal [2].

In Kathmandu, there is a significant increase in the generation of solid waste due to the increase in urbanization. These wastes from the house, that can be recycled, are sold to the rag pickers. The purchased commodities are then taken to the collection center where they are processed and are taken to the industrial areas in Nepal as well as in India. This way, rag-pickers contribute to solid waste management in Kathmandu.

The types of waste materials rag-pickers buy are metals, batteries, bottles, old furniture, old electric equipment and papers [3]. These wastes have good resell value. Due to the nature of these waste materials, rag-pickers are at occupational risk of various health hazards. Various studies have been done in neighboring India to identify the health impairments in rag pickers [4, 5].To the best of our knowledge, there is no data in the medical literature about BLL in rag pickers.

Exposure to lead can have various health effects in human beings, including impaired hemoglobin synthesis, chronic damage to the gastrointestinal system, joints, reproductive system, kidneys and nervous system [6, 7]. Children are prone to also developing neuropsychological effects [8].

Given the fact that there are limited studies on blood lead levels in various population groups in Nepal, a study of blood lead level in rag-pickers will help in monitoring and raising awareness.

The current study was done to evaluate blood lead levels in rag-pickers working in the selected area of Kathmandu valley.

The correlation of BLL with various hematological and biochemical parameters was also performed. Monitoring of BLL in this vulnerable group of people could help in planning further clinical studies.

METHODS

Study population

The cross-sectional observational study was carried out in the 50 rag-pickers working in (Balaju) northwest area of Kathmandu. Sample size was calculated using the expression for sample size, i.e., n= 4 pq/l^2 where, p denotes the prevalence of 2.8 % and q as (1-p) for lead toxicity among labourers with total allowable error (l) as 0.05 and standard normal variate as four. [9]. Ethical approval to conduct the study was taken from Nepal Health Research Council (Reg. no. 326/2019) and local government body (ward office) in the selected area. Sample was collected from those participants who were available at the time and venue, mentioned in the formal notice issued to the rag pickers collection centre. Written consent was obtained from each participant before starting the sample collection. The predesigned data collection form was filled up by the investigator after asking a question to the participants and examining them. The form included demographic data such as age, height, weight, the number of years worked as rag pickers and types of waste they were collecting. Similarly, the clinical information in the form included blood pressure and general medical findings, past medical history and smoking/tobacco/alcohol consumption history.

Sample collection

Capillary blood was collected on-site and blood lead level was measured immediately within 30 minutes. Fasting blood, 10 ml in volume (4ml in plain tube, 3ml in fluoride tube and 3 ml in EDTA K2 tube) was collected from the brachial vein under aseptic condition, and then transported to the Samyak Diagnostic Pvt Ltd clinical laboratory within one hour under the condition specified by this accreditated laboratory. This sample was used for the analysis of hematological and biochemical parameters.

Measurement of blood lead

Measurement of BLL was done using the Lead Care II instrument (Magellan Diagnostics Inc., N. Billerica, Massachusetts, USA). Lead care II is a CLIA waived point of care system that relies on electrochemistry to detect BLL in whole blood. After mixing the whole blood with treatment reagent, the red blood cells are lysed and lead is released and collected on sensor by the potential applied from analyzer [10]. After 3 minutes the analyzer measures the amount of lead collected on sensor and displays result in µg/dL [10]. The quality control (QC) in the present study was maintained with two levels of controls provided (Table 1). The target range for QC material was specified by the manufacturer. Value at or above 5 µg/dl was considered as an elevated blood lead level.

Measurement of biochemical parameters

Measurement of various biochemical parameters in rag-pickers were taken in an accredited clinical laboratory of Samyak Diagnostic Pvt Ltd, Kathmandu. All the biochemical analytes were measured using the RX Imola auto-analyzer (Randox Laboratories Ltd).

Table 1 Result of quality control for blood lead

QC level	Result (µg/dL)	Target range (µg/dL)
Level 1	9.9	5.5-11.5
Level 2	25.6	21.6-29.6

Table 2 Biochemical parameters and method of measurement

Analytes	Method of measurement
Plasma glucose	Glucose oxidase-peroxidase method
Serum creatinine	Jaffe's reaction
Serum alanine aminotransferase (ALT)	International federation of clinical chemistry (IFCC) method
Serum aspartate aminotransferase (AST)	IFCC method
Serum total bilirubin	Jendrassik and Grof method

Daily maintenance for this auto analyzer was conducted and internal quality control sample from Bio-Rad was run which were found to be within the acceptable range. The biochemical parameter along with its principal of measurement is shown in Table 2.

Measurement of hematological parameters

2 ml of whole blood was used for complete blood count test which includes hemoglobin, red blood cells, white blood cells, platelets and various red blood cell indices such as MCV, MCH, PCV and MCHC. Sysmex automated hematology analyzer XN 330 (Sysmex, Milton Keynes, UK) was used for this measurement. Two-level control material provided by Randox laboratories was used as internal quality control material which was found within a normal range.

Whole blood was also screened for the presence of hemoglobin variants, in cases with abnormal RBC indices (low hemoglobin, high RBC count with low MCV and MCH), with the use of the *Bio-Rad D10* instrument in variant mode using the principle of ion-exchange high-performance liquid chromatography (HPLC).

Statistical analysis

Data was analyzed using the Statistical Package for Social Sciences (SPSS 20, IBM Corporation, New York, USA). Data were normally distributed. Results were expressed as mean ± standard deviation. Pearson correlation was applied to evaluate the association between hematologic, biochemical parameters and blood lead level. 2-sided P value<0.05 was considered statistically significant.

RESULT

All the participants of this study were men. Baseline characteristics along with laboratory parameters in rag-pickers are shown in Table 3.

The mean value of all the biochemical and hematological parameters in rag picker was in normal range except blood lead level (11.68 ± 7.23µg/dL) and the eosinophil count (8.27 ± 5.49 %) both of which were higher than the normal range. In four workers, who also had very high level of blood lead, screening for hemoglobin variants

Table 3 Baseline characteristics along with biochemical and hematological parameters in rag pickers

Parameters	Mean ± SD	Reference range in men
Age (years)	32.56 ± 12.51	
Number of years worked (years)	9.67 ± 7.30	
BMI (kg/m2)	20.12 ± 3.21	18.5 – 24.9
Systolic blood pressure (mm Hg)	115.51 ± 9.33	120
Diastolic blood pressure (mm Hg)	74.73 ± 8.31	80
Blood lead level (µg/dL)	11.68 ± 7.23	Less than 5
Fasting blood glucose (mg/dL)	83.89 ± 27.64	70 – 100
AST (U/L)	27.64 ± 7.98	17 – 59
ALT (U/L)	25.87 ± 12.09	10 – 45
Creatinine (mg/dL)	0.86 ± 0.11	0.6 – 1.4
Total bilirubin (mg/dL)	0.53 ± 1.53	0.3 – 1.9
Hemoglobin (gm %)	15.48 ± 1.53	13.5 – 16.9
Platelet count (G/L)	158 ± 89	150 - 450
Leukocyte (G/L)	7.24 ± 2.01	4 - 11
Basophil (%)	0.6 ± 0.5	Less than 2
Eosinophil (%)	8.27 ± 5.49	1 – 8
Neutrophil (%)	49.13 ± 8.39	40 – 70
Monocyte (%)	7.96 ± 1.98	2 – 15
Lymphocyte (%)	34.13 ± 8.12	20 - 45
MCV (fl)	82.31 ± 1.1	81.8 – 95.5
MCH (pg)	29.03 ± 1.22	27 – 32.3
MCHC (g/dL)	34.11 ± 1.52	32.4 - 35

Table 4 Types of solid waste collected by the rag pickers

Group	Types of solid waste	Number of rag-pickers collecting these items	Mean blood lead level (µg/dL)
1	Paper and metals only	3 (6%)	7.35
2	Plastic, Metals and Paper	6 (12%)	8.23
3	Plastic, Metals, Paper and Paint containers	11 (22%)	8.47
4	Plastic, Metals, Papers, Paint container and electronic waste	30 (60%)	12.89

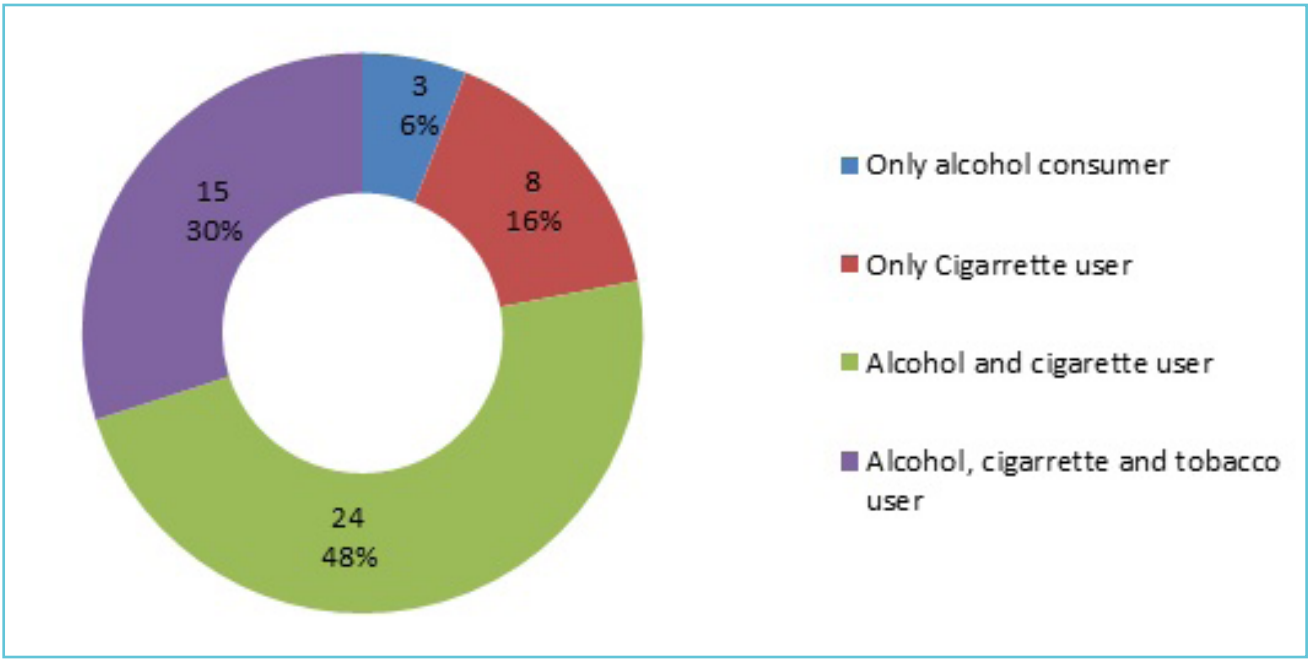
was done because of abnormal red blood cell indices. The minor variant of beta-thalassemia was found in all of them.

Rag-pickers were divided into four groups according to the types of solid waste they are collecting. Mean BLL in each group was calculated as shown in Table 4. None of the workers were found picking only one type of waste.

Smoking, tobacco and alcohol consumption habit in rag-pickers is shown in Figure 1.

When the number of work-years were correlated with the blood lead level using Pearson correlation, no significant association was found (P value=0.556). Similarly, no significant association was found between BLL and other biochemical and hematological parameters.

Figure 1 Pie chart depicting a number of rag-pickers with smoking, tobacco and alcohol consumption habit



DISCUSSION

In this study, the mean blood lead concentration in rag-pickers was found to be higher (11.68 µg/dL) than the cut off value designated by the Centre for Disease Control (CDC). The common items that rag-pickers collect are metals, plastic ware, batteries, paint buckets, tins and glass bottles. It has been reported that metals, household dust and lead-based paints are the potential source of lead exposure [11]. It has also been reported that the majority of rag-pickers in Kathmandu process the collected waste by themselves to get high resell price [5]. This act of processing solid waste possesses a potential risk of lead exposure among rag-pickers because none of these workers use protective clothing.

