IFBLS

INTERNATIONAL JOURNAL OF BIOMEDICAL LABORATORY SCIENCE

Volume 10 • Number 2/2021 • Pages 36 - 124 • www.ijbls.org

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Front page photo: Nichola H Lawrence

IJBLS e-Journal Published by International Federation of Biomedical Laboratory Science
Online ISSN: 2308-7706

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Editorial

International Journal of Biomedical Laboratory Science (IJBLS): Research, Reviews, Technical Series and Special Features

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

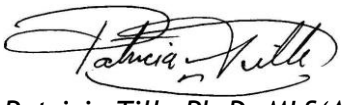
This edition of the International Journal of Biomedical Laboratory Science (IJBLS) includes a variety of valuable and timely information. As the Editor in Chief in conjunction with the International Federation of Biomedical Laboratory Science (IFBLS) Board of Directors, we are pleased to hear all the positive feedback we received following the publication of the first issue of the newly formatted IJBLS. I am confident the readers will find this edition as valuable as it includes a variety of information and article types including primary research, mini-reviews of technical standards, reviews of workforce challenges, a wonderful series in hematology and more!

The special features in this edition include a hematology series of articles that provides readers with a better understanding of the Philadelphia (Ph) chromosome negative myeloproliferative neoplasms (MPNs). The Ph negative MPNs are a closely related group of chronic hematologic diseases. In this introductory article the three Ph negative MPNs will be presented as a group while the following articles will provide a more in-depth review of each individual disease. The development of this series has been the primary goal of the new Hematology Advisory Group of the IFBLS. Inaugural group members selected this hematology topic due to its worldwide applicability and recent molecular discoveries leading to a better understanding of the pathogenesis of these diseases. The Hematology Advisory Group is an international group of academic and scientific biomedical laboratory scientists who are specialists in hematology.

Additionally, there is a special edition position paper on the role of biomedical scientists during the Covid-19 pandemic. This includes an executive summary along with the full manuscript, compiled by a group of professionals from the European Biomedical Scientists in the European member states. As with the first edition, you will see another laboratory spotlight on the Medical Laboratory Services in Nigeria. Our hope is to continue to spotlight member countries in all editions of the journal. If you are interested in sharing your laboratories story, please feel free to reach out to me.

Finally, as we expand the journal and continue to grow, we have added several Associate Editors to ensure we can continue to include current and accurate information that is of interest to all laboratory professionals across the globe. I am excited to introduce you to associate editors in this edition.

Yours truly, IJBLS Editor in Chief,



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

Editorial

Introducing: Dr. Aziz - International Journal of Biomedical Laboratory Science Associate Editor of Education and Administration

Hassan A. Aziz PhD, FACSs, MLS(ASCP)^{cm}

Dr. Hassan Aziz is the Dean of the College of Nursing and Health Sciences at Texas A&M University – Corpus Christi. Dr. Aziz is the President for the American Society for Clinical Laboratory Science (ASCLS). He is a Fellow of the Association of Clinical Scientists (ACS) and of the Institute of Higher Education at the University of Georgia (UGA). He is a certified Green Belt Six Sigma and TEDx speaker.

Dr. Aziz has extensive international experience in higher education, medical laboratory operation, consulting and advisory work. He is an external examiner for several international academic programs and an adjunct faculty to numerous institutions. He is an active member of national and international professional and scientific societies and organizations. He represents ASCLS at the International Federation of Biomedical Laboratory Science (IFBLS) and he is a member of the Scientific Network of Experts. He founded the Qatar Advisory Board of the American Society for Clinical Pathology (ASCP) in 2013 and is a mentor and a consultant for the ASCP Global Outreach Program. He is a VIP mentor to new program directors through the National Accrediting Agency for Clinical Laboratory Science (NAACLS) as well as a site visitor for clinical laboratory programs.

In addition to numerous international conference presentations and guest speaking engagements, Dr. Aziz has over 75 peer-reviewed publications. In 2019, he was the recipient of the prestigious ASCP Lifetime Achievement Award and the Distinguished Author Award by ASCLS. He is a consulting editor for Clinical Laboratory Science (CLS) published by ASCLS, and on the Development Board of Critical Values published by ASCP.

Editorial

Introducing: Dr. Hesse - International Journal of Biomedical Laboratory Science Associate Editor of Transfusion Medicine

Camilla Hesse, PhD; lic BLS

Dr. Camilla Hesse is a trained biomedical scientist (lic) and has worked in the field of transfusion medicine for many years. She holds a PhD in neurochemistry with a focus on Alzheimer's disease and diagnostic methods.

During the last 10 years or so, Dr. Hesse has focused her work on education and is currently head of the biomedical laboratory science program at the University of Gothenburg, Sweden. Camilla has received several pedagogical awards as well as funding for pedagogical projects. She has participated in external evaluation of both national and international education programs and has several positions of trust within academia. She has extensive international experience and has participated in several exchange programs. She has served as coordinator for exchange programs and received funding from Erasmus as well as national funds.

Dr. Hesse current research focuses on blood components and transfusion safety. She has been part of the research and development team at Transfusion medicine at Sahlgrenska University Hospital in Gothenburg.

Dr. Hesse has published 42 peer-reviewed papers and numerous presentations from national and international conferences. She has also supervised a numerous bachelor and master's thesis projects and co-supervised four Ph.D. students.

Editorial

Introducing: Dr. Reynolds - International Journal of Biomedical Laboratory Science Associate Editor of Histopathology

Gary M. Reynolds Ph.D.

Dr Gary Reynolds is the Cellular and Molecular Pathology Lead Scientist for the Liver Unit, Queen Elizabeth Hospital Birmingham, UK. He holds honorary positions at Birmingham Children's Hospital Liver Unit and University of Birmingham, Institute of Immunology and Immunotherapy as the Senior Research Fellow. He sits on a numerous national committees and advisory groups, including recently, the UK Coronavirus Immunology Consortium and the International Federation of Biomedical Laboratory Science Scientific Network of Experts, Histopathology.

Based at the Centre for Liver and Gastrointestinal Research and NIHR Biomedical Research Centre, his role is as a clinical and academic Biomedical Scientist. He performs an advanced role as a non-medical pathologist and develops and performs specialist diagnostic tests. As Principal Investigator, he holds numerous grants and has accumulated over 100 publications, with interests in liver machine perfusion, B-cells, viruses and tumor pathobiology. In 2016, he was honored with a Life Membership of the Institute of Biomedical Science for his contributions.

Editorial

Introducing: Dr. Singh International Journal of Biomedical Laboratory Science Associate Editor of Hematology

Indu Singh, Ph.D. MAIMS, FIBMS

Dr. Indu Singh is an experienced Medical Scientist and Program Director of the Medical Laboratory Science Program at Griffith University, Australia. She has a demonstrated history of working in higher education and Pathology for more than 37 years in India, Hong Kong and Australia including 20 years of academic tertiary teaching and doctoral research supervision. Dr. Singh has been recognized with multiple teaching, industry and research awards. Her teaching expertise and focus is on Hematology, Transfusion Science and Laboratory Medicine. She supervises and manages research and clinical trials in platelet, hemostasis, thrombosis, diabetes, lifestyle induced oxidative stress and antioxidants area. She has widely shared her research at conferences and published both scholarly and research scientific articles, book chapters and books. She is also an advisor for different Laboratory Medicine Programs and professional organizations globally.

Editorial

Introducing: Dr. Smit - International Journal of Biomedical Laboratory Science Associate Editor of Biochemistry

Francois Christiaan Smit, PhD

Dr. Smit has been in private medical pathology for 20 years beginning his career in the laboratory as a medical technologist and currently serves in a senior managerial position. His field of expertise ranges from Clinical Pathology to Clinical Chemistry. Dr. Smit has been involved in student training as well as a part time lecturer in Clinical Chemistry at the Cape Peninsula University of Technology, Biomedical Department. He has experience in planning and setting up student curriculum for the HPCSA Board examination as a member of the Society for Medical Laboratory Technology of South Africa (SMLTSA). Dr. Smit serves as the Scientific Advisory Committee chair for Clinical Chemistry at SMLTSA. He completed his PhD in Biomedical Science in December 2019 with published articles in local and international peer reviewed journals.

Editorial

Introducing: Dr. Tveten - International Journal of Biomedical Laboratory Science Associate Editor of Molecular Biology

Ann-Kristin Tveten, Ph.D.

Dr. Ann-Kristin Tveten is an associate professor at the Department of Biological Sciences at the Norwegian University of Science and Technology (NTNU) in Aalesund. She is the program manager for the bachelor program in biotechnology and works closely with the biomedical laboratory sciences program. She is a certified biomedical laboratory scientist (BLS) and teaches BLS students in microbiology, molecular biology and bioinformatics. She has a passion for innovative teaching methods, and especially teaching methods for simulation and practical skills training.

Dr. Tveten worked with diagnostics in medical genetics before completing a Ph.D. in microbiology. Her research work previously focused on tick-borne pathogens, while currently she is working with metagenomics analysis using next-generation sequencing and the characterization of marine microflora. Her extensive work with polymerase chain reaction (PCR) methodology has given her a great interest in developing new methods for use in teaching and projects associated with stress related gene expression. She enjoys developing new areas of collaboration and uses much of her expertise in research projects for the welfare in aquaculture.

Dr. Tveten has been a member of Norwegian Society of Engineers and Technologists (NITO) and Norwegian Institute of Biomedical Science (BFI) since her studies and has been actively involved in disseminating the knowledge related to biomedical laboratory science.

Editorial

The European Association for Professions in Biomedical Science

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

The International Association For Biomedical Laboratory Science (IFBLS) was established in 1954 as the world's widest reaching international organization for Biomedical Laboratory Scientists, bringing the profession, the professionals and health priorities to the world stage.

Following the conclusion of World War II in 1945, the leaders in Europe sought to ensure that such a calamity would never happen again. In 1957 the Treaty of Rome created the European Economic Community (EEC), or 'Common Market,' a grouping of 6 nations. In 1973 this group expanded to 9 countries and by 2013 the group had grown to 28 countries. Each of these countries elects members to a European Parliament and a Commission, essentially the administration of the Union, now termed European Union or EU.

One of the principles of the EU is mobility of its citizens. There is an EU Directive specifically laying down the principles relating to the free movement of professionals between member states.
The European Association for Professions in Biomedical Science (EPBS) was formed in May 1999 at The Hague, Netherlands. This International Non-Profit Association (AISBL) is committed to promoting best practice and ethics for Biomedical Laboratory Scientists throughout Europe. It was officially registered under the Belgian law in Brussels in 2006 as an international non-profit association. It now represents more than 250 000Biomedical Scientists from 22 countries in Europe

The aims of the EPBS are to:

- promote the maintenance of the highest possible standards of practice within biomedical science
- develop the ethical and professional values of the biomedical scientist
- support the training and education of the biomedical scientist in order to improve health care provision
- foster co-operation between member societies in areas of education, continuing professional development, competences and research
- liaise with EU Commission on all issues relevant to biomedical science
- utilise this shared knowledge between the societies to the benefit of all.

The two organizations IFBLS and EPBS both work towards similar objectives, one with a global remit and the other focusing on the role and education for biomedical scientists in Europe. They have a Memorandum of Understanding outlining their relationship. The membership of each organisation is drawn from the professional bodies representing biomedical scientists. Many members are members of both organizations and indeed many Presidents of IFBLS have been drawn from the profession in Europe with reputations within EPBS.

EPBS has furthered its aims by producing a policy of education of Biomedical Scientists for practice which can form the basis for the free movement of biomedical scientists in Europe. In 2021, a long-time goal has been achieved with the launch of a European Joint Master Degree in Biomedical Science offered by a consortium of colleges in 4 countries (Austria, Ireland, Portugal and Sweden).
In May 2021 members of EPBS, mindful of the continuing evolution of the Covid-19 pandemic globally and within Europe decided that a position paper outlining the issues involved in providing a service for the safe testing for the Sars-CoV-2 virus and highlighting the pivotal role of Biomedical Scientists in this service should be prepared. This position paper could be used by EPBS and its members to petition the EU Commission and their national health services to ensure that the testing provided meets quality standards and ensures the safety of European citizens.

A task force led by Anneke Geurts-Moespot and Neven Sucic from the EPBS management body, along with the past-president Marie Culliton with representation from Iceland, Portugal, Malta, and Sweden worked to prepare the executive summary and position paper which is published in this issue.

Editorial: Laboratory spotlight

Spotlight on Medical Laboratory Services in Nigeria

Dr. Bassey Enya BASSEY[1]
Dr. Casmir Ifeanyichukwu Cajetan IFEANYI[2]

How is healthcare delivered in Nigeria?

In Nigeria, healthcare service delivery is carried out in two major subsectors -- the public health service and private health service. There are three tiers of healthcare service delivery in Nigeria namely: Primary, Secondary and Tertiary healthcare service levels. Healthcare service delivery are constitutionally under the concurrent legislation in Nigeria, that is, the Federal and state governments can make regulations and policy for the efficient and effective healthcare service delivery. Healthcare services are departmentalized according to the different professions that comprise the health sector (Medical Laboratory Services, Physiotherapy, Radiography/Radiology, Pharmacy, Nursing Services, Physicians etc.). In Nigeria, each profession is distinct and strictly under the purview of their respective regulatory agencies. In the discharge of their duties, all healthcare professionals work in synergy for the interest of patients.

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

Medical laboratory services are central to the health care delivery system in Nigeria. They operate in either of the three modes stated in the previous section. In the public health institutions, laboratories are a part of the core clinical services. In the private sector, they operate independently, owned by qualified individual persons (proprietors). Recently, following the wave of private-public partnership (**PPP**) some vested interests in government are pushing for policy statements that encourage the takeover of public laboratories by private conglomerates within public hospitals.

A private MLS practitioner operating a hematology analyzer

What are the education requirements for the Biomedical Laboratory Scientists?

The basic educational requirement and minimum standard and skill to be attained by persons seeking to practice as a Medical Laboratory Scientist in Nigeria is the completion of a Bachelor of Medical Laboratory Science (BMLS) degree obtained through a five-year course in an accredited university. The BMLS degree is a prerequisite for registration and issuance of a practicing license from the Medical Laboratory Science Council of Nigeria (MLSCN). MLSCN regulates medical laboratory science practice in Nigeria. After obtaining these basic educational and professional requirements, one is mandated to increase one's scientific knowledge and expertise annually, by participating in compulsory continuous professional development (CPD) programs, scientific conferences and workshops organized across the country and elsewhere across the globe. These are conditions for renewing one's practicing license. However, there is a new curriculum pending approval by Nigeria Universities Commission (NUC) for Medical Laboratory Science departments in Nigeria Universities. It is aimed at upgrading the departments to faculties. And it entails the commencement of a six-year program leading to the award of the Doctor of Medical Laboratory Science (MLSD) which will over time replace the BMLS degree. Presently, only two universities are piloting (test-running) the Faculty structure in the country.

What title is used for Biomedical Laboratory Scientists?

In Nigeria, Medical Laboratory Scientists are simply called ***Scientists***, ***Laboratory Scientists***, or ***Medical Laboratory Scientists***. All are under the designation of **MLS** an acronym for Medical Laboratory Scientist.

MLS practitioners working in the molecular laboratory at the University of Nigeria Teaching Hospital, Enungu, Nigeria

What are the strengths of the health care delivery system?

Nigeria's health care system is very poorly ranked. The various tiers and service components are operating below standard in line with international best practices. For instance, while other African counties like South Africa have more than three hundred ISO-accredited laboratories and hospitals, Nigeria is struggling with barely six such laboratories with a meagre two hospitals (one private and military) meeting the standards. This means that health care service delivery in Nigeria is relatively substandard. There is also a disproportionate patient to healthcare personnel ratio. And that is why medical tourism is a trending phenomenon among the elites and Nigeria government officials.

What are the challenges facing the healthcare delivery system?

There are myriad of challenges facing the health care delivery system in Nigeria. These range from poor infrastructure to poor personnel development. Government has over the years neglected the system and it has continued to decay. This accounts for an increased mortality rate; with its attendant shortened life expectancy rate.

Prior to 2000, life expectancy of Nigerians was 80 (±5) years for females and 70 (±5) years for males. But today we sorely face the decline of these indices to 50 (±5) for both genders. Worse still, the United Nations Population Fund (UNFPA) released a heart-rending figure on Nigeria's life expectancy in 2019. It placed Nigeria as a country with the world's third lowest life expectancy rate of 55 years. This means Nigeria is only better than countries like Sierra Leone, Chad and the Central African Republic. All these indices explain the sorry state of Nigeria's health sector.

More so, the unhealthy antagonism between physicians and other healthcare professionals over undue role usurpation especially in specialties like laboratories between pathologists and MLS is another obstacle that contributes to the challenges bedeviling the health sector in Nigeria.

1. Formerly National President, Association of Medical Laboratory Scientist of Nigeria.
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Executive Summary

COVID-19 Pandemic and the Role of Biomedical Scientists

European Association for Professions in Biomedical Science (EPBS)

Key Points

- **SARS-CoV-2 is the greatest health challenge to the world of the century.**
- **Testing, vaccination and public health measures are the cornerstones for recovery.**
- **Testing must be undertaken and interpreted accurately.**
- **Failure to provide a quality assured testing program will lead to the failure of the recovery of commerce, travel and a return to a normal way of life.**
- **As the virus mutates there is an increasing requirement for nucleic-acid sequencing capacity.**
- **Biomedical Scientists are the experts in clinical diagnostic testing. They have, and will continue to, provide this quality assured service.**

The emergence of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection continues to present the greatest health challenge to Europe and to the rest of the world, which began in 2019. Management of this pandemic has challenged Governments, Health Authorities and the Medical and Nursing professions. One other profession, normally hidden from public view, the Biomedical Scientist, has stepped into the limelight and their contribution to the diagnosis and monitoring of the progression of this disease has been, and will remain, critical for SARS-CoV-2 management. The profession of Biomedical Science is regulated within Europe. The regulations confirm that the scientists undertaking the testing in laboratories have the knowledge, skills and competencies to verify the testing system, undertake the analysis and ensure the results are fit for purpose.

While much has been written about the healthcare staff on the front line during this pandemic, those in patient facing roles, the contribution of Biomedical Scientists to the control of this pandemic must not be underestimated. For a virus almost unknown in 2019 there have been almost 3 billion tests carried out within 18 months by Biomedical Scientists. This testing has been performed while continuing to maintain the routine clinical diagnostic laboratory workload required to ensure adequate population health.

In addition to the analysis, the Biomedical Scientists have worked with clinical colleagues to ensure that the correct specimens are submitted for analysis and that the microbiologists and infectious disease teams are provided with analytical results and surveillance data. They have interacted with public health teams. They have provided the statistical data necessary for health providers and governments to manage the pandemic and make informed decisions.

The pace with which analytical methods were developed for SARS-CoV-2 was remarkable. Clinical Diagnostic Laboratories in Europe are configured in different ways with different funding models than other countries. These variations meant that the capacity to respond for the required testing varied. The *In Vitro* Diagnostic (IVD) industry worked with remarkable speed to prepare testing kits for use on existing platforms. Virology laboratories, with research capacity, quickly developed 'in house' methods for testing. Scientists all over the world worked cooperatively to identify the genetic material and share primers to develop robust molecular testing methods. This, perhaps, led to the impression that this is easy to do. It is not. Staff trained in

specimen collection are trained to collect these samples from patients such as Doctors, Nurses and Biomedical Scientists. If other groups, or indeed individuals, are drawing samples they should be trained by competent staff, either directly or via viewing material placed online by the World Health Organization. The specific knowledge skills and competencies of a Biomedical Scientist are required to ensure that the testing systems are verified as fit for purpose in a given testing environment. All clinical diagnostic analysis must be subject to Internal Quality Control and External Quality Assurance. Irrespective of the setting where testing is provided the same guiding principles must apply for both patient safety and quality health outcomes.

In the beginning there was a lack of preparedness and the ability of healthcare systems to respond to this pandemic across the globe. This was particularly true in terms of clinical diagnostic capacity. Over the past decades there has been an assumption that this specialty is becoming simplified with the introduction of automated instruments and integrated information technology (IT) systems. There has been a trend to use non-professionally qualified staff and to reduce investment in the professional development and career pathways for Biomedical Scientists. This has been a mistake. As the pandemic unfolded it was evident that there were insufficient qualified Biomedical Scientists in Europe to undertake the range and volume of testing required. As waves of the virus pass through populations and mutations occur, it is clear the concept of zero SARS-CoV-2 is no longer a possibility. The world must all learn how to live with this virus. It is clear the virus can, and will, mutate to maintain an infectious advantage. It is likely that there will be a continued need to provide testing services for both symptomatic and asymptomatic cases, for contact tracing, to monitor the mutations of the virus and to establish immunity to permit normal life to be re-established safely.

The impact of and response to SARS-CoV-2 (COVID-19) in Europe has not been uniform, it should be consistent at a national, regional and global level. It needs to be informed by, and adapt to, the evolving evidence and science. Without robust testing systems and a European wide coordinated approach to testing regimens the resumption of normal life and commerce will be delayed.

