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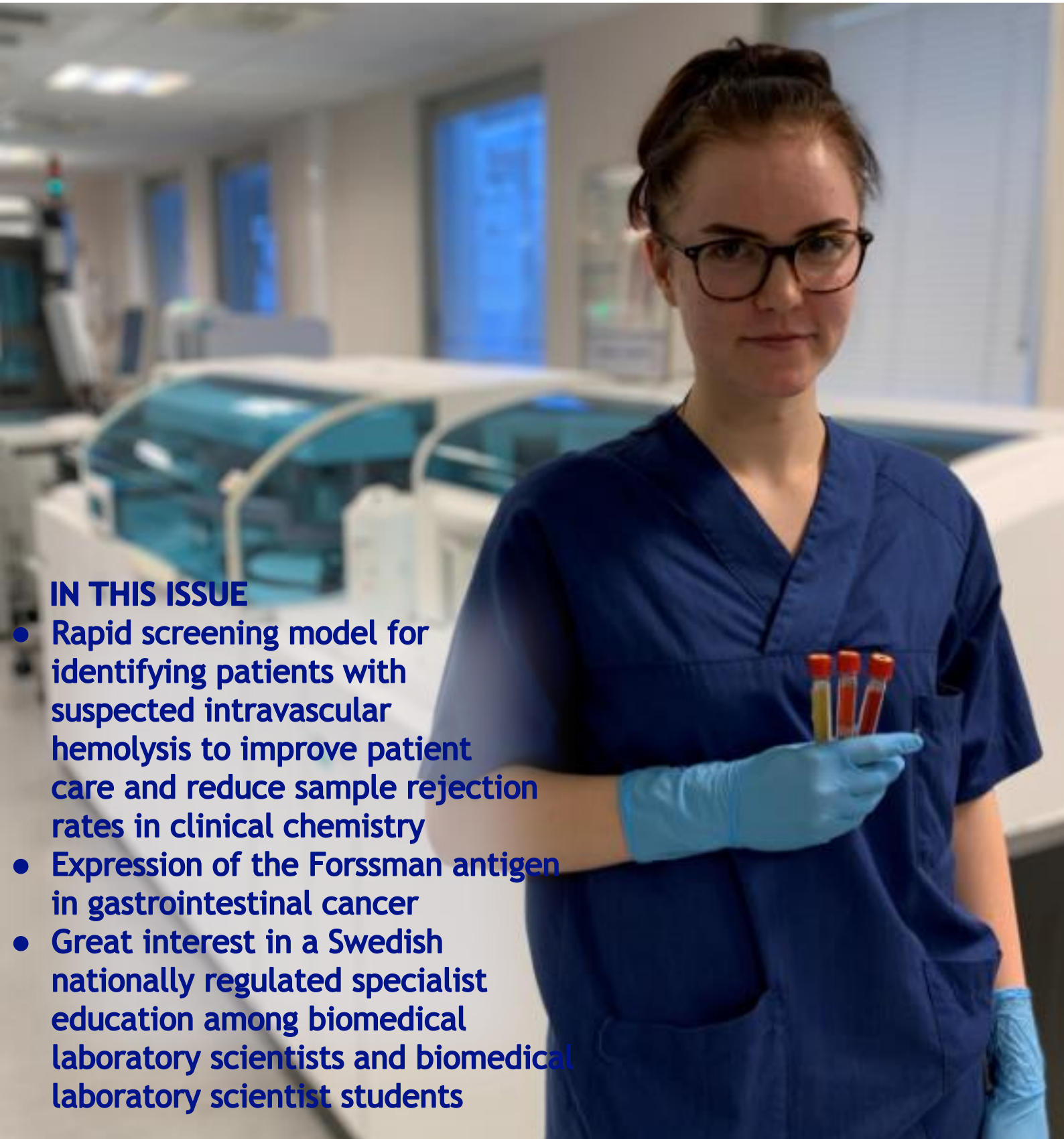

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Editorial

International Journal of Biomedical Laboratory Science (IJBLS): Quality Information, Peer-reviewed and Relevant Knowledge!

Patricia Tille Ph.D MLS(ASCP) AHI (AMT) FASC
Editor in Chief, IJBLS

Welcome to the New International Journal of Biomedical Laboratory Science (IJBLS). As the Editor in Chief, I have worked with the IFBLS Board of Directors to develop a new format for the bi-annual publication of the journal. The journal is a peer reviewed publication intended to disseminate information and knowledge to the international laboratory community by accepting a variety of manuscripts for publication. Those manuscripts should be original research articles, literature or mini-reviews, case studies, brief communications and letters to the editor describing original investigations in all fields of biomedical laboratory sciences. The journal covers several major disciplines: clinical chemistry, molecular diagnostics, clinical serology and immunology, hematology, transfusion medicine, clinical virology, clinical bacteriology and mycology, clinical microscopy, cytology, quality control, laboratory management, education and ethnics, laboratory safety and any important topics or issue related to the biomedical laboratory science.

We are very pleased to publish the first issue of the newly formatted IJBLS. As the world of laboratory science evolves across the global environment and the continued awareness of the impact of laboratory professionals on health care, IJBLS and the IFBLS Board of Directors will continue to ensure the distribution of relevant, quality information to the laboratory science community.

Along with the new format of the IJBLS, we have updated and expanded the author guidelines for publication. These are available on the IJBLS website along with my contact information. If you have any questions, please feel free to reach out to me for assistance and consider contributing to the journal.

Yours truly, IJBLS Editor in Chief,

Patricia Tille Ph.D. MLS(ASCP) AHI(AMT) FACSc

Editorial

Have your talent recognized, publish your research

Alan Wainwright CSci, FIBMS
President,
International Federation of Biomedical Laboratory Science

The International Journal of Biomedical Laboratory Science (IJBLS) was launched 2010 and until 2020 was been hosted by Taiwan Society of Laboratory Medicine (TSLM), with Professor Chuan-Liang Kao as the first Editor-in-Chief. The new Editor-in-Chief is Dr. Patricia Tille who, in conjunction with the IFBLS Management Committee is setting up the Editorial Board, identifying peer reviewers and further developing article categories, submission, publication as well as working to recruit more articles for the journal.

The aim is to develop, expand and publish bi-annually a high-quality international peer-reviewed journal relevant to biomedical laboratory science. To provide a journal that offers advice and guidance in order to empower our members to contribute to the development of areas of biomedical laboratory science and its practice internationally.

This cannot be achieved without your input. Covid-19 continues to have a huge impact on the work of Biomedical Laboratory Scientists across the world. Advances in biomedical scientific education and research continue to guide and inform developments in laboratory techniques. These advances have prepared biomedical scientists to respond to the Covid-19 challenge, to develop and deliver the required testing to diagnose this disease and to support the innovation in clinical treatment and improvements to global healthcare.

As public awareness of biomedical laboratory diagnostics has grown many of our member associations have gained recognition for the value their work brings to the diagnosis of disease. But recognizing the need for increased testing and accurate diagnosis is not new to us. We understand the importance of contributing effectively to innovation, and to influence decisions through sound, evidence-based scientific knowledge. This is what we all commit to in our profession, it defines us as biomedical laboratory scientists.

The re-launch of the IJBLS provides us with an opportunity to take this further. Through published articles we can demonstrate internationally how biomedical laboratory science makes major contributions to healthcare. We can show what can be achieved through collaborative research, through working together to share ideas and promote our professional identify.

Greater engagement with all members is crucial to achieving our shared ambitions so I invite you to share your expertise: to increase our pool of scientific knowledge through published articles in the IJBLS; to influence development in biomedical laboratory science; to raise the international profile of our profession and to increase the value of our contribution to global

Editorial: Laboratory spotlight

Healthcare and Biomedical Laboratory Science Practice in the Philippines

Evangeline R. Castillo, *RMT*[1]
Rommel F. Saceda, *RMT*[2]

The Philippines is an archipelago composed of 3 major islands and approximately 7,100 small islands. Most of its population of 110 million reside in key cities and urban municipalities making the delivery of healthcare challenging.

How is healthcare delivered in the Philippines?

The healthcare delivery system in the Philippines is dominated by the public sector (regional, provincial, municipal, and barangay level) while being supported by private healthcare service providers. The implementation of Universal Health Care (UHC) is already driving the demand across all sectors of healthcare. However, the Philippines still requires a strong focus on infrastructure and skilled manpower.[1]

The government established PHILHEALTH, the Philippines insurance corporation, with the vision that by 2020 100% of citizens would be enrolled. To date approximately 92% are enrolled. Although the insurance system gives citizens access to healthcare, however the lack of infrastructure and human resources are major concerns. For example there are not enough hospital beds in a major part of the country. Individuals who can afford to go to a private hospital have access to advanced diagnostic methodologies and care, whereas others who cannot afford the fees charged in private hospitals seek care at government operated hospitals.

There are three levels of healthcare facilities: primary, secondary and tertiary. Primary level healthcare facilities include clinics for specific diseases such as malaria and tuberculosis, clinics for employees of large companies, community hospitals. Secondary level health care facilities are smaller hospitals focusing upon specific diseases and conditions that include emergency and regional hospitals. Tertiary healthcare facilities provide are sites where highly sophisticated and technological services are offered by medical centers, large hospitals and specialized national hospitals

How are laboratories organized within the healthcare delivery system? Are they integrated, separate, independent?

There are three classifications of laboratories in the Philippines: (1) integrated laboratories are under the management of the hospital; (2) partnership laboratories are located inside the hospital however the management is independent from the hospital and have profit sharing; and (3) free standing laboratories which are outside of the laboratory and independently managed.

Furthermore, testing performed in laboratories is different: primary - where only basic laboratory procedures are performed; secondary where some advanced testing such as serology and microbiology testing are performed; and lastly, tertiary which are laboratories licensed to perform all laboratory procedures including research.

What are the education requirements for the Biomedical Laboratory Scientists? What title is used for Biomedical Laboratory Scientists?

In the Philippines, the professionals who work in laboratories or diagnostic medicine are called Medical Technologists. Our profession was established under the Republic Act 5527 of 1969 also known as the "Medical Technology Act". Section 6 of this Act states the minimum required education for Medical Technologists is at least 4 years with 12 months satisfactory internship at an accredited laboratory.

In order to be eligible for licensure, applicants for the medical technologist examinee need to have successfully completed an accredited medical technology program. Once eligibility has been verified, applicants must complete an online application, pay the fees, and prepare for and take the examinations. After passing the licensure exam they will earn the title as Registered Medical Technologist/ RMT.

The shortage of qualified personnel still exists and is considered to be a huge problem, especially in high-skill fields such as radiology, pharmacy, nursing and medical technology. The private sector is playing a significant role in helping the government address the gaps in healthcare services by allowing more colleges and universities to offer courses in these professions. Medical Centers and hospitals are permitting more medical technology students to complete their internships, providing them more experiences in the field.

What are the strengths of the health care delivery system?

Overall, the healthcare system in the Philippines, including diagnostic medicine, is of a high standard. Filipino medical staff, especially medical technologists are expertly trained, but the facilities may not be as impressive as those found in developed countries.

The quality of the Philippines' state-subsidized public healthcare, although good, varies widely between rural and urban areas. Private healthcare in the Philippines provides much more consistent care and facilities tend to be better equipped than public ones. English is also spoken throughout the Philippines, meaning that there should be few language barriers preventing tourists and foreigners from accessing healthcare.[2]

The government allocated $3.2 billion to healthcare last year but due to the pandemic most of the funds were realigned to the prevention and control of the Covid-19 disease. Private hospitals and laboratories strengthen delivery of quality healthcare especially in the areas where government services are not available due to budget constraints. Private hospitals have invested in increasing infrastructure and training which decreased the burden and influx of patients in the limited accommodation for patients in government medical centers. Because private hospitals cater to citizens who can afford to pay for their healthcare the government focuses on the poor and less privileged.

On a positive note, the Covid-19 pandemic has increased collaboration of different stakeholders in healthcare leading to an improvement in the delivery of healthcare for all. Collaboration has helped medical technologists face the challenges of diagnosis and detection of Covid-19 as well as control of the spread of the infection and distribution of information. This has been particularly important to provide these services to people who do not reside in populated areas or the main islands.

What are the challenges facing the healthcare delivery system?

Medical Technologists working in public hospitals are highly proficient; however diagnostic medicine in public hospitals faces some limitations. Despite having achieved universal healthcare, the Philippines still struggles with unequal access to medical care. As such, the standard of public healthcare in the Philippines generally varies from excellent in urban centers to poor in rural areas. Public healthcare also faces strain both from treating the large number of Filipinos who rely on public healthcare and from the trend of Filipino medical staff (including medical technologists) migrating to western countries.[3] This has resulted in understaffing in some hospital laboratories and patients may experience delays in testing and releasing of results.