In the majority of rag-pickers in the present study, high eosinophil count was found. This can be due to allergic skin disease and parasitic infestation from poor hygiene, environmental pollution and overcrowding. High eosinophil count among workers of solid waste management has also been reported from other developing nation [12, 13]. Eosinophilia leads to a potential risk of chronic allergic diseases like dermatitis, asthma, bronchitis, chronic cough in these workers.

All of these workers who had a minor variant of beta-thalassemia were from the tropical part of eastern Nepal or neighboring village in India. In a study published in Nepal, most people with haemoglobinopathies were from tropical region [14]. Our finding is consistent with the report that was published earlier and is important for reproductive age group workers to rule out haemoglobinopathies in their partners that could potentially risk transmission to their newborn children.

The mean platelet count in this study was found to be 158 ± 89 G/L. The low value in mean platelet count is attributed to the intake of non steroidal anti-inflammatory drugs by these workers to relieve their body ache. Thrombocytopenia is also due to alcohol intake by these workers.

The rag-pickers who collect electric appliances along with other commodities were found to have higher mean BLL than the workers who collect only other commodities.

Lead is a major component of electronic equipment such as cathode ray tubes inside computers or television, circuit boards, cables and batteries.

There are a number of studies where significant high BLL was found among residents of electronic waste recycling area [15, 16]. Rag-pickers working in Kathmandu, who collect and process electronic waste, are at increased risk of developing lead toxicity if urgent measures on safe working instructions are not taken.

In the present study, an insignificant association of BLL with biochemical and hematological parameters was found which is attributed to the absence of liver and kidney injury in these workers. A similar finding has also been reported in previous studies [17, 18, 19].

We found the mean BLL of 11.68 µg/dL in rag-pickers sampled in Kathmandu, with the highest value of 38 µg/dL, which is insufficient to derange other laboratory parameters.

It has been found that the heme biosynthesis is decreased and anemia occurs once the lead in the blood reaches the level of 55.0 µg/dL [20]. Renal effects in the form of chronic nephropathy occur at the blood lead level of 60.0 µg/dL [21]. Liver injury in the form of suppression of cytochrome P450 enzyme and an increase in alkaline phosphatase enzyme occur at the blood lead level above 70.0 µg/dL [22,23].

The limitation of the present study is that the concentration of lead in solid waste or in the working environment was not measured. It is essential to investigate the content of lead and other heavy metals in working site of rag-pickers and access the comprehensive health effects in rag-pickers using larger samples.

CONCLUSION

The findings of this study suggest that the rag-pickers working in Kathmandu are at increased risk of lead toxicity and this occupational exposure to lead is attributed to the use of bare hands for processing solid waste. Working instructions with solid waste should be prepared and trained to this vulnerable group.

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Conflict of Interest: The authors declare that there is no conflict of interest in the publication of this manuscript.

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Consent: Written informed consent was obtained from each participant.

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Survey on stat tests in Catalan clinical laboratories

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ABSTRACT

Introduction

The Catalan Association of Clinical Laboratory Sciences (ACCLC) conducted a survey on the vast majority of hospital clinical laboratories in Catalonia. In order to establish a debate on the emergency laboratories and aspects related to the stat tests.

Materials and methods

An online survey was distributed by ACCLC to 69 hospital laboratories in Catalonia. A 30-question survey was designed with 9 different issues. The questionnaire examined general information regarding the hospital and laboratory model, stat laboratory workload, laboratory information system, quality control, critical values results, authorization/validation of results, laboratory report and human resources, among others. The results were reported in number of laboratories and in percentage (%).

Results

The total survey response rate was 59 %. 68.3 % stat laboratories biochemistry, haematology and microbiology

departments were integrated. The majority (60.9%) of the stat tests were integrated in part with laboratory core. All laboratories employed laboratory information system and are using barcode system. In 75.6% of laboratories all requests were made electronically. 43.9% of laboratories did not give results in international system, only in conventional units. All laboratories participated in internal and external quality assessment programs. Internal quality controls are processed more than once a day in 80.5% of laboratories. The vast majority of laboratories reported critical results (97.6%). 75% of laboratories have a medical specialist (biochemistry or analysis). The average number of laboratory technicians was 4.

Conclusions

Our study highlighted the variation in how emergency laboratories and stat test are run across Catalonia.

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INTRODUCTION

The last few decades have seen a significant change in clinical laboratories. The laboratory management information system has allowed improvements with patient identification, turnaround times, manual transcription data, automated procedures for data validation, reporting on critical values, etc., reducing error and improving patient safety.

A great variability exists among different laboratories; each laboratory is a world of its own. There are different emergency laboratory models related to the size and type of hospital or institution in which they are employed. Ordinary and stat tests integrated or separated. Biochemistry, haematology and microbiology departments integrated or independent among them. There are different characteristics of each laboratory: the number of request and tests per day, laboratory information system (LIS), aspects of quality control, reporting and receiving critical values, validations of results, laboratory report, human resources... etc.

Within the scope of the IX European Symposium of Clinical Laboratory and *in vitro* Diagnostic Industry entitled "Stat Tests in Clinical laboratory", Catalan Association of Clinical Laboratory Sciences (ACCLC) [1] conducted a survey on the vast majority of hospital clinical laboratories in Catalonia. In order to establish a debate on the emergency laboratories and aspects related to the stat tests, to know the *state of the art* and new trends on stat tests. Stat (from the Latin statim, immediately, but also considered as an acronym for "short turnaround time") identifies laboratory tests that should be made available within a defined, as short as possible, time according to clinical necessity [2-3]. Stat analytes were tests ordered when the results were in urgent need, typically for patients from emergency department, intensive care unit (ICU) patients whose condition change suddenly, and inpatients with serious diseases or whose condition change suddenly.

Using a national survey, ACCLC has collected information on the workload and roles of different clinical laboratories in Catalonia in order to present a picture of current practice across Catalonia.

MATERIALS AND METHODS

In 2017, an online survey was distributed by ACCLC using Google Surveys tool to 69 hospital laboratories from Catalonia. A 30-question survey was designed with 9 different issues. The questionnaire examined general information regarding the hospital and laboratory model, stat laboratory workload, laboratory information system, quality control, critical values results, authorization/validation of results, laboratory report and human resources, among others. The

questions and format of the survey are provided as supplementary data. The questionnaire was administered in an online format (https://www.google.com/forms/). A web link to the survey was distributed to the laboratory medical specialist responsible for each stat laboratory with an invitation to participate in the survey. The survey link was made available up till April 2017.

The collected information was analysed and the results were reported in number of laboratories and in percentage (%).

RESULTS

There were 49 responses to the online survey, of which 41 were included in analysis of the objective data (59%). Eight responses were excluded as they were duplicates from laboratories already represented in the data.

Twenty-nine respondents represented laboratories in Barcelona province, nine of which were situated in Barcelona. Four laboratories in Girona, three laboratories from Lleida, three from Tarragona, one in Balearic Islands and one in Andorra were also represented.

All respondents were laboratory medical specialists. All stat laboratories were in a hospital setting. There were 8 tertiary hospitals (19.5%) with more than 400 patients per day in the emergency department and there were 21 secondary hospitals (51.2%) with more than 170 patients per day. The majority of hospitals (68.3%, 28 hospitals) involved in this study were teaching hospitals with medical training for residents. 63.4% (26 hospitals) had an intensive care unit. 53.7% were public hospitals. In 28 stat laboratories (68.3%), biochemistry, haematology and microbiology units were integrated. In 10 laboratories (24.4%), biochemistry and haematology units were integrated with an independent microbiology unit. Only two laboratories (4.9%) had the three units unintegrated. In majority of the laboratories (25 laboratories, 60.9%), the stat tests were integrated in part with core laboratory.

The number of daily requests and the number of tests per day can be found in Table 1. The average number of tests per request in the stat laboratories was 8 (range: 4-14).

All laboratories employed laboratory information system. The LIS employed are: Eyra (Laboratori Referència Catalunya®) by 10 laboratories, Servolab (Siemens®) by 7 laboratories, Modulab (Werfen®) by 6 laboratories, Omega (RocheDiagnostics®) by 4 laboratories, Lumen Software® by 3 laboratories, Infinity (RocheDiagnostics®) by 2 laboratories, Link It (Cegeka®) by 2 laboratories, OpenLab (Nexus®) by 2 laboratories, and Indra (GestLab®) and LabSuite® by 1 laboratory each.

Table 1 Stat laboratory workload. Number of laboratories (n labs) and percentage (%)

Requests/day	n labs (%)	Tests/day	n labs (%)
<100	12 (29.3 %)	<1000	13 (31.7 %)
100-300	19 (46.3 %)	1000-3000	20 (48.8 %)
300-500	9 (22.0 %)	3000-5000	6 (14.6 %)
>500	1 (2.4 %)	>5000	2 (4.9 %)

All laboratories delivered the final reports to the hospital information system.

All participating laboratories used the barcode system to ensure accuracy and timeliness of the transmission of test reports of the results of biochemistry, blood gas, and haematology tests. In 31 laboratories (75.6%) all requests were made electronically and in 9 laboratories only part of them were electronical. Only one laboratory processed manual requests.

In the final test reports, 46.3% of laboratories were using international and conventional units and 43.9% of laboratories did not give results in international system, and were using conventional units only. Only 4 laboratories (9.8%) expressed their final test report in the international system units.

All laboratories participated in internal and external quality assessment programs. In stat laboratories, 80.5% internal quality controls are processed more than once a day. The vast majority of laboratories reported critical results (97.6%). Only one laboratory did not report critical values. Table 2 presents survey responses on dealing with critical values. The responses about the authorization or validation of results can be found in Table 3.

Human resources, i.e., stat laboratory staff are shown in Table 4. In majority of stat laboratories there was one medical specialist (biochemistry

Table 2 Critical values in stat laboratories. Number of laboratories (n labs)and percentage (%)

		n labs (%)
How were the critical value limits established?	By literature, laboratory and consensus with clinicians	24 (59.0 %)
	Only by literature	10 (24.4 %)
	By Laboratory and consensus with clinicians	5 (12.2 %)
	Only by laboratory	2 (4.9 %)
Notification procedure	By telephone	28 (68.2 %)
	By telephone & email	11 (26.8 %)
	By telephone & hospital information system	2 (4.9 %)
Responsible for receiving the critical value notification	Clinician or nurse	19 (46.3 %)
	Only clinician	15 (36.6 %)
	Only nurse	6 (14.6 %)
	Administrative staff	1 (2.4 %)

Table 3 Validation of stat laboratory results. Number of laboratories (n labs) and percentage (%)

		n labs (%)
Who performs the validation of patients' results?	Clinical validation by Laboratory medical specialists	1 (2.4 %)
	Technical validation	10 (24.4 %)
	Technical validation & Clinical validation	13 (31.7 %)
	Technical validation & Autovalidation	6 (14.6 %)
	Technical validation & Clinical validation & Autovalidation	8 (19.5 %)
	Other options	3 (7.3 %)
If the validation is not done by laboratory medical specialist, is there a pre-report or a final report of patient results?	Yes (pre-report)	8 (19.5 %)
	No (final report)	3 (7.3 %)

or analysis) (75%) and in some laboratories also there was additionally a microbiology or haematology specialist. The average number of laboratory technician staff was 4 (range: 5-8).