The COVID-19 pandemic has had a huge impact on international travel, including in Europe. As part of the European strategy to re-establish free movement the Digital COVID Certificate (EUDCC) was developed and implemented. The purpose of these digital certificates is to show that an individual can travel and cross borders without a (tangible) risk of carrying the virus. For safety of the citizens it is imperative that all testing processes are quality assured. Without this assurance the EUDCC cannot succeed, and free movement will be prohibited. It is the position of the European Association for Professions in Biomedical Science (EPBS) that only regulated healthcare professionals, ideally Biomedical Scientists, should carry out such testing for the issuing of these certificates. The European Centre for Disease Prevention and Control (ECDC) is supporting scaling up of nucleic-acid sequencing and neutralization assay capacity in European Union/European Economic Area Member States.[1] This development is both welcome and vital for the coordinated European response to the pandemic. It will also require investment in the education and training of additional Biomedical Scientists in Europe to be sustainable.

The Biomedical Scientists of Europe, represented by EPBS, are the diagnostic partners in this fight. We will continue to work for the health benefit of our countries and together we can harness a resource that is at the disposal of the EU. Work with us, take advantage of our knowledge skills and competencies, give us the tools we need to deliver the service required bring us into the discussion and, as we have demonstrated, we will deliver.

(1) European Union/European Economic Area (EU/EEA): The EU is an economic and political union of 27 countries in Europe, collaborating with an additional three countries as members of the EEA. EU/EEA operates an internal market, which allows free movement of goods, capital, services and people between member states.

EPBS Position paper

COVID-19 Pandemic and the Role of Biomedical Scientists

Marie Culliton[1,2*], Anneke Geurts-Moespot[1,3], Ian Brincat[1,4], Alda M Hauksdottir[1,5], Lucilia Vicente[1,6], Neven Sucic[1,7], Maysae Quttineh[1,8,9], Michel Silvestri[1,9], Fernando Mendes[1,6].

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Introduction

Although a pandemic has been predicted several times over the past decades, little was done to prepare for it in Europe. Now we are paying the price. A worldwide outbreak with many sick people, many hospitalizations and, tragically, many deaths. Management of this pandemic has challenged Governments, Health Authorities and the Medical and Nursing professions. One other profession, normally hidden from public view, the Biomedical Scientist, has stepped into limelight and their contribution to the diagnosis and monitoring of the progression of this disease has been, and will remain critical, for SARS-CoV-2 management. Without robust testing systems and a European wide coordinated approach to testing regimens the resumption of normal life and commerce will be delayed.

The emergence of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection presents the greatest health challenge to Europe and to the rest of the world. It is clear the virus can, and will, mutate to maintain infectious advantage.[1] The response to this virus needs to be consistent at a national, regional and global level. It needs to be informed by, and adapt to, the evolving evidence and science. The global response has been innovative with rapid development and deployment of testing platforms and novel vaccines that would normally take many years to bring to market. These programs of testing, tracing, and vaccination are proving effective but, as the virus continues to evolve, full immunity has not been established. Indeed, some seven months post rollout of comprehensive vaccination programs evidence of breakthrough infection is emerging.[1] It is likely that there will be a continued need to provide testing services for both symptomatic and asymptomatic cases, for contact tracing, to monitor the mutations of the virus and to establish immunity to permit normal life to be re-established safely.

This paper, prepared by the European Association for Professions in Biomedical Science (EPBS), outlines the considerations required for provision of safe testing for SARS-CoV-2 within Europe. It is not a scientific treatise rather a discussion paper for European decision makers, outlining considerations, to assist them forge the right course. In this regard it is important that there is a coordinated European response from all nations within Europe.

Through the course of COVID-19 infection, viral replication, immune response, and inflamma-

Accepted: September 11, 2021, corrected October 25, 2021

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tory outcome are dynamic events that can change quickly, causing different outcomes so it´s very important to use the most appropriate laboratory diagnostic tests for the situation.

Clinical Diagnostic Testing Guidelines

Traditionally clinical laboratory diagnostic tests are provided in a clinical laboratory by scientists, qualified, trained and competent in the analysis. Point of care testing may also be used in a hospital or doctor office setting to provide rapid analysis without the benefit of scientific expertise. Patients with chronic diseases, such as diabetes, may also undertake self-testing. Irrespective of the setting where testing is provided the same guiding principles must apply for both patient safety and quality health outcomes:

- The testing method and equipment used must be fit for the purpose.
- The individual undertaking the test must be trained in how to do the test correctly using the equipment.
- There must be appropriate quality assurance of the entire testing process.
- There must be traceability from patient to result.
- There must be an appreciation of the factors that can influence the test.

Clinical diagnostic laboratories work within a quality management system, many are accredited to the International standards ISO 15189. The standard ISO 17025 attests to the quality of point of care testing systems.

The profession of Biomedical Scientist is regulated within Europe and this confirms that the scientists undertaking the testing in laboratories have the knowledge, skills and competencies to verify the testing system, undertake the analysis and ensure the results are fit for purpose.

There are 3 phases for testing: pre-analytical, analytical and post-analytical.

- **Pre-Analytical** refers to the steps in patient preparation for the test, the correct sampling, sample identification and transport to the testing area. Pre-analytical manipulation of the sample prior to its analysis may also occur.
- **Analytical** refers to the testing system itself; the choice of method, its verification quality assurance and performance
- **Post-Analytical** refers to how the result will be interpreted which must consider the patient clinical details in addition to an understanding of the limitations of the testing process.

The Biomedical Scientist has a major role in each of these phases, either through direct action or the provision of advice.

Pre-Analytical Phase: Sample Collection

SARS-CoV-2 is a respiratory virus. The samples used for testing are either a combination of a throat swab and nasopharyngeal swab or a nasopharyngeal swab alone. The quality of the final analytical result is dependent on the quality of sample submitted for testing. The best analytical method will not produce the correct result unless the sample is fit for testing. It therefore stands to reason that those collecting the specimen are appropriately trained.

Given the contagious nature of the virus it is important that the staff collecting the sample are trained properly and equipped with the appropriate personal protective equipment. Correct sampling of a nasopharyngeal swab is not easy. The swab must be inserted in through the nasal cavity until it reaches the nasopharyngeal area. Once there it must sweep the area to obtain the sample. The sample must be uniquely labelled with a combination of patient and sample identifiers. Each sample taken from an individual must be uniquely identifiable. This unique identifier must follow the sample from collection through testing and reporting to contact tracing. The sample transport medium must be appropriate for the test method being used. The sample must be analysed within a prescribed time from the time of collection to completion for the result to be valid. It is important that all material used in the collection of samples are correctly disposed of in accordance with bio safety regulations.

Staff trained in specimen collection are best placed to collect these samples from patients such as Doctors, Nurses and Biomedical scientists. If other groups, or indeed individ-

uals, are drawing samples they should be trained by competent staff, either directly or via viewing material placed online by the World Health Organisation (WHO) and others via YouTube.

Analytical Phase

The choice of analytical method and setting is a critical step in provision of a testing service. In Europe the *In Vitro* Diagnostic (IVD) manufacturers must validate methods and ensure their 'conformité européenne' (CE) marking is acceptable. This validation ensures traceability of testing components to a standard.

Within the testing centre the method must be fit for purpose. In choosing the method the Biomedical Scientist must consider:

- The purpose of testing: screening or diagnostic.
- The volume of specimens to be tested which may dictate the testing methodology and platform to be used.
- The turnaround time required for the result to be available.
- Disease prevalence.
- The skill of the biomedical scientist or other tester required for testing.

The manufacturer validated method must then be verified for use in the diagnostic setting using the equipment and staff who will perform the test. In addition, the sample matrix must be considered. Can the sample be analysed from any transport medium or must a specific matrix be used?

The specific knowledge skills and competences of a Biomedical Scientist are required to ensure that the testing systems are verified as fit for purpose in a given testing environment. In assessing any testing platform and method for use consideration must be given to many factors:

- **Precision** - The reproducibility of testing method. If the same sample is measured repeatedly, how likely is it that the same result will be achieved.
- **Accuracy** - How close is the reported result to the correct or true result? This considers the systematic error of the analysis.
- **Uncertainty of Measurement** - No testing is exact. A measurement result is only complete if it is accompanied by a statement of the uncertainty in the measurement. Measurement uncertainties can come from the measuring instrument, from the item being measured, from the environment, from the operator, and from other sources. Such uncertainties can be estimated using statistical analysis of a set of measurements and using other kinds of information about the measurement process.[2]

Precision, accuracy and uncertainty of measurement are a function of a combination of the robustness of the analytical method employed, the instrumentation and the competence of the analyst. The most reliable results are obtained when analysis is performed by Biomedical Scientists rather than by others who are not specialists in this area.

Quality Assurance

All clinical diagnostic analysis must be subject to quality assurance. Within clinical diagnostic laboratories this can be broken down into internal quality control and external quality assurance.

Internal Quality Control (IQC)

This is a process whereby the same sample is analysed multiple times, daily with each batch of tests or at defined intervals. The same result should be achieved within an agreed tolerance or standard deviation from the mean. This confirms that a given testing process operates satisfactorily and provides assurance regarding the method, instrumentation and testing personnel.

External Quality Assurance (EQA)

This brings IQC to a different level comparing results from a testing centre to external peers. This confirms consistency of results across testing platforms, testing centres and countries.

There are two major method options for measurement of SARS-CoV-2 commonly known as polymerase chain reaction (PCR) and antigen (or lateral flow) testing. The choice of analytical method must take into consideration the factors outlined above as well as the disease prevalence. It is also clear that

whatever testing methodology is used that the detection and management of this disease is a public health issue, both nationally and globally. Therefore, all testing systems must have a clear reporting route to public health for case management and contact tracing.

Post-Analytical Phase: Interpretation

Interpretation of results of analysis is not straightforward. It is dependent of the capacity of the analytical method to detect the disease and the prevalence of the disease in the population.

The following must be considered.

- **Sensitivity.** The ability of the test to identify those with the disease
- **Specificity.** The ability of the test to identify those without the disease
- **Predictive Value.** This is the chance that a positive test result indicates disease, and a negative result indicates absence of disease. These values are based on a combination of the sensitivity and specificity of the testing method along with the prevalence of the disease in the population

Results of analysis for SARS-CoV-2 should be reported as 'Detected' or 'Not Detected'. Each of these results must be interpreted considering the clinical presentation of the individual. It is most important that it be understood that a 'Not Detected' result is not synonymous with 'absence of infection'. It may be that the sample was incorrectly collected, transported, or analysed. It may also be that the sample was collected too early in the infection life cycle.

Choice of Analytical Method for Detection of COVID-19

The pace with which analytical methods were developed for SARS-CoV-2 (COVID-19) was remarkable. Scientists all over the world worked cooperatively to identify the genetic material and share primers to develop robust molecular testing methods. This, perhaps, led to the impression that this is easy to do. It is not. There are, essentially, two method types for detection of the virus. The first method detects the presence of viral RNA and the second detects the presence of viral antigens. In addition, there are antibody tests which detect the immune response to the infection.[3]

Detection of Viral RNA

Viral ribonucleic acid (RNA) can be detected using nucleic acid amplification (NAAT) methods. NAAT detect genetic material (nucleic acids). NAATs for SARS-CoV-2 specifically identify the RNA sequences that comprise the genetic material of the virus. NAAT first amplifies (make multiple copies) of the virus's genetic material. Amplifying the nucleic acids enables NAATs to detect very small amounts of SARS-CoV-2 RNA in a specimen, making these tests highly sensitive for diagnosing COVID-19. The most common NAAT used in diagnosis is the reverse transcriptase polymerase chain reaction or RT-PCR, typically referred to as a PCR test. Optimal diagnostics consist of a NAAT assay with at least two independent targets on the SARS-CoV-2 genome.

PCR testing is the method generally employed in clinical diagnostic laboratories. Methods have been developed by the IVD manufacturers to run on large, automated platforms capable of analysing several thousand samples per day. The genetic material must be extracted from the virus, mixed with primers to facilitate the amplification of the target nucleic acid sequences and then subjected to the amplification process with a detection system to identify the presence of the amplified target. There are smaller, semi-automated platforms which run nucleic acid extraction and the amplification as separate processes. These platforms can process from 50 to 500 specimens daily depending on the configuration of the analyser. The process of sample preparation through extraction, amplification and detection can take up to 6 hours. Typically, these automated methods are interfaced to laboratory information systems facilitating the logging and tracing of specimens from receipt to the final report. Compiled reports can be sent directly to public health disease surveillance systems for case management and contact tracing.

Rapid PCR methods are available in clinical laboratories where a result can be available within one hour. Some of these analysers are sufficiently portable to be used in the field for

outbreak management or where there are high risk individuals who are resistant to attending at large sampling centres. The cost per test of the sample analysis varies. The rapid detection PCR tests can cost between €50 to 100 per test whereas the automated platforms can run at less than €20 per test.

Detection of Viral Antigen

These are commonly referred to as lateral flow tests (LFT) or rapid antigen tests (RAT). With a COVID-19 LFT, a nasopharyngeal sample is placed on a small absorbent pad, which is then drawn along the pad via a capillary line to a strip coated with antibodies, which bind to SARS-CoV-2 proteins. If these proteins are present, this will appear as a colored line on the test, indicating infection. Results are typically available within 15 minutes.

The arrival of RATs suggested that testing could be decentralized and devolved to work places, schools, sports venues and even homes. While performance of the test may be apparently simple the same attention to detail is required. The sample must be correctly collected and identified. The pre-analytical manipulation of the sample must be performed correctly. All individuals that are in contact with the samples must be provided with, and wear, personal protective equipment. Sample manipulation should be performed in a safety cabinet with all materials disposed of in accordance with biosafety requirements.

The sensitivity of RATs is variable. This variability depends on the specific formulation of the test kit and the operator competence. The World Health Organization (WHO) offers clear guidance on their use.[4,5] The minimum performance requirements of ≥80% sensitivity and ≥97% specificity compared to a NAAT reference assay are required before a RAT can be used to diagnose SARS-CoV-2 infection in a range of settings where NAAT is unavailable or where prolonged turnaround times preclude clinical utility. Tests should only be carried out by trained operators.

A Cochrane review of the use of RATs published in March 2021 concluded: *"Antigen tests vary in sensitivity. In people with signs and symptoms of COVID-19, sensitivities are highest in the first week of illness when viral loads are higher. The assays shown to meet appropriate criteria, such as WHO's priority target product profiles for COVID-19 diagnostics ('acceptable' sensitivity ≥ 80% and specificity ≥ 97%), can be considered as a replacement for laboratory-based RT-PCR when immediate decisions about patient care must be made, or where RT-PCR cannot be delivered in a timely manner. Positive predictive values suggest that confirmatory testing of those with positive results may be considered in low prevalence settings. Due to the variable sensitivity of antigen tests, people who test negative may still be infected. Evidence for testing in asymptomatic cohorts was limited. Test accuracy studies cannot adequately assess the ability of antigen tests to differentiate those who are infectious and require isolation from those who pose no risk, as there is no reference standard for infectiousness. A small number of molecular tests showed high accuracy and may be suitable alternatives to RT-PCR. However, further evaluations of the tests in settings as they are intended to be used are required to fully establish performance in practice."* [6]

While the cost of RATs is less than that of the PCR tests, the overall cost can be considerable if used indiscriminately. The reported sensitivity of detection concerns for RATs means that consideration must be given to the probability of incorrect results and the potential impact for the individual and public health. These tests have a role when in a program of repeated testing likely to identify individuals when viral loads are high.

Antibody Testing

Antibody tests do not identify the virus, rather they indicate that the body has mounted an immune response to the virus. These antibodies appear in circulation one to two weeks after infection. They can therefore indicate that an individual has been exposed to the virus, either via infection or through vaccination. Given that it is unclear how long immunity remains post infection or vaccination it is possible that antibody testing may be used to assist decisions on booster vaccination.

Detection of Variants of SARS-CoV-2

Viruses mutate. Mutations occur when the virus is replicating in a host. The more the virus is

replicating the greater the chance of mutations. Mutations that have a competitive advantage will flourish and eventually become dominant. There have been waves of infection from different variants over the past year, specifically the alpha and delta variants. Each of these variants has been more infectious than the previous one.

As infection cycles wane and a new variant is becoming dominant it is important that this change is identified quickly, and appropriate measures put in place. This can only be done if detected cases are subject to whole genome sequencing. The WHO and European Centre for Disease Prevention and Control (ECDC) have a joint paper addressing the requirement for monitoring of variants.[7] They note that several variants of SARS-CoV-2 have emerged which are of concern. Emerging variants are classified as either Variants of Interest (VOI) or Variants of Concern (VOC). Monitoring of VOC in all countries is key. This requires sequencing of the viral genome. The cost and expertise required for this sequencing makes it impracticable for routine application.

Using alternative solutions such as the use of variation in response to multiple targets in PCR assays can triage those samples to be subjected to nucleic acid sequencing. In addition to identifying new variants by sequencing the impact of the variant must also be established; viral infectivity, its associated morbidity and mortality and the efficacy of vaccines. The impact of the variant on vaccine efficacy can be established using methods such as neutralization assays. The assays for sequencing and neutralisation require more sophisticated equipment and bio containment facilities that may not be available in routine clinical diagnostic laboratories.

COVID: The European Dimension

The impact of and response to COVID in Europe has not been uniform. Initially the infection presented in Italy with devastating loss of life. As the tragedy unfolded other countries had time to build defences. The healthcare authorities put triage systems in place and worked to ensure the hospitals were not overrun. The virus is primarily a respiratory virus, but it was some time before it became clear that the main route of transmission was aerosolized droplets.

Clinical diagnostic laboratories in Europe are configured in different ways with different funding models. These variations meant that the capacity to respond for the required testing varied. The scientists in China worked collaboratively with their global colleagues and the genomic sequence of the virus was published permitting preparation of primers for molecular assays. The IVD industry worked with remarkable speed to prepare testing kits for use on existing platforms. Virology laboratories, with research capacity, quickly developed 'in house' methods for testing.

The early period of the pandemic was characterized by a shortage of reagents for testing. In some cases, this led to a rationing of testing and conservation of supplies. Countries with large laboratories and large budgets were able to use their purchasing power. Differing testing protocols were used with some countries instituting mass testing in dedicated laboratories and others using distributed testing throughout their existing clinical diagnostic laboratory network. Many countries did not have the required testing capacity and there was cooperation between countries with many availing of capacity available in Germany. Specimen transport was facilitated by air forces.

European Country Responses

Members of the European Association for Professionals in Biomedical Science (EPBS) have recorded their national responses highlighting the essential role of biomedical scientists in the response.

Croatia

In Croatia, the fight against the COVID-19 pandemic is like that in most European Union (EU) countries. The government, Ministry of Health and the Civil Protection Headquarter issue recommendations and directives on measures to combat the pandemic. PCR tests are performed in most hospitals and the Institutes of Public Health, while antigen tests are performed in primary health care centres. The central hospital for the care of the most difficult patients as well as for the sequencing and coordination of laboratories that perform

PCR tests is the Clinic for Infectious Diseases in Zagreb.

Biomedical scientists at all levels have, as before, borne the greatest burden in laboratories for testing on SARS-CoV-2 because their knowledge, skills and competencies could contribute to the diagnosis of COVID-19. The status of Master of Biomedical Science has unfortunately not changed. Although in this pandemic, they have proven to be a valuable part of the team of healthcare workers. It is hopeful that the Government and the Ministry of Health will correct this injustice and that those with Master of Biomedical Science will improve in status like in most countries of Europe.

Iceland

In Iceland, the first quarantine orders were given in February 2020. The first COVID-19 domestic infection was confirmed in March 2020 and the first restrictions followed. From the beginning of the pandemic the screening and analysing of the Covid samples was manage by the heads of the Clinical Microbiology department at the University Hospital Landspítali (SVEID) who are a Biomedical Scientist and Doctor both specialists in Infectious Diseases. They had the technology, isolation and PCR equipment needed to analyse COVID. When Icelandic authorities decided to start screening, on a large scale, for COVID at the border as well as in the community, assistance was received from a private company with access to their facilities, equipment and, in the beginning, some professionals. Capacity at SVEID was insufficient in the beginning but it changed when they received improved facilities and new research equipment in late 2020. With new analysers productivity multiplied. The laboratory could not fill all the positions needed with Biomedical Scientists, so they also hired biologists, other scientists, engineers and students from scientific fields to work screening-related jobs for Covid-19.

Only two official and one private laboratory do Covid PCR tests and serum antigen tests. A few private laboratories do rapid antigen test which are all on the “Common list of rapid antigen tests” and one of them does serum antigen test as well.

There has been a shortage of Biomedical Scientists in Iceland for many years as in other European countries. Biomedical Scientists are a highly competent profession. Our work is interesting, diverse, demanding, joyful and requires specialized knowledge. According to Icelandic law from 2006 Biomedical Scientists in Iceland are authorized to own, run and manage laboratories. According to our operating license we have the authority to perform, interpret, validate, and approve results. But it is always doctors that diagnoses people. All information on COVID-19 available on covid.is

Ireland

Using Ireland as an example the response of laboratories was one of collaboration rather than competition. Initial testing was performed by the National Virus Reference Laboratory using an ‘in house’ method developed using internationally supplied primers. The biomedical scientists managing the laboratories came together, meeting weekly and, with the assistance of management consultants and the health service executive, identified the testing platforms needed for a hub and spoke model of testing. The platforms chosen depended on the size of laboratory and the testing catchment area. Following failure to deliver agreed testing kits by some companies, there was diversification of methods across the country with each hub laboratory having access to both batch analysis and rapid molecular testing. As the capacity was being commissioned, the samples from mass testing of the population were outsourced to Germany. Such was the dedication and commitment of the biomedical scientists to this project, that individuals drove across the country to ensure colleagues had the reagent supplies they needed. The scientists from research institutions, universities and veterinary testing laboratories were harnessed into the testing program. Each different group brought their own expertise from molecular testing of researchers to the clinical laboratory organization and sample tracking of biomedical scientists. The response to this virus highlighted the shortage of qualified biomedical scientists in Ireland.