Laboratories in the private healthcare sector are well-established and growing in the Philippines. Although medical technologists in private hospitals are as good as medical technologists practicing in the public sector, private facilities are much better equipped and testing is typically completed faster. Private services are considered to be expensive by locals, but are relatively inexpensive by most foreigners and tourists. The relative affordability of private healthcare can be seen in the increasing popularity of the Philippines as a medical tourism destination.[4]

1. Treasurer, Philippine Association of Medical Technologists
2. President, Philippine Association of Medical Technologists

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Brief Communication

World Health Organization Essential *in vitro* Diagnostics 2020

Marie Culliton, MSc, MBA, FACSLM
President Elect,
International Federation of Biomedical Laboratory Science

Introduction

In March 2017, the World Health Organization (WHO) Expert Committee on Selection and Use of Essential Medicines recommended that a list of Essential Diagnostics (EDL) be developed. WHO created an EDL Secretariat, which drafted the first edition of the EDL in consultation with colleagues in the various WHO disease programs. The EDL was then posted online for open consultation. WHO also created a Strategic Advisory Group of Experts on *In Vitro* Diagnostics (SAGE-IVD) to support the development of the EDL and to advise on other IVD policies and initiatives.

WHO published its first EDL[1] in 2018. This was a list of diagnostic tests that it considered essential for every healthcare system in the world. Apart from the standard haematology, biochemistry and urinalysis tests it focused on tests for diseases that WHO considered highest priority: human immune deficiency virus (HIV), hepatitis, tuberculosis, malaria, human papilloma virus (HPV) and Syphilis.

Many of these tests have been available for many years but their use has been inconsistent across countries. This edition examined the use of diagnostic tests in a range of settings from primary care through to a clinical diagnostic laboratory. The EDL is not intended to be prescriptive rather a guide to healthcare systems and laboratory managers.

One very important statement in the preface of this 1st edition is in keeping with the objectives of International Federation of Biomedical Laboratory Science (IFBLS): "While the EDL provides a list of important tests required at various levels of the health care system, it is important to note that the EDL itself cannot have an impact without an integrated, connected, tiered laboratory system, with adequate human resources, training, laboratory infrastructure, and regulatory/quality assurance systems."[1]

The EDL identifies diagnostic tests by category and is complementary to the "prequalified lists"(PQ) which include priority IVDs which have been assessed by WHO and are identified by brand. Within the disease specific categories where a WHO PQ or endorsed product exists it is cross referenced, along with WHO policies.

Open session with Stakeholders on the 2nd meeting of the WHO SAGE IVD, Geneva, Switzerland, March 18 2019. Photo: Marie Nora Roald

The second edition of the EDL was published in 2019.[2] New categories were added with general laboratory tests, anatomical pathology tests and therapeutic drug monitoring expanding the EDL from 62 to 122 categories. The disease specific tests were extended to include cancer tests. A new anatomical pathology section was added and consideration to blood safety was addressed by the addition of 7 test categories intended for screening of blood donations. Tests are further categorised to indicate if the test is used for screening, diagnosis, aid to diagnosis, monitoring, prognostic, surveillance or staging.

For countries where HIV is not endemic, the inclusion of flow cytometry as a test for primary care without laboratory may seem unusual, however the document is clear that every diagnostic repertoire depends on circumstances.

Selection and use of IVD

The 3rd edition, launched in January 2021[3], is a more substantial document compared to earlier versions. It includes a report of the third meeting of the WHO Strategic Advisory Group of Experts (SAGE-IVD) on *In Vitro* Diagnostics, 2020. The document acknowledges, by naming, the members of SAGE-IVD who provide WHO with technical advice on global policies and strategies related to priority, essential and neglected IVDs. The EDL is updated yearly, following a consensus process which includes face to face meetings, expert review and public consultation. It is remarkable that in the year of a pandemic that this document could be reviewed and expanded so thoroughly and that it has included considerations of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) tests.

The members of SAGE are drawn from a range of academics, public health officials, pathologists and biomedical laboratory scientists. Members of SAGE are appointed for a period of 2 years and there are calls for membership each year.

The International Federation of Biomedical Laboratory Scientists (IFBLS) were consulted prior to the launch of the first edition and have provided input to numerous entries thereafter.

Innovations

Do Not Do

Perhaps as important as the recommendations on what tests are essential is the list of tests that are not useful in informing clinical management, performing surveillance or informing critical aspects of population health status: a list of "Do Not Do"

eEDL

It is intended that the EDL 3 be released in electronic format as well as in print. This will make it more accessible, searchable and facilitate updates. While it was intended that this be published in 2020 the beta version remains under review.

Harmonisation

Work is ongoing to align the EDL with the International Disease Classification system (ICD-11) and other global and regional nomenclature systems. It is intended that the EDL will also link into the Universal Health Coverage (UHC) compendium. This is a single interactive database which will facilitate searching for diagnostic tests, clinical interventions and essential medicines for any condition.

Health Technology Assessment (HTA)

It is recognized that all countries must undertake some level of assessment before introducing new services. Factors to be considered include clinical effectiveness, ethics, social issues and organizational frameworks. It is intended that the EDL will assist countries with this task.

As mentioned earlier one of the objectives of the EDL is to assist countries. SAGE considered options for achieving this by embedding the prioritisation into the EDL or developing a multi decision criteria and methodology. No decision on this was made and preferred method is to be researched prior to the 4th edition.

Applications for Addition to the EDL

The EDL is a living, evolving document and the 3rd edition is more substantial than the first two.

Table 1: Sectional Layout of the EDL

EDL Section	Description
Section 1a	General IVDs for community settings and health facilities without laboratories
Section 1b	Disease-specific IVDs for community settings and health facilities without laboratories
Section 11a	General IVDs for use in clinical laboratories
Section 11b	Disease-specific IVDs for use in clinical laboratories
Section 11c	Disease-specific IVDs for blood screening laboratories

Applications for additions, revisions and "do not do" recommendations to the EDL were submitted by academia, industry and WHO technical departments. Each is presented according to the section of EDL (Table 1).

Each proposal is organized and reviewed under the following headings:

- Proposal
- Applicant
- WHO Technical Department
- Background
    - Disease condition and impact on patients
    - Does the test meet a medical need?
    - How the test is used
- Public health relevance
- WHO or other clinical guidelines relevant to the test
- Basic test characteristics
- Evidence for diagnostic accuracy
- Evidence for clinical usefulness and impact
- Evidence for economic impact and/or cost–effectiveness
- Ethics, equity and human rights issues
- Summary of evidence evaluation
- Summary of SAGE IVD deliberations
- SAGE IVD recommendations
- References

The reviews are very thorough and informative. Decisions and the reasons for them are clear. Based on the current pandemic and the impact of testing for the Sars-CoV-2 virus on all biomedical laboratory scientists the specific applications relating to their inclusion are discussed below.

Sars-CoV-2

Given the global pandemic SAGE prioritized and fast-tracked consideration of testing under two sections. Section 1b Disease-specific IVDs for community settings and health facilities without laboratories where the introduction of SARS-CoV-2 antigen was considered and Section 11a.

Sars-CoV-2 antigen testing

The SARS-CoV-2 antigen testing was under consideration as an aid in the diagnosis of COVID-19 infection in symptomatic and asymptomatic individuals with known close contact with a confirmed case or to aid in the identification and investigation of outbreaks and community spread of COVID-19.

In considering medical need it was noted that; "WHO guidance on the use of rapid antigen tests recommends use in settings where "NAT is unavailable or where prolonged turnaround times preclude clinical utility". Given the generally lower sensitivity of these tests compared to reverse transcription polymerase chain reaction (RT-PCR), they should only be used to identify COVID-19 infection in patients who are within 5–7 days of the onset of symptoms."[3]

In considering the usefulness of the test, it is recommended that all negative tests do not rule out infection and should be confirmed by RT-PCR or repeat antigen test where the test is not available.

The caveats relating to negative tests are clearly described. However, it is evident that there is political pressure to use these tests in situations where the efficacy of the test is not confirmed.

Biomedical laboratory scientists should use their knowledge and competence to advise on the correct use of the test where possible.

SARS-CoV-2 nucleic acid test (NAT)

This application relates to the use of SARS-CoV-2 NAT to diagnose infection by SARS-CoV-2 in symptomatic and asymptomatic individuals suspected of exposure.

There can be no biomedical laboratory scientist who is unaware of the serious nature of this pandemic, its impact on patients and the role of the clinical diagnostic laboratory in the detection and monitoring of cases.

In considering the medical need for this test it is noted that; "The clinical utility of SARS-CoV-2 infection testing lies in early identification and isolation of cases, but also in choosing the right therapeutic approach in a clinical picture that can mimic several other entities."[3]

"Because SARS-CoV-2 is a global pandemic pathogen, in most areas the positive predictive value (PPV) of a SARS-CoV-2 diagnostic test based on PCR is high, especially for patients in high-risk groups."[3]

It is also noted the benefits of testing for case isolation and containment of infection spread and the proper use of personal protective equipment (PPE).

SAGE acknowledges the information is preliminary. However, it recommends that SARS-CoV-2 NAT be included in the third EDL using the NAT format. The test should be used on individuals suspected of being exposed to the virus, whether symptomatic or asymptomatic.

The specific evidence reviewed was for RT-PCR tests. Other nucleic acid tests require further evidence and review.

It might have been useful if some guidance was provided regarding the use of cycle threshold (CT) ratio, or other markers of viral load, in the interpretation of the results of analysis.

Applications for Modifications

Numerous requests were made to modify the entry in the EDL, some of these provided significant evidence and others did not. SAGE itself suggested some modifications such as the disaggregation of clinical chemistry metabolic panels, recognizing variations in practice in different countries.

While the indications for measuring D-dimers were modified their use in management of Covid-19 requires further consideration.

Conclusion

The development of the EDL through three editions demonstrates a commitment to the work by WHO and SAGE. The new edition is a very useful document which should be readily available, and consulted, in all clinical laboratories. It should be recommended reading for biomedical laboratory science students and indeed those planning services.

Biomedical laboratory scientists are advised to consult the EDL and audit their laboratory repertoire using the document. When areas are identified that need improvement, correction, addition or removal they should engage in the review process via a national process or via IFBLS.

The call for members of SAGE for the 4th Edition closed on 31st March 2021. The next of the EDL is already in gestation.

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Research Article

Rapid screening model for identifying patients with suspected intravascular hemolysis to improve patient care and reduce sample rejection rates in clinical chemistry

Gabriel Garang Pioth[1], Gay Janet Davis[2], Dr. Uba Nwose[3], Dr. Philip Bwititi[4]

School of Biomedical Science, Faculty of Science, Charles Sturt University, Wagga Wagga, New South Wales, Australia[1,4]. Chemical Pathology, Microbiology and POCT, Pathology Queensland, Health Support Queensland, Department of Health, Queensland Children Hospital, Queensland, Australia[2]. School of Community Health, Faculty of Science, Charles Sturt University, Orange, New South Wales, Australia[3].

The importance of developing a rapid screening model is critical for identifying patients with intravascular hemolysis to improve patient care and reduce sample rejection rates. Specimen hemolysis is a leading cause of spurious test results in clinical chemistry. Intravascular hemolysis releases cell-free hemoglobin into the serum and haptoglobin irreversibly binds hemoglobin to form a hemoglobin-haptoglobin complex, which is cleared in the circulation by the monocyte-macrophage scavenger receptor CD163 in the liver and spleen. The depleted serum haptoglobin in a hemolyzed sample is used to diagnose intravascular hemolysis. However, haptoglobin testing may take hours or days to confirm intravascular hemolysis. The aim of this study was to investigate the relationship between intravascular markers of hemolysis and haptoglobin in hemolyzed specimens. This study retrospectively mined archived data from the laboratory database between February 2017 and February 2018 from patients 6 months of age and above. The Statistical Package for Social Science was used for data analysis. The partial plot results showed a strong relationship between hemolysis markers and haptoglobin levels. Multiple regression models that predict serum haptoglobin levels in intravascularly hemolyzed samples were analyzed. Although the results showed a strong relationship between dependent and independent variables, the data did not demonstrate a clinically significant relationship or establish cause and effect. The use of a larger sample size along with adequate controls in preanalytical, analytical, biological and environmental variables would likely improve the clinical significance of the model. With proper modifications and validations, this model has the potential to provide a rough estimate of the haptoglobin levels and reduce cost as well as sample rejection rates. Due to the numerous hemolytic diseases, this model could be used to direct the clinicians to select the appropriate test for diagnosis of intravascular hemolysis.

Key words: haptoglobin, intravascular hemolysis, extravascular hemolysis, turnaround time, *in vivo* hemolysis, *in vitro* hemolysis, hemolysis markers.