DISCUSSION

This is the first survey ever launched to know the state of the art in stat tests laboratories in Catalonia. The majority of responses were received from Barcelona, leaving other regions of Catalonia relatively under-represented. There are few publications in the literature about laboratory clinical survey results.

Several national surveys have been published on different aspects of the laboratory in other countries: clinical authorization [4], intra-laboratory turnaround time [5] and critical results reporting [6-7] but no survey was as complete as this study.

The analysis of the completed questionnaires reveals a heterogeneous laboratory situation. The tendency is that stat laboratory serves all three units: biochemistry, haematology and microbiology. In general, in small- and medium-sized laboratories, ordinary and stat tests are integrated, and their analysis are performed in the same place using the same instrumentation. The situation in large laboratories is rather more heterogeneous, with the majority of organizations continuing to separate stat from ordinary tests, using different instrumentation, personnel and locations. An intermediate option also exists, in which stat test analyses are semi-integrated in an automated core chain with routine samples, all of which are processed at the same time [1]. In the vast majority of laboratories, the number of stat laboratory orders is 100-300 requests per day and 1000-3000 tests per day, as in most

Table 4 **Human resources. Number of laboratories (n labs) and percentage (%)**

		n labs (%)
Are there laboratory medical specialists only for Stat laboratory? *(If yes: how many?)*	Yes	13 (31.7 %)
	1	7
	2	4
	≥3	2
	No	28 (68.3 %)
Is there a laboratory medical specialist on call 24 hours/day?	Yes	31 (75.6 %)
	Physically on duty	4
	In-house Call	13
	Physically on duty / In-house duty	14
	No	10 (24.4 %)
Are there clinical residents on call 24 hours/day?	Yes	24 (58.5 %)
	With In-house call support	15
	With physically on duty support	3
	Physically on duty / In-house duty support	6
	No	17 (41.5 %)
Laboratory staff constituted by	Laboratory technicians	41 (100 %)
	Laboratory nurses	12 (29.3 %)
	Administrative staff	5 (12.2 %)
Average laboratory staff	Morning: 3-4 people Afternoon: 2-3 people Night: 1-2 people	

emergency laboratories in other countries [5]. Also it revealed that the average stat ordering is 8 tests per request.

All stat laboratories are working with LIS. The most commonly used laboratory information management system was Eyra, implemented in 10 of the laboratories that answered (n=41). Laboratory information system receives, processes and stores information generated by the laboratory workflow. It automates the workflow of all information related to total testing process [2]. It facilitates communication between laboratory and clinicians and ideally, enables faster delivery of patient reports [3].

All stat laboratories surveyed were participating in quality assessment programs. Adequate internal quality and external control assessment are parameters which enhances laboratory quality testing [8]. Despite the recommendations of the IUPAC [9], the international units system is not the most commonly used in clinical laboratories.

The definition and reporting of critical values is an important phase of the clinical laboratory testing process, and laboratories are responsible for detecting life-threatening results, for reporting them to health care providers, and also for tracking and improving the timeliness of reporting and the receipt of results. All participants indicated that they communicate critical values. The criteria for considering test results critical are still controversial, with lack of harmonization both in defining the analytes as well as low and high critical value cut-offs [10]. There is no consensus on the most reliable source of information regarding the list of critical values and clinical laboratories may follow recommendations of scientific societies, clinician' opinions in their institutions with consensus of medical laboratory specialist (59 %).

The reporting of critical values from the laboratory to caregivers is still made mainly by telephone (68.2 %). Less commonly used means of communication included email, SMS or hospital information system. A great variability exists among the professionals involved in critical values communication: in reporting and receiving the data. The vast majority of laboratories notified to physicians or nurses (97.6 %). In Italy [10] the notification is similar but in United States the notification is directed to patients in some cases [11-12]. Guidance from NHS England and the British Medical Association (BMA) acknowledge that the ordering clinician is traditionally responsible for acting upon abnormal results and life-threatening results must be communicated to him [13-14]. Finally, few laboratories have yet adopted a read-back verification of the complete test result by the person receiving the information [7].

The analysis of the validation of results reveals a heterogeneous situation. Currently, each laboratory has different approaches to the challenge of authorization as there is no comprehensive guidance available. The validation process may include a combination of technical, clinical and autovalidation. The best practice guidelines issued by the Association for Clinical Biochemistry and Laboratory Medicine (ACB) [15] acknowledges the impossibility of clinically authorizing every result generated (web). It is impractical and time consuming to clinically authorize every result, but equally the use of technical and auto-validation alone may be over-sensitive to abnormal results. The focus of clinical attention must be on the neediest of patients [16]. Only one laboratory clinically authorized normal results.

24.4 % of laboratories did not have a formal duty specialist on call 24 hours/day. A national survey of practice in the UK shows only one laboratory (1/49) that did not have a formal duty specialist [4]. In vast majority of the laboratories laboratory technicians are non-medical staff. There are fewer nurses in laboratory. Nurses have been replaced by technicians in clinical laboratories.

In daytime rotas there are more laboratory staff than on the night-time rota.

The major limitation of this study is the veracity of the data. Nonetheless, there were a large number of hospitals and laboratories from Catalonia involved in this survey and the database can provide support with suitable information. The survey can be really meaningful and conductive. We expect to expand the scope of our survey to pre-analytical, analytical and post-analytical areas and conduct a more comprehensive survey in the future.

CONCLUSIONS

Our study highlighted the variation in how emergency laboratories and stat test are run across Catalonia. This survey was helpful in order to know the state of the art in emergency laboratories in Catalonia and debate about new trends on stat tests.

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SUPPLEMENTAL DATA: STAT LABORATORY SURVEY

Thank you for participating in this survey. Please complete the following questions about your Stat Laboratory:

I. Type of your center

1. Skills of your center?
   (You can mark more than one option)
   a) Hospital
      a. Teaching
      b. No-teaching
      c. With Intensive Unit Care
      d. Tertiary hospital
      e. Secondary hospital
   b) Non-Hospital
   c) Primary Health Care
2. Number of patients in the Emergency Department for day:
3. Type of center
   a. Public
   b. Private

II. Laboratory model

4. Type of laboratory
   a. Public
   b. Private
5. Stat Laboratory
   a. Biochemistry, Hematology and Microbiology joined
   b. Biochemistry and hematology joined and microbiology independently
   c. Biochemistry, hematology and microbiology independently
6. Stat Laboratory is
   a. Independently from the rest of laboratory
   b. Semi-integrated with routine laboratory
   c. Integrated Core laboratory
   d. Point of care testing

III. Stat Laboratory Workload

7. Number of requests per day
   a. < 100
   b. 100-300

c. 300-500
d. > 500

8. Number of tests per day in Stat Laboratory:
   a. < 1000
   b. 1000-3000
   c. 3000-5000
   d. > 5000
9. Average of tests per request
   a. < 5
   b. 5-8
   c. 8-10
   d. > 10

IV. Laboratory Information System (LIS)

10. The analytical request is:
    a. Electronically
    b. Manual
11. Laboratory information system
    a. Type (Commercial or own laboratory system)
    b. Name of LIS:
12. Connections:
    a. All devices online
    b. Partially devices online
    c. Manual transcription of results
13. Is barcode system used in your laboratory?
    a. Yes
       i. Printed in extraction department
       ii. Printed in the request
       iii. Printed in the laboratory
    b. No

V. Quality control

14. Internal quality control assessment
    a. Once a day
    b. > once a day
15. Does the laboratory participate in external quality control programs?
    a. Yes
    b. No

VI. Critical values

16. Has the laboratory defined critical values?
    a. Yes
       i. By bibliography
       ii. Own laboratory
       iii. Laboratory with consensus with clinicians
       iv. Bibliography, laboratory and consensus with clinicians
    b. No
17. How laboratory report critical values?
    a. By telephone
    b. By email
    c. By SMS
    d. Writing in clinical history of patient
18. Who should receive the critical values results?
    a. Physician who requested the test
    b. Nurse
    c. Administrative staff

VII. Validation of results

19. Who perform the validation of patients' measured values?
    a. Laboratory medical specialist
    b. Laboratory technician
    c. Laboratory specialist + technician
    d. Autovalidation + laboratory medical specialist
    e. Autovalidation + laboratory technician
    f. Autovalidation + laboratory specialist + technician
20. If the validation is not by laboratory medical specialist, does exist a pre-report

of patients results or there is a final report?

a. Yes (pre-report)
b. No (final report)

VIII. Laboratory report

21. Units
    a. International system units (IS)
    b. Conventional units
    c. International system and conventional units
22. Is laboratory report recorded in patient's clinical history?
    a. Yes
    b. No

IX. Human resources

23. Are in stat laboratory medical specialists full dedicated to stat tests?
    a. Yes
        i. How many?
            1. 1
            2. 2
            3. 3
            4. >3
24. What is the specialization of laboratory medical staff?
        i. Clinical chemistry
        ii. Clinical analysis
        iii. Hematology
        iv. Microbiology
    b. No
25. Is there a laboratory medical specialist on call 24 hours/day?
    a. Physically on duty
    b. In-house call
    c. Physically on duty/ In-house duty
26. Are there laboratory clinical residents on call 24 hours/day?
    a. Yes
        i. With in-house call support
        ii. With physically on duty support
        iii. Physically on duty/ In-house duty support
    b. No
27. Laboratory staff constituted by
    a. Laboratory technicians
    b. Laboratory nurses
    c. Administrative staff
28. How many people (no medical specialist) are working in the morning rota?
    a. 1
    b. 2
    c. 3
    d. 4
    e. >5
29. How many people (no medical specialist) are working in the afternoon rota?
    a. 1
    b. 2
    c. 3
    d. 4
    e. >5
30. How many people (no medical specialist) are working in the night rota?
    a. 1
    b. 2
    c. 3
    d. 4
    e. >5

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Critical results reporting in Portuguese hospital laboratories: state-of-the-art

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ABSTRACT

Introduction

This survey aimed to assess the state-of-the-art of current practices on critical results reporting among Portuguese Clinical Pathology Laboratories. The results of the survey will set basis for future standardization and national guideline development.

Materials and methods

The survey was transmitted to 49 Clinical Pathology Laboratories among public hospitals inserted in the Portuguese National Health System. In 27 questions, laboratories were asked about their critical results procedures, critical results list, reporting and further education. Data were analyzed using Microsoft Excel v.2016 and MedCalc Statistical Software version 12.5.0.0 (Ostend, Belgium). Where applicable, the comparison of proportions was used to estimate the level of significance (P<0.05).

Results

The response rate was 44/49 (90%), including 36 participants with a defined critical results reporting proce-

dure. Among them, 31 laboratories defined a critical results list, mainly based on published literature (27/31). There was a statistically significant number of laboratories (P=0.019, 24/30) that report different critical results depending on the patient's age, but regardless of disease, ethnicity and location (P>0.05). The majority of laboratories (60%) report critical results via telephone within 15 minutes. Critical results are usually reported by clinical pathologists to physicians. Twenty-five laboratories periodically re-evaluate their critical results list.

Conclusion

Despite the fact that most of the Portuguese hospitals have a critical results policy, this survey showed high variability among the hospitals concerning critical results reporting practices and critical results list. This survey points out that nationally established procedures and guidelines are urgent step for critical results standardization.

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INTRODUCTION

Critical results of laboratory analyses indicate a high risk of major patient harm or possible death, and require immediate medical intervention and urgent patient treatment (1). These dangerously abnormal laboratory results, also known as "panic" or "alert" values, are first defined by George D. Lundberg and his colleagues in 1972. Currently, the term "panic value" has been abandoned as it represents emotional stress and disables clear communication between laboratory and physicians. However, urgent results need to be distinguished from the critical ones. Urgent results are required by physicians and they need to be processed and reported urgently, nevertheless if they are abnormal or not (2, 3). Management of critical results includes every step between finding out the critical result during laboratory analysis, informing the healthcare personnel responsible for patient care, as well as their appropriate action. Definition of laboratory parameters and their values that should be considered as critical and life-threatening is complicated due to various recommendations and different expert opinions (4, 5, 6). An appropriate definition of critical results and their compliance is needed to ensure patient safety.