Malta

In Malta, preparations for the anticipated

increased demand for laboratory services linked to the local spread of SARS-CoV-2 infections started early. The suspension of specific, non-urgent health services led to a decrease in workload which was usually received in various pathology sections. This allowed management to increase the staff working within the molecular diagnostics lab responsible for all COVID testing. Staff working in other sections were retrained and deployed to this section to increase human resources. New staff was also recruited and assigned duties related to COVID testing. Biomedical scientists working within university were also employed to ensure that every possible resource was being used. Additionally, our biomedical scientists were involved in managing and giving trainings to non-laboratory healthcare workers and moreover performing spot-checks in these remote clinics, hospitals and hubs involved with Covid-19 rapid testing. All of this has helped to keep up with the ever-increasing demand for COVID tests. Different rosters and teleworking were introduced to mitigate against any outbreaks within biomedical scientists. This was very important since laboratories located within the main public hospital are the main medical diagnostic facility and need to cater to the needs of the entire country.

Netherlands

In the Netherlands, a national network was set up in 2008 to deal with outbreaks of new infectious diseases in a coordinated way with laboratories being coordinated by the National Institute for Public Health and the Environment (RIVM). At the start of the pandemic a rapid and high-quality roll-out of the necessary diagnostic capacity and expertise were facilitated. The scaling up (rolling out of diagnostics) takes place in various phases, with the degree of scaling up depending on the expected course of an outbreak. Currently, 81 laboratories are accredited (ISO 17025 or ISO 15187 or ISO 22780) to perform molecular diagnostic tests for SARS-CoV-2. Initially, during a period of huge demand for testing, the samples were outsourced to Belgium and Germany. Biomedical scientists contribute greatly in processing laboratory results for SARS-CoV-2, they perform analyses, validate and interpret results and are responsible training of non-laboratory or less educated laboratory personnel when there are shortages. The knowledge, skills and competencies of the biomedical scientist is indispensable within the multidisciplinary team.

Portugal

In Portugal, the SARS-CoV-2 testing situation was like the rest of Europe. The government issued special directives regarding where and when massive testing could be performed, and by whom. The Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA), jointly with Health Minister and Direção-Geral de Saúde, coordinated the Portuguese answer to this pandemic. The INSA is the authority responsible for accreditation of laboratories to perform SARS-CoV-2 NAAT. Portuguese bio-medical scientists were included in these directives, recognizing their knowledge skills and competencies in this area. Regulation of this activity is fundamental, to have the right professionals acting at the different stages of analysis is crucial. Therefore, Portuguese authorities allowed biomedical scientists to act as laboratories directors, performing, inter-preting, validating and issuing results under specific conditions. This is seen in Portugal with the massive testing program run by Higher Education Institutes in collaboration with the Portuguese Red Cross.

Sweden

In Sweden, from the beginning of the pandemic outbreak the public health agency was given the authority to provide recommendations related to restrictions and testing strategies. They were also responsible for the support to the diagnostic laboratories to maintain testing assays with high performance levels. All regions in the country were following the recommendations without exceptions. As everybody knows, the restrictions in Sweden differed very much from other countries in Europe resulting in almost no lockdowns. In the beginning, the testing strategies mainly focused on the hospitalised patients but with the escalation of the pandemic the testing strategies changed very quickly to be very generous. The laboratories in Sweden were unfortunately not prepared for this quick change that forced the laboratories to prepare

for a 1000-fold increase in testing in less than one week. Fortunately, some mass testing laboratories were prepared to receive many of these samples. Within the regions, the strategy changed so that tests from primary care patients were sent to the mass testing laboratories.

In the beginning, the testing of SARS-CoV-2 was dominated by PCR-tests performed in clinical laboratories mainly by biomedical laboratory scientists (BLS) but the lack of BLS in the bigger university hospitals forced the management to use other less educated individuals but, in their opinion, still educated enough to perform the tests. Later, a large-scale implementation of rapid diagnostics for SARS-CoV-2 antigen happened again in a short time. As the tests were new and the users in many cases had limited knowledge of the type of analysis, there was a need to ensure the quality of the entire chain at the national level; from choosing a quick test supplier to handling the sampling. Equalis (EQA provider) offered to perform an external quality assurance program during this time. The results were very clear, tests performed outside the clinical laboratories had less accuracy than the same tests performed by BLS despite very good implementation programs. These results were reported nationally, once again confirming the importance of BLS in health. The results are soon going to be published in an international journal.

Europe learned from the response to testing and instituted a more coordinated approach to the purchase and supply of vaccines. This ensured that all countries within Europe have access to the vaccines and no one has been left behind. As waves of the virus pass through populations and mutations occur the concept of zero COVID is no longer a possibility, and we must all learn how to live with this virus.

European Union (EU) Regulation and the EU Digital COVID Certificate (EUDCC)

Within the EU Healthcare is a national competence and each country makes its own decisions on how it will be configured and delivered within each state. An exception to this freedom within the EU is the directive governing the free movement of professionals. This system ensures that regulated professionals in one-member state may seek to practice the profession in another. This directive balances the entitlement for free movement to address the health and safety of the public. Similarly, the threat of this pandemic requires wide response across Europe.

Part of this response, in keeping with the CE marking of testing systems, is regulating the use of rapid antigen tests for COVID-19. The Health Security Committee (HSC), with expert representatives from each member state, decides on which rapid antigen tests (RAT) should be accepted. This list is updated regularly.[8]

The COVID-19 pandemic has had a huge impact on international travel, including in Europe and at the EU level. As part of the EU strategy to re-establish free movement the EU Digital COVID Certificate (EUDCC) was developed and implemented. The purpose of these digital certificates is to show that an individual can travel and cross borders without a (tangible) risk of carrying the virus. The EUDCC comes in three forms. Especially when it comes to the test and recovery certificates the professional expertise of the registered biomedical scientist becomes clear. For epidemiological safety it is crucial that tests such as PCR and other NAAT are performed in the correct way.

EU Digital COVID Certificate

The EUDCC comes in three forms:

- **Vaccination Certificate.** Confirmation that the holder has completed vaccination.
- **Test Certificate.** Confirmation that the holder has tested Negative for COVID-19; NAAT or RAT.
- **Recovery Certificate.** Confirmation that the holder was previously confirmed infected by SARS-CoV-2 and that this infection was identified by a 'Detected' or Positive COVID-19 NAAT.

According to EU Regulation on EUDCC, all testing used for the certificate, NAAT test or RAT test must be carried out by a health professional or by skilled testing personnel in

the member state issuing the certificate. It is important that tests are performed in the correct manner according to regulation and professional ethical guidelines. All such testing must be subject to quality assurance, ideally in centres accredited as ISO 15189 or 17025. For the safety of the citizens of the EU it is imperative that all testing processes are quality assured. Without this assurance the EU Digital COVID Certificate cannot succeed, and free movement will be curtailed. It is the position of EPBS that only regulated healthcare professionals, ideally biomedical scientists, should carry out such testing for the issuing of these certificates.

European Centre for Disease Prevention and Control (ECDC) is supporting scaling up of sequencing and neutralization assay capacity in EU/EEA Member States.[7] This development is both welcome and vital for the coordinated European response to this pandemic. It will also require investment in the education and training of additional biomedical scientists in Europe to be sustainable.

While we were ill prepared to respond to this current pandemic the citizens of Europe will not look kindly on their politicians if they fail to invest now to ensure we can respond to future threats. The investment in genomic capacity and expertise will also bring dividends in characterization of cancer tumors and other genetic conditions, in addition to supporting prenatal diagnosis of genetic defects.

The Biomedical Scientist and COVID-19

Biomedical scientists are a hidden profession within healthcare. Their work is critical to diagnosis and monitoring of all diseases. They are a profession of highly skilled scientists, educated in the biological basis of disease and the analytical method for ensuring safe and consistent analysis of biological specimens. The range of analysis covers clinical biochemistry, hematology, histopathology, immunology, microbiology, transfusion and transplantation sciences and virology. Their services are provided in clinical diagnostic laboratories 24/7/365 days. The analytical methods range from microscopic observation through chemical and immunoassay to use of molecular diagnostics. Within healthcare the work of clinical diagnostic laboratories is subject to rigorous monitoring with most laboatories operating to the ISO 15189 standard.

While much has been written about the healthcare staff on the front line during this pandemic, those in patient facing roles, the contribution of biomedical scientists to the control of this pandemic must not be underestimated. For a virus almost unknown in 2019 there have been almost 3 billion tests carried out within 18 months. This testing has been carried out in addition to the routine clinical diagnostic laboratory workload required for population health.

In addition to the analysis of samples, the biomedical scientists have worked with clinical colleagues to ensure that the correct specimens are submitted for analysis, and that the microbiologists and infectious disease teams are provided with analytical results and surveillance data. They have interacted with public health teams. They have provided the statistical data necessary for health providers and governments to manage the pandemic and make informed decisions.

In our introduction we highlighted the lack of preparedness of the world and the healthcare systems to respond to the COVID-19 pandemic. This was particularly true in terms of clinical diagnostic capacity. Over the past decades there has been an assumption that this specialty is becoming simplified with the introduction of automated analysers and integrated IT systems. There has been a trend to use non-professionally qualified staff and to reduce investment in the professional development and career pathways for biomedical scientists. This has been a mistake. As the pandemic unfolded it was evident that there were insufficient qualified biomedical scientists in Europe to undertake the range and volume of testing required. We need to prioritise the investment of the education and training of this profession now to ensure we can emerge from this pandemic and prepare for the next one.

The development and delivery of vaccination has been a game changer in the fight against this virus, however, the war is not yet won. The

work of the biomedical scientist is not yet over. As we look to the future for a successful emergence from the cycles of this pandemic, we will need a European wide system for virus identification that quickly identifies emerging infections, either via virus mutation or breakthrough infections. We need a responsive sequencing capacity to identify and monitor emerging variants tracking them from interest through concern. In addition, we need ready access to monitoring of immune response to inform the program of vaccine boosters.

The legacy of this viral infection will be a challenge. Already we see many presenting with 'long COVID' and hear talk of 'brain fog'. In pregnant women we have seen cases of devastating stillbirth from COVID placentitis. The long-term monitoring of the sequelae of this infection will require the knowledge skills and competence of biomedical scientists. Of concern is the demonstrable loss of cognitive function following this infection. We must do all in our power to ensure that this is not a legacy we leave to future generations and thus the testing and monitoring of infection in children must be given more attention.

The biomedical scientists of Europe, represented by EPBS, are your Diagnostic Partners in this fight. We will continue to work for the health benefit of our countries and together we can harness a resource that is at the disposal of the EU. Work with us, take advantage of our knowledge skills and competencies, give us the tools we need to deliver the service required, bring us into the discussion and, as we have demonstrated, we will deliver.

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Mini-review

Waste Containers (Plastic Bags) for Infectious Waste Disposal in the Medical Laboratory

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The purpose of the current paper was to provide reasonably achievable guidance for the International Standard ISO 15189:2012 accredited medical laboratory to support the implementation of disposal facilities for medical waste by ensuring that usage of infectious waste bags is within acceptable specifications. Guidance documents from selected international organizations were identified: The International Organization for Standardization, the World Health Organization, and the International Committee of the Red Cross. This study identified relevant requirements from selected organizations (n = 3) associated with the support of implementation of storage and disposal facilities for infectious waste in the medical laboratory. The information could be used to develop conformity checklists for internal auditing, if required. The present paper has provided a practical contribution to established knowledge of International Standard ISO 15189:2012 accreditation compliance management in the disposal of potentially infectious wastes using waste bags by laboratory personnel.

Key words: Accreditation, management audit, quality improvement, quality management.

Contemporary situation

International Standard ISO 15189:2012 published by the International Organization for Standardization specifies that the medical laboratory is to have storage and disposal facilities for dangerous materials appropriate to the hazards of the materials (ISO 15189:2012, 5.2.3).[1] To support the implementation of such facilities in the medical laboratory, potential infectious waste management should be maintained in compliance with good practice and applicable requirements [ISO 15189:2012, 4.1.1.4 e)].[1]

How should the medical laboratory select appropriate waste containers for infectious waste disposal to support ISO 15189:2012 accreditation?

Infectious waste disposal measures

The medical laboratory must implement suitable measures to ensure relevant waste containers are provided for infectious waste disposal. The term infectious waste, defined as 'waste containing or suspected to contain human pathogenic microbiological agents' (ISO 12891-1:2015, 2.3) differs from hazardous waste, defined as 'waste that is potentially flammable, combustible, ignitable, corrosive, toxic, reactive, infectious or injurious to people or the environment' (ISO 12891-1:2015, 3.14) and medical waste, defined as 'any solid waste that is generated in the diagnosis, treatment, or immunization of human beings or animals, in research pertaining thereto, or in the production or testing of biological materials, including but not limited to isolation wastes, infectious agents, human blood and blood products, pathological wastes, sharps, body

Accepted: July 10, 2021

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parts, contaminated bedding, surgical wastes and potentially contaminated laboratory wastes and dialysis wastes' ISO 16304:2018, 3.5).[2,3] The medical laboratory should use specified leak proof containers to support the waste management implementation.

Relevant good practice guidance

Clause 4 (Management requirements) of ISO 15189:2012 and Clause 5 (Technical requirements) of ISO 15189:2012 do not provide any conformance requirements in relation to waste container physical specification, it is good practice for the medical laboratory to establish relevant infectious waste disposal practices that are in alignment with the following guidance documents from the selected international organizations: the International Organization for Standardization,[1] the World Health Organization, and the International Committee of the Red Cross.

International Standard ISO 15190:2020 published by the International Organization for Standardization provides general waste disposal information to address disposal requirements.[4] More specifically, information relating to general precautionary waste disposal measures for hazardous waste is provided (ISO 15190:2020, 17.3) and proper labelling of hazards must be maintained to ensure safety for all laboratory personnel (ISO 15190:2020, 18 i).[4]

Table 1. Biological risks, biological hazard and biohazard symbols. The symbol ISO 7000-0659 (2004-01) is the biological risks symbol that should be used to label the waste bags. The other two symbols should not be used.

Symbols	Description
	**Functional reference number:** 0659 **Referent:** Biological risks **Registration date:** 2004-01-15
	**Functional reference number:** W009 **Referent:** Warning; biological hazard **Registration date:** 2011-05-01
	**Functional reference number:** Nil **Referent:** Warning; biohazard **Registration date:** Nil

Further information can be sought from the World Health Organization. The World Health Organization provides recommendations for waste containers, including single use sharps containers and bags. The bags should be identified with a color codification scheme to support the waste segregation system. For highly infectious waste, the bag should be in yellow, marked with the symbol ISO 7000-0659 (2004-01) from International Standard ISO 7000:2019 published by the International Organization for Standardization together with supplementary safety information 'HIGHLY INFECTIOUS'; for other infectious, pathological and anatomical waste, the bag should be in yellow and marked with the symbol ISO 7000-0659 (2004-01).[5,6]

It is important to note that there are two similar symbols which should not be used for marking bags. The symbol ISO 7010-W009 (2011-05) from International Standard ISO 7010:2019 prepared by the International Organization for Standardization differs from the symbol ISO 7000-0659 (2004-01) and should not be used.[7] Another way to ensure the symbol is the recommended one is to check that it is accepted by the universal coded character set (UCS) code, as specified in International Standard ISO/IEC 10646:2017 published by the International Organization for Standardization.[8] The equivalent pictogram (UCS coded character point: 2623; Associated character name: Biohazard sign) could be used if required (ISO/IEC 10646:2017, 33.5).[8] Another biohazard symbol that is fluorescent orange red is also available for marking.[9] However, the symbol is not affiliated with the International Organization for Standardization and should not be used. In addition, the bags should be sturdy, leak proof and chlorine free.[6] The bag thickness should conform to 70 μm [when measured in accordance with Clause 8 (Procedure) of ISO 7765-1:1988].[6,10]

The International Committee of the Red Cross also recommends a similar color codification scheme based on the World Health Organization scheme to support the waste segregation system and the bag thickness of 70 μm (when measured in accordance with Clause 8 of ISO 7765-1:1988) is also recommended to support the waste segregation system.[11]

It should be noted that applicable international, national or regional requirements may also be enforceable (ISO 15189:2012, 1).[1] The medical laboratory must do what is reasonably achievable to ensure waste bags are identified and marked unambiguously for a clear allocation of the waste fractions for waste management.

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Mini-review

Single-Use Sharps Containers for Medical Waste Disposal in the Medical Laboratory

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The purpose of the current paper is to provide reasonably practicable guidance for the International Standard ISO 15189:2012 accredited medical laboratory to support the implementation of disposal facilities for medical waste by ensuring the usage of single-use sharps containers is within acceptable specifications. Guidance documents from selected international organizations: The International Electrotechnical Commission, the International Organization for Standardization, and the World Health Organization, were identified. This review identified relevant requirements from selected organizations (*n* = 3) associated with the support of implementation of storage and disposal facilities for dangerous materials in the medical laboratory. The information could be used to develop conformity checklists for internal auditing, if required. The present paper has provided a practical contribution to established knowledge of International Standard ISO 15189:2012 accreditation compliance management in the disposal of potentially hazardous sharps using single-use sharps containers by laboratory personnel.

Key words: Accreditation, management audit, quality improvement, quality management.

Contemporary situation

International Standard ISO 15189:2012 prepared by the International Organization for Standardization specifies that the medical laboratory is to have storage and disposal facilities for dangerous materials appropriate to the hazards of the materials (ISO 15189:2012, 5.2.3).[1,2] To support the implementation of such facilities in the medical laboratory, potential hazardous sharps management should be maintained in compliance with good practice and applicable requirements (ISO 15189:2012, 4.1.1.4 e).[1]

How should the medical laboratory ensure suitable type of single-use sharps containers are selected to support ISO 15189:2012 accreditation?

Medical waste disposal measures

The medical laboratory should use an acceptable waste receptacle for the disposal of potentially hazardous sharps, defined as 'objects capable of cutting or penetrating skin' (ISO 23907-1, 3.15).[3] A commonly used item for the disposal of such medical waste, defined as 'any solid waste that is generated in the diagnosis, treatment, or immunization of human beings or animals, in research pertaining thereto, or in the production or testing of biological materials, including but not limited to isolation wastes, infectious agents, human blood and blood products, pathological wastes, sharps, body parts, contaminated bedding, surgical wastes and potentially contaminated laboratory wastes and dialysis wastes' (ISO 16304:2018, 3.5), in the medical laboratory is the use of a specified puncture-resistant container, such as a single-use sharps conta-

Accepted: July 10, 2021

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iner, defined as a 'container designated by the manufacturer to be filled only once' (ISO 23907-1:2019, 3.17), to support the waste management implementation.[3,4]

Relevant good practice guidance

Clauses 4 and 5 of ISO 15189:2012 do not provide any specified conformance requirements in relation to single-use sharps container physical specifications, it is good practice for the medical laboratory to establish relevant medical waste disposal practices that are in alignment with the following guidance documents from selected international organizations, such as the International Electrotechnical Commission, the International Organization for Standardization, and the World Health Organization.[1,2]

International Standard ISO 15190:2020 prepared by the International Organization for Standardization specifies that all sharp objects must be discarded directly in specified puncture-resistant containers (ISO 15190: 2020, 17.3 c) and the containers must be positioned within arm's reaching distance, below eye level and not be filled to more than two-thirds of the capacity (ISO 15190:2020, 14.8 d).[5]

Figure 1. The medical laboratory must ensure only single-use sharps containers that meet relevant marking and performance requirements are used for the disposal of potentially hazardous sharps.

International Standard ISO 23907-1:2019 prepared by the International Organization for Standardization specifies performance specifications (ISO 23907-1:2019, 4) and marking specifications (ISO 23907-1:2019, 6) for single-use sharps containers.[3] The medical laboratory should ensure the single-use sharps containers are safe for laboratory personnel to use for medical waste disposal by non-technically inspecting the following selected specifications. First, the display of the fill line, defined as 'mark, indicator or feature on the container that represents the fill volume' (ISO 23907-1:2019, 3.3).[3] Second, the display of the word 'DANGER', defined as 'signal word used to indicate an imminently hazardous situation which, if not avoided, will result in death or serious injury' (ISO 3864-2:2016, 3.3), or the equivalent wording in the language of the country where the container is used.[6] Third, the display of the fill volume, defined as 'usable volume determined by the manufacturer and indicated by the fill line on the container' (ISO 23907-1:2019, 3.4).[3] Fourth, the display of the lot number. Finally, the display of a warning notice 'not filling above fill line and not forcing sharps into container'.[3]

Guide ISO/IEC Guide 37:2012 prepared by the International Organization for Standardization and the International Electrotechnical Commission specifies the information presentation format relating to instructions for use, defined as 'information provided by the supplier of a product to the user, containing all the necessary provisions to convey the actions to be performed for the safe and efficient use of the product' (ISO/IEC Guide 14:2018, 3.9) for reagents and consumables by containing information required by users to be able to minimize harm to people, property and the environment.[7,8] In particular, the medical laboratory should take note to storage requirements in normal use that may influence the effectiveness of usage of single-use sharps containers (ISO/IEC Guide 37:2012, 4.11).[7]

Table 1. The biological risks symbol. The symbol ISO 7000-0659 (2004-01) by the International Organization for Standardization is the graphical symbol for biological risks.