Introduction

The importance of developing a rapid screening model is critical for identifying suspected patients with intravascular hemolysis (IVH). Hemolysis is defined as the breakdown of the red blood cell (RBC) membrane to release free hemoglobin (Hb) and its contents into the extracellular fluid/ plasma.[1] Hemolyzed specimens account for 40%-70% of rejected specimens in the clinical chemical and represent 3.3% of routine specimens received in the laboratory.[2] The hemolysis can be categorized into *in vivo* and *in vitro* hemolysis. hemolysis is caused by preanalytical factors such as blood collection technique, specimen handling and delivery, or specimen storage.[3-5] In contrast, *in vivo* hemolysis, is caused by pathological conditions such as biochemical, immunological mechanisms, physical, chemical, and/or infections inside the body.[6] Biochemically, *in vitro* hemolyzed specimens are characterized by red coloration of plasma or serum, high Hb, potassium (K), lactate dehydrogenase (LD), aspartate aminotransferase (AST), and normal haptoglobin (HP) and reticulocytes (retics).[7] In contrast, *in vivo* hemolyzed samples are

characterized by elevated Hb, Bili, (unconjugated bilirubin) retics, low HP, normal K and a lack of red coloration of the plasma/serum.[8,9]

In vivo hemolysis can be further classified into IVH and extravascular hemolysis (EVH) depending on the site of RBC destruction. While both hemolytic pathways can overlap and converge during the process, it is worth noting the key difference between the two. The RBC's bound by IgM and IgG are marked by the reticuloendothelial system (monocyte-macrophage) in the spleen and liver as cells are targeted for the IVH and EVH pathways, respectively.[10]

The IVH releases free Hb and RBC content into the circulation during excessive hemolysis while in EVH, the whole RBC is lysed inside the macrophage and no RBC content is released into the plasma.[11] During normal IVH, HP irreversibly binds all free Hb to form a HP-Hb complex. The HP-Hb complex binds to the transmembrane scavenger receptor CD163 on the monocytes and macrophage in the liver and spleen where the complex is destroyed.[12,13] This suggests that, in negligible IVH instances, the IVH pathway merges into the EVH pathway. However, during increased IVH, the HP clearance mechanism is overwhelmed by excess free Hb and becomes depleted. The depleted serum HP level in a hemolyzed sample is used to diagnose IVH.[14]

Without sufficient information on the clinical notes to indicate whether the sample was a difficult collection and the lack of available guidelines, it is understandable why there is heterogeneity across all laboratories regarding the proper management and handling of hemolyzed specimens.[15,16] The dilemma that laboratory staff frequently face is whether to reject the sample to avoid an erroneous lab result resulting in an error in the diagnosis or a rejection that may lead to delay and treatment of the patient during recollection. [17-19] Rejecting *in vitro* hemolyzed specimens may be appropriate because the hemolysis interferes with analytical processes and yields spurious results which can have a deleterious impact on the quality of patient care and the laboratory's reputation.[20]

However, the identification of hemolysis related to mechanisms *in vivo*, may be important and clinically relevant to adequate patient management and care.

It appears there is no single screening and definitive test that can identify IVH with high aacuracy.[21] The routine laboratory tests used to screen for patients suspected with IVH include complete blood count, peripheral blood smear revision, total and unconjugated bilirubin, LD, retic counts, HP, ferritin, and urinalysis.[22,23] The presumptive test often requires a Coombs' test, serological testing, enzymatic testing, osmotic fragility test, hemoglobin analysis, and genetic testing to rule out/in IVH.[24-26] This testing process can be costly, time consuming and often inconvenient to the patients.

The aim of this study is to investigate the relationship between the activities of markers for hemolysis (HM) and serum HP levels in hemolyzed samples with the goal of developing a model that rapidly screens and identifies a patient suspected of experiencing intravascular hemolysis. The HM used in this study include unconjugated bilirubin (Bili), C-reactive protein (CRP), AST, Retics, LD, and alkaline phosphatase (ALP), which are often elevated in intravascularly hemolyzed specimens. The model derived from the relationship between HM and HP would provide a rapid estimated HP level.

Materials and Methods

Ethical approval

The study was conducted in the Pathology Queensland Laboratory at the Queensland Children's Hospital, Queensland, Australia and was approved by the Children's Health Queensland Hospital and Health Services Human Research Ethics Committee (Approval No: HREC/17/QRCH/244). The Charles Sturt University Human Research Ethic Committee also approved (Protocol No: H18017). The consent to

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Corresponding author: Gabriel Garang Pioth, 3 Ironpot St, Redbank Plains Queensland 4301, Australia
Email: gabpioth@gmail.com

use the data was given by data custodians, the Pathology Queensland and Public Health Act (CT_3098 & CT_2926***).***

Sample collection

The research retrospectively mined archived pathology data for tests performed between February 2017 and February 2018. The data were retrieved from patients aged over 6 months who visited or were admitted at 34 public hospitals serviced by Pathology Queensland Laboratory. The 6 months threshold was selected since HP level is detectable at six months of age.[27] The data came from patients aged from 4 to 93 with a mean age of 62, median = 65 and mode of 74. The database search input for hemolysis markers returned results of HP, Bili, CRP, AST, retics, LD, and ALP with a hemolysis index greater than two.

The first criterion for including the parameters into the study was, the analyte must be a hemolysis marker and part of a hemolytic disease screening/diagnostic test or often elevated in hemolyzed samples with a hemolysis index of 2 and above. The second selection criterion was the linear relationships between the independent variables and dependent variable (HP). The units of measurements for these parameters include: LD (U/L), HP (g/L), ALP (U/L), CRP (mg/L), retics (%), AST (U/L) and Bili (µmol/L). The data were downloaded and cleaned, transformed and processed as described by Pujari, 2001.[28] The data were de-identified; thus, no patient' identifiable information was retrieved. The analytes were measured in a continuous scale (scale ranging from 0 to over 100).

Data analysis

The statistical package for social sciences (SPSS) software was used to produce multiple linear regression coefficient[29] and correlation coefficient analyses between HP and each of the independent variables as described. A linear regression was chosen because it permits the assessment of the extent of each relationship among the independent variables and dependent variable.[30] The following regression equation was expected: $\hat{Y} = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_5 + \beta_6X_6 + \varepsilon$. Where β_0 is y-intercept, $\hat{Y}$ is predicted serum HP level and $\beta_{1\text{-}6}$ are the slopes of Bili, LD, CRP, Retic, ALP and AST, respectively. The R^2 and adjusted R^2 were reported and used to determine the level of variance in the HP that is explained by the Bili, LD, CRP, retic, ALP and AST as described.[31]

The *t*-test and F-test determined the significance of the predictors while the beta coefficients determined the magnitude and direction of the relationship.[32] F-test was the preferred tool to test the strength of the impact of LD, ALP, CRP, Retics, AST and Bili results on HP values.

For statistically significant models, for every 1 unit increase in the independent variable, the dependent variable increases or decreases by the number of unstandardized beta coefficients. The assumptions of linearity and homoscedasticity were assessed by examining the partial plots. The multiple regression equation derived from this study can be used to: (a) predict new values for the serum HP when the independent variables results are known; and (b) determine the variation in the HP explained by the independent variable.

Results

The study examined 175 archived pathology data. The preliminary review resulted in excluding 11 data with very high values and leaving 164 data (87 males, 77 females). The data was from patients aged 4- 93 years old. The patients mean age was 62, mode = 65 and median = 74. The results came from 34 different public hospitals spread across the state. The criteria used to exclude a patient data set was based on the distance away from the statistical mean, mode, median that fell out of three standard deviations (3SD). After removal of these outliers, the statistical analysis was completed. The data was then screened to meet multiple regression assumptions. Dependent and independent variables were measured on a continuous scale (from 0 to over 100). The sample residuals were independent from the sample mean model. There were linear partial correlations between each of the predictors and the HP level. The study shows presence of homoscedasticity of residuals around the model and no evidence of multicollinearity among independent variables. There were no significant outliers, high leverage points or highly influential points in the data as attested by Cook's distances, tolerance, and variance inflation factors (VIF) values. The errors (residuals) around the mean model were normally distributed.

Hemolysis markers and haptoglobin

The study investigated the presence of multicollinearity among the independent variables. Visual inspection of the collinearity statistics shows that none of the independent variables have correlation values greater than 0.7, which is the minimum threshold required to prove the presence

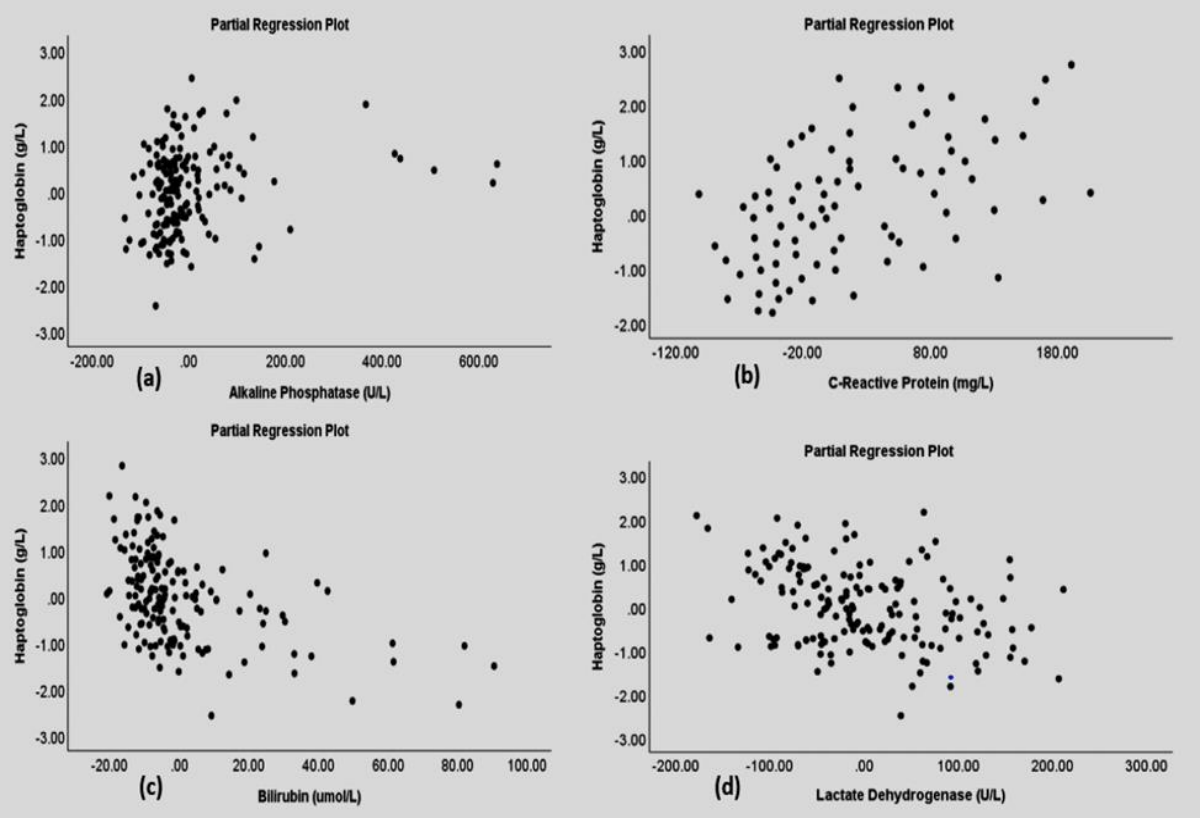

Figure 1: Haptoglobin Correlation Studies

of multicollinearity. The criteria to rule out multicollinearity is tolerance (>0.1) and VIF (<10).

The SPSS Statistics produced tolerance values greater than 0.1 (the lowest is 0.919) and VIF values are <2. The following partial correlation depicted a linear relationship between HP and CRP, Bili, LD and ALP (Figure 1. (a), (b), (c) and (d)). The partial correlation plots showed a strong relationship between HP and ALP, CRP, Bili, and LD, respectively. ALP and CRP have strong positive relationship with HP (Figure 1. (a) & (b). The ALP surges as HP levels increase. The serum elevation of Bili and LD contributed a negative impact on serum HP level (Figure 1. (c) and (d).

Normality test of sample residuals

To determine statistical significance of the model, the study used two methods: histogram with superimposed normal curve and P-P plot. The histogram with superimposed normal distribution showed the standardized residuals to be more-or-less distributed normally around the mean (Figure 2) the Distribution of Residuals Around the Mean Model. The mean of the model is on point zero on the histogram. The residuals are within ±3 standard deviation (SD). Any value outside the ±3SD were excluded from the model. In Figure (2.b), the P-P plot depicted the behaviors of residuals along the line of best fit. The observed and expected cumulative probability of residuals are lined up on a straight line, which shows that residuals are normally distributed along the mean model (Figure 2.b).