Various practices, different terminologies, parameters included in the critical results list and their values, reporting pathways and communication with physicians and other healthcare personnel affect the quality of critical results management (5, 7). Despite many recommendations, it is evident that many aspects such as lack of standardization and quality indicators are still challenging issues in this area (1, 6).

Furthermore, surveys conducted all over the world discovered a lack of harmonized practices, both internationally and within the same country (7, 8, 9, 10, 11). We hypothesized that a similar situation would be in the Clinical Pathology Departments of the Portuguese Public Health System. According to the Clinical and Laboratory Standards Institute (CLSI) GP47 guideline, each laboratory should develop a certain strategy for critical results management (4). To do so, it is crucial to identify current critical points, possibilities for improvement and set basis for future standardization and guideline development. Therefore, we aimed to assess the state-of-the-art of current practices on critical results reporting among Portuguese Clinical Pathology Laboratories.

MATERIALS AND METHODS

In order to evaluate the status of current practices on critical results reporting, a comprehensive survey was created and transmitted to 49 Clinical Pathology Laboratories among public hospitals inserted in the Portuguese National Health System. A survey was sent to all Laboratory

Directors with a deadline for answer and those who did not respond on time were excluded. The survey was conducted between November and December 2018. Questionnaires were distributed by an e-mail and data were collected using Google Forms.

Survey development

A survey composed of 27 questions (multiple choice questions and yes/no responses) comprised essential topics for the laboratory management of critical results – "Characteristics of Participating Laboratories", "Characteristics of Critical Result Policies", "Characteristics Relating to Critical Result Practices", "Analytes Included on Critical Result List" and "Education" as shown in Tables 1-5. The last section ("Education") was aimed to establish the attitude of laboratories regarding further improvement on this issue. Confidentiality was assured to all participating laboratories in order to preserve their privacy rights, although the results were never intended to be presented individually or to reveal the identity of the hospital.

Data analysis

The analysis was carried out using Microsoft Excel v.2016 and MedCalc Statistical Software version 12.5.0.0 (MedCalc Software, Ostend, Belgium). Most data were presented as percentages or ratios when the total number of observations was low. For some proportions, the level of significance has been estimated ($P<0.05$) using the MedCalc statistical test "comparison of two proportions" (e.g. we estimated if there is statistically significant difference between critical result management between accredited and non-accredited laboratories).

RESULTS

Out of 49 pathology laboratories in Portugal, 44 participated in the survey, thus giving the response rate of remarkably high 89.8%. Among them, 36 reported that they have a certain

Table 1 First part of the survey composed questions about the "Characteristics of participating laboratories"

	Questions	Answers
1.	Is your laboratory accredited?	- Yes - No
2.	Does your laboratory report Critical results?	- Yes - No (end of questionnaire)

Table 2 Second part of the survey referred to the "Characteristics of critical result policies"

	Questions	Answers
1.	Does your laboratory have a defined list of critical results?	- Yes - No

2.	How many critical results are included in the critical results list?	- More than 20 - 15 to 20 - 14 to 10 - 9 to 5 - Up to 5
3.	Which was the primary resource for your list of critical results? (more than 1 answer is permitted)	- Published literature/textbooks - Consensus with physician - Manufacturer's recommendation - Internal study of healthy individuals
4.	Are there critical results for different age groups?	- Yes - No
5.	Are there critical results for different populations based on disease type?	- Yes - No
6.	Are there critical results for different populations based on ethnicity?	- Yes - No
7.	Are there different critical results for out- and inpatients?	- Yes - No

Table 3 **Third part of the survey composed questions about "Characteristics relating to critical result practices"**

	Questions	Answers
1.	Who is involved in routine notification of critical results to caregivers?	- Clinical Pathologist - Superior LaboratoryTechnician - LaboratoryTechnician - Others
2.	How are critical results reported to caregivers?	- Mobile phone - Department Phone - Electronic communication of critical values
3.	Which is set timeframe of critical results reporting in your laboratory?	- Up to 15 minutes - Up to 30 minutes - Up to 1 hour - More than 1 hour

4.	Before reporting critical result, the analysis is repeated?	- Yes - No
5.	Is there an automatic critical result notification system in your laboratory?	- Yes - No
6.	Is the reporting of a critical result documented?	- Yes - No
7.	How is reporting of a critical result documented in your laboratory?	- Comment in the computer system - Written on the result form - Both above
8.	Are the recorded data of a reported critical result easily accessible for all laboratory staff?	- Yes - No
9.	Is the list of critical results periodically evaluated?	- Yes - No
10.	Does your laboratory have the perception of the total number of critical results actually reported?	- Yes - No
11.	Who can receive a critical result?	- Ordering physician - Nurse - On-call physician/resident - Other
12.	Does your laboratory have a “read back” policy implemented?	- Yes - No

Table 4 **Fourth part of the survey about Clinical Pathology areas and “Analytes included on critical result list”**

	Questions	Answers
1.	For which areas of Clinical Pathology have your laboratory established critical results?	Microbiology - Yes - No Hematology - Yes - No Clinical Chemistry - Yes -No

2.	Which chemistry parameters are included in your critical results list?	- Ammonia - Bilirubin - Creatinine - Glucose - Ionized calcium - Lactate - Lipase - Magnesium - Myoglobin - Pancreatic amylase - Phosphate - Potassium - Procalcitonin - Reactive C Protein - Sodium - Therapeutic drugs - Thyroid-stimulatinghormone (TSH) - Total calcium - Troponin - Urea
3.	Which hematology parameters are included in your critical results list?	- Blasts in peripheral smear - Erythrocyte Sedimentation Rate (ESR) - Hemoglobin - Malaria parasites in peripheral smear - Platelets - Total White blood count - Total Neutrophils count
4.	Which microbiology parameters are included in your critical results list?	- Acid-alcohol-resistant bacilli - Carbapenemase-producing Enterobacteriaceae (CPE) positive in screening - Detection of Clostridium difficile - Fungus in blood cultures - Gram negative bacils in blood culture - Gram positive cocci in two set of blood cultures - Gram positive cocci in one set of blood cultures - Legionella urinary antigen - Methicillin-resistant Staphylococcus aureus (MRSA) - S. pneumococcal urinary antigen - Type A or B Influenza

Table 5 Fifth part of the survey asked about future "Education"

	Questions	Answers
1.	Do you consider that professional qualification on critical results is important in Portugal?	- Yes - No
2.	Do you consider important to implement consensus on reporting critical results in Portugal?	- Yes - No

procedure for critical results reporting. Half of the surveyed laboratories were not accredited (23/44), but there was no statistically significant difference in critical result management despite laboratory accreditation status (P=0.695).

The majority of laboratories (31/36, P<0.0001) indicated that they have defined a list for critical results reporting. For almost half of these laboratories (13/31), their defined list included more than 20 critical risk results for different analyses. The numbers of Portuguese laboratories which report critical results of particular parameters in each area of clinical pathology – clinical chemistry, hematology and microbiology are shown in Figure 1, Figure 2 and Figure 3. Twenty-six laboratories report critical risk results in all three main areas of clinical pathology. Most reported chemistry parameters are potassium (28/29), sodium (25/29), glucose (27/29) and creatinine (23/29). Regarding hematology parameters, all surveyed laboratories report critical results of hemoglobin (28/28) and most of them report critical results of platelets (25/28). A*cid-alcohol-resistant* bacilli is the most reported parameter in the microbiology area as 23 out of 26 laboratories report its critical result.

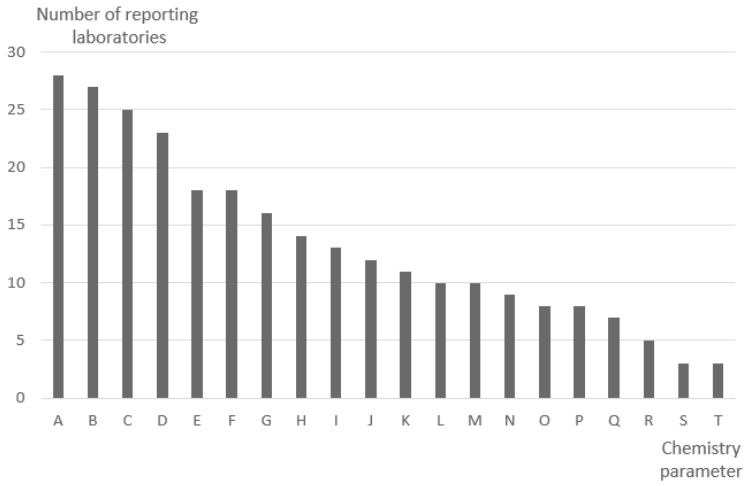
Out of 31 laboratories, 28 used only one resource to define their critical results list – previously published literature (24/28) or consensus with physicians (4/28). Remaining three laboratories combined these two resources. Moreover, 25 out of these 31 laboratories stated that their critical results list is periodically evaluated.

There was a statistically significant difference in the number of laboratories that report different critical results depending on the patient's age (24/30, P=0.019). However, they report the same critical results regardless of disease type (20/30, P=0,1684), location (in- and outpatients; 21/30, P=0.103) and ethnicity (29/30, P=0,083).

In 25 out of 36 laboratories, critical result information is reported by technicians or clinical pathologists, while in other 11 laboratories it is done by clinical pathologists exclusively. Critical risk results are mainly reported via telephone (35/36) to physicians (32/36) or nurses (4/36). Moreover, some laboratories (13/36) implemented a "read back" policy for critical risk results reporting. Only 4 out of 36 hospitals have implemented automated notification systems between laboratory and clinical departments. However, in the majority of laboratories (31/36) reported critical risk result is also documented in the laboratory information system (LIS) and easily accessible for all laboratory staff (31/36).

Half of the surveyed laboratories re-analyze critical results before reporting (19/36, P=0.785). Regards to timeframe limits for critical result reporting, 60% of surveyed laboratories have set 15 minutes timeframe, 33% have set 30 minutes, and only 7% of laboratories report critical results within one-hour timeframe. All 44 surveyed

Figure 1 Number of laboratories that report critical results of certain parameters in clinical chemistry*



Legend	Chemistry parameter	Ratio of reporting laboratories
A	Potassium	28/29
B	Glucose	27/29
C	Sodium	25/29
D	Creatinine	23/29
E	Therapeutic drugs	18/29
F	Total calcium	18/29
G	Magnesium	16/29
H	Troponin	14/29
I	Bilirubin	13/29
J	Urea	12/29
K	Thyroid-stimulating hormone	11/29
L	Ammonia	10/29
M	Lactate	10/29
N	Phosphate	9/29
O	Ionized calcium	8/29
P	Lipase	8/29
Q	Pancreatic amylase	7/29
R	Reactive C Protein	5/29
S	Myoglobin	3/29
T	Procalcitonin	3/29

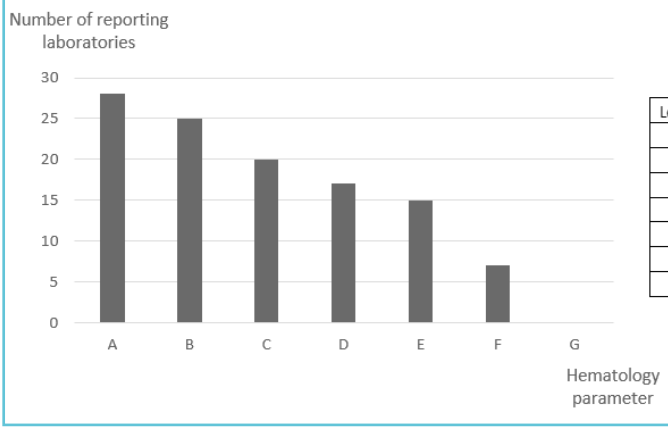
**Total number of reporting laboratories in Portugal is 36;*
Out of them, 29 laboratories report critical results in clinical chemistry area.

laboratories stated that it is important to implement national guidelines concerning reporting critical risk results in Portugal. Moreover, they also indicated that professional qualification on critical risk results is fundamental. Considering that the privacy of survey participants has been assured, all laboratories were consent with this publication.