Symbol	Description
	**Reference:** ISO 7000-0659 **Referent:** Biological risks **Registration date:** 2004-01-15

The World Health Organization has a segregation scheme for sharps disposal for

puncture-proof containers.[9] The medical laboratory should ensure the single-use sharps containers are safe for laboratory personnel to use for medical waste disposal by non-technically inspecting the following selected specifications.[9] First, the container is yellow. Second, the display of the word 'SHARPS'. Finally, the display of the symbol ISO 7000-0659 (2004-01)[10] (Table 1).

It should be noted that applicable international, national or regional requirements may also be enforceable (ISO 15189:2012, 1).[1] The medical laboratory must do what is reasonably practicable to implement relevant safety features to the normal usage of single-use sharps containers to ensure the safety of laboratory personnel during the disposal of potentially hazardous sharps (Figure 1).

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Mini-review

Warning Markings for Laser Products in the Medical Laboratory

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The purpose of this paper was to provide reasonably feasible guidance for the International Standard ISO 15189:2012 accredited medical laboratory to support the implementation of relevant equipment hazard information provision to laboratory personnel by ensuring the usage of laser warning markings is within acceptable specifications. Guidance documents from selected international organizations were identified: the Institute of Electrical and Electronics Engineers, the International Electrotechnical Commission, and the International Organization for Standardization. This study identified relevant requirements from the selected organiZations (*n* = 3) associated with implementation of laser warning markings in the medical laboratory. The information could be used to develop conformity checklists for internal auditing, if required. The present paper has provided a practical contribution to established knowledge of International Standard ISO 15189:2012 accreditation compliance management in the provision of relevant equipment hazard information relating to laser hazard warning markings to laboratory personnel.

Key words: Accreditation, management audit, quality improvement, quality management.

Contemporary situation

International Standard ISO 15189:2012 prepared by the International Organization for Standardization (ISO) specifies that the laboratory director [or designate(s)] is to 'implement a safe laboratory environment in compliance with good practice and applicable requirements' (ISO 15189:2012, 4.1.1.4 e).[1,2]

How do lasers differ from light emitting diodes?

The term 'laser', an acronym for light amplification by stimulated emission of radiation, defined as 'any device which can be made to produce or amplify electromagnetic radiation in the wavelength range from 180 nm to 1 mm primarily by the process of controlled stimulated emission' (IEC 60825-1:2014, 3.44).[3] This differs from a 'light emitting diode', defined as 'any semiconductor p-n junction device which can be made to produce electromagnetic radiation by radiative recombination in the semiconductor in the wavelength range from 180 nm to 1 mm' (IEC 60825-1:2014, 3.52).[3]

How should the medical laboratory deliver safety messages to laboratory personnel relating to laser safety to support International Standard ISO 15189:2012 accreditation?

Equipment hazard warnings.

The medical laboratory must provide relevant equipment hazard information to laboratory personnel. Clauses 4 and 5 of ISO 15189:2012 do not provide any practical guidance on how to address the hazard warning issues. However,

Accepted: July 10, 2021

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the International Standard ISO 15190:2020 prepared by the ISO specifies that appropriate approved signs must be displayed (ISO 15190:2020, 9.51).[1,4] In addition, the International Standard IEC/IEEE 82079-1:2019 prepared by the International Electrotechnical Commission (IEC) and the Institute of Electrical and Electronics Engineers specifies that relevant safety-related information must be provided in the equipment instructions for use.[2,5] This is defined as 'information provided by the supplier of a product to the user, containing all the necessary provisions to convey the actions to be performed for the safe and efficient use of the product' (ISO/IEC Guide 14:2018, 3.9), supplied by the manufacturer (IEC/IEEE 82079-1:2019, 7.11.2) to support hazard communication (ISO 15189: 2012, 5.3.1.3).[1,6,7]

Laser warning markings

The equipment instructions for use should contain an appropriate level of laser safety information for laboratory personnel. The information can be further supported by the International Standard IEC 60825-1:2014 prepared by the IEC.[3] IEC 60825-1:2014 classifies lasers into eight classes according to the increasing order of ocular hazard (IEC 60825-1:2014, 4.3).[3] Each laser product must carry appropriate labels (IEC 60825-1:2014, 7).[3,] Appropriate warning labels, explanatory labels that contain recommended wordings and alternative labels must be included for each class of laser product (Figure 1 and Table 1).

Class 1. The alternative label is a combination product safety label, defined as 'combination of product safety sign and/or supplementary safety information and/or hazard severity panel on one rectangular label' (ISO 3864-2:2016, 3.2) and (IEC 60825-1:2014, 7.2).[3,8]

Class 1M. The alternative label is a combination product safety label that contains a hazard severity panel, defined as 'area of a combination or multiple product safety label that communicates the category of risk associated with a hazard' (ISO 3864-2:2016, 3.7).[8,] The label must also contain the degree of hazard severity 'CAUTION' defined as a 'signal word used to indicate a potentially hazardous situation which, if not avoided, could result in minor or moderate injury' (ISO 3864-2:2016, 3.1) and (IEC 60825-1:2014, 7.2).[3,8]

Table 1. Warning labels. Warning, explanatory and alternative labels for Class 1 to Class 4 laser products.

Classes	Warning and explanatory labels	Alternative labels
1	CLASS 1 LASER PRODUCT	LASER 1
1M	LASER RADIATION DO NOT EXPOSE USERS OF TELESCOPIC OPTICS CLASS 1M LASER PRODUCT	CAUTION LASER 1M
1C	LASER RADIATION FOLLOW INSTRUCTIONS CLASS 1C LASER PRODUCT	CAUTION LASER 1C
2	LASER RADIATION DO NOT STARE INTO BEAM CLASS 2 LASER PRODUCT	LASER 2
2M	LASER RADIATION DO NOT STARE INTO BEAM OR EXPOSE USERS OF TELESCOPIC OPTICS CLASS 2M LASER PRODUCT	CAUTION LASER 2M
3R	LASER RADIATION AVOID DIRECT EYE EXPOSURE CLASS 3R LASER PRODUCT	CAUTION LASER 3R
3B	WARNING – LASER RADIATION AVOID EXPOSURE TO BEAM CLASS 3B LASER PRODUCT	WARNING LASER 3B AVOID EXPOSURE TO BEAM
4	DANGER – LASER RADIATION AVOID EYE OR SKIN EXPOSURE TO DIRECT OR SCATTERED RADIATION CLASS 4 LASER PRODUCT	DANGER LASER 4 AVOID EYE OR SKIN EXPOSURE TO DIRECT OR SCATTERED RADIATION

Figure 1. The medical laboratory must ensure relevant warning labels are displayed at all times. In this case, the plate displays information relating to laser classification and warning in accordance with American National Standard ANSI Z136.1-2014 prepared by the American National Standards Institute and European Standard EN 60825-1:2007 prepared by the European Committee for Electrotechnical Standardization. The medical laboratory must implement the most relevant warning requirements that are in alignment with International Standard ISO 15189:2012 accreditation requirements.

Class 1C. The alternative label is a multiple product safety label, defined as 'product safety label that contains two or more safety signs on the same rectangular label and, if used, the supplementary safety information and/or the hazard severity panel' (ISO 3864-2:2016, 3.8), that contains a hazard severity panel with the degree of hazard severity 'CAUTION' (IEC 60825-1:2014, 7.3).[3,8,]

Class 2. The alternative label is a combination product safety label that contains a hazard severity panel (IEC 60825-1:2014, 7.4).[3]

Class 2M. The alternative label is a combination product safety label that contains a hazard severity panel with the degree of hazard severity 'CAUTION' (IEC 60825-1:2014, 7.4).[3]

Class 3R. The alternative label is a combination product safety label that contains a hazard severity panel with the degree of hazard severity 'CAUTION' (IEC 60825-1:2014, 7.5).[3]

Class 3B. The alternative label is a combination product safety label that contains a hazard severity panel with the degree of hazard severity 'WARNING', defined as a 'signal word used to indicate a potentially hazardous situation which, if not avoided, could result in death or serious injury' (ISO 3864-2:2016, 3.18), with a separate supplementary safety information text panel (IEC 60825-1:2014, 7.6).[3,8]

Class 4. The alternative label is a combination product safety label that contains a hazard severity panel with the degree of hazard severity 'DANGER', defined as 'signal word used to indicate an imminently hazardous situation which, if not avoided, will result in death or serious injury' (ISO 3864-2:2016, 3.3), with a separate supplementary safety information text panel (IEC 60825-1:2014, 7.7).[3,8]

It should be noted that applicable international, national or regional requirements may also be enforceable (ISO 15189:2012, 1).[1] The medical

laboratory must do what is reasonably practicable to ensure the relevant laser hazard information is communicated to laboratory personnel.

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Mini-review

Personal Protective Measures for Laboratory Personnel in the Medical Laboratory

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The aim of this paper was to provide reasonably practicable guidance for the International Standard ISO 15189:2012 accredited medical laboratory to support the implementation of personal protective measures for laboratory personnel. Guidance documents from selected international organizations were identified: The Institute of Electrical and Electronics Engineers, the International Commission on Occupational Health, the International Electrotechnical Commission, and the International Organization for Standardization. This study identified relevant recommendations and requirements from the selected organizations (*n* = 4) associated with implementation of relevant personal protective measures in the medical laboratory. The information could be used to develop conformity checklists for internal auditing, if required. The present paper has provided a practical contribution to existing knowledge of International Standard ISO 15189:2012 accreditation compliance management in personal protective measure provision to laboratory personnel in the medical laboratory.

Key words: Accreditation, management audit, quality improvement, quality management.

Contemporary situation

International Standard ISO 15189:2012 prepared by the International Organization for Standardization (a Type B international organization according to the Union of International Associations) specifies that the laboratory director (or designate/s) is to 'implement a safe laboratory environment in compliance with good practice and applicable requirements' [Subclause 4.1.1.4 e) of ISO 15189:2012].[1,2]

How should the medical laboratory deliver safety messages to laboratory personnel relating to mandatory action to support International Standard ISO 15189:2012 accreditation?

The medical laboratory may wish to provide safety information to laboratory personnel by using safety signage in the areas of responsibility. A safety sign, defined as a 'sign giving a general safety message, obtained by a combination of a color and geometric shape and which, by the addition of a graphical symbol, gives a particular safety message' (Item 3.3 of ISO 7010:2019), may signify information of a mandatory action relating to the application of personal protective measures.[3] While Clause 4 (Management requirements) of ISO 15189:2012 and Clause 5 (Technical requirements) of ISO 15189:2012 do not provide any practical guidance on how to address the use of safety signs to indicate specific action relating to personal protective equipment, defined as a 'variety of barriers including clothing and respi-

Accepted: July 25, 2021

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rators used alone or in combination to protect mucous membranes, airways, skin, and clothing from contacts with infectious or hazardous agents' (Item 3.1.36 of ISO 15190:2020), in the medical laboratory.[1,4]

International Organization for Standardization.

International Standard ISO 7010:2019 prepared by the International Organization for Standardization specifies information to address the provision of safety signage in the medical laboratory.[3] ISO 7010:2019 classifies safety signs into five categories: Category E [signs indicating an evacuation route, the location of safety equipment or a safety facility, or a safety action (safe condition signs)], Category F (fire equipment signs), Category M (mandatory action signs), Category P (prohibition signs) and Category W (warning signs) [Subclause 4.3 (Categorization of safety signs) of ISO 7010:2019].[3] Category M signs (mandatory action signs) are the ones providing personal protective measures information (Table 1). The relevant symbols (*n* = 7) are described below:

Face and body protection.

The symbol (ISO 7010-M004) (2011-05) implies the wearing of a face shield, defined as a 'protector that is worn directly or indirectly on the head and covers the eyes and all, or a substantial part, of the face' (Item 3.5.1.6 of ISO 4007:2018), and the symbol (ISO 7010-M013) (2011-05) implies the wearing of goggles, defined as a 'protector that fully encloses the orbital area and fits firmly on the face' (Item 3.5.1.7 of ISO 4007:2018), in the medical laboratory.[3,5]

Footwear.

The symbol (ISO 7010-M008) (2011-05) implies the wearing of safety footwear, defined as 'footwear incorporating protective features to protect the wearer from injuries that could arise through accidents' (Item 3.1 of ISO 20345:2011), in the medical laboratory.[3,6]

Gloves.

The symbol (ISO7010-M009) (2011-05) implies the wearing of gloves, including protective gloves against cold, protective gloves against dangerous chemical risks, defined as 'protective gloves which form a protective barrier to dangerous chemicals' (Item 3.3 of ISO 374-1:2016), protective gloves against ionizing radiation and radioactive contamination, protective gloves against micro-organisms, defined as 'protective gloves which form a protective barrier to microbiological agents' (Item 3.1 of ISO 374-5:2016), in the medical laboratory.[3,7,8]

Table 1. Safety signs (Category M).[3] Mandatory action signs.

Symbols	Information
	**Functional reference number:** M004 **Referent:** Wear eye protection
	**Functional reference number:** M008 **Referent:** Wear safety footwear
	**Functional reference number:** M009 **Referent:** Wear protective gloves
	**Functional reference number:** M010 **Referent:** Wear protective clothing
	**Functional reference number:** M013 **Referent:** Wear a face shield
	**Functional reference number:** M016 **Referent:** Wear a mask
	**Functional reference number:** M017 **Referent:** Wear respiratory protection

Protective clothing.

The symbol (ISO 7010-M010) (2011-05) implies the wearing of protective clothing, defined as 'clothing including protectors which cover or replace personal clothing, and which is designed to provide protection against one or more hazards' (Item 3.5 of ISO 13688:2013), in the medical laboratory.[3,9]

Respiratory protection.

The symbol (ISO 7010-M016) (2011-05) implies the wearing of a medical face mask, defined as an 'item of protective clothing designed to protect portions of the wearer's face, including at least the mucous membrane areas of the

wearer's nose and mouth, from contact with blood and other body fluids during medical procedures' (Item 3.6 of ISO 22609:2004), in the medical laboratory.[3,10] The symbol (ISO 7010-M017) (2011-05) implies the wearing of respiratory protective device, [3, 11,12] defined as 'personal protective equipment designed to protect the wearer's respiratory tract against inhalation of hazardous atmospheres' (Item 3.203 of ISO 16972:2020), in the medical laboratory.[13]

Concurrently, International Standard ISO 15190:2020 prepared by the International Organization for Standardization specifies the relevant actions relating to personal protective measures that the medical laboratory should take to address the compliance requirements [Subclauses 15.2 (Protective clothing in the laboratory), 15.4 (Face and body protection), 15.5 (Gloves), 15.6 (Footwear), and 15.7 (Respiratory protection) of ISO 15190:2020].[4] This is supported by International Standard ISO 11014:2009[1] prepared by the International Organization for Standardization.[4] ISO 11014:2009 classifies a safety data sheet under 16 document headings [Clause 5 (Contents and general layout of an SDS) of ISO 11014:2009] where Section 8 provides recommendations relating to appropriate personal protection equipment when handling chemical related risks [Annex A.9 (Section 8 — Exposure controls and personal protection) of ISO 11014:2009].[14]

Institute of Electrical and Electronics Engineers and International Electrotechnical Commission.

This is further supported by the instructions for use information provision requirements in International Standard IEC/IEEE 82079-1:2019 prepared by the International Electrotechnical Commission (a Type C international organization according to the Union of International Associations) and the Institute of Electrical and Electronics Engineers (a Type F international organization according to the Union of International Associations) that specifies the location of instructions for use where relevant safety-related information is to be displayed to the users.[2,15] Relevant safety-related information is to be provided to users if the task requires the use of personal protective equipment [Subclause 7.11.2 (Location of safety-related information) of IEC/IEEE 82079-1 :2019] and recommendations in Guide ISO/ IEC Guide 37:2012 prepared by the International Organization for Standardization and the International Electrotechnical Commission that specifies the provision of special protective measures that are required to protect bystanders and users (Subclause 4.7 of ISO/IEC Guide 37:2012).[15,16]

International Commission on Occupation Health.

The International Commission on Occupational Health (a Type B international organization according to the Union of International Associations) has relevant information relating to the selection and use of personal protective equipment for respiratory protection, eye protection, head protection, foot protection, and hand protection.[2,17]

It should be noted that applicable international, national or regional requirements may also be enforceable [Clause 1 (Scope) of ISO 15189: 2012].[1] The medical laboratory must do what is reasonably practicable to ensure relevant information relating to personal protective measures in the medical laboratory is properly identified to support accreditation compliance management.

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Literature Review

Position of Laboratory Scientist, Analyst, and Technologist in Standard Occupation Classification

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Terms for medical laboratory personnel were researched using code names in the standard occupational classification and the job titles for a total of 46 countries including the International Federation of Biomedical Laboratory Science (IFBLS) and European Association for Professions in Biomedical Science (EPBS) through Google search. In the case of the technologist or technician type, the identities used by medical laboratory personnel include biomedical laboratory health technician, clinical diagnostic laboratory technician, clinical laboratory technologist, medical laboratory technologist, medical laboratory technician, medical technologist, biomedical analysis technician, clinical analysis technician, and medical analytics technician. For the analyst type, professional titles include bio analyst, biomedical analyst, and medical analyst, whereas for the scientist type, professional designations include biomedical scientist and medical laboratory scientist. Additionally, other professional titles may include bioengineer and medical technical laboratory assistant. In most countries, medical laboratory technologists and technicians belong to the Major Group 3 Technicians and Associate Professionals in the International Standard Classification of Occupations 2008 (ISCO-08). Biomedical scientists or medical laboratory scientists in the United Kingdom (UK), Ireland, Australia, and New Zealand are categorized as Major Group 2 Professionals according to their standard occupational classification. Medical laboratory personnel must be distinguished in the International Standard Classification of Occupations because they have different education levels, experience levels, and responsibilities. Medical laboratory personnel with a bachelor's degree qualification should be moved to the Major Group 2 Professionals. Medical laboratory personnel with an associate degree or diploma qualification should be designated as Major Group 3 Technicians and Associate Professionals. A medical technologist and a medical technician or a medical laboratory technologist and a laboratory medical technician are not properly recognized by individuals with similar terms. This review proposes a new professional designation of "Medical Laboratory Analyst and Biomedical Analyst" as unified terms for medical laboratory personnel with a bachelor's degree qualification (excluding the title of medical laboratory scientist and biomedical scientist).

Key words: Associate professionals, Biomedical analyst and Medical laboratory analyst, Biomedical scientist and Medical laboratory scientist, Medical laboratory technologist and technician, Professionals

Introduction

The International Standard Classification of Occupations, abbreviated as ISCO, is an international classification under the responsibility of the International Labor Organization (ILO) for organizing jobs into a clearly defined set of groups according to the tasks and duties associated with the position.

Accepted: July 20, 2021, corrected November 3, 2021

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The ISCO is intended both for use in compiling statistics and for client-oriented uses such as the recruitment of workers through employment offices, the management of migration of workers between countries and the development of vocational training programs and guidance.[1]Founded in 1954, the International Association of Medical Laboratory Technologists (IAMLT) was revised to the International Federation of Biomedical Laboratory Science (IFBLS) at the general assembly in 2002.[2] The IFBLS introduces the occupations of member countries as medical laboratory scientists and technologists or as biomedical laboratory scientists and technologists or as biomedical scientists and laboratory technicians.[2] Even the name of the laboratory professional occupation is not standardized, like "clinical laboratory scientist, clinical laboratory technologist, medical laboratory scientist, medical laboratory technologist, medical technologist, bio analyst, biomedical analyst, medical laboratory scientist, biomedical scientist", and so on.[4,5] The present study compares the job titles of medical laboratory personnel registered to ISCO-08 and the formal qualifications of each country, and accordingly, proposes terms for medical laboratory personnel for which identities have been established.[1]

Materials and Methods

Terms for medical laboratory personnel were researched using code names in the standard occupational classification and the official titles for a total of 46 countries including the IFBLS and EPBS using Google search.[2,3] A complete listing of the subject search organizations and associations is provided in Table 1.