Hypothesis testing

The null hypothesis (H_0) states that the elevated retic, CRP, Bili, ALP, LD, and AST level in hemolyzed samples have no effect on the serum HP level. Low serum HP level in hemolyzed specimens may be due to a random effect and this implies that the relationship between HP and each of the independent variables is equal to zero; H_0: $\beta_1=\beta_2=\beta_3=\beta_4=0$, where β is the slope of the line of best fit. The alternative hypothesis argued that at least one of the slopes is greater than zero i.e. H_0: $\beta_1 = \beta_2 = \beta_3 = \beta_4 \neq 0$. In this study, the standardized beta coefficients are: CRP = 0.511, Bili = 0.407, LD = 0.274 and ALP = 0.165. The study set the level of significance at $\alpha = 0.05$; the mean critical value of 0.05 is 1.65. The *t*-statistic results for CRP = 7.875, Bili = 6.925, LD = 3.837 and ALP = 2.432, demonstrated that these critical values are greater than 1.65. The null hypothesis was rejected at a critical value greater than 1.65. The SPSS generated four models numbered 1 to 4. The model 1 is based on a relationship between a CRP and HP. This model has lower *R*2, adjusted *R*2 and high standard error of estimate (table 1).

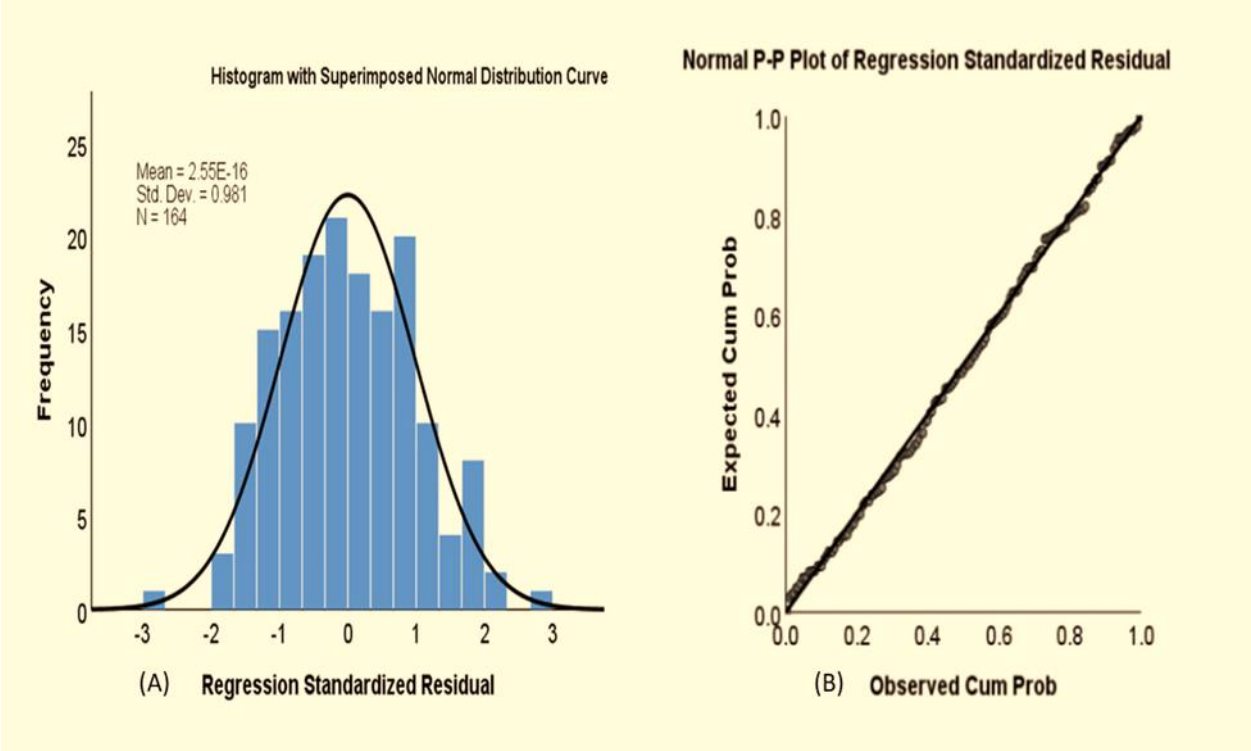

Figure 2: Test for the normality of sample residuals. (a) Histogram Depicting the distribution of residuals around mean model. (b) shows residuals lining along the line of best fit. Both demonstrated that the residuals are normally distributed.

In model 2, Bili was added to CRP and the effect on HP prediction was observed. Model 3 has CRP, Bili, and LD added as predictors. As more independent variables were added to model 1 and their effects on model 3 are observed, prediction of serum HP improved. CRP, Bili, LD, and ALP were added in Model 4 and yielded less standard error of estimates, improved *R*, *R*2 and adjusted *R*2. The model 4 has better prediction than model 1, 2 and 3. Thus the model 4 is the preferred model.

Table 1: Multiple regression models and predictors represented by superscript letters

Model Summary[e]						
Model	**Constant**	**R**	**R^2**	**Adjusted R^2**	**SEE**	**Durbin-Watson**
1	1.374	0.419[a]	0.175	0.170	1.043	
2	1.838	0.588[b]	0.345	0.337	0.932	
3	2.840	0.646[c]	0.418	0.407	0.882	
4	2.678	0.666[d]	0.444	0.430	0.864	2.151

a. Predictors: (Constant), C-Reactive Protein (mg/L); b. Predictors: (Constant), C-Reactive Protein (mg/L), Bilirubin (µmol/L); c. Predictors: (Constant), C-Reactive Protein (mg/L), Bilirubin (µmol/L), Lactate Dehydrogenase (U/L); d. Predictors: (Constant), C-Reactive Protein (mg/L), Bilirubin (µmol/L), Lactate Dehydrogenase (U/L), Alkaline Phosphatase (U/L); e. Dependent Variable: HP (g/L); SEE -standard error of estimate. The smaller the SEE, the closer the residuals are to the line of best fit.

Table 2: Analysis of variance

ANOVA[a]						
	Model	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig. F**
1	Regression	37.451	1	37.451	34.454	.000[b]
	Residual	176.088	162	1.087		
	Total	213.539	163			
2	Regression	73.772	2	36.886	42.490	.000[c]
	Residual	139.766	161	0.868		
	Total	213.539	163			
3	Regression	89.161	3	29.720	38.232	.000[d]
	Residual	124.378	160	0.777		
	Total	213.539	163			
4	Regression	94.818	4	23.704	31.747	.000[e]
	Residual	118.721	159	0.747		
	Total	213.539	163			

Key: df -degree of freedom, F -F-test, Sig -significant. The df is calculated by Nk-1, where k is number of variables. All four equations have been tested and have been found to statistically predict HP level in hemolysed samples.

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The decision to retain the null hypothesis (H_0) was set at alpha (α) greater than 0.05 and the decision to reject null hypothesis was set at p-value <0.05. The study found the p-values for CRP, ALP, LD and Bili to be less than 0.05 and therefore the null hypothesis was rejected at p-value <0.005. The standardized beta coefficient for each marker were: Bili = -0.407, CRP = +0.511; LD = -0.274 and ALP = +0.165. On the other hand, the unstandardized beta coefficient results suggest that for every unit change in CRP, ALP, Bili and LD, the HP level decreases by 0.009, 0.002, 0.025 and 0.004 g/L, respectively, provided that all the other variables are constant. The overall model produced is: Predicted HP – 2.678 + 0.009* CRP 0.025 * Bili-0.004* LD + 0.002 * ALP.

Discussion

The results from this research support the alternative hypothesis that the increase in levels and activities of serum Bili, CRP, LD, and ALP, respectively in intravascularly hemolyzed samples have a significant impact on serum HP levels. The results are significant at F (4,159), 31.75; p <0.005. The null hypothesis was rejected using critical and p-value approaches. Therefore, these findings provide an alternative technique for estimating serum HP results from pre-existing HM results and in the absence of IVH, EVH can be inferred from the results. The Bili, CRP, LD, and ALP have shown to be valuable parameters to assess reduction of HP level in hemolysis.

The study found CRP level and ALP activity to have positive correlation with HP. Like HP, CRP and ALP are acute phase proteins (APP), produced in the hepatocytes under the influence of cytokines. The role of CRP and ALP in tissue injuries/damages are to mediate and modulate inflammatory responses, respectively.[33] CRP has been shown to activate complement pathways, which exacerbate the intravascular hemolysis.[34] For instance, a sudden increase in serum CRP level at the start of IVH suggests that immune systems are responding to cell injury and can contribute to a higher rate of hemolysis. The cytokines-APP interactions trigger a surge in serum HP level with clinical and pathological consequences. The HP on the other hand controls the accumulation of cell-free hemoglobin levels. A sharp increase in the level of HP in a hemolyzed sample suggests a pending inflammatory situation. CRP and ALP alone would not necessarily suggestion present of IVH according to the model.

In contrast, the study found that high LD and Bili levels have a negative impact on serum HP level. Bilirubin and LD activity surge several days after the initiation of a hemolytic episode. LD has five isoenzymes and the most predominant isoenzymes in the RBC membrane are LD1 and LD2. [35,36] These isoenzymes spill into peripheral blood during IVH and increase the serum LD activity. In addition, high serum LD level strongly correlates with high level of cell-free Hb.[37,38] As free Hb level is catabolized, it yields globin and heme. The heme breakdown leads to a high Bili concentration. By the time the Bili and LD start to surge, the serum HP level is already significantly declining. The results showed that high LD and Bili concentration contribute to the rapid depletion of serum HP level. These results suggest that the Bili and LD model have a high probability of showing the extent of IVH impact on patients. In other words, the variables that negatively decrease HP level, are better candidates for a model that can accurately estimate HP level in hemolyzed samples and diagnose IVH.

The next plausible question is how best these variables accurately estimate HP level. This study showed that 44% coefficient of determination (R^2 = 0.44) of HP results variation above the HP mean model were accounted for by independent variables. For instance, a unit rise of serum Bili level, contributes to a 0.025 g/L drop of serum HP level provided other independent variables are held constant (Table 1, model 4). Bilirubin has the greatest impact on HP level compared with the other independent variables. When all the independent variables are added into the model, the *R2* dropped slightly from 44% to 43%. This slight decrease of adjusted R^2 improves the error of prediction of the model. The remaining variance of the model can be accounted for by systemic and random errors due to mechanical, preanalytical or biological factors.[39] These results are consistent with a previous study which showed that positive biases are expected in a normal population.[40] The study assessed the cross-talks (multicollinearity) between independent variables and no evidence of such relationship was found among the independent variables. This suggests that there is high degree of certainty in the model to accurately estimate serum HP in hemolyzed samples.[41]

The tools used to assess multicollinearity include tolerance, variance inflation factors (VIF) and Pearson's r. All the Pearson's (r) results are below 0.7, which suggest that there is no multicollinearity effect on the model. In addition, the tolerance and VIF results are greater than 0.1 and less than 2, respectively. The tolerance and VIF values are below the minimum threshold required to confirm multicollinearity among the independent variables, which suggested that model has not been affected by any collinearity.[43,44] The model is likely to accurately estimate the serum HP level in hemolyzed samples. After addressing the question of multicollinearity, the student attempted to address the question of closeness of results to reference or normal population.

The study found the residuals to be normally distributed around the mean model as depicted by histogram with superimposed normal curve and P-P plot (Figure 2.a & 2. b). These residuals' graphs

demonstrated that the residuals behave naturally and are free from external biases, implying that the model produced by this study is free of statistical noises. The

research excludes retics and AST from the model as they contributed no effect on the model. As expected, retics and AST elevation are often associated with bone marrow compensation and hepatocellular conditions, respectively. It is likely that AST and retic elevations in hemolyzed samples and concomitant decrease of HP level are due to extravascular factors other than IVH. The data were sampled from 34 public hospitals spread across the state and from diverse patient demographics and conditions.

Although, the research found statistically significant impact of Bili, LD, CRP and ALP on HP level, the results did not provide clinical significance or establish a cause and effect relationship. This model can be improved to demonstrate clinical significance by carrying out further studies with a larger sample size and controls applied to the preanalytical, analytical, biological, and environmental variables.

Conclusion

The present study shows a strong association exists between elevated independent variables and dependent variable in hemolyzed samples. The results also indicated that high levels of Bili and LD have negative relationship with HP while CRP and ALP significantly increase serum HP level in hemolyzed specimens. Negative association variables demonstrate an excellent model for the prediction of the HP level and diagnosing IVH. The parameters produced multiple regression model that can predict serum HP level from levels of hemolysis markers. Due to great variation of hemolytic diseases, the results of this model should be interpreted in the context of the patient's medical condition. With further study, the model has the potential to produce an estimated level of HP, which can be used to make diagnostic decisions, send sample for a confirmation of the HP level and to reduce sample rejection rates. The study utilized the data from a diverse patient's pool, which supports the validity of the study.