DISCUSSION

This survey aimed to assess the state-of-the-art of critical results reporting among Portuguese laboratories. Results show that 82% of surveyed laboratories have a certain procedure for critical results reporting. However, they also reveal that practices, timeframes, analytes, and values

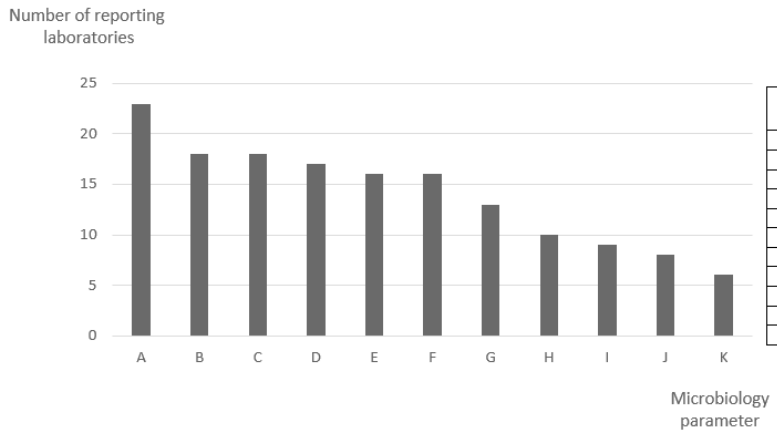
Figure 2 Number of laboratories that report critical results of certain parameters in hematology*



Legend	Hematology parameter	Ratio of reporting laboratories
A	Hemoglobin	28/28
B	Platelets	25/28
C	Blasts in peripheral smear	20/28
D	Total White blood count	17/28
E	Malaria parasites in peripheral blood smear	15/28
F	Total Neutrophils count	7/28
G	Erythrocyte Sedimentation Rate (ESR)	0/28

**Total number of reporting laboratories in Portugal is 36;*
Out of them, 28 laboratories report critical results in hematology area.

Figure 3 **Number of laboratories that report critical results of certain parameters in microbiology***



Legend	Microbiology parameter	Ratio of reporting laboratories
A	Acid-alcohol resistant bacilli	23/26
B	Carbapenemase-producing Enterobacteriaceae	18/26
C	Legionella urinary antigen	18/26
D	Methicillin-resistant Staphylococcus aureus (MRSA)	17/26
E	Detection of Clostridium difficile	16/26
F	Gram negative bacilus in blood culture	16/26
G	Fungus in blood cultures	13/26
H	Gram positive cocci in two set of blood cultures	10/26
I	S. pneumococcal urinary antigen	9/26
J	Type A or B Influenza	8/26
K	Gram positive cocci in one set of blood cultures	6/26

**Total number of reporting laboratories in Portugal is 36;*
Out of them, 26 laboratories report critical results in microbiology area.

vary widely among laboratories in Portugal. Considering an impressively high response rate, this survey provides valuable insight into the heterogeneity of critical result laboratory management.

Consistent with some other international surveys, our results show great variability in critical risk results, and even the number of analytes included in predefined lists (8, 9, 10). Half of the participating laboratories include more than 20 critical results of different parameters and different areas of clinical pathology. Wagar et al. described that there are advantages to a relatively limited list of critical results (12). The long and complex list often includes some parameters of which critical results are not necessarily "life-threatening". These kinds of lists require increased laboratory personnel investment and confuse the importance of critical results. Moreover, we were unable to confirm that laboratory management of critical results depends on the accreditation status of the laboratory as non-accredited laboratories in Portugal have a similar practice in reporting and developing their critical results lists. According to the CLSI GP47 guideline for critical results management, the critical result list development should reflect professional consensus and sources should always be documented (4). The majority of the laboratories in our survey used previously published literature to develop their critical results list, and only 7 laboratories consulted with physicians. These results reflect those reported previously in Spain and China (10, 11). Lam Q. et al. also emphasized about "published literature" as commonly cited resource for critical results list development, unfortunately that literature is usually not quoted nor further explored (1). Guidelines also recommend that each laboratory should develop customized critical result list suitable for the clinical needs of their patient populations in every healthcare environment (4). Sonjic et al. recently investigated the physicians' attitudes about unique critical results list in one hospital in Croatia, and stressed out the need for different approaches for each hospital department (13). As reported by Salinas et al., decision making and critical results reporting efficiency are improved if individual patient characteristics are observed.

Unfortunately, Portuguese laboratories only report critical results in accordance with patient's age, but regardless of patient location, disease type or ethnicity. Very similar results are previously reported in a survey conducted by College of American Pathologists (CAP) (14). This survey revealed a gap for the improvement and set the basis for Portuguese laboratories to take action. Laboratories should develop consensus with physicians in order to customize their critical result lists according to groups of patients in different departments and their individual clinical needs.

The fact that most of the surveyed laboratories in Portugal report critical results of potassium, sodium, and glucose broadly reflects on the situation in Europe. The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) also found that these three parameters are included in critical results lists of 90% surveyed laboratories in 30 countries among Europe (1). However, the obvious lack of agreement is present in other parameters and critical result values (9, 13, 15).

Results related to communication practices show that the majority of critical results are reported by clinical pathologists to referring physicians which is previously proved to be the most effective pathway for immediate medical intervention and treatment (10, 11, 16). However, reporting by telephone remains main communication channel which diverts and burden laboratory workload, especially in situation when referring physician can not be contacted (6). Several recent guidelines and accreditation standards require the laboratory to establish critical result communication strategy and reporting protocol (1, 4, 6, 16). The International Council for Standardization in Hematology proposed alternative electronic pathways that will ease laboratory work but still be effective and fast (17). Half of the laboratories in Portugal reanalyze critical results before reporting which confirms controversy in recent surveys and recommendations. The most recent study conducted in Spain suggest analytical repetition in their notification protocol, while other studies, stated that it contributes to the unnecessary delay of critical results reporting (18, 17, 7). Moreover, CLSI guidelines also emphasized that this repeat examination practice should be carefully evaluated for its usefulness (4). According to CLSI GP47 reporting timeframe classification, all timeframe limits for critical results reporting, set by surveyed laboratories in Portugal, have been "acceptable" (within 60 minutes) (4). Moreover, 60% of Portuguese laboratories report critical results within 15 minutes and thus are classified as "timely". In a survey conducted by the College of American Pathologists, the reporting time for inpatients and outpatients was also within 15 minutes (9, 12, 19), despite that reporting within 30 minutes was also considered acceptable. Interestingly, communication was much faster using computerized options rather than the telephone (12).

Although the majority of surveyed laboratories stated that reported critical results have been documented in LIS, automated notification system and "read-back" policy has been underestimated in Portugal. The implementation of these practices has also been inconsistent in other countries among Europe (1, 17). According to the accreditation norm ISO 15189 (requirement 5.8.2.), the laboratory should keep documentation of critical results for a certain period and continuously monitor reporting performance (6, 16, 17). CLSI also state that entire chain of communication should be well documented in real time (4). Thus, the laboratory can make corrective actions and improvements in reporting and critical results list content. It is noteworthy that 80% of laboratories in Portugal periodically re-evaluate their critical results list. Moreover, all surveyed laboratories stated that further education and

development of national guidelines are substantial in this kind of manner.

This survey also has some limitations. In Portugal most Clinical Pathology Departments do not carry out haemostasis and coagulation studies, which is why INR was not included in this survey. This parameter is performed by Blood and Transfusion Departments. Moreover, laboratory management of critical results highly depends on patient population, therefore these results are not transferable to other countries. Further studies in different healthcare environments are still needed.

To the best of our knowledge, this is the first study on laboratory management of critical results in adults in Portugal. Despite most of the Portuguese hospitals having a critical results policy, this survey shows high variability among the hospitals concerning critical results policies, critical results practices and even critical results list.

Standardization of laboratory management of critical results is a necessary and urgent step, which will improve the diagnostic efficiency and reduce the delay in the identification of patients at risk. Thus, the urgent need for nationally and/or locally established policies and procedures for the management of critical results is evident.

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Diagnostic challenges with acyclovir crystalluria – a case study

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acute kidney injury, adverse drug reaction

ABSTRACT

Background/objective

Marked to abundant crystalluria may cause significant morbidity due to acute renal injury. Intravenous acyclovir administration may result in a pathologic crystalluria, especially in cases with increased renal concentration of the drug. It is important that clinical laboratory staff recognize and communicate the presence of abundant crystalluria to clinical staff to avoid irreversible kidney injury.

Methods

We report a case of crystalluria in a patient treated empirically with intravenous acyclovir for possible viral meningitis.

Results

Opaque “milky” urine was submitted for urine analysis which showed abundant long needle-shaped brightly birefringent crystals under polarized light microscopy and was diagnosed as acyclovir crystalluria.

Conclusions

Any case of moderate to abundant crystalluria should be reported in a timely manner to the clinical staff to facilitate treatment modification to reduce the risk of acute kidney injury. Laboratory staff should be aware and recognize acyclovir treatment as a possible cause of pathologic crystalluria.

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INTRODUCTION

Rapid identification of abundant crystals in urine is a clinically important skill for laboratory staff because prompt treatment actions can avoid acute kidney injury and crystalluria may be the first clinical indication of a serious adverse drug reaction (1). Drug-induced crystalluria and acute kidney injury can be reduced by discontinuation of the drug, enhanced drug clearance by patient hydration, urine alkalization, or dialysis (in some cases). Detection of trace amounts of common urine crystals such as calcium oxalate, struvite (triple phosphate) and uric acid are often transient observations with little clinical sequelae. Identification and reporting moderate abundance of urine crystals are useful clinical correlates that may support diagnoses. The most common agents causing crystalluria are sulfonamide antibiotics, ethylene glycol, high dose ascorbic acid, methotrexate and antiviral protease inhibitors (2). It is important to distinguish the rare observation of milky white urine with a pathologic crystalluria from the common milder events.

Pathologic crystalluria is usually associated with administration of pharmaceuticals with limited water solubility to patients at risk with intravascular volume depletion or pre-existing kidney or liver disease, who are predisposed to high urine concentrations of drug and drug metabolites (2-4). This scenario is particularly common among patients that receive intravenous medications and are not able to access drinking water to maintain their hydration status such as pediatric patients, patients verbally impaired and patients that experience intravascular volume contraction due to acute fluid loss (e.g. diarrhea, vomiting).

Acyclovir is a common antiviral drug used to treat herpes simplex infection or varicella zoster and is administered orally, as a cream, or intra-venously (5). As a purine derivative, it has limited water solubility and 60-90% is excreted unchanged in the urine. It is known to cause nausea and diarrhea and should be used with caution in patients with poor liver or kidney function. Three recent studies reported the incidence of acute kidney injury among patients administered parenteral acyclovir to be 5.1-10.5%, 13%, and 17.5% (6, 7, 8). It is critical for laboratories to identify and report detection of acyclovir crystalluria to alert clinicians, so they can make immediate therapeutic changes to avoid irreversible acute kidney injury (9, 10). We report a recent case to highlight this importance.