Table 1. Subject Search Categories. Complete listing of the subject search organizations and associations

International organizations
International Standard Classification of Occupations (ISCO)
International Standard Classification of Education (ISCED)
International professional associations
International Federation of Biomedical Laboratory Science (IFBLS)
European Association for Professions in Biomedical Science (EPBS)
Professional associations based in Africa
Cameroon Association for Medical Laboratory Sciences (CAMELS)
Ghana Association of Medical Laboratory Scientists (GAMLS)
Association of Kenya Medical Laboratory Scientific Officers (AKMLSO)
Association of Medical Laboratory Scientists of Nigeria (AMLSN)
Society of Medical Laboratory Technologists of South Africa (SMLTSA)
Biomedical Society of Zambia (BSZ)
Professional associations based in America
American Society for Clinical Laboratory Science (ASCLS)
American Medical Technologists (AMT)
Canadian Society for Medical Laboratory Science (CSMLS)
Professional associations based in Asia
Hong Kong Institute of Medical Laboratory Sciences (HKIMLS)
All India Medical Laboratory Technologists Association (AIMLTA)
Japanese Association of Medical Technologists (JAMT)
Korean Association of Medical Technologists (KAMT)
Malaysian Institute of Medical Laboratory Sciences (MIMLS)
Myanmar Medical Technologist Association (MMTA)
Medical Laboratory Technologists Association of Pakistan (MLTAP)
Philippine Association of Medical Technologists (PAMET)
Singapore Association for Medical Laboratory Sciences (SAMLS)
Association of Medical Technologist of Thailand (AMTT)
Taiwan Association of Medical Technologists (TAMT)
Taiwan Society of Laboratory Medicine (TSLM)
Professional associations based in Europe
Austrian Association of Biomedical Analysts (AABA/OBBA)
Belgian Association of Laboratory Technologists (BALT/BVLT)
Croatian Chamber of Health Professionals (CCHP)
Danish Association of Bio analysts (DAB)
Estonian Association of Bio analysts (EAB)
France Association of Medical Laboratory Technicians (FAMLT/AFTLM)
Finnish Association of Bio analysts (FAB)
Association for Medical Technologists and Analysts – Germany (AMTA/DVTA)
Greek Association of Medical Laboratory Technologists (GAMLT/PETIE)
Association of Biomedical Scientists – Iceland (ABS/FL)
Academy of Clinical Science and Laboratory Medicine – Ireland (ACSLM)
Medical Laboratory Scientists Association – Ireland (MLSA)
Italian Scientific Society of Biomedical Laboratory Technicians (ISCBLT/SITLab)
Netherlands Association of Medical Laboratory Employees (NAMLE/NVML)
Norwegian Engineers and Technologists Organization (NETO_DB/NITO_BFI)
Portuguese Association of Clinical Analysis Technicians (PACAT/APTAC)
Spanish Association of Laboratory Technicians (SALT/AETEL)
Institute of Biomedical Laboratory Science – Sweden (IBL)
Swiss Association of Biomedical Analysts (SABA)
Institute of Biomedical Science – UK (IBMS)
Professional associations based in Oceania
Australian Institute of Medical and Clinical Scientists (AIMS)

Results

Categorical classification of medical laboratory personnel by HISCO

The first ISCO version, known as ISCO-58, was adopted in 1957 by the 9th International Conference of Labor Statisticians; subsequent versions were ISCO-68 (17th International Conference of Labor Statisticians, 1966), ISCO-88 (14th International Conference of Labor Statisticians, 1987) and the recent ISCO-08, adopted in December 2007. Medical laboratory personnel were classified as 0-53 Medical Technicians when ISCO-58 was enacted, as 0-54.30 Medical Science Technicians in the ISCO-68 revision, as 3211 Life Science Technicians in the ISCO-88 revision, and as 3212 Medical and Pathology Laboratory Technicians in the ISCO-08 revision.[6] (Table 2)

In most countries, medical laboratory personnel are categorized as "major group: technicians and associate professionals > sub major group:

health associate professionals > minor group: medical and pharmaceutical technicians > unit group: medical and pathology laboratory technicians (examples: medical laboratory technicians, pathology technicians)," but in the UK, Australia, and New Zealand, they are characteristically registered as "major group: professionals > sub major group: science and engineering professionals > minor group: life science professionals > unit group: biologists related profession (examples: biochemists, biologists, biomedical scientist, medical laboratory scientists)."

Table 2. Categories of medical laboratory personnel in HISCO

Version	Category
ISCO-58 (1958)	0-5 Professional Medical Workers Not Elsewhere Classified and Medical Technicians 0-53 Medical Technicians
ISCO-68 (1968)	0-5 Life Scientists and Related Technicians 0-54 Life Sciences Technicians 0-54.30 Medical Science Technicians
ISCO-88 (1988)	3 Technicians and Associate Professionals 32 Life Science and Health Associate Professionals 321 Life Science Technicians and Related Associate Professionals 3211 Life Science Technicians
ISCO-08 (2008)	2 Professionals 21 Science and Engineering Professionals 213 Life Science Professionals 2131 Biologists, Biologists, Botanists, Zoologists and Related Professionals 3 Technicians and Associate Professionals 31 Science and Engineering Associate Professionals 314 Life Science Technicians and Related Associate Professionals 3141 Life Science Technicians (excluding Medical) 32 Health Associate Professionals 321 Medical and Pharmaceutical Technicians 3212 Medical and Pathology Laboratory Technicians

Abbreviation: HISCO, Historical International Standard Classification of Occupations.

Job titles of medical laboratory personnel in 45 countries

History of job titles in UK medical laboratory personnel

Medical laboratory personnel in the UK were pathological and bacteriological laboratory assistants in 1912, medical laboratory technicians in 1943, medical laboratory scientific officers in 1974 before being designated as biomedical scientists in 1994 .[7] The European Federation of Clinical Chemistry and Laboratory Medicine (EFCC) defines "specialists in laboratory medicine" as medical doctors, pharmacists, and scientists and includes biomedical scientists in the UK at the scientist level. Biomedical scientists are academically trained laboratory professionals (master's degree) without specialist training unlike physicians.[8]

History of job titles of US medical laboratory personnel

In 1926, the ASCP (1922 American Society for Clinical Pathologists; 2001 American Society for Clinical Pathology) established the Committee on Registration of Technicians (1928 Board of Registry; 2009 Board of Certification) to address the need for formally registering and regulating the field of medical technology. Registration was open to practicing medical and laboratory technicians. According to the ASCP, registration for laboratory technicians and medical technologists began in 1931. With college graduates being granted the qualification of medical technologist in 1935, the title laboratory technician was automatically discontinued. In 1969, a qualification examination was established for medical laboratory technicians.[9] In 2009, the ASCP Board of Registry and the National Credentialing Agency for Laboratory Personnel (NCA) merged into a single credentialing agency, the ASCP Board of Certification. The two-major national certifying agencies (ASCP and NCA) agreed to provide a single credential under the ASCP Board of Certification, combining titles of medical technologists and clinical laboratory scientists. The official title from ASCP was renamed medical laboratory scientist.[9] The NCA certification clinical laboratory technician was replaced with the title medical laboratory technician.[9] There are currently three major certification agencies in the United States of America for medical laboratory personnel. They are the American Society for Clinical Pathology (ASCP), the American Medical Technologists (AMT), and the American Association of Bio analysts (AAB).[10]

Unit group and Job titles

The qualification for medical laboratory personnel was designated at the skill level 3-4 in the ISCO-08. In the case of the technologist

or technician type, the identities used by medical laboratory personnel include biomedical laboratory sanitary technician, clinical diagnostic laboratory technician, clinical laboratory technologist, medical laboratory technologist, medical laboratory technician, medical technologist, biomedical analysis technician, clinical analysis technician, and medical analytics technician. For the analyst type, the professional titles include bio analyst, biomedical analyst, and medical analyst, whereas for the scientist type, the titles include biomedical scientist and medical laboratory scientist. Additional designations include the bioengineer and medical technical laboratory assistant.[11-54] (Table 3)

Table 3. Job titles of medical laboratory personnel on ISCO-08

SOC version	Examples of job title according to unit group*	Reference
SCIENTIST REGISTRATION		
UK 2020	P: 2113 Biomedical Scientist AP: 3111 Laboratory Technician (ex: Medical Laboratory Assistant)	[11]
Ireland 2010	P: 2112 Medical Laboratory Scientist AP: N/A	[12]
Iceland (unk)	P: 2212 Biomedical Scientist AP: N/A	[13]
Australia 2013	P: 2346 Medical Laboratory Scientist AP: 311213 Medical Laboratory Technician	[14]
New Zealand# 2013	P: 2346 Medical Laboratory Scientist AP: 311213 Medical Laboratory Technician	[14]
Saudi Arabia# 2019	P: 213115-06 Medical Laboratory Scientist AP: 321201 Medical Laboratory Technician	[15]
Singapore 2020	P: 2134-02 Medical Laboratory Scientist (ex: Epidemiologist) AP: 3212 Medical Laboratory Technologist	[15]
AFRICA		
Cameroon (unk)	P: N/A AP: 3212 Medical Laboratory Scientist; Medical Laboratory Technician	[17]
Ghana 2010	P: N/A AP: 3212 Medical Laboratory Scientist; Medical Laboratory Technician	[18]
Kenya (unk)	P: N/A AP: 3212 Medical Laboratory Technologist; Medical Laboratory Technician	[19]
Nigeria (unk)	P: N/A AP: 3212 Medical Laboratory Scientist; Medical Laboratory Technician	[19]
RSA 2012	P: N/A AP: 3212 Medical Laboratory Scientist; Medical Technologist; Medical Technician	[20]
Zambia (unk)	P: N/A AP: 3212 Biomedical Scientist; Biomedical Technologist; Biomedical Technician	[21]
AMERICA		
US 2018	P: N/A AP: 29-2010 Clinical Laboratory Technologist; Clinical Laboratory Technician 29-2011 Medical and Clinical Laboratory Technologist (ex: Medical Laboratory Scientist, Medical Technologist) 29-2012 Medical and Clinical Laboratory Technician (ex: Medical Laboratory Technician; Medical Laboratory Assistant+)	[22]
Canada 2016	P: N/A AP: 3211 Medical Laboratory Technologist AP: 3212 Medical Laboratory Assistant+	[23]
Mexico# 2011	P: N/A AP: 2813 Medical Laboratory Technician	[24]
Brazil# 2010	P: N/A AP: 3242-05 Clinical Pathology Technician (ex: Clinical Analysis Technician) AP: 3242-10 Clinical Pathology Technical Assistant+	[25]
ASIA		
China# 2015	P: N/A AP: 20507-04 Clinical Laboratory Technologist; Clinical Laboratory Technician	[26]
Hong Kong (unk)	P: N/A AP: 3212 Medical Laboratory Technologist	[27]
Pakistan# 2015	P: N/A AP: 3212 Medical Technologist; Medical Laboratory Technician	[28]
India 2015	P: N/A AP: 3212 Medical Laboratory Technologist; Medical Laboratory Technician	[29]
Sri Lanka 2011	P: N/A AP: 3212 Medical Laboratory Technologist	[30]
Myanmar (unk)	P: N/A AP: 3212 Medical Technologist; Medical Laboratory Technician	[31]
Malaysia 2020	P: N/A AP: 3212 Medical Laboratory Technologist	[32]
Thailand# (unk)	P: N/A AP: 3212 Medical Technologist	[33]
Philippines 2012	P: N/A AP: 2227 Medical Technologist	[34]
Japan 2009	P: N/A AP: 143 Clinical Laboratory Technician (ex: Medical Technologist)	[35]
Korea 2017	P; N/A AP: 2451 Clinical Laboratory Technologist (ex: Medical Technologist)	[36]
Taiwan 2010	P: N/A AP: 3212 Medical Laboratory Technologist (ex: Medical Technologist)	[37]
EUROPE		
Belgium# (unk)	P: N/A AP: 3212 Medical Laboratory Technologist	[38]
Greece (unk)	P: N/A AP: 3212 Medical Laboratory Technologist	[39]
France# 2017	P: N/A AP: 433a Medical Laboratory Technician	[40]
Spain 2011	P: N/A AP: 3314 Clinical Diagnosis Laboratory Technician	[41]
Croatia 2010	P: N/A AP: 3212 Health Laboratory Technician	[42]

Germany 2010	P: N/A AP: 8121-02-103 Medical Technical Laboratory Assistant	[43]
Portugal 2010	P: N/A AP: 321201 Clinical Analysis Technician	[44]
Poland# 2014	P: N/A AP: 3212 Medical Analytics Technician	[45]
Netherlands# 2014	P: N/A AP: 3212 Medical Analyst	[46]
Italy 2011	P: N/A AP: 321302 Biomedical Laboratory Sanitary Technician	[47]
Switzerland# 2010	P: N/A AP: 862-08 Biomedical Analyst (Biomedical Analysis Technician)	[48]
Austria 2011	P: N/A AP: 3212 Biomedical Analyst	[49]
Sweden 2012	P: N/A AP: 3212 Biomedical Analyst	[50]
Denmark 2010	P: N/A AP: 321210 Biomedical Analyst	[51]
Estonia 2021	P: N/A AP: 32120101 Bio analyst	[52]
Finland 2010	P: N/A AP: 3212 Bio analyst	[53]
Norway 2011	P: N/A AP: 3212 Bioengineer	[54]
Hungary# (unk)	P: N/A AP: 3324 Medical Laboratory Assistant (ex: Medical Laboratory Analyst)	[63,64]

* Entry-level education: Bachelor's degree (3, 2+2, 4 years); Associate degree or Diploma (2-3 years).
\+ Certificate (4-6 months, various)
\# IFBLS Non-member country

Abbreviations: ISCO, International Standard Classification of Occupations; SOC, Standard Occupational Classification; P, Professionals; AP, Associate Professionals; RSA, Republic of South Africa; US, United States of America; UK, United Kingdom; unk, unknown; N/A, Not Applicable.

Adapted from Koo et al. Korean J Clin Lab Sci. 2010;53(1):105-121.

Discussion

Ambiguity regarding medical technologist's professional titles

ISCO-08 distinguishes the types of identity for science occupations as scientist, technician, and assistant, and for engineering occupations, engineer, engineering technician, and drafter. What's unusual is how the standard occupational classification in the United States (US) and Canada distinguishes between technician and engineering technician on the one hand and technologist and engineering technologist on the other regarding occupations in science, medicine, and engineering, whereas the ISCO-08 and most countries simply use the term technician. Technologist and engineering technologist refers to an individual who has completed 3 years of college or 3-4 years at a university. This is not reflected in the standard occupational classification. The term medical technologist has the disadvantage of lacking identity due to the various professional titles unlike medical doctor, pharmacist, nurse, and physical therapist. In the case of medical technologist and medical laboratory technician in the US, individuals use casual, non-professional terms such as "med tech, lab tech, or tech" in referring to laboratory experts.[55] Medical laboratory technicians work under the supervision of a medical technologist, who performs more complex testing. The difference between the medical technologist and the medical laboratory technician is the complexity of tests performed, the level of judgment needed, and the amount of responsibility each has. It is the difference in the amount of education and job skill that allows one to perform at the medical technologist or medical laboratory technician level. Technicians require the completion of a two-year associate degree, while a technologist requires the completion of a bachelor's degree. There is more of a difference between a medical technologist and a technician career than many people realize. The similar-sounding labels of "medical technician, medical technologist," as well as the overlap between the acronyms, was yet another reason for the migration to the more descriptive title of "medical laboratory scientis."[56]

Medical technologist is a term that occupationally requires renaming. Medical technologists are recognized as medical laboratory technicians working in clinical laboratories, but in most countries, they are introduced as including medical radiation technologists, nucleated medicine technologists, and medical sonographers. In Korea, medical service technologists (formerly medical technicians) are be classified into medical technologists, radiological technologists, physical therapists, occupational therapists, dental technicians, and dental hygienists .[57]

Ambiguity regarding medical technology's occupational title

Medical technology can be defined as the application of science to develop solutions to health problems or issues such as the prevention or delay of onset of diseases or the promotion and monitoring of good health .[58] In the World Health Organization (WHO), a health technology is the application of organized knowledge and skills in the form of devices, medicines, vaccines, procedures and systems developed to solve a health problem and improve quality of lives.[59] In a comprehensive way, medical technology includes medical and surgical procedures, drugs, equipment and facilities, and the organizational and supportive systems within which care is provided.[60] Even the academic name for the field of study for medical technologists, according to International Standard Classification of Education-Fields of Education and Training 2013 (ISCDE-F 2013) of the United Nations Educational, Scientific and Cultural Organization (UNESCO), is medical laboratory technology, not medical technology.[61] Moreover, the course of study and scope of business for medical laboratory technology have expanded following changes in the medical environment so that medical laboratory science and biomedical laboratory science have been used instead.

Preliminary Study of Name Change by the KAMT

In the Korean Association of Medical Technologists (In Korea, medical technologists are called “Clinical Pathology Technologists”) in 2021, the organization was charged with conducting research concerning the potential for a new professional title using 22,638 full members as subjects.[5] The survey was distributed to all members. The results indicated that “Diagnostic Laboratory Analyst” was the most preferred alternative selected by the largest proportion of respondents (34.73%), followed by “clinical laboratory analyst” (24.57%), “biomedical pathology technologist” (10.89%), “biomedical analyst” (10.49%), “biomedical laboratory analyst” (10.03%), and “clinical laboratory scientist” (9.26%).

The view regarding the need for a new professional title is thought to have been impacted by the fact that diagnostic tests (or diagnostic laboratory test) were popularly recognized during the past two years due to the coronavirus (COVID-19) pandemic. It is also important to note that medical, veterinarian, pharmaceutical, and chemical facilities and specialized laboratories employ such analysts to work in all types of laboratory environments. Depending on the industry, laboratory analysts can test and evaluate materials ranging from biological samples such as DNA and blood to environmental samples such as water and industrial waste.[62]

Cases to Move the Occupational Classification

Depending on the discipline, some occupations were already classified in Minor Group 226, Other Health Professionals (ISCO-88 examples: 3223 Dieticians and Nutritionists, 3224 Optometrists and Ophthalmic Opticians, 3226 Physiotherapists, 3229 Occupational Therapists, 3229 Audiologists and Speech Therapists). The skill level and essential qualifications for medical laboratory personnel have increased because of advances in scientific technology. In some countries, medical laboratory personnel now fall under science professionals to reflect the advancement of the discipline. Biomedical scientists or medical laboratory scientists in the UK, Ireland, Australia, and New Zealand are categorized as life scientists along with biologists, biochemists, and microbiologists according to their standard occupational classification. According to the standard occupational classification in the US, life scientists consist of Board Group 19-1020 Biological Scientists (examples: biologists, biochemists, microbiologists) and Board Group 19-1040 Medical Scientists (examples: epidemiologists, toxicologists). If medical laboratory scientists in the US are to be registered in the standard occupational classification, a detailed occupation must be created for Board Group 19-1040 Medical Scientists, or Board Group 10-1050 Medical Laboratory Scientists. Especially, “scientist” types of title are officially being recognized

Table 4. Proposal new titles for medical laboratory personnel

Unified job titles	ISCO-08 Skills (1-4)	ISCED 1997 Groups (1-6)	ISCED 2011 Groups (1-8)
Medical Laboratory Scientist, Biomedical Scientist	4	5a	6
Medical Laboratory Analyst, Biomedical Analyst	4*	5a*	6*
Medical Laboratory Technologist and Technician	3	5b	5
Medical Laboratory Assistant	3	4 (PNDA)	4 (PNDA)

*Bachelor's degree or equivalent

Abbreviations:
ISCO, International Standard Classification of Occupations;
ISCED, International Standard Classification of Education;
PNDA, postsecondary non-degree award.

and regulated by the Government Departments (examples: Ministry of Education, Ministry of Health).

Recommendation for Renaming and Realigning Laboratory Professionals

Medical laboratory personnel must be distinguished in the International Standard Classifica-tion of Occupations because they have differe-nt education levels, experience levels, and responsibilities. Medical laboratory personnel with a bachelor's degree qualification should be moved to Major Group 2 Professionals, and medical laboratory personnel with an associate degree or diploma qualification should be composed as Major Group 3 Technicians and Associate Professionals.