Declaration

The datasets generated during and/or analyzed during the current study are not publicly available due to sensitive nature of the data but are available from the corresponding author on reasonable request.

Acknowledgements

GP conceived the idea, designed the project, mined the data, performed data analysis using SPSS and wrote the manuscript. GD assisted in the navigation of ethical approval processes while PB and UN provided professional advice and guidance. We thank the technical assistance provided by BS at Pathology Queensland Laboratory, Queensland Children's Hospital, Queensland, Australia.

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Research Article

Expression of the Forssman antigen in gastrointestinal cancer

Tiago Silva[1], Diana Martins[1,2,3,4], Paulo Teixeira[1,5], Cristiana Mourato[1], Rúben Nunes[1], Carolina Melo[1], Clara Rocha[6,7], Simone Oliveira[8], Inês Mónica[9], Lucília Conceição[8], Daniel Almeida[10], Inês Oliveira[10], Fernando Mendes[1,2,3,4,11]

Politécnico de Coimbra, ESTeSC, DCBL, Rua 5 de Outubro - SM Bispo, Apartado 7006, 3046-854 Coimbra, Coimbra, Portugal[1]. University of Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR) area of Environment Genetics and Oncobiology (CIMAGO), Biophysics Institute of Faculty of Medicine, Coimbra, Portugal[2]. University of Coimbra, Center for Innovative Biomedicine and Biotechnology (CIBB), Coimbra, Portugal[3].Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal[4]. Pathological Anatomy Service from Coimbra Hospital and Universitary Center[5]. Polytechnic of Coimbra, ESTeSC, Department Complementary Sciences, ESTeSC, Rua 5 de Outubro, SM Bispo, Apartado 7006, 3046-854, Coimbra, Portugal[6]. Institute for Systems Engineering and Computers at Coimbra, Coimbra, Portugal[7]. Surgical Service, Hospital Distrital da Figueira da Foz, E.P.E., Figueira da Foz, Portugal[8]. Medical Service, Hospital Distrital da Figueira da Foz, E.P.E., Figueira da Foz, Portugal[9]. Anatomopathological Diagnosis Center(CEDAP)[10]. European Association for Professions in Biomedical Sciences[11].

Forssman (FORS) system is a new histo-blood group system with only one antigen. This Forssman (FORS1) antigen is expressed in erythrocytes, body fluids, several cells types and organs according to the species involved. Humans are Forssman negative, so the occurrence of antibodies Anti-FORS1 is highly common. However, there are individuals that are Forssman positive. Several studies suggest that the FORS1 antigen might have a key role in carcinogenesis. In this study, the aim was to determine FORS1 antigen expression in gastrointestinal cancer samples and compare the expression in normal and neoplastic tissue.

The expression of FORS1 antigen was analyzed using immunohistochemistry on gastrointestinal tumor and normal samples. The present study compared FORS1 expression in normal and tumor tissues, and the associated FORS1 expression pattern of differentiation with the therapeutic regimen.

The results demonstrated that from the 12 cases studied, 8 cases presented weak expression, 2 cases presented moderate expression of FORS1 antigen and 5 showed strong expression of FORS1 antigen in the cytoplasm of tumor cells. The results demonstrated that the intensity and extension of immunostaining differ according to the differentiation profile, suggesting that areas with a well-differentiated tumor showed higher expression of FORS1 antigen, whereas poorly differentiated areas presented less expression of FORS1 antigen. Samples from patients who performed chemotherapy regimens showed less Forssman expression, compared to patients who underwent surgery, suggesting that FORS1 antigen may have a relevant role in gastrointestinal carcinogenesis.

Key words: Forssman Antigen, gastrointestinal cancer, immunohistochemistry, antibodies.

Introduction

John Frederick Forssman discovered the Forssman (FORS1) antigen (Ag) in 1911, after injecting rabbits with a suspension of kidney tissue from a guinea pig. The antibodies produced by rabbits were found to lyse erythrocytes in the presence of the complement proteins.[1] FORS1 antigen is a heterophile glycosphingolipid antigen, structurally characterized as GalNAcα 1- 3GalNAcβ 1-3Galα 1-4Galβ 1-4Glcβ 1-Cer.[2] In 2012, the International Society of Blood Transfusion recognized the Forssman system as a new histo-blood group system with only one antigen.[3,4] During the eighties, three unrelated English families were reported with a blood subgroup called Apae which reacted strongly with *Helix pomatia* lectin, weakly with polyclonal anti-A Ab, but not with monoclonal anti-A antibodies.[5] However, Svensson *et al.* (2012) demonstrated that the erythrocytes from people of subgroup *Apae* didn't have A antigen but expressed FORS1 glycolipids instead.[6] Although the glycosyltransferases from group ABO and Forssman were related they have distinct substrate specificity, and FORS1 antigen is synthesized by Fs-synthase (globoside 3-α-N-acetyl-D-galactosaminyltransferase), which is codified by the gene *GBGT1* located on chromosome 9.[7] Canine

GBGT1 gene cDNA encoding FORS1 was cloned by Haslam and Baenziger and *GBGT1* and *ABO* genes were found to be paralogous genes that were derived from the same ancestral gene through gene duplication and subsequent divergence.[8]

Humans have been classified as FORS1 negative, so the occurrence of expression of antibodies (anti-FORS1) is common. However, there are some cases of individuals that are FORS1 positive. These individuals have a *GBGT1* gene containing the Arg296Gln substitution, which makes the FORS1 glycolipid synthase capable of catalyzing the final step of FORS1 biosynthesis.[9,10] The FORS1 antigen is widely but unevenly distributed in the animal kingdom. It is present on sheep erythrocytes and a variety of tissues of different animals such as cat, dog, guinea pig, mouse, horse, chicken, pigeon, and turtle, but it is absent in other animals such as rabbit, rat, pig, cow, monkey, and frog. Thus, species have been classified as FORS1 positive and FORS1 negative depending on the expression of the antigen.[1,11,12]

A previous study suggested that FORS1 antigen might have a key role in carcinogenesis. FORS1 antigen was described as present in gastric, colon and lung cancers and also that the expression in tissues could be related with the antibody titer in the patient's plasma.[13,14] Gastrointestinal cancers are one of the most prevalent cancers and consequently a major public health problem.[15] Ono *et al.* demonstrated the presence of FORS1 antigen in the cytoplasm of colon goblet cells, especially those in the transitional mucosa adjacent to a carcinoma. It was demonstrated that 69 of the 70 patients contained the FORS1 antigen.[16] Hirayama *et al.* demonstrated that patients with cancer showed levels of antibodies anti FORS1 lower than the age-matched, sex-matched, and blood type-matched control groups. The study demonstrated that patients with gastric cancer presented lower levels of antibodies than patients with non-gastric cancer. The serum levels of antibodies are also influenced by the histological type of cancer, with serum levels lower among those with differentiated adenocarcinoma and higher among those with poorly differentiated adenocarcinoma. The study also demonstrated that the levels of FORS1 antibodies increased post-surgically and when there was a recurrence of cancer, the levels of antibodies decreased again.[17]

The aim of this study was to identify the FORS1 antigen expression in gastrointestinal cancers and compare the expression of Forssman antigen between normal and neoplastic tissues.

Materials and Methods

Sample Characterization

Tissue samples were from the Hospital Distrital da Figueira da Foz E.P.E. (HDFF, E.P.E.), collected by the surgical team between September 2018 and March 2019. As inclusion criteria, the tissue samples were from gastrointestinal tumors, previously diagnosed as adenocarcinoma as primary tumors. Exclusion criteria, samples could not be from another organ tissue nor from another type of cancer nor have another diagnosed pathology.

All subjects who agreed to participate in the study were briefed about the aim of the study and signed an informed consent document. The study followed the principles of the Declaration of Helsinki for scientific research. The Ethics Committee of HDFF, E.P.E. approved the protocol.

Anti-Forssman antibody concentration

The primary antibody produced by cell line clone M1/22.25.8.HL (ATCC® TIB-121™) was concentrated using a solution of ammonium sulfate (0,55g/mL). Then, transferred to a tube containing 1 mL of the Ab supernatant. One mL of ammonium sulfate solution (Panreac Barcelona, Spain) was slowly added to the tube. The tube was then incubated for 2 hours at 4ºC. After incubation, the tube was centrifuged for 20 minutes at 3000 g. At the end of the centrifugation, the supernatant was removed, and the pellet was re-suspended in a solution of 200 mL of phosphate-buffered saline.[18]

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue specimens from 12 patients with gastrointestinal adeno-carcinoma were used for immunohistochemistry staining.

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Corresponding author: Fernando Mendes, Rua 5 de Outubro - SM Bispo Apartado 7006, 3046-854 Coimbra, Portugal
Email: fjmendes@estescoimbra.pt

Through paraffin blocks containing both cancerous and adjacent noncancerous tissue, 3 μm sections were obtained in the microtome and placed on immunohistochemistry positively charged slides.[19,20]

To increase the tissue adhesion to the slides, the sections were heat fixed in a laboratory stove at 60ºC for 1 hour. Next, the slides were dewaxed in xylol and rehydrated in a graded series of ethanol. Before starting immunostaining, and to increase accessibility to the antigen, the slides were incubated with Ultra Cell Conditioning Solution (Ventana Medical Systems, Tucson Arizona, USA), a buffer solution was used in the antigenic retrieval of tissues for 40 minutes at 95ºC, pH 8. The activity of endogenous peroxidase was blocked by incubation of the tissues with "Peroxidase Inhibitor" (Ventana Medical Systems, Tucson Arizona, USA) for 8 minutes.

After incubation, a blocking nonspecific antigen binding solution was used to reduce background staining, with a hyperproteic solution "Discovery antibody diluent" (Ventana Medical Systems, Tucson Arizona, USA) for 8 minutes. The slides were then incubated for 1 hour with a primary antibody clone M1/22.25.8.HL (ATCC® TIB-121) which reacts with human and mouse tissues.

The detection of the Ab was made using a multimer "Horseradish peroxidase Multimer" (Ventana Medical Systems, Tucson Arizona, USA) which provides signal amplification, for 8 minutes. After that, a solution of DAB Chromogen (Ventana Medical Systems, Tucson Arizona, USA) and DAB H_20_2 (Ventana Medical Systems, Tucson Arizona, USA) in equal parts, was placed on the slides for 4 minutes. All incubations were followed by a rinse in a phosphate-buffered Saline solution, "Reaction Buffer 10x" (Ventana Medical Systems, Tucson Arizona, USA) and all the steps in this protocol took place at room temperature, except as noted.[19,20]

Next, the slides were incubated for 4 minutes with a solution containing copper sulfate (5.0 g/L) in an acetate buffer to amplify DAB signal, "DAB Copper" (Ventana Medical Systems, Tucson Arizona, USA). Finally, nuclear counterstaining was performed for 2 minutes using a hematoxylin solution (Ventana Medical Systems, Tucson Arizona, USA) followed by incubation for 1 minute with a solution to bluing the hematoxylin, DAB Bluing (Ventana Medical Systems, Tucson Arizona, USA). The slides were rinsed in water, dehydrated, cleared, and mounted. Then the intensity of the immunostaining was observed with a microscope. All slides were classified by three independent observers.

Evaluation of immunostaining

The intensity of immunostaining was evaluated using a scale from 0 to 3, where 0 corresponds to a negative expression of FORS1 antigen, 1 corresponds to a weak expression of FORS Antigen, 2 corresponds to a moderate expression of FORS1 antigen and 3 corresponds to a strong expression of FORS1 antigen.

Statistical analysis

The IBM SPSS ® v.24 (National Opinion Research Center, Chicago, USA) was used for statistical analysis. The Chi-Square test was used to correlate the intensity and differentiation profile.

The One-Way ANOVA was used to relate the extension of immunostaining and the differentiation profile. The differences between the groups were considered statistically significant when assuming a random error of $p>0.05$, with a confidence level of 95%.

Results

This study included 12 patients that ranged in age from 57 to 89 years old, including 5 females and 7 males diagnosed with gastrointestinal cancer, properly staged according to the TNM system, as well as the therapeutic approach (Table 1).[21] The respective slide of tissue from each patient was observed and classified according to the differentiation phenotype, as described in Table 2. Of the 12 cases, 5 have differentiated adenocarcinoma, 2 have poorly differentiated adenocarcinoma and 3 have some areas

Table 1 – Characterization according to gender, age, TNM and therapeutic approach of the patients.