CLINICAL-DIAGNOSTIC CASE

A 53-year-old man living in a long-term care facility presented to the emergency department with fever (39.4° C) and decreasing level of consciousness (Glasgow coma score = 8). This gentleman had a history of multiple cerebral infarctions and an intraventricular arteriovenous malformation (AVM) with baseline left sided deficits and epilepsy and was verbally impaired. The patient had chronic Hepatitis C infection.

The patient was taking 250 mg phenytoin daily for epilepsy. Phenytoin toxicity, new cerebral infarction, and meningitis were differential diagnoses in this case. He was treated empirically for meningitis with 3.375 g of piperacillin-tazobactam IV, 1 g of vancomycin IV, 500 mg azithromycin IV, and 500 mg of acyclovir IV in the

emergency department. A lumbar puncture was performed and Varicella Zoster Virus (VZV) and Herpes Simplex Virus (HSV) PCR was assessed. The non-contrast CT of the brain was consistent with previous imaging; no acute infarctions were identified.

Table 1 Serum Chemistry and Hemoglobin Results Days 1-4

Test	Day 1	Day 2	Day 3	Day 4	Reference Interval
Sodium	147	148	151	150	135-146 mmol/L
Potassium	3.8	3.5	3.2	3.0	3.5-5.1 mmol/L
Chloride	103	109	114	117	100-110 mmol/L
Total CO2	30	26	29	26	22-31 mmol/L
Urea	12.8	16.7	11.1	7.1	3.7-7.0 mmol/L
Creatinine	58	81	49	44	60-104 µmol/L
Hemoglobin	168	150	136	130	135-180 g/L

Table 2 Day 3 Automated Urinalysis and manual Urine Microscopy

Collection type	Catheter	WBCs Urine	Present
Appearance Urine	Cloudy	RBCs Urine	Present
Color Urine	Cloudy	Crystals Urine	-
Specific Gravity	-		
pH Urine	6.5	Needle-shaped crystals	
Leukocyte Esterase crystals	6.5	Presumptive identification: Acyclovir	
Nitrites Urine	Negative		
Protein Urine	0.25 g/L (+)		
Glucose Urine	Normal		
Glucose Urine	Negative		
Hemoglobin Urine	250 (++++)		

On admission, the patient had normal electrolytes (Table 1), elevated urea and normal creatinine. As the patient became better hydrated during this admission, urea, hemoglobin and creatinine concentrations fell (Table 1).

The unusually low creatinine level may be attributed to the chronic hepatitis C infection. With continued fluids, antipyretics, and antibiotic/antiviral treatment, the patient's fever settled over the next few hours and his level of consciousness improved to baseline.

On admission day 3, the catheter derived urine specimen grossly appeared pale, cloudy, and milky (Table 2).

Automated urinalysis (Roche Diagnostics Canada, Laval, Quebec, Canada; u6500 analyzer) showed presence of leukocyte esterase, protein and blood. The specimen was too turbid for determination of specific gravity. The specimen was flagged for manual microscopy, which showed very abundant needle-shaped crystals. These were initially reported as "needle-like crystals,

Figure 1 **Acyclovir crystalluria: Light microscopy of a wet mount of urine sediment showed abundant sharp needle-shaped transparent crystals (400 × magnification)**

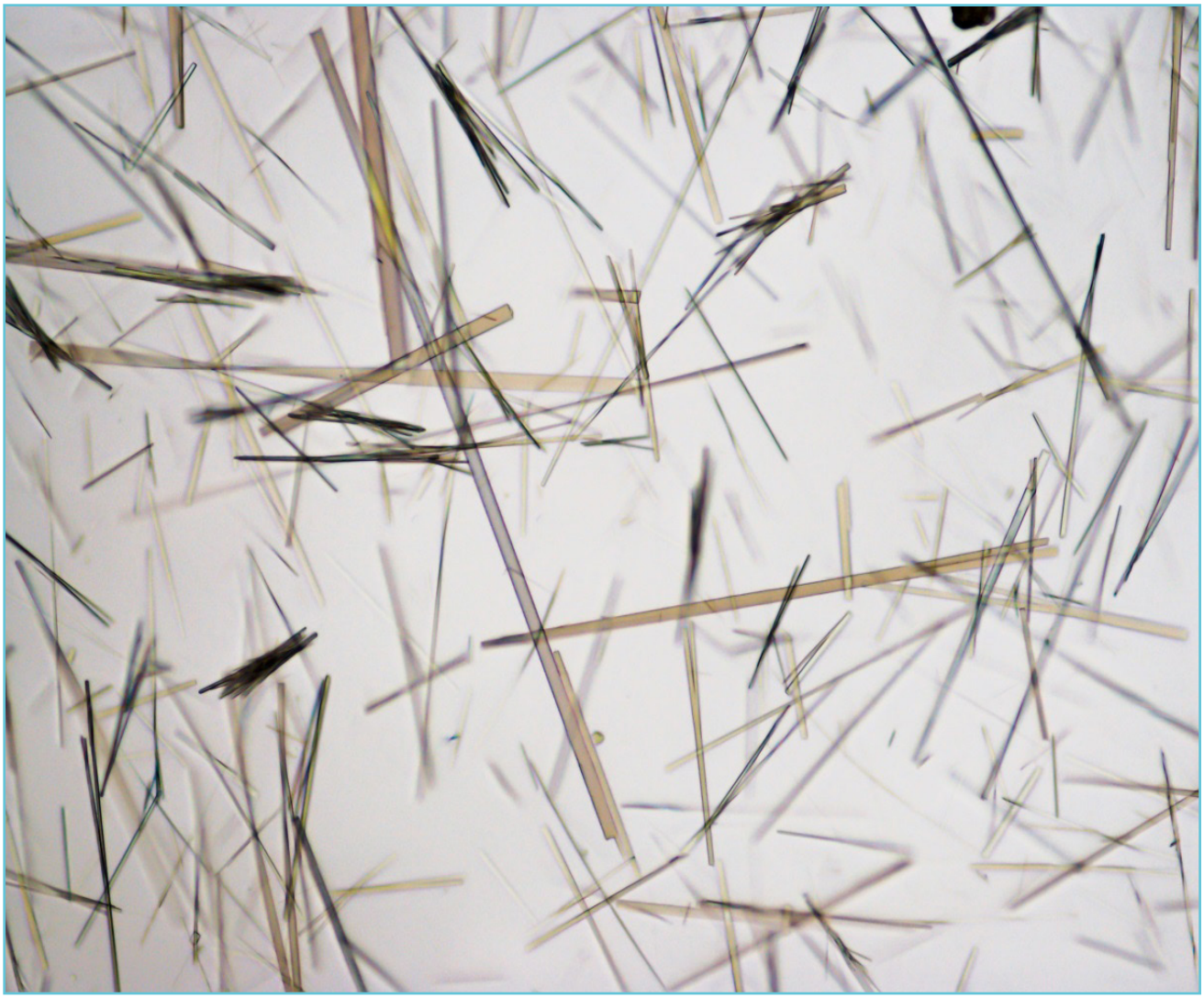
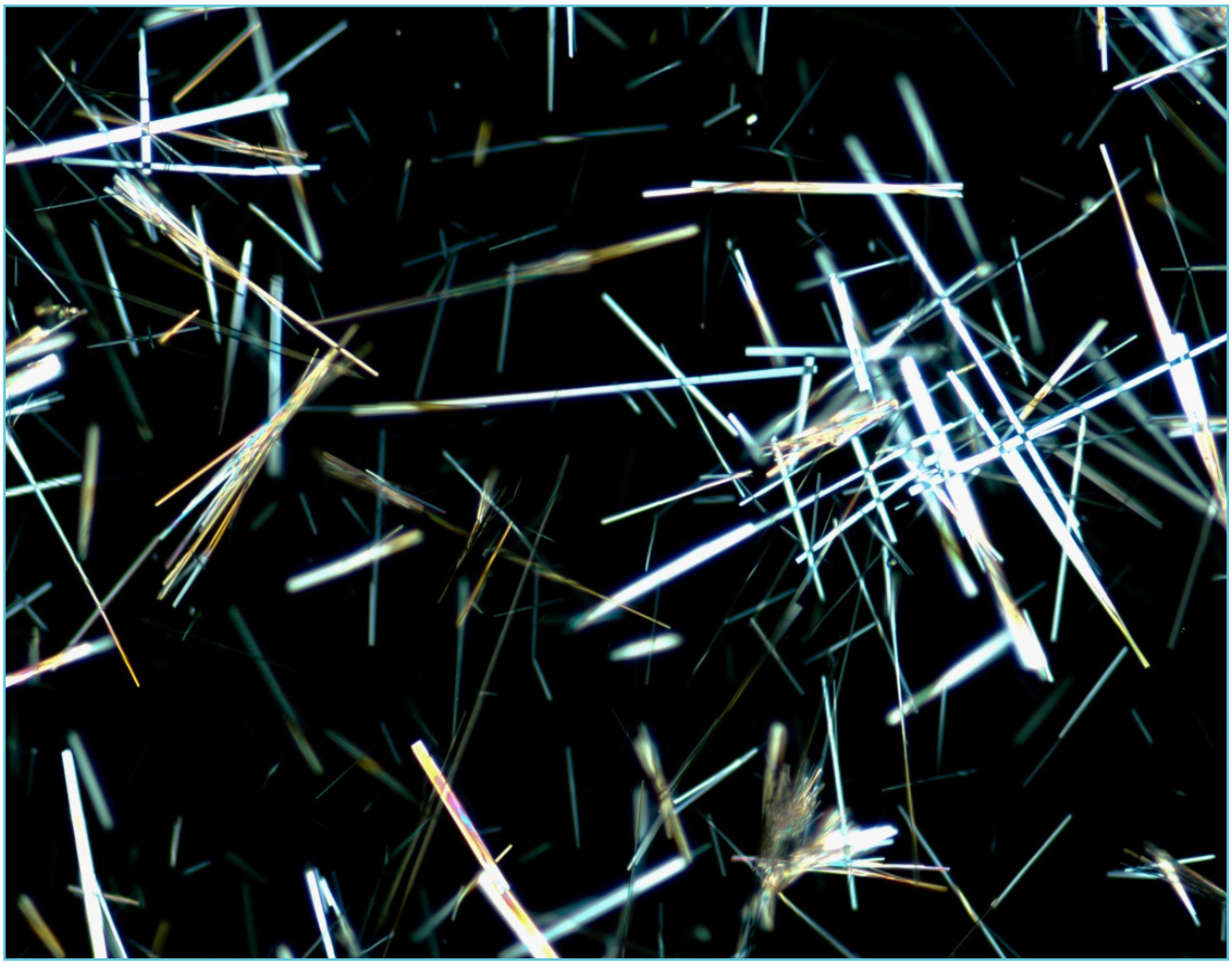


Figure 2 Acyclovir crystalluria: Polarized light microscopy of a wet mount of urine sediment. With crossed polarization filters the needle shaped crystals are very bright and birefringent (400 × magnification)



unidentified" and the biochemist was called to review the specimen. The crystal morphology resembled previous cases of acyclovir crystalluria encountered (Figure 1 and Figure 2), and the report was modified to reflect this. The on-call pharmacist as well as the attending physician caring for the patient were notified and informed of the abundant crystals present in the urine and the risk of renal injury. At this time the viral PCR results had been reported as negative for VZV and HSV, and the patient's acyclovir was discontinued. Evidence of renal insufficiency was not present at follow up based on serum creatinine levels.