This paper proposes a new professional designation of "Medical Laboratory Analyst and Biomedical Analyst" as a unified term for medical laboratory personnel with a bachelor's degree qualification, excluding the title of medical laboratory scientist and biomedical scientist. (Table 4)

Table 5. Proposal new categorical classification for medical laboratory personnel

Current group in ISCO-08
2 Professionals
22 Health Professionals
221 Medical Doctors
222 Nursing and Midwifery Professionals
223 Traditional and Complementary Medicine Professionals
224 Paramedical Practitioners
225 Veterinarians
226 Other Health Professionals
2261 Dentists
2262 Pharmacists
2263 Environmental and Occupational Health and Hygiene Professionals
2264 Physiotherapists
2265 Dieticians and Nutritionists
2266 Audiologists and Speech Therapists
2267 Optometrists and Ophthalmic Opticians
2269 Health Professionals Not Elsewhere Classified
3 Technicians and Associate Professionals
32 Health Associate Professionals
321 Medical and Pharmaceutical Technicians
3211 Medical Imaging and Therapeutic Equipment Technicians
3212 Medical and Pathology Laboratory Technicians
3213 Pharmaceutical Technicians and Assistants
3214 Medical and Dental Prosthetic Technicians
322 Nursing and Midwifery Associate Professionals
323 Traditional and Complementary Medicine Associate Professionals
324 Veterinary Technicians and Assistants
325 Other Health Associate Professionals
Proposed group
2 Professionals
22 Health Professionals
221 Medical Doctors
222 Nursing and Midwifery Professionals
223 Traditional and Complementary Medicine Professionals
224 Paramedical Practitioners
225 Veterinarians
226 Dentists
227 Pharmacists
228 Allied Health Professionals
2281 Physiotherapists
2282 Dieticians and Nutritionists
2283 Audiologists and Speech Therapists
2284 Optometrists and Ophthalmic Opticians
2285 Occupational Therapists
2286 Radiographers and Sonographers
2287 Medical Laboratory Analysts and Biomedical Analysts
2288 Orthotists and Prosthetists
2289 Other Allied Health Professionals
2291 Environmental and Occupational Health and Hygiene Professionals
2292 Allied Health Professionals Not Elsewhere Classified
3 Technicians and Associate Professionals
32 Health Associate Professionals
321 Medical and Pharmaceutical Technicians
3211 Radiography Technicians and Sonography Technicians
3212 Medical Laboratory Technicians and Pathology Technicians
3213 Pharmaceutical Technicians and Assistants
3214 Medical and Dental Prosthetic Technicians
322 Nursing and Midwifery Associate Professionals
323 Traditional and Complementary Medicine Associate Professionals
324 Veterinary Technicians and Assistants
325 Other Health Associate Professionals

The recommendation from the present study is to separate the medical technologist and medical technician qualification in Unit Group 3211, 3212, and 3214, and subsequently include medical technologists as a new group of health professionals. (Table 5) First, the new group will be established to include dentists and pharmacists in Minor Groups 226 and 227. Second, the existing Minor Group 226 Other Health Professionals will be changed to 228 Allied Health Professionals. Allied health professionals will include physical therapists, occupational therapists, audiologists and speech therapists, dieticians and nutritionists, optometrists and ophthalmic opticians,

radiographer and sonographer, medical laboratory analysts and biomedical analysts, and orthotists and prosthetists. Third, environmental and occupational health and hygiene professionals will be included in the newly established Minor Group 229. Renamed Minor Groups 3212 Medical Laboratory Technicians and Pathology Technicians perhaps should be categorized in the same category as occupations such as assistant nurses, assistant midwives medical and dental technicians, physiotherapy technicians and assistants, pharmaceutical technicians and assistants, and veterinary technicians and assistants in revisions after ISCO-08. Medical laboratory technicians and pathology technicians can also be described as clinical pathology technicians, medical biology technicians, and histopathology technicians, etc.

Conclusion

Name changes are not just a change of designation but signify the occupational growth of a specialized job and the growth of its social value. In conclusion, the proposal for name changes for medical technologist grade can shed new light on the roles and responsibilities of the medical laboratory expert in education, research, and laboratory management.

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Literature Review

An Introduction to Myeloproliferative Neoplasms

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The Philadelphia chromosome negative myeloproliferative neoplasms (MPNs) are a rare group of chronic hematological diseases that are closely related. They arise from one of three disease-initiating driver mutations that cause over-activation of the JAK-STAT pathway resulting in disease of the hematopoietic system. These three MPNs have overlapping clinical and diagnostic features making diagnosis challenging. The World Health Organization (WHO) diagnostic criteria continues to evolve as more advances are made in understanding these complex diseases. As such, prognostic models for risk stratification are also evolving and newer models (e.g., Mutation-Enhanced International Prognostic Score System) incorporate genetic and molecular features. The major and most common complications include thrombohemorrhagic manifestations and progression to acute leukemia. The development of Janus kinase inhibitors has changed the treatment landscape of MPNs, yet treatment options are at this time still limited due to the complexities of these diseases.

Key words: Myeloproliferative neoplasm, polycythemia vera, essential thrombocythemia, primary myelofibrosis.

Introduction

The classical myeloproliferative neoplasms (MPNs) are a group of chronic hematological diseases composed of chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). The four classical MPNs are characterized by somatic mutations that occur in the hematopoietic stem cell resulting in excessive and chronic production of mature blood cells.[1] As MPNs are myeloid diseases, the excessive proliferation is restricted to erythrocytes, granulocytes, monocytes, and megakaryocytes.

CML is the only one that is Philadelphia (Ph) chromosome positive and this translocation between chromosomes 9 and 22 results in the BCR-ABL1 gene. The BCR-ABL1 gene is the driver (disease-initiating) mutation in the majority of CML patients and leads to unrestricted hematopoietic stem cell proliferation.[1,2] The other three diseases are Ph negative and are often classified as a group also known as the BCR-ABL1 negative MPNs. They are primarily the result of driver mutations in the Janus Kinase 2 (JAK2), calreticulin (CALR), or myeloproliferative leukemia (MPL) genes.[3,4]

As the focus of this article is on the Ph negative MPNs, any reference to MPN going forward will designate these 3 diseases. PV is characterized by excessive production of cells of the erythroid lineage, ET is characterized by excessive production of cells of the megakaryocytic lineage, and PMF is characterized by bone marrow fibrosis and megakaryocytic proliferation.[1] These diseases have shared clinical, diagnostic, molecular, and pathologic features that will be overviewed in this introductory article and further developed in the following articles.

Accepted: August 24, 2021

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PMF was the first MPN to be described in 1879 followed by PV in 1892 and ET was the last to be formally described in 1934.[5-8] It was not until 1951 that these diseases (including CML) were proposed to be interrelated. Dr. William Dameshek was an American hematologist and was the first to classify the diseases as "myeloproliferative disorders".[2] The term "neoplasm" replaced "disorders" in 2008 to emphasize the clonal nature of MPNs.[9]

Pathogenesis

Discoveries associated with CML (1960s-1990s), such as the Ph chromosome and resulting fusion gene BCR-ABL1, led researchers to question the possibility of undiscovered disease-initiating driver mutations in the Ph negative MPNs.[2] In 2005, research groups identified an acquired mutation in JAK2 gene that is present in more than 95% of patients with PV and over 50% of patients with ET or PMF.[1,10] The second most frequent driver mutation is CALR found in over 25% of patients with ET or PMF.[1,10] The last driver mutation, MPL, is the least frequent and found in over 3% of patients with ET or PMF.[1] About 10% of MPN patients lack a detectable driver mutation (JAK2, CALR, nor MPL) and are denoted as triple negative MPN.[3,11]

These driver mutations over-activate the JAK-STAT (Janus kinase-signal transducer and activator of transcription) signaling pathway which is essential for cytokines and growth factors that regulate differentiation, proliferation and survival of hematopoietic stem cells.[12] Thus, the pathway also plays a role in regulating hematopoiesis. The over-activation of the JAK-STAT pathway leads to impairment of hematopoiesis regulation resulting in various disorders of the hematopoietic system.[11,12] For MPNs, the result is hematopoietic stem cells that are hypersensitive to these cytokines and growth factors resulting in over proliferation of the myeloid cell line.

JAK2 assists with the regulation of growth factors: erythropoietin, granulocyte-colony stimulating factor (G-CSF), and MPL (the thrombopoietin receptor) thus affecting erythroid, granulocytic, and megakaryocytic lines.[3,11] As mutations of JAK2 are found in the majority of MPN patients, the resulting hematological characteristics include erythrocytosis, thrombocytosis and leukocytosis.[11] CALR and MPL mutations expressed in hematopoietic stem cells affect megakaryocytic lineage thus explaining their presence in ET and PMF but rarely found in PV.[11]

Beyond the three disease-initiating MPN driver mutations, additional non-driver mutations are found in many MPN patients. The most common of these mutations affect genes involved in epigenetic regulation, such as the TET2 gene.[3] Other affected genes include those involved in metabolic pathways, signaling cascades and splicing factors. Many of the non-driver mutations may be found in other hematological diseases and malignancies. They are also believed to play a role in disease development such as secondary myelofibrosis and transformation to acute leukemia.[1,3]

Clinical and Diagnostic Findings

MPNs are classified as a rare disease due to the low incidence rates.[6,9] They are often found in middle to advance-aged adults yet can occur in younger populations.[6,9] Clinical findings in MPNs range from asymptomatic to those associated with a debilitating disease thus making management a challenge.[3] The shared clinical features include hypercellular bone marrow, splenomegaly, thrombotic complications, and transformation to acute myeloid leukemia.[4,6] Due to these overlapping clinical features, diagnosis can present a challenge, yet an accurate diagnosis is critical for prognosis and treatment.

As mentioned above, the interrelatedness of the MPNs were described in 1951 by Dr. Dameshek who also referred to them as myeloproliferative disorders. Over the past seven decades, the research and resulting discoveries have guided the classification system as presented in Figure 1. The WHO, in collaboration with the Society for Hematopathology and the European Association for Haematopathology has published the fourth edition of *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* in 2008 and revised this

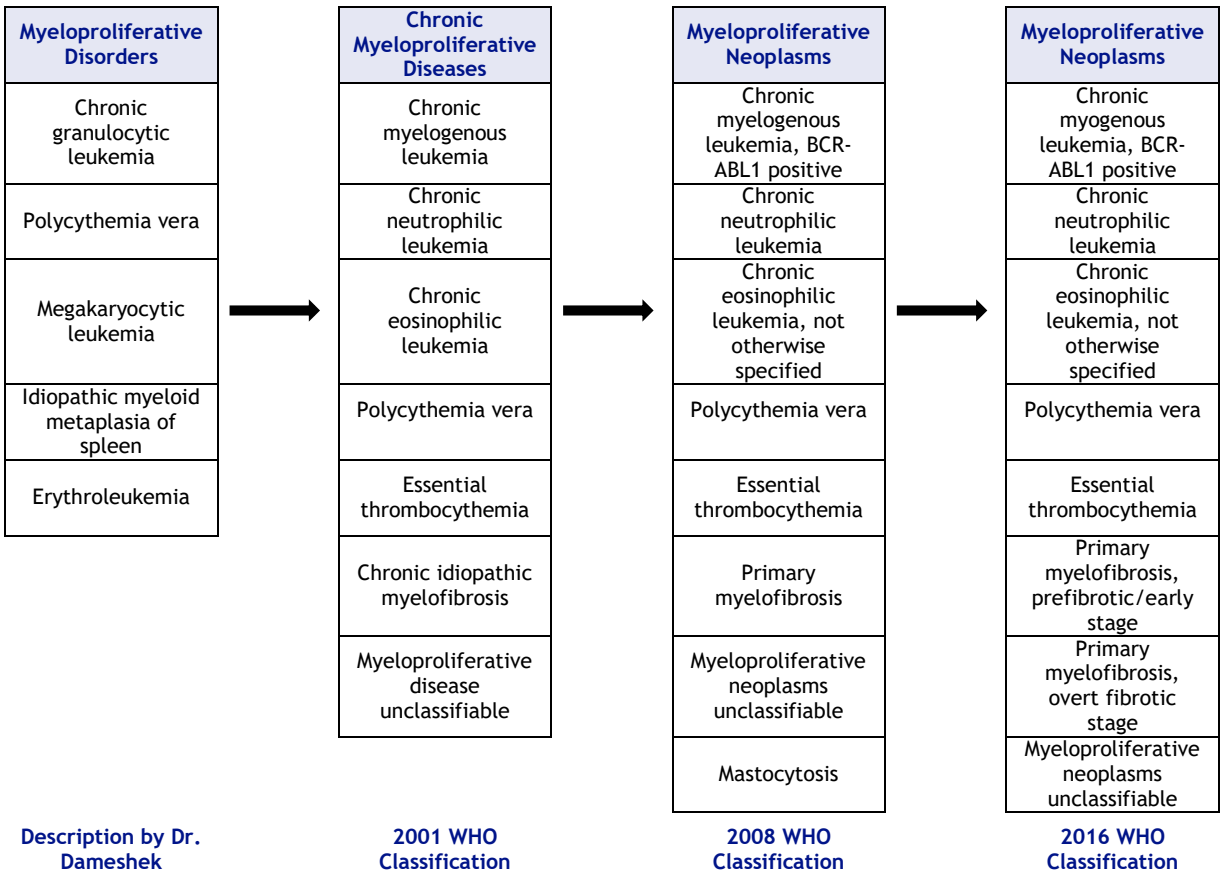

Figure 1. World Health Organization (WHO) classification evolution of myeloproliferative neoplasms. Adapted from Shallis et al. 2020.[2]

edition in 2016. The MPN category was revised due to discoveries of new mutations and one of the major revisions was to subcategorize PMF into prefibrotic or early stage PMF (prePMF) and overt fibrotic stage PMF.[13] This subcategorization is designed to help differentiate ET from prePMF.

PV is characterized by erythrocytosis and is suspected in patients with an elevated hemoglobin or hematocrit, hypercellular bone marrow indicating trilineage proliferation (panmyelosis), and the presence of a JAK2 mutation.[9,14] ET is characterized by thrombocytosis with a platelet count greater than 450 x 10^9/L, bone marrow showing proliferation of the megakaryocyte lineage, and presence of JAK2, CALR, or MPL mutation.[9,14] PMF is characterized by bone marrow proliferation of the megakaryocytic lineage and is accompanied by reticulin and/or collagen fibrosis, and presence of JAK2, CALR, or MPL mutation.[9,14] In addition, cytogenetic abnormalities are found in approximately 30-50% of PMF patients thus cytogenetic analysis contributes to diagnosis and prognosis.[15]

Major complications found in MPN patients include thrombosis, bleeding, and transformation to acute myeloid leukemia. For PV and ET patients, progression to secondary myelofibrosis is another complication of concern.[6] In order to assess the associated risks, multiple prognostic models have been developed specific to each MPN to aid in clinical decision-making.[9] Traditionally these prognostic models for risk stratification have been based on clinical and hematologic parameters however newer models are incorporating molecular and genetic parameters. A more in-depth review of the diagnostic criteria, complications, and prognostic models will be provided in the following articles.

Treatment

Current treatments for MPNs are primarily directed at prevention of complications and alleviation of symptoms as there are no curative drug therapies. For PV and ET patients, treatment has consisted of low-dose aspirin, therapeutic phlebotomy and cytoreductive drugs (e.g., hydroxyurea). As

PMF is associated with anemia, treatment also includes corticosteroids and androgens in addition to cytoreductive drugs.[1,14] The discovery of the driver mutations led to a pivotal moment in the treatment of MPNs, the development of JAK2 inhibitors. Ruxolitinib was the first approved targeted treatment for PMF and has improved symptoms and blood cell counts, and reduced spleen size.[3] Currently several novel drugs and targeted therapies (inhibitors) are being investigated for use in the treatment of MPNs.[16]

Conclusion

The three Ph negative MPNs have overlapping features yet are distinct hematological diseases and are presented in more detail in the following articles. PV is the most common Ph negative MPN with the hallmark clinical finding of an elevated hematocrit. Whereas, PMF has the hallmark findings of bone marrow fibrosis and splenomegaly with a bleak prognosis in comparison to the other Ph negative MPNs.

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Literature Review

Essential Thrombocythemia

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Essential Thrombocythemia (ET), a clonal hematopoietic stem cell disorder, is one of the classic Philadelphia negative myeloproliferative neoplasms and is characterized by thrombocytosis with bone marrow megakaryocytic hyperplasia. Mutations in Janus kinase 2 (JAK2), calreticulin *(*CALR), or myeloproliferative leukemia (MPL) are found in approximately 80-90% of patients. Over the past decade, new molecular and clinical knowledge in ET has led to a significant improvement in the diagnostic, prognostic, and therapeutic processes. Despite these advancements, many uncertainties remain regarding clinical decision-making. In the 2016 revised World Health Organization (WHO) classification true ET requires meeting all 4 major criteria described below or 3 major criteria and one minor criterion. Because of the revised WHO classification, study data has been re-evaluated and the revised International Prognostic Score of Thrombosis for Essential Thrombocythemia risk stratification was devised allowing clinicians to assign patients to the appropriate risk group in a 4-tiered system. In ET patient's transformation to acute myeloid leukemia and/or post-ET myelofibrosis are rare events. Current treatment in ET is primarily indicated for the purposes of preventing vascular complications which have been reported to be the leading cause of death. Thrombotic complications can occur in up to 24% of patients with 13% developing a vascular event before diagnosis. Mutational status has an impact on thrombotic risk with a lower rate of thrombosis seen in CALR-mutants as compared to JAK2 V617F/MPL mutants and triple-negative cases. Mutations other than JAK2, CALR, or MPL have been found in approximately 53% of patients with ET with the most frequent being TET2, ASXL1, DNMT3A, and SF3B1.

Key words: Essential Thrombocythemia, JAK2 mutation, CALR mutation, MPL mutation, Platelets.

Introduction

Essential thrombocythemia (ET) is a rare but serious myeloproliferative neoplasm (MPN) characterized by thrombocytosis with bone marrow megakaryocytic hyperplasia and a tendency to develop thrombotic and hemorrhagic complications. Essential thrombocytosis (primary thrombocythemia) is a nonreactive, chronic myeloproliferative neoplasm predominantly occurring in the age group of 50-60 years equally in the male and female population. A sustained megakaryocyte proliferation leads to an increase in the number of circulating platelets. Mutations in Janus kinase 2 (JAK2*)*, calreticulin (CALR), or myeloproliferative leukemia (MPL) are found in approximately 90% of patients with essential thrombocytosis.[1] As in primary myelofibrosis (PMF) and polycythaemia Vera (PV),

Accepted: August 24, 2021

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dysregulated signalling in the JAK pathway plays a role in the pathophysiology of ET. Over the past decade, new molecular and clinical knowledge in ET has led to a significant improvement in the diagnostic, prognostic, and therapeutic processes. Despite these advancements, many uncertainties remain regarding clinical decision-making.

Epidemiology, Etiology and Pathogenesis

The incidence rate refers to the number of new cases of a disease diagnosed within a specific time. Annual incidence rate for ET has been reported to be 1.03 per 100,000 in Europe and North America with higher rate in males.[1,2] Prevalence is the number of cases of a disease in the population at a specific point in time. The prevalence rate of ET has been found to be 11.00 to 42.52 per 100,000.[1] Female to male incidence is 2:1, generally with median age of about 60 years.[3] Most frequent recurrent chromosomal defects seen in ET patients include trisomy 1q, trisomy 8, trisomy 9, del(13q) and del(20q). ET generally has a better prognosis compared to other MPN with an expected survival rate of 18 – 19.8 years. Survival is significantly better in patients with a lower risk of thrombosis and the incidence of blast and fibrotic transformation in ET is lower than other MPN.[4]

Three main mutations seen in most MPN including PV, ET and PMF involve JAK2 gene or MPL gene that provides instructions for making the thrombopoietin receptor protein and CALR. However, their absence does not rule out ET diagnosis, as about 20% ET patients are triple negative.[5] JAK2 is a member of the tyrosine kinase family of enzymes and is involved in signal transduction for erythropoietin, thrombopoietin, and granulocyte colony-stimulating factor (G-CSF). JAK2 gene is located on chromosome 9p24. The V617F is the most frequent mutation of JAK2.[6] MPL gene, also known as myeloproliferative leukemia or thrombopoietin receptor gene, is on chromosome 1p34 and has a mutation cluster in exon 10 including MPLW515L/K and MPLS505N which is both a germline and somatic (ET) mutation resulting in hereditary thrombocythemia. CALR gene is on chromosome 19p13.2 and is responsible for a multifunctional Ca^{2+} binding protein chaperone mostly localized in the endoplasmic reticulum. These mutations are also found in cases with thrombocytosis other than MPN such as myelodysplastic syndrome with ring sideroblasts (MDS-RS), MDS/myeloproliferative neoplasm with RS and thrombocytosis (MDS/MPN-RS-T) and prefibrotic PMF (pre-PMF). Hereditary thrombocytosis has also been reported with germline JAK2 mutation (JAK2V617I) and associated with vascular events but not fibrotic/leukemic progression. JAK2 mutated patients are usually older with either higher hemoglobin or platelet count and abnormal serum erythropoietin levels. These patients are more likely to develop a thrombosis.[5-7]

The JAK2V617F mutation occurs in about 55% of patients with ET, CALR mutation is seen in 15%-24% of the ET patients and MPL gene mutation occurs in about 4% of affected patients.[5,8,9] CALR or MPL mutations may co-exist in some patients. Unfortunately, the clinical significance of the coexistence of multiple mutations is still unclear.[3] Mutations other than JAK2, CALR, or MPL have been found in approximately 53% of patients with ET with the most frequent being the tet-methylcytosine dioxygenase 2 (TET2) [16%], ASXL transcription regulator 1 (ASXL1) [11%], DNA-methytransferase 3A (DNMT3A) [6%], and component of the U2 snRNP (SF3B1) [5%].[1]

The most common risk for patients with ET is thrombosis. This increased risk may also be due to the presence of giant platelets that may lead to microvascular occlusion and large vessel thrombosis. Bleeding may also result in patients with extreme thrombocytosis. The reason could be due to acquired deficiency of von Willebrand Factor (VWF).