Cases	Gender	Age	TNM	Treatment
G1	Male	62	T3N1cM1	Surg + Adjv QT
G2	Female	89	T3N2M1	Surgery
G3	Male	82	T4aN1M0 IVL	Surg + Adjv QT
G4	Male	78	T1N0M0	Surgery
G5	Female	81	T3N0M0	Surg + Adjv QT
G6	Male	73	T4N3bM0 IVL	Surg + Adjv QT Neoadjuvant QT
G7	Female	72	T3N0M0	Surg + Adjv QT
G8	Female	83	T3N0M0	Surgery
G9	Male	76	T2N0M0	Surgery
G10	Male	62	T3N0M0	Surg + Adjv QT
G11	Female	78	T3N2M0	Surg + Adjv QT
G12	Male	57	T4N2bM0	Surg + Adjv QT

Key: IVL – Invasion lymphovascular; T – Size of tumor; N – Number of lymph nodes invaded by tumor cells; M – Metastases; Surg – Surgery; Adjuvant; Chemotherapy – QT

with differentiated adenocarcinoma and poorly differentiated adenocarcinoma (Table 2). Regarding

Table 2 – Characterization according to differentiation tumor phenotype**.**

Cases	Differentiation phenotype of the tumors
G1	Differentiated Adenocarcinoma
G2	Poorly Differentiated Adenocarcinoma
G3	Poorly Differentiated Adenocarcinoma
G4	Differentiated Adenocarcinoma
G5	Differentiated Adenocarcinoma
G6	Poorly Differentiated Adenocarcinoma
G7	Differentiated Adenocarcinoma
G8	Poorly Differentiated Adenocarcinoma / Differentiated Adenocacinoma
G9	Differentiated Adenocarcinoma
G10	Differentiated Adenocarcinoma
G11	Poorly Differentiated Adenocarcinoma / Differentiated Adenocacinoma
G12	Poorly Differentiated Adenocarcinoma / Differentiated Adenocacinoma

the FORS1 antigen expression, it was observed that five cases showed weak expression, one case moderate expression of FORS1 and three have strong expression of FORS antigen in the cytoplasm of tumor cells. In two cases areas with weak and strong intensity were observed, cases G8 and G12, respectively. G11 showed a weak and moderate intensity of FORS1 antigen expression (Table 3).

Table 3 – Intensity and extension of FORS1 antigen expression in tumor tissues

Cases	Gender	Age
G1	Weak	40%
G2	Weak	20%
G3	Moderate	90%
G4	Weak	50%
G5	Strong	80%
G6	Weak	90%
G7	Weak	60%
G8	Weak / Strong	30% / 70 %
G9	Strong	70%
G10	Strong	80%
G11	Weak / Moderate	40% / 90%
G12	Weak / Moderate	50% / 90%

Figure 1 shows negative and positive controls. The negative control was a tonsil sample (Fig 1a) and the positive controls were colon adenocarcinoma, lung adenocarcinoma and gastric adenocarcinoma, Figures, 1b, 1c and 1d, respectively. The results suggested that the intensity and the extension of the immunostaining were associated with the differentiation profile, $p<0,038$ and $p<0,003$, as presented in Figure 2, which

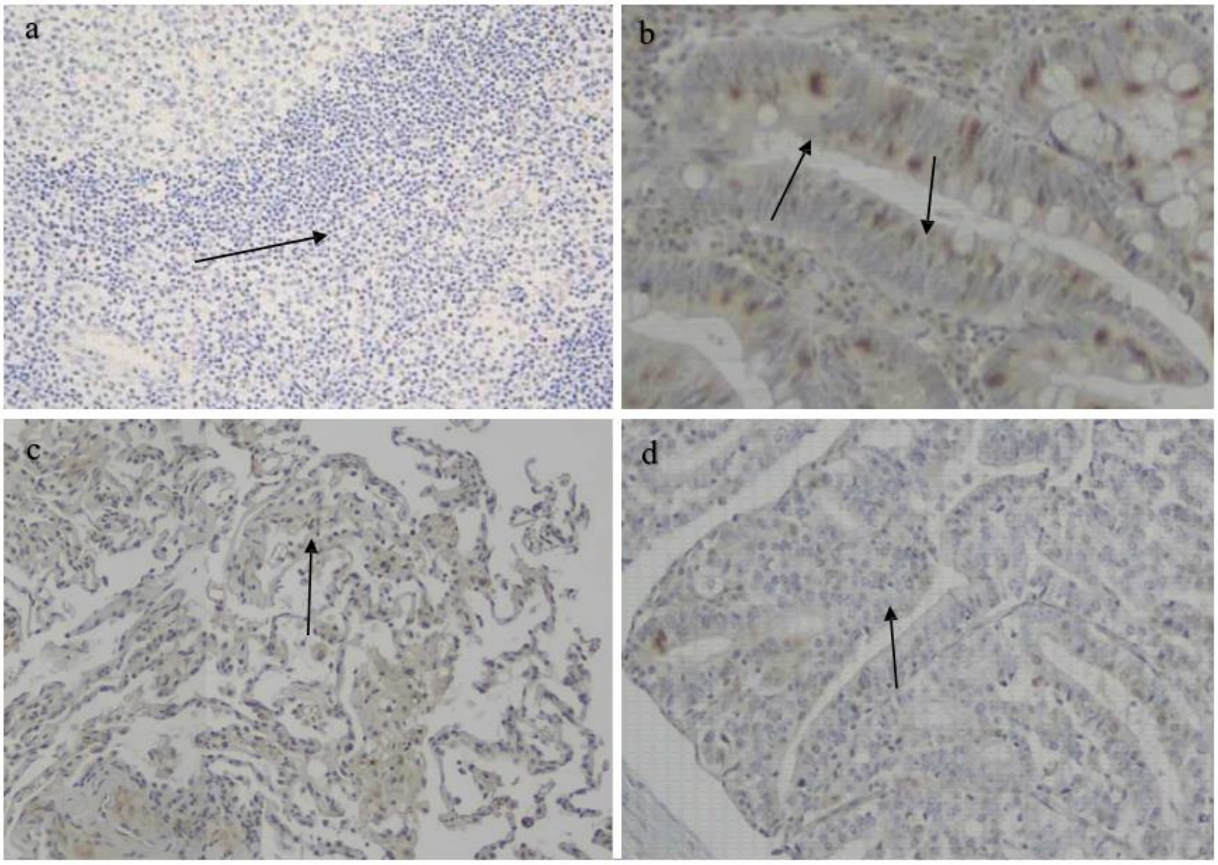

Figure 1: Immunostaining of Negative control and Positive control of FORS1 antigen. The slides were observed under the Olympus U-D30 optical microscope (Olympus, Tokyo, Japan), 100x magnification and photographed with an Olympus SC30 camera (Olympus, Tokyo, Japan); a) negative control of FORS1 antigen using normal tissues (arrow- blue cytoplasm of cells); b) positive control of FORS1 antigen using colon adenocarcinoma (arrow- brown cytoplasm of cells); c) positive control of FORS1 antigen using lung adenocarcinoma (arrow- brown cytoplasm of cells); d) positive control of FORS1 antigen using gastric adenocarcinoma (arrow- brown cytoplasm of cells).

is representative of FORS1 antigen expression. Interestingly the results also suggest that areas with well-differentiated tumor showed higher expression of FORS1 antigen, while areas poorly differentiated showed lower expression of FORS1 antigen. Surprisingly, three samples of the same tumor well-differentiated areas with higher expression of FORS1 antigen, and poorly differentiated areas showed lower expression of FORS1 antigen.

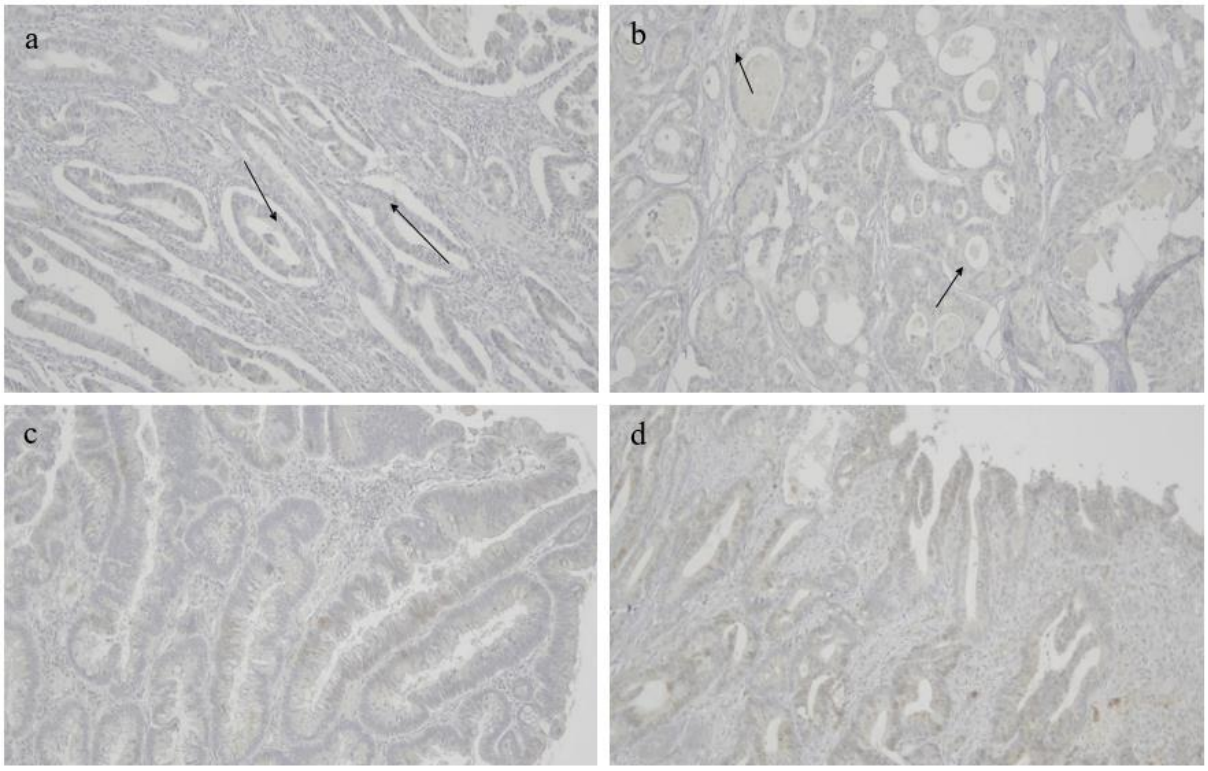

Figure 2: Figure representative of FORS1 antigen expression. The slides were observed under the Olympus U-D30 optical microscope (Olympus, Tokyo, Japan), 100x magnification and photographed with an Olympus SC30 camera (Olympus, Tokyo, Japan); a)The picture shows the presence of a weak expression of FORS1 antigen in the cytoplasm of cells of a poorly differentiated adenocarcinoma in Case G1 (T3); b) The picture shows the presence of a moderate expression of FORS1 antigen in the cytoplasm of cells of a differentiated adenocarcinoma in Case G3 (T4); c) The picture shows the presence of a strong expression of FORS1 antigen in the cytoplasm of cells of a differentiated adenocarcinoma ins Case G9 (T2); d) The picture shows the presence of a weak/strong expression of FORS1 antigen in the cytoplasm of cells of a differentiated adenocarcinoma in Case G8 (T3).

Discussion

Most people have naturally occurring anti-FORS1 in their plasma, however the expression of FORS1 antigen in tissues has been a challenge. FORS1 antigen was described for the first time in gastric, colon and lung cancer tissues.[13] The results obtained in the present study confirmed the presence and the expression of FORS1 antigen in tumor tissues of gastrointestinal cancer.

A previous study had suggested a correlation between the FORS antibody titer in the blood and the expression of the FORS1 antigen in tumor tissue, reporting that the titer of FORS antibodies could be associated with the adenocarcinoma differentiation pattern and with antibody serum levels.[17] Lower levels of antibodies were associated with differentiated adenocarcinoma and higher levels of FORS antibodies were observed in cases with poorly differentiated adenocarcinoma.[17]

Based on the results observed in the previous study, this study hypothesized that the variation of antibody concentration in the blood could influence the expression of the FORS1 antigen in tissues of tumors. When the FORS antibody titer in the blood is high, the expression of the FORS1 antigen in the tumor tissue should be weak. When the FORS antibody titer in the blood is low, the expression of the FORS1 antigen should increase. The results of this study support the an inverse relationship between FORS antibody levels and FORS1 antigen expression in gastrointestinal cancers. Furthermore, besides the correlation between FORS1 antigen expression and FORS antibody levels with the differentiation pattern (differentiated adenocarcinoma and poorly differentiated adenocarcinoma), the association with the tumor staging also occurred (T1 to T4). In fact, patients within group T1 and T2 exhibit lower antibody levels, and higher expression of FORS antigen in tumor tissues. Patients in group T3 and T4 presented higher levels of FORS antibody, but lower

expression of FORS1 antigen in tumor tissues. The subset of patients in the study underwent different therapeutic regimens, namely surgery or adjuvant chemotherapy. The intensity and extent of FORS1 antigen expression in tumor tissues was different between the patients who underwent surgery and the group of patients who underwent surgery plus adjuvant chemotherapy. Tumor tissues obtained from patients who underwent chemotherapy showed a weaker FORS1 antigen expression than patients who underwent only surgery. This decrease in the expression of the FORS1 antigen may be associated with the death of the tumor cells that express FORS1 antigen suggesting that there is a relationship between the therapy regimen and the expression of the FORS1 antigen in the tumor tissue. Based on the above, FORS1 antigen expression could be associated with the prognostic and therapeutic approach in cases of gastrointestinal cancer.