DISCUSSION

This case highlights several important points. Patients presenting to the emergency department with vague non-specific symptoms may have a broad differential diagnosis which may be treated with initial empiric pharmaceutical therapies while awaiting confirmatory laboratory results. Volume status and renal function can significantly impact the adverse effects of these

pharmaceutical agents. In this case, a transient period of hemo-concentration likely secondary to dehydration may have contributed to a markedly elevated concentration of acyclovir in the urine.

Acyclovir crystalluria is a rare laboratory observation due to extensive clinical utilization warnings from the drug manufacturers and pharmacists which encourage maintaining patient hydration, monitoring renal function, and dose adjustments for patients with known renal impairment (11). Substitution with alternative antiviral agents (e.g. valacyclovir or famciclovir) that are also effective against HSV or VSV and have lower risk of crystalluria to reduce the risk for acute kidney injury is another common strategy (12).

Laboratory technologists often do not have access to patient charts or medication records. However, timely reporting of moderate to abundant crystalluria by the laboratory to clinical staff, even if the crystals have not been specifically identified, is important to raise concern for the risk of acute renal injury. Crystalluria secondary to pharmaceutical products may not have distinct morphology. Laboratory procedures including assessment of the reaction of the crystals to the addition of water, saline, acid, and base, as well as the use of a polarizer for microscopic examination may be useful. Direct communication with the clinical team and a review of the patient chart including prescribed pharmaceutical agents by the biochemist is extremely useful in elucidating the etiology of urinary crystals.

TAKE HOME MESSAGES/ LEARNING POINTS

1. Laboratory staff should recognize that milky white urine with abundant crystalluria is a clinically critical observation that needs to be immediately reported to medical staff, even if the initial report indicates an abundant crystalluria with an unidentified crystal.
2. Acyclovir crystalluria is often observed following intravenous administration of the drug.
3. Acyclovir crystals do not have a unique morphology. However, long needle shaped crystals, with bright birefringence under polarized light microscopy that readily dissolve when diluted by water, saline, acid or base in the urine of patients receiving acyclovir IV is most likely to acyclovir crystalluria.
4. Physicians and pharmacists treating patients with acyclovir crystalluria may consider alternative antiviral therapies to reduce the risk of acute kidney injury.

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Author disclosures & contributions

A. R. Andrews: Reviewed the clinical case, conducted microscopic photography and literature review, wrote and reviewed the manuscript.

D. Yu: Directed photography, wrote and reviewed the manuscript.

A. W. Lyon: Reviewed the clinical case, wrote and reviewed the manuscript.

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Case report on paediatric nephrotic syndrome

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ABSTRACT

Nephrotic syndrome (NS) is a glomerular disorder typically characterized by gross proteinuria, hypoalbuminemia, hyperlipidemia, and peripheral oedema. We report the case of a 2-year-old male toddler weighing 15 kg with a 1-week history of swelling around the eyes and both legs, and generalized body swelling. She had a history of fever, cough and decreased urine output. Examination revealed bilateral pedal oedema (pitting type).

Laboratory investigations showed protein in urine, reduced serum albumin (2.0 g/dL) with elevated lipid levels. Although kidney biopsy could not be performed due to economic problem of the family, a diagnosis of idiopathic nephrotic syndrome (NS) was made based on clinical and laboratory findings.

The patient was mainly treated with furosemide, prednisolone and enalapril. Urine I/O charting (Intake/Output chart for assessing fluid intake and ability to pass urine in adequate amounts) was done daily until optimal results were obtained.

INTRODUCTION

Primary nephrotic syndrome (PNS), also known as idiopathic nephrotic syndrome (INS), is associated with glomerular diseases intrinsic to the kidney and not related to systemic causes. The subcategories of INS are based on histological descriptions, but clinical-pathological correlations have been made (1). Diagnosis is generally based on clinical features and investigations including blood tests, renal imaging, and biopsy (2). The incidence of idiopathic nephrotic syndrome (INS) is 1.15–16.9 per 100 000 children, varying by ethnicity and region. The cause remains unknown but the pathogenesis of idiopathic NS is thought to involve immune dysregulation, systemic circulating factors, or inherited structural abnormalities of the podocyte. Genetic risk is more commonly described among children with steroid-resistant disease. The mainstay of therapy is prednisone for the vast majority of patients who are steroid responsive; however, the disease can run a frequently relapsing course, necessitating the need for alternative immunosuppressive agents. Infection and venous thromboembolism are the main complications of NS with also increased risk of acute kidney injury. Prognosis in terms of long-term kidney outcome overall is excellent for steroid-responsive disease, and steroid resistance is an important determinant of future risk of chronic or end-stage kidney disease (3).

CASE REPORT

Clinical features

A 2-year-old male toddler weighing 15 kg presented with a history of fever which is high grade continuous type associated with chills and rigors. The patient had cough (wet cough more in amount) whitish colour sputum not foul smelling. Swelling over face was present which initially started around peri-orbital (which is more during morning) and gradually progressed to face which decreases by evening. The toddler had decreased urine output (oligouria). The baby was delivered by C-section and weighed 2.75 kg after birth. On examination pitting type of oedema was present over lower limbs and swelling over face was present. Based on these clinical presentations, nephrotic syndrome was suspected and specific laboratory testing was performed to establish diagnosis.

Laboratory findings

The urine dipstick indicated for proteinuria, no signs of haematuria. Blood testing showed a significantly depressed C3 level of 0.638 g/L (reference interval 0.9-1.8 g/L) and hypoalbuminaemia of 2.0 g/dL (reference interval 3.5-5.5 g/dL) indicating nephrotic syndrome (NS). The urine creatinine level was – 620 mg/L (reference interval 400-3000mg/L) and APTT was prolonged- 47.7 *Sec* (reference interval 24-30 *Sec*). Serologic testing for active infections: anti-streptolysin-O titer was positive. The lipid levels were markedly increased as outlined in the Table 1. LDL was measured and calculated by enzymatic selective protection (Direct). The urine protein/creatinine ratio was found to be high (7.3). Mantoux test was done before administration of steroids which was negative.

Clinical course

After establishing diagnosis, optimal supportive treatment including Enalapril p.o., Prednisolone p.o., intravenous albumin, furosemide, low salt intake, high caloric and protein diet were given along with Ceftriaxone and Ascoril-LS. The urine output and blood pressure was monitored.

Successful control of peripheral oedema with the administration of albumin and diuresis with furosemide was seen. The peri-orbital oedema and leg swelling reduced, and there was a concomitant increase in serum protein levels. The lipid levels also gradually decreased in due course of time without any medication.

Table 1 Laboratory parameters

Parameters	Result	References
Urine		
Creatinine	620 mg/L	400-3000 mg/L
Protein	4574 mg/L	< 100 mg/L
Protein Creatinine ratio	7.3	< 0.2
Serum electrolytes		
Serum Sodium	131 mmol/L	136-145 mmol/L
Serum Chloride	96 mmol/L	98-107 mmol/L
Serum Potassium	4.6 mmol/L	3.4-4.7 mmol/L
Serum lipid profile		
Total Cholesterol	342 mg/dL	< 170 mg/dL
LDL Cholesterol	196 mg/dL	< 110 mg/dL
Triglycerides	329 mg/dL	< 75 mg/dL
Others		
APTT (plasma)	47.7 sec	24-30 sec
ASO titer (serum)	400 IU/mL	< 200 IU/mL
Serum Albumin	2 g/dL	3.5-5.5 g/dL

DISCUSSION

The hallmark of INS is massive proteinuria, leading to decreased circulating albumin levels. The initiating event that produces proteinuria remains unknown. However, strong evidence suggests that INS, at least in part, has an immune pathogenesis.

The classical explanation for oedema formation is a decrease in plasma oncotic pressure, as a consequence of low serum albumin levels, causing an extravasation of plasma water into the interstitial space. The resulting contraction in plasma volume (PV) leads to stimulation of the renin-angiotensin-aldosterone axis and anti-diuretic

hormone secretion. The resultant retention of sodium and water by the renal tubules contributes to the extension and maintenance of oedema.

A more recent theory of oedema formation posits that massive proteinuria leads to tubule-interstitial inflammation, release of local vasoconstrictors and inhibition of vasodilation. This leads to reduction in glomerular filtration rate and sodium and water retention[4].

INS is accompanied by disordered lipid metabolism. Apolipoprotein (apo)-B–containing lipoproteins are elevated, including very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoproteins (LDL), with resultant increases in total cholesterol and LDL-cholesterol. Elevations in triglyceride levels occur with severe hypoalbuminemia. Also contributing to the dyslipidemia of INS are abnormalities in regulatory enzymes, such as lecithin-cholesterol *acyltransferase, lipoprotein lipase,* and cholesterol ester transfer protein (4,5).

Nephrotic syndrome is a hypercoagulable state; the increased risk of thrombosis can be attributed to two basic mechanisms:

1. urine losses of antithrombotic proteins and

2. increased synthesis of prothrombotic factors.

Abnormalities described in INS include decreased antithrombotic factors and increased synthesis of pro-thrombotic factors (6).

Risk of infection may be increased in INS because of low immunoglobulin IgG levels, which do not appear to be the result of urinary losses. Instead, low IgG levels seem to be the result of impaired synthesis, again pointing to a primary disorder in lymphocyte regulation in INS. The medications used to treat INS, such as corticosteroids and alkylating agents, further suppress the immune system and increase the risk of infection (7). The ASO test done in this patient had a positive result.

CONCLUSION

We have presented a case of idiopathic NS successfully managed with corticosteroid, albumin, furosemide and enalapril. We could not perform kidney biopsy but could make a diagnosis based on clinical features and investigations, and fortunately our patient recovered and attends monthly follow-up visits.

TAKE HOME MESSAGES/ LEARNING POINTS

- In order to establish the presence of nephrotic syndrome, laboratory tests should confirm nephrotic-range proteinuria, hypoalbuminemia, and hyperlipidemia.
- A 3+ proteinuria on dipstick is highly suggestive of nephrotic syndrome to be confirmed by appropriate laboratory work-up.
- Serologic testing for active infections should be done as the patients with NS are more prone to it.
- Mantoux test [purified protein derivative (PPD)] should be performed prior to steroid treatment to rule out TB infection.

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Contribution of authors

Shireen and Dr. Naresh conceived the idea and wrote and edited the manuscript.

Dr. Tulasi was the paediatrician managing the patient and contributed to the manuscript.

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Aerococcus urinae spondylodiscitis: an increasingly described localization

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ABSTRACT

Aerococcus urinae is currently more frequently identified since the introduction of MALDI-TOF MS technique in routine laboratories. Serious infections such as endocarditis and spondylodiscitis are increasingly reported in the literature.

This is a case of septic spondylodiscitis and bacteremia due to *Aerococcus urinae* with a urinary starting point.

INTRODUCTION

Aerococcus urinae is a Gram-positive bacteria growing in clusters but in contrast to staphylococci they are catalase negative. Due to their similarities with staphylococci, streptococci and enterococci, correct species determination has been difficult in the past and aerococci have been thought to be rare causes of human infection until the recent introduction of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Aerococcus urinae is found in human skin and/or fecal flora and can cause urinary tract infections, or in some cases (elderly with comorbidities) it can cause invasive infections such as sepsis or infective endocarditis, or musculoskeletal infections. We report the case of spondylodiscitis caused by *Aerococcus urinae*.

PATIENT AND OBSERVATION

A 77 years old male presented to the ER with complaints of fever, hematuria and lower back pain. His medical history was significant for a benign prostatic hypertrophy and osteoarthritis; he had no history of smoking, alcohol or drug use and didn't have any history of nephropathy or any other chronic diseases. He started having hematuria and nocturnal fever three days prior his admission; the back pain appeared only on the morning of his admission.