Clinical and Diagnostic Findings

The revised 2016 World Health Organization (WHO) diagnostic criteria for ET include four major and one minor criteria. Diagnosis of ET requires meeting all 4 major criteria or the first 3 major criteria including peripheral thrombocytosis, large megakaryocytes in bone marrow, Philadelphia negative MPN or positive

Table 1. Essential Thrombocythemia WHO diagnostic criteria

	Diagnostic Criteria	Major	Minor
1	Platelet count ≥450 × 109/L.	X	
2	Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyper-lobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers.	X	
3	Not meeting WHO criteria for *BCR-ABL1*+ CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms.	X	
4	Presence of *JAK2, CALR,* or *MPL* mutation.	X	
5	Presence of a clonal marker or absence of evidence for reactive thrombocytosis.		X

JAK2, CALR, or MPL mutation. The minor criterion demonstrates the presence of a clonal marker or absence of evidence for reactive thrombocytosis (Table 1).[3,10,11]

Peripheral blood morphology and bone marrow examination are often necessary to make an accurate morphologic diagnosis of ET and distinguish it from other myeloid neoplasms, like pre-PMF. Platelets in peripheral blood show anisocytosis with small to giant platelets which may be hypo granular or agranular (Figure 1).

Megakaryocytes in ET are large and mature-appearing and form loose clusters (Figure 2), whereas those in pre-PMF display abnormal maturation with hyperchromatic and irregularly folded nuclei and form tight clusters.[12] Patients may present with microcytic hypochromic blood picture due to impaired platelet function resulting in chronic blood loss from the gastrointestinal tract. Generally, bone marrow is hypercellular showing increase in large mature megakaryocytes with hyper-lobulated nuclei, often forming clusters within the bone marrow. If ET is triple negative, that is all three common mutations are absent (about 12% ET patients fall in this category), in these cases the bone marrow may show an increase in pleomorphic megakaryocytes forming loose clusters (Figure 3). Similar megakaryocytic clusters are also seen in the bone marrow of patients with CALR positive ET (Figure 4). These can also be seen with CD61(cluster of differentiation marker found on thrombocytes) immunohistochemical stain (Figure 5).

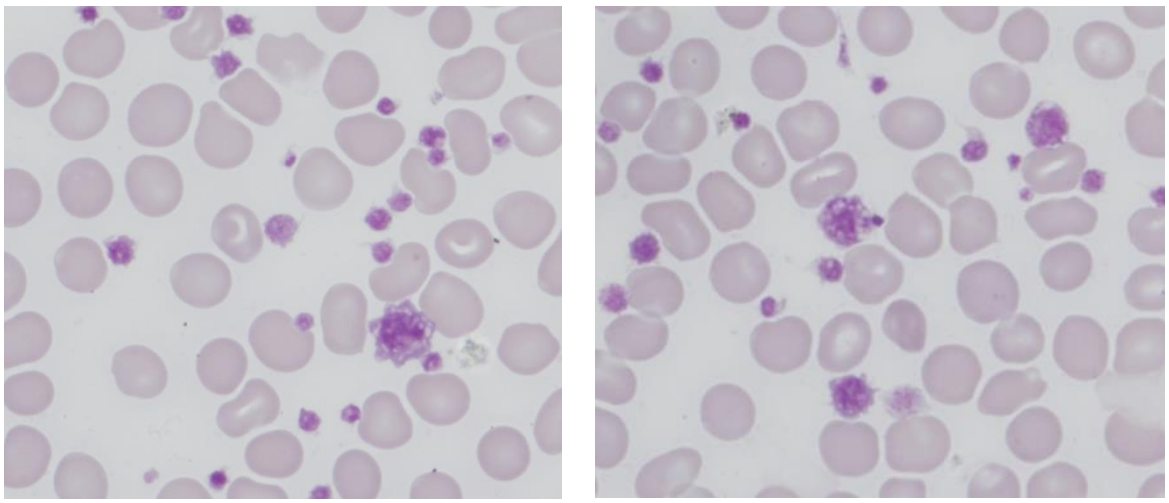

Figure 1. Peripheral Blood (magnification x600) JAK2 positive ET. Platelet count 1287x109/L with platelet anisocytosis including small and large platelets.

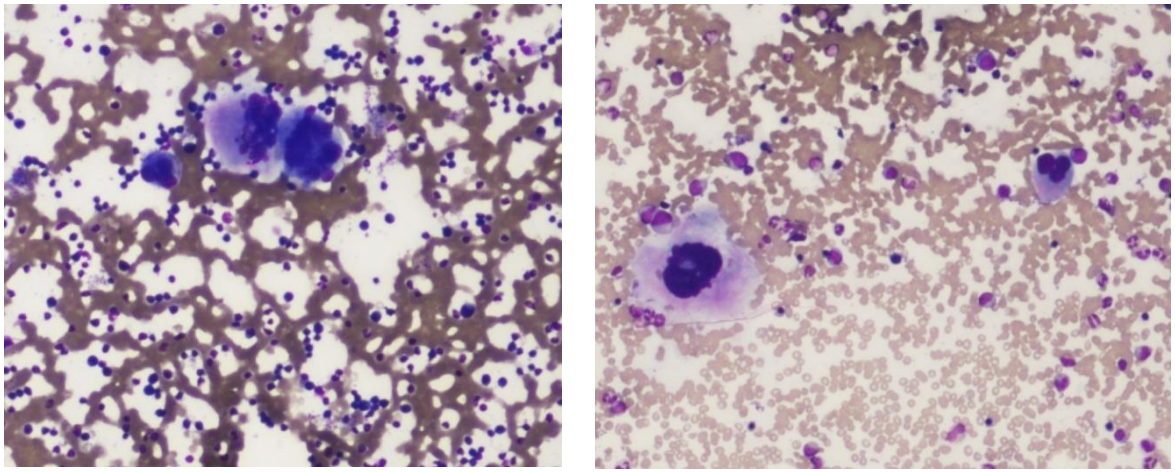

Figure 2. Aspirate (magnification x100) JAK2 positive ET. Increased numbers of megakaryocytes can be seen.

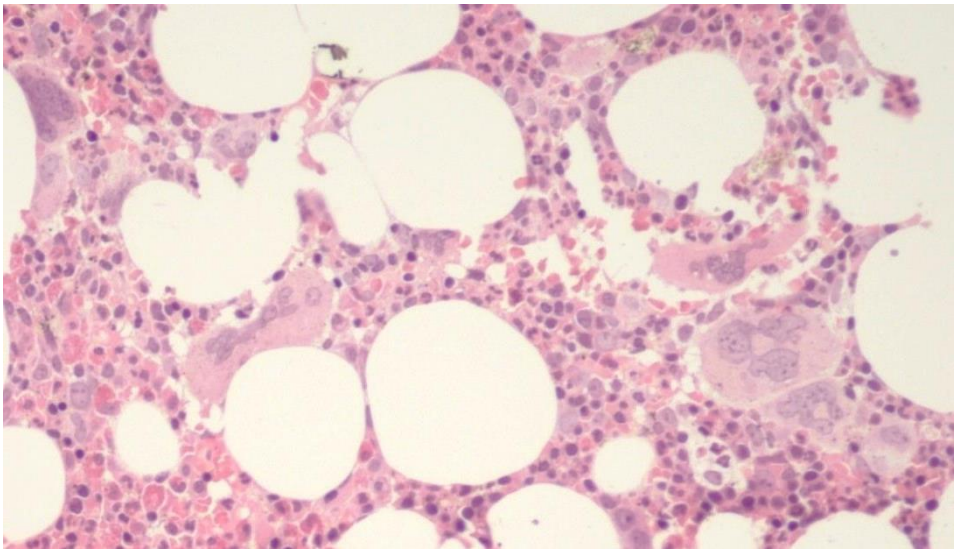

Figure 3. Triple negative ET (Magnification x200) Increase in pleomorphic megakaryocytes forming loose clusters.

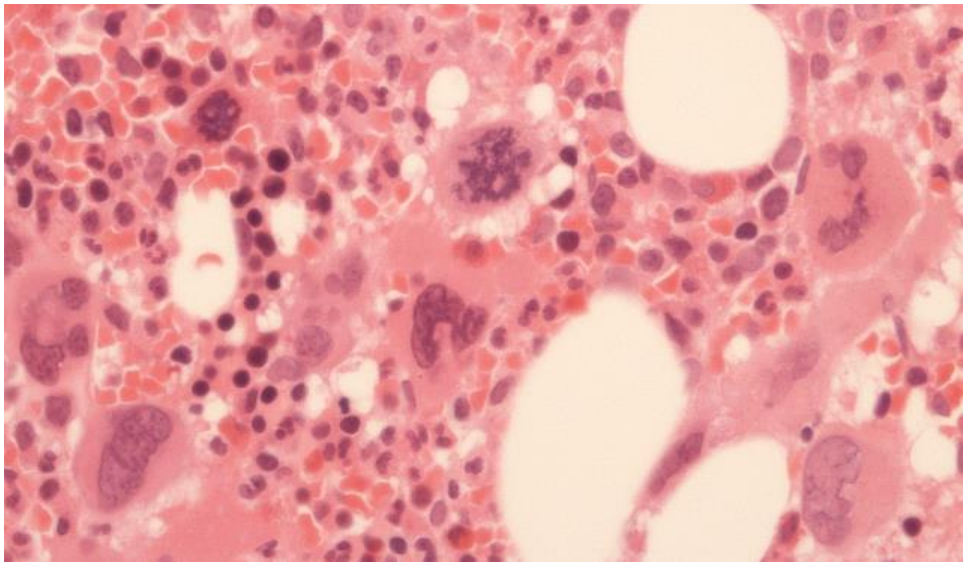

Figure 4. CALR positive ET (Magnification x400) Increase in pleomorphic megakaryocytes forming loose clusters.

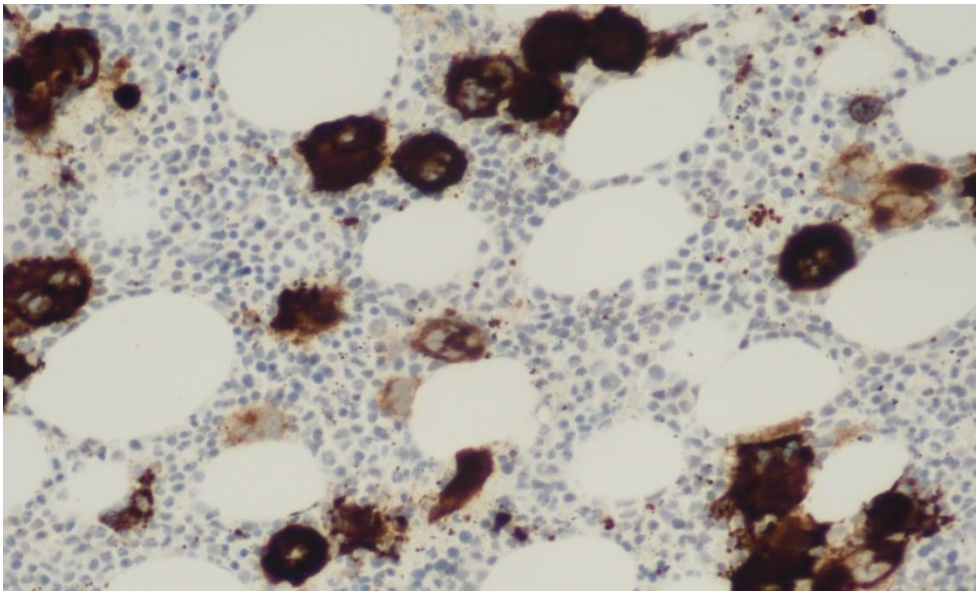

Figure 5. CALR positive ET (Magnification x200) CD61 immunohistochemical stain demonstrating increased numbers of pleomorphic megakaryocytes.

Risk Stratification

Risk stratification is an early step that follows diagnosis and is critically important to guide clinicians towards appropriate therapeutic interventions.[13] Historically, risk stratification looked at two risk parameters; whether the patient was >60years of age and if there was a history of thrombosis. Patients who met these criteria were high risk. The absence of both risk factors i.e. <60 years of age and no history of thrombosis were classified as low risk. A new 3-tiered score, which included an intermediate risk group, was proposed in 2012 and was called the "International Prognostic Score of Thrombosis for Essential Thrombocythemia" (IPSET-Thrombosis model).[14] The IPSET-Thrombosis score accounted for the presence of cardiovascular risk factors and JAK2V617F mutation.[15] Previously, classification had allowed a degree of bone marrow fibrosis and morphologic features more resembling myelofibrosis. With the revised WHO classification true ET was characterized by lower white blood cell (WBC) counts, lower hemoglobin levels, lower lactate dehydrogenase levels in plasma, and more importantly a better prognosis, with close to normal survival rates.[16] The data was re-evaluated, and the revised IPSET risk stratification was devised. The revised IPSET-Thrombosis score changed to a 4-tiered system of very-low-risk, low-risk, intermediate risk, and high-risk categories (Table 2) and included the integration of MPL-mutation status.[17] According to the revised 2016 WHO criteria, in triple-negative patients,

Table 2. IPSET-Thrombosis revised risk groups

Risk Group	Criteria
Very-low-risk	No thrombosis history, age ≤ 60 years and *JAK2*/*MPL*-unmutated
Low risk	No thrombosis history, age ≤ 60 years and *JAK2*/*MPL*-mutated
Intermediate risk	No thrombosis history, age > 60 years and *JAK2*/*MPL*-unmutated
High-risk	Thrombosis history or age >60 years with *JAK2*/*MPL*-mutated

Adapted from Awada et al 2020.[13]

testing by next generation sequencing (NGS) for the most frequent additional mutations (e.g., ASXL1, DNMT3A, TET2, EZH2 [histone-lysine-N-methyltransferase enzyme], IDH1/2 [isocitrate dehydrogenase 1 or 2], SRSF2 [serine and arginine rich splicing factor 2]*)* may be helpful to determine the clonal nature of the disease and complement the morphological criteria.[16] Another advantage of using NGS for mutation detection in triple-negative MPNs is the possibility of simultaneous testing of rare variants in JAK2, CALR, or MPL otherwise not detected by conventional assays.[16]

Prognosis and Disease Progression

In ET patient's, the transformation to acute myeloid leukemia (AML) and/or post-ET myelofibrosis (post-ET MF) are rare events. Risk of transformation to acute leukemia was

reported at 2-3% at 10 years and 5% at 15 years.[18] A history of thrombosis and male sex were independent predictors of death.[19] ET is considered to have a favorable prognosis A 2014 study reported the median survival of patients with ET to be 19.8 years, inferior to that of the age- and sex-matched United States population and unaffected by driver mutation status.[20] Life expectancy in ET for patients younger than 60 years of age is mildly compromised with median survival approaching 33 years. A more recent study in 2019 concluded ET has a similar or slightly lower survival than the general population.[18] The distinction made in 2016 by the WHO in pre-PMF and overt myelofibrosis has improved the prognosis, as several cases of ET have been reclassified to pre-PMF which has a slightly worse prognosis.[18]

The prognostic relevance of the somatic mutations by NGS has been investigated in large ET cohorts of patients.[1] SH2B3/LNK (lymphocyte adapter protein), SF3B1, U2AF1 (U2 small nuclear RNA auxiliary factor 2) , TP53 (tumor protein p53), IDH2, and EZH2 have been shown to impact on overall, leukemia-free, and myelofibrosis-free survival based on age-adjusted multivariable analysis. Their presence was associated with poor survival in ET (median 9 years vs. 22

Table 3. Clinical-Molecular Prognostic Scores in Essential Thrombocythemia

Variables	Points	Risk Categories	Median Survival (years)
Leukocyte count ≥11 x10^9/l	1	Low (0-1)	34.3
Age >60 years	4	Intermediate (2-5)	14.1
Male sex	1	High (6-8)	7.9
SRSF2, SF3B1, U2AF1, TP53	2		

Adapted from Tefferi et al 2020.[22]

years). A study of 502 molecularly annotated ET patients allowed mutational information to be incorporated into a new prognostic model, the Mutation-Enhanced International Prognostic Scoring System (MIPSS) specific for ET (Table 3). [22]

Overall survival was adversely affected by spliceosome mutations SF3B1 and SRSF2 while U2AF1 and SF3B1 adversely affected myelofibrosis-free survival; earlier studies showing TP53 to be predictive of leukemic transformation were confirmed.[1] The number of somatic mutations has also been reported to impact on overall survival with reported hazard risk values of 6.6 for 3 mutations, and hazard risk of 2.2 for 1 or 2 mutations.[21]

Data on the thrombotic risk associated with MPL mutations are scant, primarily due to low overall frequency. In a 2013 study, the 5-year cumulative incidence of thrombosis was estimated to be around 9% while in the 2008 prospective primary thrombocythemia-1 cohort MPL mutation (4.1% of ET patients) was not predictive of thrombosis.[23,24] However, a larger 2008 study, (N=994) in which 3% of subjects had the MPL mutation, demonstrated that it was associated with higher risk of thrombosis when compared to JAK2 mutation or wild-type MPL.[25]

In an analysis of 576 ET patients, 15.5% of whom had CALR mutations, the 10-year cumulative incidence of thrombosis was 5%. The thrombotic risk of CALR mutated patients was similar to that of triple-negative ones and lower than JAK2- and MPL-mutated patients.[26] Later studies confirmed these findings, with a 2015 study of 217 patients with WHO-defined ET or early PMF demonstrating that the lower incidence of thrombosis in patients with CALR mutation vs. JAK2 mutation persisted at 15 years (9.1% vs. 21.7% respectively P=0.04).[27]

CALR-mutated ET shows clinical features different from JAK2 V617F-positive ET with CALR-mutated patients presenting with a higher platelet count at diagnosis, a lower hemoglobin and WBC count, coupled with a lower thrombotic risk if compared to JAK2 V617F-positive ET patients.[28,29]

An extreme thrombocytosis may result in minor bleeding or major hemorrhagic complications as a platelet count >1000 x 10^9/l can induce an acquired von Willebrand syndrome (AVWS), a rare but probably underestimated bleeding disorder. AVWS is usually associated with a range of underlying disorders and according to

the International Society on Thrombosis and Hemostasis registry, 15% of cases are linked to an underlying myeloproliferative neoplasm.[30] AVWS is caused by the proteolytic reduction of VWF multimers due to the passive adsorption to the platelet membrane with an inverse relationship between platelet count and the plasma defect of high molecular weight multimers.[13]

ET may develop into PMF or PV in small number of patients probably due to pathophysiological continuum, starting from ET and ending in MF. The fact that made the continuum theory attractive was the detection of the V617F mutation in the tyrosine pseudokinase region of the JAK2 gene, being a gain-of-function mutation, resulting in uncontrolled cellular growth in the hematopoietic compartment. The presence of a JAK2V617F mutation indicates MPNs but does not differentiate between them.

Treatment

Current treatment in ET is primarily indicated for the purposes of preventing vascular complications which have been reported to be the leading cause of death.[13] Thrombotic complications can occur in up to 24% of patients with 13% developing a vascular event before diagnosis. Mutational status has an impact on thrombotic risk with a lower rate of thrombosis seen in CALR-mutants as compared to JAK2 V617F/MPL mutants and triple-negative cases. Thrombosis can occur in unusual sites such as the splanchnic vessels (Budd-Chiari syndrome) or cerebral venous sinus. These thrombotic events in unusual sites are more frequent among JAK2 V617F carriers and may represent the first sign of disease onset.[13]

In patients with ET, the risk of thrombosis is about twice as high in those with the JAK2 mutation compared to those without [odds ratio (OR) 1.83-1.92]. The risk is increased for both arterial (OR range 1.68-2.59) and venous thrombosis (OR range 2.09-2.5).[31] The JAK2 V617F mutation burden also influences thrombotic risk. JAK2 V617F homozygosity is rare in ET patients but it is thought to confer an increased risk of thrombosis when compared to a heterozygous or wild-type condition.[32] A 2015 study showed that an allele burden of 20-25% or higher independently predicted the risk of arterial and venous thrombosis. Another study found that among WHO-defined ET or early PMF a high JAK2 allele burden (>50%) positively correlated with the thrombotic risk regardless of the WHO diagnosis.[33,26]

Treatment for patients with ET varies according to individual risk stratification, and ranges from the use of aspirin (acetylsalicylic acid [ASA]) to that of cytoreductive therapy. Current treatments are not intended to be curative but rather directed at reducing the thrombo-hemorrhagic risk.[34]

There is general agreement that high risk patients with ET should be treated with low-dose ASA with most doctors giving ASA to practically all ET patients.[16] However, the European Leukemia Net consensus recommendations advise a more restrictive approach with ASA only being given to patients who have microvasculature symptoms and those with cardiovascular risk factors.[14] Antiplatelet therapies should be used with caution in patients with platelet counts >1000x10^9/L due to the possibility of the acquired von Willebrand syndrome. In patients with marked thrombocytosis and previous hemorrhages, the use of antiplatelet therapy should be avoided.[16]

Worldwide, hydroxyurea [HU (also known as hydroxycarbamide)] is the most widely used cytoreductive agent for patients with ET.[35] It works by inhibiting the enzyme ribonucleotide reductase, thereby decreasing the production of deoxyribonucleotides. It has long been considered first-line treatment for ET, given its favorable toxicity profile. The most common side effects, which are generally mild, include hyperpigmentation of the skin, oral ulcers, and rashes. A cessation rate of about 10% has been reported due mostly to the presence of leg ulcers.[16] HU produces macrocytosis, and dysplasia can be seen in the bone marrow due to its action on DNA formation. This is usually disregarded as clinically irrelevant HU has been suspected of increasing the risk for leukemia transformation.[36] The issue is contentious, as various studies over several decades either give support for or minimize the leukemic risk.[16] It

should be noted that HU is teratogenic. For patients wishing to maintain their fertility interferon therapy may be a more appropriate treatment.