The authors are aware of the limitations of the study related to the small number of clinical cases and the inability to measure FORS antibody levels in the blood due to lack of patient's serum.

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Research Article

Great interest in a Swedish nationally regulated specialist education among biomedical laboratory scientists and biomedical laboratory scientist students

Quttineh M[1,2], Heldestad V[1,3], Wijkmark T[1], Rehnberg M[1], Lillsunde Larsson G[1,4,5]

IBL (The Swedish Institute of Biomedical Laboratory Science), SE-11426 Stockholm, Sweden,[1] Department of Laboratory Medicine, Region Jönköping County, SE-551 11 Jönköping,[2] Department of Clinical Microbiology, Umea University, SE-901 87 Umea, Sweden,[3] School of Health Sciences, Örebro University, SE-701 82 Örebro, Sweden,[4] Department of Laboratory Medicine, Faculty of Medicine and Health, Örebro University, SE-701 82 Örebro, Sweden.[5]

Biomedical laboratory scientists (BLS) work in many different disciplines but one common denominator for all the fields within the profession is the rapid development in biomedicine and the corresponding increase of advanced technology. A nationally regulated specialist training for BLS is a way for the profession to gain advanced skills and to create career opportunities. From a larger study set, the aims of this sub-study were to investigate BLS student's and professional's view on education, choice of workplace, career development, advanced studies and a potential nationally regulated specialist training program. Two surveys were designed using webbenkater.com. The surveys were sent to BLS student members (n=483) and professional members (n=2083) of The Swedish Institute of Biomedical Laboratory Science (IBL), the professional organization of BLS´s in Sweden. Response rate was 57% (276/483) for the student sub-survey and 44% (n=923/2083) for the professional sub-survey. Students from all semesters (1-6, n=272) were represented, with a majority from semester 2, 4 and 6. Top reasons for choosing the BLS education were; easy to get a job (65%), stimulating work tasks (59%) and a good education for further studies (39%). A majority of the students planned for further advanced academic studies (64%) and 54% percent were interested in a potential nationally regulated specialist training. Among professionals, 21% stated there were explicit career paths at their workplace. The individual interest for a potential nationally regulated specialist training was 53% and most responders (93%) stated a need for such an education in Sweden. Among IBL members, there is great interest in a nationally regulated specialized training among both future and present professionals in Sweden. In relation to a future shortage, we also show that in order to attract students to BLS training, we need to be able to offer advanced training as well.

Key words: Medical Laboratory Personnel, Medical Laboratory Science, Education.

Introduction

The biomedical laboratory scientist (BLS) is a young profession compared to several of the other health professions.[1] The BLS education and practical training make the profession unique compared to other professions in the medical laboratory, in terms of knowledge within quality assurance, evaluation of pre-analytical conditions, and assessment and validation of medical laboratory analysis.[2] The current curriculum in Sweden is three years of academic studies resulting in a professional exam as biomedical laboratory scientist and a Bachelor of Science degree. Within this program, curriculum in all courses are based on learning outcomes regulated at a national level and related to knowledge and understanding, skills and abilities as well as judgement and attitude/approach.[3] Biomedical laboratory scientists today work in many different disciplines and BLS in Sweden can either work in laboratory medicine or in the field of clinical physiology. While BLS in laboratory medicine are educated and work within molecular biology, immunology, transfusion medicine, pathology, microbiology, hematology, chemistry etc. BLS in clinical physiology are more directly patient oriented, and educated in clinical physiology, clinical neurophysiology, ultrasound and nuclear medicine. The two educations in Sweden are separate, although some initial basic courses are common. One common denominator for all the fields within the profession is

the rapid development in biomedicine and the corresponding changes in methodology and increase of advanced technology. Here, the BLS´s knowledge throughout the analytical chain has contributed to the desired efficiency improvement that takes place.[4] In order to keep up with changes in methodology and high technology, professionals are expected to have a continuous professional development (CPD) throughout their entire working life.[5]

Today there is a shortage of BLS in Sweden, with large expected retirements within the next few years. According to a recent national report[6], 17 out of 21 county councils reported that there is a shortage of BLS. In addition, the largest age group of BLS, according to the same report, are within the age range of 60-64 years. At the same time, not enough of BLS students are examined to make up for the retirees. The number of required training places within the education has increased, but unfortunately, many students do not carry out the full training. Only 51% of all students who started a BLS education in the 2012/2013 academic year in Sweden graduated as of academic year 2017/2018, according to a report from the University Chancellor's Office. Among those, 71% graduated within the normal time span (3 academic years). Apart from recruiting newly examined students, it is of outmost importance to also maintain the existing workforce in Swedish health care.[7]

The national organization for biomedical laboratory scientists in Sweden, the Swedish Institute of Biomedical Laboratory Science (IBL), has for decades been working for the development of the profession e.g. educating BLS in different fields and working towards a nationally regulated specialist training to create career opportunities in order to make the profession more attractive. The existence of a nationally regulated specialist training program ahead could also motivate students for applying to the BLS programs in the future. A specialist training program can specialize BLS skills and abilities within specific areas since the BLS scientist degree/bachelor degree in Sweden today provides the student with general skills in all disciplines within each field.

Today, guidelines for career development (qualification ladder) for BLS in Sweden is present at some regional levels. With continuous professional development and experience, the BLS should be able to gradually increase his/her knowledge and responsibility in the laboratories. Besides the clinical profile, education and research are other areas or career paths for the BLS.

In order to plan for the future work within the organization of IBL it is important to get both BLS students and professional BLS opinions on current topics of interest. Also, from a wider perspective, it is important to get input from future and present professionals on professionally related issues that impact this workforce in Sweden today.
From a larger study set, the aims of this sub-study were to;
- Investigate BLS students view on BLS education, choice of future workplace, advanced studies and a potential nationally regulated specialist training program.
- Investigate BLS professionals view on choice of workplace, career development, advanced studies and about a nationally regulated specialization program.

Materials and Methods

General study overview

Names and e-mail contact details of all the BLS professionals and BLS students in Sweden registered at the Swedish Institute of Biomedical Laboratory Science were collected. We designed two surveys using (webbenkater.com), one to use among the students at the BLS programs in any of the ten universities in Sweden and one to use among professional members of the IBL. We sent an email invitation to complete the survey to all the individuals for whom we had email addresses. Registration of e-mail address is voluntary in the IBL directory. We issued several re-invitations to maximize response rates.

The student survey was sent to 483 e-mail addresses (at the time there were 544 student members in the register) in February 2018. We also encouraged the student members to spread the survey to their student colleagues that were non-members. There are

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Corresponding author: Gabriella Lillsunde Larsson, E-mail gabriella.lillsunde-larsson@oru.se

currently 10 universities in Sweden with a BLS program. Six of them offers both orientations; laboratory medicine and clinical physiology, and four laboratory medicine only.

The invitation for the survey for professional members was sent to 2083 e-mail addresses (2541 professional members in the register) in September 2018. We also provided a link on the IBL homepage (https://ibl-inst.se/) to use for answering the survey.

In both surveys, participants could choose what questions to answer; hence, everyone who responded did not answer all questions. Only one question, year of graduation, in the professional study was compulsory. In total, the student survey consisted of 18 questions and the professional survey consisted of 41 questions.

From this larger study set-up, questions related to the study aim, were analyzed as of sub-study set up.

Sub-study set-up

Thirteen (of 18) questions from the student survey were selected in this sub-study. We selected questions related to BLS education, choice of future workplace, advanced studies and a potential nationally regulated specialist training program. None of the selected questions were compulsory to answer.

Twelve (of 41) questions from the survey to the professional members were selected in this sub-study. We selected questions related to choice of workplace, career development, advanced studies and about a nationally regulated specialization program. None of the selected questions were compulsory to answer.

Results

The student sub-study

Below, we report results from 13 questions answered by the students, relevant to the scope of this report. For the 13 questions, 3265 answers were collected. Two hundred and seventy-six students responded to one or more questions in the sub- study, response rate 57% (276/483). Most responders were women (80%, n=221/276) and between 19-24 years of age (59%, n=162/276). Students from all semesters (1-6, n=272) were represented, with a majority from semester 2 (28%, n=77/272), 4 (32%, n=87/272) and 6 (36%, n=99/272). Most students were enrolled in the laboratory medicine orientation (62%, n=166/269), 24% (n=66/269) listed their program as containing both laboratory medicine and clinical physiology and 14 % studied clinical physiology (n=37/269).

The majority of the students had BLS as a first choice when applying to higher education (84%, n=227/271). There was a significant difference between student groups regarding BLS as a first choice. Students enrolled in clinical physiology more often had another first choice when applying to higher education, compared to students enrolled in laboratory medicine orientation (Pearson's Chi-square: p=0.021). There were no differences between students in terms of age (Pearson´s Chi-square: p=0.59) or sex (Pearson´s Chi-square: p=0.84) regarding BLS as first choice. Students that listed BLS as a second choice, listed medical doctor and other educations within health care and biomedicine as firsthand choices. Most of the students found the education to correspond to their expectations in terms of academic level (62 %, n=165/267).

Top reasons (multiple choices possible) for choosing the BLS education were; easy to get a job (65%, n=173/267), stimulating work tasks (59%, n=157/267) and a good education for further studies (39%, n=105/267).

A majority of students already had plans for further advanced academic studies (64%, n=170/265), 11% (n=28/265) immediately after bachelor graduation (figure 1). Despite no significant difference between the groups (Pearson´s Chi-square: p=0.18), more women (66%) compared to men (55%) had plans for further academic studies (yes results combined). Students enrolled in clinical physiology answered more often that they had plans for further advanced academic studies (81%) compared to students enrolled in laboratory medicine orientation (59%, Pearson's Chi-square: p=0.013). Students in the age groups of 30-34 and 35-39 had the lowest interest for further academic studies, (48% and 33% respectively), while interest was higher in age groups 19-24 (71%), 25-29 (62%) and above age 40 (58%). The interest for advanced academic studies was significantly different between students at different semesters. Among semester 2 students, 19% (n=14/75) intended to continue with advanced academic studies directly after graduation while only 1% (n=1/91) of students from

semester 6 answered the same (Pearson's Chi-square: p=0.000085). Fifty-eight term 2 students (77%) were interested in advanced studies either directly after graduation or later after graduation (Yes-answers combined) while 49 of the term 6 students answered the same (54%, Pearson's Chi-square: p= 0.0017) (figure 1).

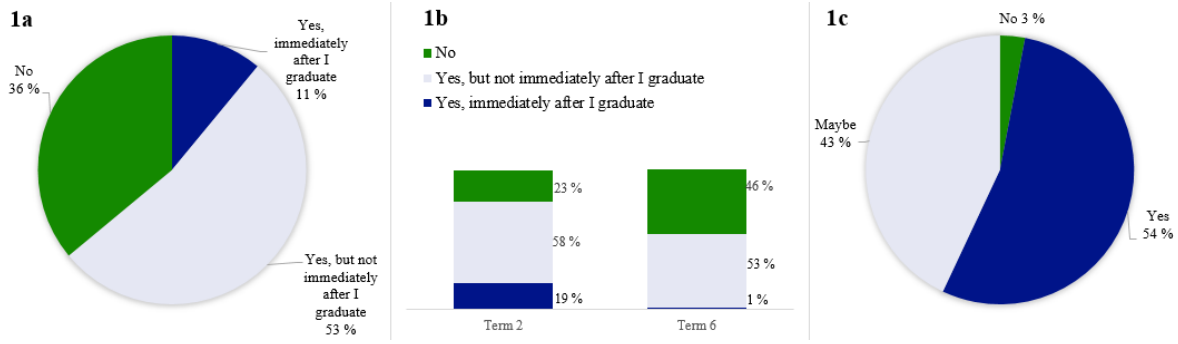

Figure 1: BLS student view on advanced studies and a nationally regulated specialist training program. From the student sub-study conducted by the Swedish Institute of Biomedical Laboratory Science (IBL), most students were interested in further advanced studies (1a). There were however differences between students at different semesters (1b). Term 6 students were less interested in further advanced studies compared to term 2 students both in terms of immediately after graduation (1% compared to 19%; Pearson's Chi-square: p=0.000085) or combined with later after graduation (54% compared to 77%; Pearson's Chi-square 0.0017). A majority of the students also showed interest in a potential nationally regulated specialist training program (1c).