Upon initial examination the patient was awake and responsive; his vital signs included a temperature of 38.5 °C, heart rate of 94 bpm, oxygen saturation of 97% on room air, and blood pressure of 140/78 mm Hg.

Rheumatologic examination revealed lower lumbar para-vertebral pain, exaggerated by anterior and lateral flexion and spinal stiffness, neurological examination was unremarkable.

Urological examination found a hypertrophied homogenous prostate, urine dipstick was positive for nitrites (+), leukocyte esterase (+), and hemoglobin (++) and was negative for protein, ketone and bilirubin.

The reminder of the physical examination was unremarkable.

Biology showed a high CRP (=98 mg/L), hyperglycemia (6.9 mmol/L), leukocytosis (WBC= 14 300/mm^3, PNN=10 000/mm^3), urinalysis showed numerous white blood cells (=360/mL), red cells (>1000/mL) and gram-positive cocci arranged in clusters.

Thoracolumbar radiography showed spinal osteoarthritis with spondylolysthesis and ultrasound showed no abnormalities (no obstructive syndrome or renal mass syndrome. It was concluded that the patient has acute prostatitis, blood culture samples were taken then he was put under empiric antimicrobial therapy (Ceftriaxone) and antalgics (tramadol and paracetamol); he was then transferred to polyvalent medicine department for further investigation.

Urine and blood culture showed small alpha-hemolytic colonies which were identified by MALDI-TOF mass spectrometry system as *Aerococcus urinae*.

Given the persistence of lumbar pain of inflammatory nature, we decided to complete the investigation by performing a thoracolumbar MRI which showed L3/L4 and L5/S1 spondylodiscitis with para-vertebral soft tissue infiltration and epiduritis with no signs of fluid collection.

The patient underwent a percutaneous tomography-guided intervertebral disc biopsy which was performed after seven days of therapeutic window and it was also positive to *Aerococcus urinae*. Susceptibility testing was performed with the disk diffusion method; it came back susceptible to Penicillin 1U, Amoxicillin 20 µg, Ciprofloxacin 5 µg, Levofloxacin 5 µg, and Vancomycin 5 µg.

Endocarditis was ruled out after performing a transesophageal echocardiography.

The patient was diagnosed with *Aerococcus urinae* spondylodiscitis with urinary starting point. He was immediately put under monotherapy with intravenous Amoxicillin (200mg/Kg/day) for 2 weeks, then Amoxicillin (150mg/Kg/day) PO for 6 weeks.

The patient remained in strict decubitus in bed, with rehabilitation and muscle reinforcement until CRP was back to normal and the pain was resolved. Then the patient was discharged after two weeks of IV therapy and continued the oral treatment at a PM&R center.

DISCUSSION

Aerococcus urinae is a newcomer to clinical and microbiological practice. The first report on A. urinae was published in 1989 (1) and the name designated in 1992 (2). Isolates were originally recognized by the cell-morphology (Gram-positive cocci growing in clusters), a negative catalase reaction, Alpha-hemolytic colonies on blood-agar, and a consistent susceptibility to β-lactase and, resistance to sulfonamides and aminoglycosides.

However, the Aerococcus species shared these characteristics with other Gram positive cocci, including Streptococci, Staphylococci, and Enterococci and was therefore often mistaken for these, many studies showed that the true incidence of Aerococci infections is underestimated due to this confusion (3,4,5). Since the emergence of new sophisticated identification systems such as mass spectrometry and genomic sequencing, the incidence of Aerococcus haven't ceased growing.

The three Aerococcus species which are mostly responsible for human infections are *A. viridans, A. urinae,* and *A. sanguinocola*. Other Aerococcus species, such as *A. christensenii* and *A. urinae hominis*, have also been described but their pathogenic role in humans still remains uncertain.

A. urinae can cause invasive infections, mostly in older males (>65 years old) with underlying urinary tract disease, particularly those with a urinary tract catheter; the most frequently found are bacteriemia followed by infectious endocarditis. Other less common invasive infections have been described; among them, only a few musculoskeletal infections have been documented (6). In a majority of cases, A. urinae urinary tract infection was suspected, and although the prognosis is relatively favorable, some fatal cases of infectious endocarditis described have been recorded (7).

To date, six cases of spondylodiscitis due to *Aerococcus urinae* were found in the literature based on a PubMed® research using "Aerococcus" and "Discitis" MeSH.

One case was excluded for the lack of a reliable identification technique (8).

Among these cases, five were men; most of these patients were over 65 years and had comorbidities and almost all of them presented an underlying urinary tract disease.

Spondylodiscitis was always located in the lumbar spine. Regarding the antimicrobial approach, the previous cases were treated mostly by beta-lactams for very different treatment durations from one case to another; ranging from 6 weeks, in our case, to 28 weeks.

The outcome was generally good, the resolution of back pain and the normalization of inflammatory parameters took from 2 weeks to 2 months with a mean of 4.1 weeks. (Table 1)

Reviews about other *A. urinae* invasive infections reported good sensitivity to beta- lactams. However, the beta-lactam-aminoglycoside combination is not entirely clear because, although this combination has been shown to be synergistic in vitro for *A. urinae* isolates,

Rasmussen et al. could only demonstrate this synergy in a few cases (7) .

In this way, we treated our case with intravenous Amoxicillin for 2 weeks then switched to oral treatment for 6 weeks. The choice of antibiotic was made based on its side effects and its level of bone penetration.

CONCLUSION

In conclusion, spondylodiscitis seems to have become a recurrent localization of *A.urinae* infections in the literature, which suggests that spondylodiscitis should be suspected, especially in older males with previous history of urinary tract disease.

Table 1 Treatment regimen and clinical outcome

Case	Treatment	Total duration	Outcome
Astudillo et al. 2003	Amoxicillin + Clindamycin IV/1 month Amoxicillin + Clindamycin PO/1 month Amoxicillin PO /5 months	28 weeks	Resolution of back pain and normalization of inflammatory parameters after 2 months.
Tekin et al. 2007	Gentamycin IV+ Penicillin G/4 weeks PO antibiotic (no data)/4 weeks	8 weeks	Resolution of back pain after 3 weeks of therapy.
Torres-Mortos et al. 2017	Ampicillin IV/3 weeks Amoxicillin/5 months	23 weeks	Resolution of back pain after 3 weeks of therapy.
Degroote et al. 2017	Penicillin G IV/2 weeks Ciprofloxacin + Clindamycin PO/8 weeks	10 weeks	Resolution of back pain after 3 weeks of therapy.
Rougier et al. 2018	Amoxicillin + Clindamycin IV Levofloxacin + Clindamycin PO	6 weeks	Resolution of back pain and normalization of inflammatory parameters.
Our case	Amoxicillin IV/2 weeks Amoxicillin PO/6 weeks	8 weeks	Resolution of back pain and normalization of inflammatory parameters after 2 weeks.

This emphasizes the importance of a reliable identification technique and calls for a standardization of antibiotherapy and its duration.

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Authors' contributions

Dr. Amina LYAGOUBI: conception, data collection, literature review and writing.

Dr. Chahrazad SOUFFI: supervision and critical review.

Dr. Victoria BAROILLER: data collection and critical review.

Dr. Eric VALLEE: critical review.

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A tarnished toy story

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LETTER TO THE EDITOR

Little children frequently put things in their mouth or even forgo washing their hands before putting those hands in their mouth. It is hence intuitive that any form of transferable lead in toys is highly unadvisable. Children are far more prone than adults to the adverse effects of lead. Lead poses an environmental hazard especially in relation to cumulative exposure. Environmental lead is a significant public health concern. Lead exposure is associated with lower IQ and cognitive decline. Some researchers suggest there may be a connection with increased crime and childhood lead exposures.

In 1929, the Dutch Boy lead paint promotions by the National Lead Company were ongoing. Ironically the promotional booklets of the time such as 'A Magical Trip to Paint Land with the Dutch Boy Painter' and 'The Dutch Boy conquers Old Man Gloom' comprised the images of a young boy using the 91% pure lead paint. The Dutch boy painter was marketed portraying children who were seen handling the lead paint. The teachers in turn were given a decorative receptacle

made of lead and some chocolates. Lead paint was discontinued in 1978. The discontinuation came numerous years after there was data linking lead to toxicity.

Lead was extensively used in ancient Rome. More recently it has still been used in tetraethyl lead added to petrol (from the 1920s) before subsequently being phased out. Beyond the fun and games that toys tend to bring, some have been found to have high toxic lead content. The harbinger of joy, in those cases, being also the harbinger of potential toxicity.

Toddlers may lick paint on their toys and chew others. Colouring agents containing lead have frequently been implicated in toxicity. Lead even tastes sweet which does not deter further licking or inappropriate handling. Even charms and trinkets containing lead have been reported, and where small may be ingested by toddlers with disastrous consequences.

Toys should meet Regulatory thresholds and be compliant with Consumer Safety Product Commission (CPSC) regulations (1). Instances of tainted lead toys has been recorded for a while (2) and continued in various shapes or forms even beyond initial reports (3). A toy's lead that comes in contact with saliva, sweat, or if ingested, gastric juices can be quite hazardous to the developing child.

Various studies were conducted to assess the degree of lead tainting. Plastic toys were gathered from day care centers to assess for lead contamination. In this study, the colour yellow derived from lead chromate was hypothesized to be the colour most associated with contamination, as was increased risk with polyvinyl chloride plastics (4). In a separate study, from 460 toys screened from daycare centers 56 tested as above 100 ppm (5). In yet another study with other toys published in 2016, they all met the European Committee for Standardization and EU Directive requirements (6).

At one point in the United States toys tainted with lead were recalled (7). In Colombia a random toy sample from the Bogota market were analysed further. Brown paint and being manufactured in Columbia were factors that gave a higher lead level likelihood (8). Toys from three major online Chinese stores were also investigated (9). A select 100 toys were looked into and it emerged that there was a preponderance of lead tainting from one online selling platform versus the other two. Atomic absorption spectrophotometer was used to assess 24 different children's toys (10). Toys have also been tested with migration tests for lead and cadmium (11).

A toy necklace may also cause lead poisoning after inadvertent ingestion by a child (12, 13). Some such medallions were obtained from toy vending machines. A toy in question was subsequently recalled throughout a nation (14). Lead may not be found just in toy paint but also in colored crayons (15) as well as salvaged windows and even antique items (16). Cadmium and arsenic have also been reported in toys (17).

In spite of precautions, regulatory oversight and recalls, some such toys still lay lurking in old toy boxes and others may have remained on shelves. Like there is a sea of people, there also is a sea of toys. From vintage toys found in grandma's basement, to newer models. After a mass recall of a million of Mattel's toys some cautious parents said they would check online the safety of a toy they are purchasing for their child. From the variety of toys on the shelves to choose from, a certain train toothbrush and curious fireman toy were just two of the identified culprits which had originally appeared innocuous to unsuspecting parents.

The 4-year-old boy from Minneapolis who died of lead poisoning in 2006 after inadvertent ingestion of a lead trinket led to greater publicity of the issue. Some stuffed toys came with warning labels that they contain lead. Leeching

of lead from certain dishes into food has also been reported. The outer part of children's toys should have no more than 90 ppm lead content. Some noted that yellow and reddish-brown paints were more common to be culprits in certain investigations.

Lead poisoning may present with insidious symptoms and may be either acute or chronic. Symptoms may include cognitive decline, neurobehavioral deficits, headaches, tremor, slurred speech, poor co-ordination, weight loss and abdominal pain, amongst others. Lead binds preferentially to sulfhydryl groups interfering with protein folding. It also interferes with cell signaling and nerve conduction.

Toys are used habitually, unknowingly a handful of mothers were left regretting the toxic gift with hidden dangers they gave their kid… in a dreaded, leaded toy story.

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