Fifty-four percent (142/264) were interested in a potential nationally regulated specialist training and 43% stated that they might be interested, thus only 3% stated they were totally uninterested (figure 1). There was no difference in interest for a specialist education (yes/no) in terms of sex (Pearson´s Chi-square: p=0.68) or orientation of studies (Pearson´s Chi-square: p=0.88). Students in the age groups of over 40 had the lowest interest for a potential nationally regulated specialist training (yes/no, 82%), while interest was higher in all other age groups 19-24 (95%), 25-29 (96%), 30-34 (89%) and 35-39 (100%). The interest for a potential nationally regulated specialist training was not significantly different between students at semester 2 (84%) or semester 6 (92%, Pearson´s Chi-square: p=0.85).

Good colleagues and a nice working community were top reasons for choosing a future workplace (69%, n=184/266 multiple choices possible). Interesting working tasks were second (46%, n=122/266) followed by a high salary (44%. n=116/266) and possibilities for career development (41%, n=108/266). The choice of sector was irrelevant for most students (62%), n=165/265, 12% (n=32/265) answered the public health care, and 23 % (n=60/265) the private sector. Most students saw themselves working in health care (49%, n=129/264), followed by in research (17%), n= 45/264, pharmaceutical industry (10% n=26/264), and veterinary medicine (10%, n=25/264).

The professional sub-study

Below, we report results from 12 questions answered by the professionals, relevant to the scope of this report. For the 12 questions, 10 874 answers were collected.

Nine hundred and twenty-three professionals responded to one or more questions in the sub-study, response rate 44% (n=923/2083). Again, most participants were women (94%, n=870/922). Responders were from all age groups (19-24, 25-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60-65, >65), with the lowest participation in age groups 19-24 (2%, n=22/923) and >65 (2%, n=16/923). The highest participation rate was seen in age groups 55-59 (17%, n=191/923) and 60-65 (18%, n=171/923). Professionals were from all parts of Sweden, with a majority from the county of Stockholm (13%, n=117/922), Västra Götaland (18%, n=167/922) and Skåne (10%, n=96/922). Participation from the other counties varied between 1 and 7 %.

Most professionals were from laboratory medicine (90%, n=816/910) while 10% (n=94/910) worked in clinical physiology. In addition, most responders were from the public health care sector (86%, n=789/921), less were working in private care (8%, n=74/921), at universities (5%, n=43/921) and other. Employees were in majority (92%, n=839/914), 6% (n=52/914) stated themselves as managers.

Good colleagues and interesting working tasks were top reasons for choosing a future workplace among the professionals (62% n=576/923 and 55% n=506/923 respectively, multiple choices possible). Alternate working tasks were third (38%, n=350/923) followed by career development (CPD) (34%, n=316/923). A high salary and good management were stated as important among 25% and 26% (n=233/923, 237/923) of the responders.

Twenty-one per cent (n=193/918) in total stated that there were explicit career paths at their workplace while 68% (n=622/918) stated the lack thereof. Eleven per cent (n=103/918) were unsecure in regards of career paths. Professionals working in clinical physiology, compared to professionals working in laboratory medicine, more frequently answered that there were explicit career paths at their working place (43% compared to 23%, yes/no answers only). Professionals at universities and other governmental institutes more often answered that there were career paths (56%), compared to professionals working in public health care (23%), private sector (26%), municipality (7%) and other (11%) (Pearson's Chi-square: p= 0.000031, yes/no answer only).

Eighty-two per cent (n=729/884) answered that they´ve had continuous professional development at the workplace and there was no significant difference between professionals from clinical physiology (89%) or from laboratory medicine (81%, Pearson´s Chi-Square: p=0.057) nor between different sectors (private 78%, public health care 83%, universities and other governmental institutes 82% and municipality 81%, Pearson´s Chi-Square: p=0.77).

Seventeen per cent (n=148/885) of the professionals planned for advanced academic studies in general. Among professionals, more men (52%) compared to women (25%) had plans for further academic studies (yes results combined and compared to no-results, Pearson's Chi-square: p= 0.0015). Also, professionals from clinical physiology more often stated their interest for advanced academic studies compared to professionals working in laboratory medicine (35% and 19% respectively, Pearson's Chi-square: p= 0.034).

Is there a need for a regulated specialist education?

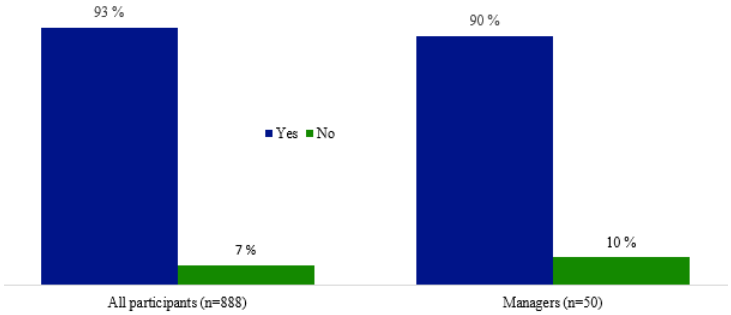

Figure 2: BLS professional view on a nationally regulated specialist program in Sweden. From the professional sub-study conducted by the Swedish Institute of Biomedical Laboratory Science (IBL), professional employees and managers both agreed on the need for a nationally regulated specialist program in Sweden. There was no significant difference regarding managers and non-managers opinions on the need for a nationally regulated specialist training (Pearson's Chi-square: p= 0.38).

The individual interest for a potential nationally regulated specialist training was 53% (n=470/884) while 26% stated that they might be interested and most responders also stated a need for such an education in Sweden, 93% (n=809/868). There were no significant differences between men or women in regards of interest for a nationally regulated specialist training (71% and 80% respectively, yes results combined and compared to no-results (Pearson´s Chi-Square: p=0.24) nor between professionals from clinical physiology or laboratory medicine (71% and 80% respectively, yes results combined and compared to no-results, Pearson´s Chi-Square: p=0.14). Of participants who answered that they work as managers, 90% (n = 45/50) consider that a regulated specialist training is needed (figure 2). There was no significant difference regarding their managers and non-managers opinions on the need for a nationally regulated specialist training (Pearson's Chi-square: p= 0.38).

Discussion

The biomedical laboratory scientists is in the crossroads between the health disciplines and has a deep understanding of technology for diagnostic purposes. The high rate of technical development in combination

with new evidence in both the diagnostic and treatment field requires a continuous and deep knowledge to meet the future challenges in health care.[4]

Here, we present data from BLS students and professionals on their view on the education, choice of workplace, career development, advanced studies and a potential nationally regulated specialist training program. Response rates for the two surveys were 57% (student sub-study) and 44% (professional sub-study). Two hundred and seventy-six students gave their important opinions and although we had hoped to include more participants, we are grateful for their interest. IBL as a professional organization has for many years offered free membership for students to include future professionals and make sure they have impact on the development of the organization. In Sweden 2018, 294 students graduated (personal communication C Hesse, University of Gothenburg) with a professional and a bachelor's degree from the ten universities. IBL, as many other organizations in Sweden, struggle to keep former students as members once the free period has ended. At the time of the survey, 544 student members were registered in IBL. There are an estimated 1257 number of students enrolled in the BLS education throughout Sweden.[8]

For the professionals, at the time of the survey, we had 2541 members and e-mail addresses to 2083 of them. Nine hundred and twenty-three contributed, a substantial number but unfortunately approx. only 1/10 of all BLSs in Sweden.[9] Despite declining numbers due to retirees and perhaps a general lack of interest in organizational work, IBL works hard to change this development. We want to be the obvious choice of organization for all Swedish BLSs, no matter their specialization, discipline or working platform. Since this was a survey aimed mostly for member students or member professionals, response rates can only be seen within the frames of the organization. No register is present to reach all BLS in Sweden, hence this is an attempt to provide observational data from this group of professionals.

Despite moderate response rates, we were able to draw some general conclusions from the participants that took part in the study. Most responders in both groups were women. This is in accordance with the distribution in the general working BLS population. Also, most participants were from laboratory medicine. There are fewer BLS in clinical physiology nationally, and this is reflected in both the number of educational places and clinics at the hospitals. Among the 466 educational slots in Sweden 2018, 112 were in clinical physiology (personal communication C Hesse, University of Gothenburg). Most professional responders were in the age-groups 55-59 and 60-65. This is also in line with national data.[9]

The student survey indicates that the shortage of BLS in the field was the main reason for applying to the program. Other top reasons were stimulating working tasks and the possibility to continue with advanced studies. Despite a promising job market, we know that many students do not carry out the full training potentially due to several reasons. The current survey did not include questions on this topic, and we have no follow-up data on student flow-through for this group. We acknowledge that their opinions would have been of high value to understand the reasons for leaving the programs. One speculation from the results is that although the majority of the students had BLS as a first choice when applying, some that listed BLS as a second choice mentioned medical doctor and other educations within health care and biomedicine as first hand choices. It is possible that they later gained admission to their firsthand choices. Others may have chosen the program without prior knowledge on the education and/or profession, and if not in agreement with their own expectations, they´ve exited before graduation. Interestingly, students enrolled in clinical physiology more often had another first choice besides the BLS program, compared to students enrolled in laboratory medicine orientation.

When it comes to choosing working place, students and professionals here agree on the importance of good colleagues and interesting working tasks. High salary was more important for students (44%) compared to professionals (25%). This may reflect a wish from the students on future working conditions. However, among professionals, experience could have pointed to other factors as more important. A good career development was listed high in both groups. It is interesting that students also recognize this, already during training. It is alarming that 68% of the professionals listed a lack of career paths at their own working place. There were however differences between workplaces and between laboratory disciplines; professionals of clinical physiology and professionals in universities and other governmental

sectors more often reported that were career paths present at the workplace. The lack of clear carrier opportunities in health care can also be one reason for BLS to quit employments and apply for other jobs in e.g. the diagnostic industry for example, thereby adding to the shortage.

Many professional BLS in this study had however been offered some continuous professional development. A good career development that includes CPD could be a tool to attract and to keep employees at the laboratories, in times of shortage of working force. One of IBLs major objects is to offer CPD in different areas and hence educate BLSs in different fields. Also, IBL has provided a competence document including examples of competence levels as example to be used at the working places.[5]

Many of the students responded that they already planned for advanced studies (64%) once they have graduated from the bachelor program. Students that planned for advanced studies were more often women and in clinical physiology orientation. There was a very high interest in the lower semesters while semester 6 students seemed to be more interested in maybe trying on the working life before continuing with advanced studies. It would be of great interest to do a follow-up study aiming to better understand their thoughts on this and why this is different between the semesters. Compared to the students, more men than women among professionals planned for further advanced studies, and numbers were also substantially lower (17%). In agreement, professionals and BLS students in clinical physiology more often planned for advanced studies compared to professionals and students in laboratory medicine.

Most students and professionals who responded to the study were interested in a potential nationally regulated specialist training. The interest was highest among the students (97% were interested or maybe interested) compared to the professionals (79% were interested or maybe interested). Among the professionals, 93% of all responders stated a need for such an education in Sweden, the numbers among managers was 90%. There is no regulated specialist education in Sweden today, despite long-term efforts from IBL and others for many years. There are master programs available, some in specific specializations, open for BLS. However, without a regulated national program recognized in society and health care, students must argue the importance and value in the workplace themselves and will not benefit from agreements open for nurses for example. Without a national consensus, positions for specialized BLS can be rare and different between regions and hospitals. IBL has for several years been inviting managers and educators to work on a plan for a core curriculum for specialized training competences and to design an education that benefit the needs in healthcare but also to investigate how an experienced and specialized BLS can get specific positions. One good example area is pathology. Here, the experienced and well-educated BLS can perform gross cutting of selected specimens for example, in order to save pathology consultant time. Thereby, pathology consultants can be empowered to cope with increasing workloads and/or be able to contribute and participate more to the always increasing number of services, quality and development within the pathology field.[10] Other areas in Sweden where a specialist program for BLS is of relevance is for example within molecular diagnostics, diagnostic cytology and point-of care testing. Also in clinical physiology/ neurophysiology there is a need for future specialists, i.a. ultrasound and intraoperative neurophysiology monitoring since there is a lack of physicians.

There are some limitations with the two sub-studies. If response rate would have been higher, results and conclusions would of course have been more powerful. There is also a possibility of bias if only BLS in favor of advanced studies and a national regulated specialist program answered the surveys.

In conclusion, we show that among the participating students and professionals, there is great interest in a nationally regulated specialized training. Students were also highly interested in further studies on an advanced level. In relation to a future shortage, since the possibilities of further studies was important already for students, we also show that in order to attract students to basic BLS training, we need to be able to offer advanced training as well.

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