Anagrelide, an inhibitor of cyclic adenosine monophosphate (AMP) phosphodiesterase III, originally designed as an anti-platelet agent was subsequently found to inhibit both megakaryocytic differentiation and proliferation.[37] At higher doses, anagrelide acts through AMP phosphodiesterase to inhibit platelet aggregation. At lower doses, it also works by decreasing platelet counts.[38] Anagrelide is generally considered for patients where HU or interferon fails or causes unacceptable toxicities. Studies have evaluated anagrelide as a first-line therapy for ET. However, there is conflicting evidence on the efficacy and safety. One study suggested that anagrelide was not inferior to HU.[39] In another study, patients receiving anagrelide experienced higher incidences of arterial thrombosis, bleeding complications, and fibrotic progression.[40] Similarly, non-controlled studies have suggested that more than a quarter of patients receiving anagrelide therapy become anemic while a lesser percentage experience renal insufficiency.[41] Anagrelide is considered to have a significant side effect profile related to its vasodilator action. Risks reported include fluid retention, headaches, heart palpitations and cardiomyopathy. Cardiac evaluation is recommended before treatment.[13,38,42]

Interferon-alfa (Interferon-α) has been used successfully for the treatment of ET for many years, with consistently high hematologic as well as molecular remission rates. Interferon-α may result in approximately 80% hematological responses as defined by reduced hematocrit, WBC and platelet counts.[34] Interferon-α targets the malignant clone to reduce the colony-forming capabilities of erythroid, granulocytic, and megakaryocytic progenitors. It is an effective agent for treating both ET and PV.[38] Interferon-α is able to clear not just JAK2- but also CALR-mutated clones, suggesting its potential as a true disease-modifying agent, although there is preclinical evidence that CALR-mutated ET may be less responsive to interferon-α than JAK2-mutated disease.[35] Pegylated interferon-α (P-IFN-α) treatment in ET, where interferon-α is conjugated with polyethylene-glycol, is often the second line therapy of choice in patients who are intolerant or refractory to HU. Pegylation allows the interferon-α to stay in the body longer before it is broken down and eliminated. P-IFN-α has been shown to be relatively safe and effective and has been associated with both clinical (70-80%) and molecular (10-20%) remissions in some patients, especially in the presence of CALR gene mutations.[41] However, side effects have been reported with P-IFN-α and include depression, flu-like symptoms, headache, malaise, fevers, arthralgia, pruritus, injection-site reactions, gonadal toxicity, and thyroid dysfunction.[13] The average rate of discontinuation due to side effects is approximately 25% with patients younger than 60 years tending to tolerate this agent better than older patients.[38] Because of the significant side effects, Interferon-α is usually reserved for younger patients or those who are pregnant.[34]

While considered a relatively safe treatment, Ruxolitinib is an expensive therapy. It is also an immunosuppressant with an increased propensity for infection and development of skin cancers, especially in those predisposed. The use of Ruxolitinib, not currently licenced for use in ET, needs further evaluation, in part due to the fact ET patients have near-normal life expectancy and exposure to this treatment could be over several decades.[34]

With an increased incidence of ET in women over men (approximate ratio of 2:1) and 20% of patients receiving a diagnosis at less than 40 years of age, there is the potential for the need to manage pregnancy in female patients with ET.[18] A study of 234 patients with ET identified 20.49% of females that were fertile at the time of diagnosis.[18] In these patients, the risk of first-trimester fetal loss (about 3.5-fold) and placental complications (e.g. abruption, pre-eclampsia) is increased if compared to healthy women. Risk factors include previous pregnancy complications and possibly the presence of a JAK2V617F mutation. Venous thrombosis may occur, particularly in the

postpartum period. The risk is also higher in patients with a history of vascular events. Current treatment recommendations in young women wishing to be pregnant or are pregnant include once-daily ASA for "very low-risk" or "low-risk" disease and P-IFN-α for high-risk disease. Both ASA and Interferon-α therapy have been shown to be safe for use during pregnancy and might be associated with lower miscarriage rates in women with ET.[41] Interferon-α is not associated with gonadal toxicity and has no teratogenic effects, and therefore is not contraindicated in pregnancy, unlike HU that is teratogenic and requires a recommended 3-6 month washout period for patients wishing to conceive.[38]

Table 4: Treatment options

Risk Category	Therapy
Very low (Age ≤60 years, *JAK/MPL2* wild type, no prior thrombosis)	Management of cardiovascular risk factors, observation, or low-dose aspirin, unless contraindicated
Low (Age ≤60 years, *JAK2* V617F/*MPL* positive, no prior thrombosis)	Management of cardiovascular risk factors and low-dose aspirin unless contraindicated. Higher dose aspirin may be used if cardiovascular risk factors present
Intermediate (Age >60 years, *JAK2/MPL* wild type, no prior thrombosis)	Management of CV risk factors and cytoreductive therapy plus low-dose aspirin unless contraindicated. Higher dose aspirin without cytoreductive therapy if no cardiovascular risk factors
High (Age >60 years and *JAK2* V617F/*MPL* positive or prior thrombosis	Management of cardiovascular risk factors and cytoreductive therapy plus low-dose aspirin

Key: JAK2 – Janus kinase 2 gene, MPL myeloproliferative leukemia gene, a thrombopoietin receptor.

The patient's clinical history, circumstances, and life events remain the main influences on the treatment of choice for the physician. Table 4 summarizes the potential therapeutic approaches based on the different revised IPSET-Thrombosis model risk categories.

Conclusion

ET is an acquired MPN characterized by the thrombocytosis but relatively benign prognosis. In the WHO classification, it is defined as a Philadelphia negative MPN. ET is most often caused by JAK2, CALR and MPL mutations. The mutational status and risk of thrombosis dictate the therapeutic regime. Focus of the treatment is to suppress the megakaryocytic proliferation with busulfan, HU, Interferon-α and Anagrelide, and to reduce the risk of thrombosis using antiplatelet therapy such as ASA. Early detection reduces risk of thrombosis and improves prognosis. Despite significant improvement in the diagnostic, prognostic, and therapeutic clinical knowledge about ET, there are still unanswered questions about uncertainties associated with clinical decision making particularly when there is increased incidence of vascular complications and a tendency to progress to pre-PMF, myelofibrosis or acute myeloid leukemia.

Acknowledgement

Images used in figures were provided by Nichola H Lawrence BSc MSc CSci FIBMS, taken at University Hospitals of North Midlands NHS Trust.

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Literature Review

Primary Myelofibrosis

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Medical Laboratory Science, West Virginia University, West Virginia, USA [1]. School of Science and Technology, Nottingham Trent University, Nottingham, UK [2]. Medical Laboratory Science Program, Griffith University, Queensland, Australia [3]. Pathology, University Hospital of North Midlands NHS Trust, Staffordshire, UK [4].

Primary myelofibrosis (PMF) is a Philadelphia negative myeloproliferative neoplasm characterized by bone marrow fibrosis, splenomegaly, anemia, constitutional symptoms, and extramedullary hematopoiesis. As a clonal hematopoietic stem cell disorder, it is often accompanied by a disease-initiating driver mutation and shortened survival. Diagnosis is often based on bone marrow findings. Diagnosis is supported by the presence of janus kinase 2 (JAK2), calreticulin (CALR), or thrombopoietin receptor protein (MPL) mutation, found in approximately 90% of patients. In 2016, the World Health Organization divided PMF into pre-fibrotic and overt categories to aid in distinguishing PMF from essential thrombocythemia. Several prognostic systems, using a variety of clinical and genetic features, have been developed to aid in therapeutic decision-making. Treatment focuses on alleviation of symptoms and an increase in overall survival. Treatment options have historically been limited. However, the therapeutic landscape is changing with the development of new JAK inhibitors.

Key words: Myelofibrosis, myeloproliferative neoplasm, prefibrotic myelofibrosis, primary myelofibrosis, overt myelofibrosis.

Introduction

Primary myelofibrosis (PMF) is the least frequent Philadelphia chromosome negative (Ph negative) myeloproliferative neoplasm (MPN).[1] PMF is an aggressive and chronic hematologic disease characterized by bone marrow fibrosis resulting in extramedullary hematopoiesis and splenomegaly.[2,3] Additional disease features include anemia, inflammatory cytokine production, constitutional symptoms, and transformation to acute leukemia.[3,4]

In 1951, hematologist William Dameshek included PMF among a group of diseases that he termed myeloproliferative disorders.[5,6] In the past, PMF has had several names including agnogenic myeloid metaplasia, chronic idiopathic myelofibrosis, and myelofibrosis with myeloid metaplasia with the latest being primary myelofibrosis.[5] The 2008, 4th edition, of the World Health Organization (WHO) *Classification of Tumors of Hematopoietic and Lymphoid Tissues* modified the term myeloproliferative disorders to myeloproliferative neoplasms. The disease name chronic idiopathic myelofibrosis was replaced with PMF.[7] In addition, MPNs were classified based on bone marrow morphology, clinical features, and genetic information.[7]

In 2016, the WHO revised the 4th edition resulting in changes to the classification of MPNs. As described above, the classification of MPNs are still based on bone marrow morphology, clinical features and genetics, yet the revised edition has more integration of molecular genetic data due to the discovery of new somatic mutations.[8] PMF is further subcategorized into prefibrotic/early primary

Accepted: August 24, 2021

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myelofibrosis (pre-PMF) and overt PMF.[8] This subcategorization allows for distinction between "true" essential thrombocythemia (ET) and pre-PMF.[9,10] Additionally, myelofibrosis can follow a diagnosis of ET and polycythemia vera (PV) and is known as post-ET/post-PV MF (or secondary myelofibrosis).[9]

Epidemiology

The annual incidence rate of PMF is approximately 1 per 100,000 based on reports in Australia, Europe, and North America making it the least frequent Ph negative MPN.[6] The prevalence rate of PMF ranges from 1.76 to 4.05 per 100,000.[11] PMF has been reported in all ages, yet it is most often found in middle aged and elderly patients with the majority of patients greater than 50 years old at diagnosis.[1,2] Some studies have indicated that men are affected more frequently than women, however other studies indicate that both sexes are nearly equally affected.[1] In general, median overall survival for PMF is 5-7 years post-diagnosis with primary causes of death including leukemia transformation, vascular events, and infections.[1,6]

Etiology and Pathogenesis

The majority of PMF patients have one of three disease-initiating driver mutations that over-activate the janus kinase 2 and signal transducer and activator of transcription (JAK2-STAT) pathway resulting in unregulated myeloproliferation. Approximately 50-65% of PMF patients have a mutation in the JAK2 gene, specifically the JAK2V617F exon 14 mutation.[12-14] These patients are associated with older age, higher hemoglobin levels and white blood cell (WBC) counts, and lower platelet counts. A mutation in the calreticulin (CALR) gene is found in 20-30% of PMF patients and associated with younger patients, lower hemoglobin and WBC counts with a higher platelet count.[12-14] The least frequent driver mutation, found in about 10% of PMF patients, is a mutation in the myeloproliferative leukemia (MPL) gene .[12-14] An estimated 10% of PMF patients do not have any of the three driver mutations and are known as "triple negative" cases .[12,13] Triple negative PMF patients can be difficult to distinguish from other myeloid diseases and have the poorest prognosis. In addition, there are several non-driver mutations associated with PMF patients that are believed to contribute to disease development and transformation to acute leukemia (Table 1).[4]

Table 1.
Frequent Non-driver Somatic Mutations in PMF

Mutation	Mutational Frequency
TET2 (TET oncogene family member 2)	~17%
SRSF2 (Serine/arginine-rich splicing factor 2)	~17%
U2AF1 (U2 Small Nuclear RNA Auxiliary Factor 1)	~16%
ASXL1 (Additional Sex Combs-Like 1)	~13%
EZH2 (Enhancer of zeste homolog 2)	~7%
DNMT3A (DNA cytosine methyltransferase 3a)	~7%
SF3B1 (Splicing factor 3B subunit 1)	~7%
IDH1/IDH2 (Isocitrate dehydrogenase 1 and 2)	~4%
TP53 (Tumor protein p53)	~4%

These non-driver mutations play a role in DNA methylation (TET2, DNMT3A, and IDH1/IDH2), RNA splicing (SF3B1), chromatin modifications (ASXL1, EZH2), and DNA repair (TP53).[1] In triple negative patients, the presence of one or more of these non-driver mutations may be useful in diagnosis and often indicate a poorer prognosis.[12]

The overexpression of hematopoietic cytokines and growth factors associated with the over-activation of the JAK2-STAT pathway lead to megakaryocyte hyperplasia ultimately resulting in the classic features of PMF – bone marrow fibrosis and abnormal megakaryocytes. Abnormal megakaryocytes and other bone marrow cells release cytokines promoting bone marrow fibrosis. Extramedullary hematopoiesis is associated with the extensive bone marrow fibrosis which contributes to the splenomegaly seen in PMF patients.[6]

Clinical and Diagnostic Findings

The clinical manifestations in PMF vary, however most patients present with anemia, constitutional symptoms (fatigue, fever, and night sweats), and splenomegaly. Splenomegaly is a hallmark finding in PMF patients

and it often seen in approximately 90% of patients.[1] Splenomegaly results from extramedullary hematopoiesis leading to abnormalities in splenic architecture and increased presence of megakaryocytes.[6] It is often a debilitating symptom and a contributing factor in morbidity. Other findings include changes in platelet counts, bleeding, bone pain, headache, hepatomegaly (40-70% of patients), thrombosis, and weight loss.[1-4] Symptoms are often due to the production of cytokines during disease progression. Clinical findings may vary and approximately 30% of patients are asymptomatic at diagnosis.[1]

Diagnosis of PMF is based upon the 2016 WHO criteria and includes a combination of clinical and laboratory findings (Table 2). Pre-PMF was integrated within the PMF category as a variant and was first mentioned in the 2001 WHO classification of tumors.[7,15] Patients with pre-PMF often present with thrombocytosis (increased platelet count) and a lack of bone marrow fibrosis, thus they were often misdiagnosed as having ET. In ET patients, the differentiating bone marrow findings include granulocytic and erythropoietic cells that are in regular ratio with normal megakaryocytes.[16] In pre-PMF, the most profound peripheral blood finding is thrombocytosis often resembling ET. Anemia may be present however tear-drop red blood cells (RBCs) are rare.[17] A slight leukocytosis is common, however an increase in peripheral blood blasts may or may not be present.[17,18]

In overt PMF (classical fibrotic stage), peripheral blood findings include leuko-erythroblastosis with tear-drop RBCs and abnormal platelets due to the release of abnormal cells from sites of extramedullary hematopoiesis. Leuko-erythroblastosis leads to the presence of nucleated RBCs and immature

Table 2. 2016 WHO Diagnostic Criteria for PMF (Diagnosis requires meeting all 3 major criteria and at least 1 minor criterion that is confirmed in 2 consecutive determinations.)

PREFIBROTIC/EARY PMF	OVERT PMF
MAJOR CRITERIA	
1. BONE MARROW MORPHOLOGY: Megakaryocytosis with atypical features, lack of reticulin fibrosis > grade 1[a], accompanied by increased age-adjusted bone marrow cellularity, granulocytic proliferation, and often decreased erythropoiesis	1. BONE MARROW MORPHOLOGY: Megakaryocytosis with atypical features, accompanied by either reticulin and/or collagen fibrosis (grade 2 or 3)[a]
2. CLINICAL: Not meeting WHO criteria for *BCR-ABL1*+ CML, PV, ET, myelodysplastic syndromes or other myeloid neoplasms	2. CLINICAL: Not meeting WHO criteria for *BCR-ABL1*+ CML, PV, ET, myelodysplastic syndromes or other myeloid neoplasms
3. GENETIC: Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these 3 major clonal mutations, presence of another clonal marker or absence of minor reactive bone marrow reticulin fibrosis[b]	3. GENETIC: Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these 3 major clonal mutations, presence of another clonal marker or absence of reactive myelofibrosis[c]
MINOR CRITERIA	
• Anemia not attributed to a comorbid condition	• Anemia not attributed to a comorbid condition
• Leukocyte count ≥ 11 x 10^9/L	• Leukocyte count ≥ 11 x 10^9/L
• Palpable splenomegaly	• Palpable splenomegaly
• Serum LDH level above the upper limit of institutional reference range	• Serum LDH level above the upper limit of institutional reference range
	• Leuko-erythroblastosis

Table adapted from Passamonti and Maffioli 2016, and Abner et al. 2016.[9,10]

Key: WHO, World Health Organization; PMF, primary myelofibrosis; CML, chronic myeloid leukemia; PV, polycythemia vera; ET, essential thrombocythemia; LDH, lactate dehydrogenase

[a]See Table 3.

[b]Minor (grade 1) reticulin fibrosis secondary to infection, autoimmune disorder or other chronic inflammatory condition, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies.

[c]Bone marrow fibrosis secondary to infection, autoimmune disorder or other chronic inflammatory condition, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies.

myeloid cells. Often megathrombocytes and megakaryocyte fragments are seen. Anemia is common while the platelet and WBC count can be variable.[1,6]

In pre-PMF, the bone marrow is often hypercellular with an increase in the proliferation of megakaryocyte and granulocytic cells with a decrease in erythropoietic cells. There is usually no bone marrow fibrosis or minimal reticulin fibrosis at this stage but atypical megakaryocytes and micromegakaryocytes may be present. The bone marrow findings may resemble ET, yet in ET the megakaryocytes appear normal and mature.[1,19] Extramedullary hematopoiesis is minimal if present (Tables 2 and 3).

Table 3.
WHO 2008 Criteria for Grading Reticulin Fibers

Grade	Description
MF-0	Scattered linear reticulin with no intersections (crossovers) corresponding to normal bone marrow
MF-1	Loose network of reticulin with many intersections, especially in perivascular areas
MF-2	Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of collagen and/or focal osteosclerosis
MF-3	Diffuse and dense reticulin with extensive intersections and coarse bundles of collagen, often associated with osteosclerosis

Table adapted from Abner et al. 2016.[10]
Key: WHO, World Health Organization

Due to the hallmark of bone marrow fibrosis in overt PMF, successful bone marrow aspiration is often not achieved resulting in a dry tap requiring a trephine bone marrow biopsy. Bone marrow findings include significant collagen and/or reticulin fibrosis, patches of hematopoietic cellularity, and an increased number of abnormal mega-karyocytes often found in clusters (Tables 2 and 3, and Figures 1-3).

Approximately 30%-50% of PMF cases demonstrate cytogenetic (karyotypic) abnor-malities at diagnosis.[1,6] Cytogenetic analysis is important in the diagnosis and prognosis but it can be challenging due to bone marrow fibrosis. Some of the more common chromo-somal abnormalities detected in PMF include deletions of the long arms of chromosomes 13 and 20, abnormalities of chromosomes 1, 7, and 9, and trisomy 8 and 9. Of these abnormalities, those associated with a favorable prognosis include deletions 13q and 20q, and trisomy 9. A normal karyotype is also associated with a favorable prognosis. Those associated with an unfavorable prognosis include deletions of 7q, trisomy 8, and complex karyotypes.[1,6] These abnormalities are not specific for PMF as they are found in the other Ph negative MPNs and other myeloid malignancies.

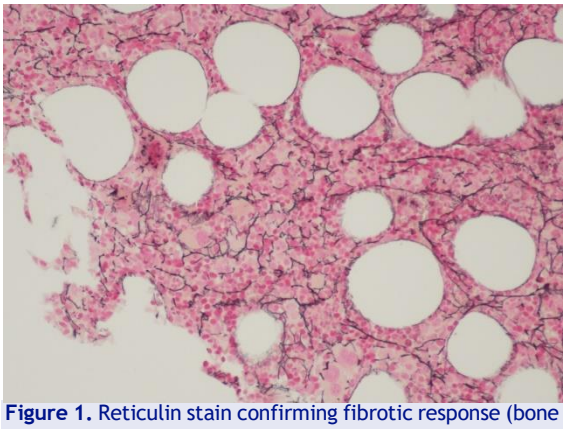

Figure 1. Reticulin stain confirming fibrotic response (bone marrow biopsy - magnification x100).

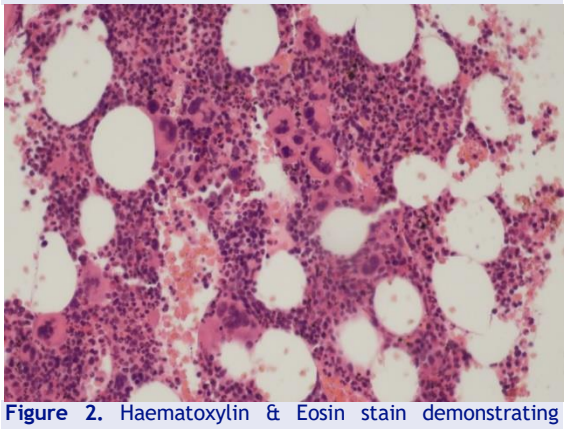

Figure 2. Haematoxylin & Eosin stain demonstrating clusters of abnormal megakaryocytes (bone marrow biopsy – magnification x200).

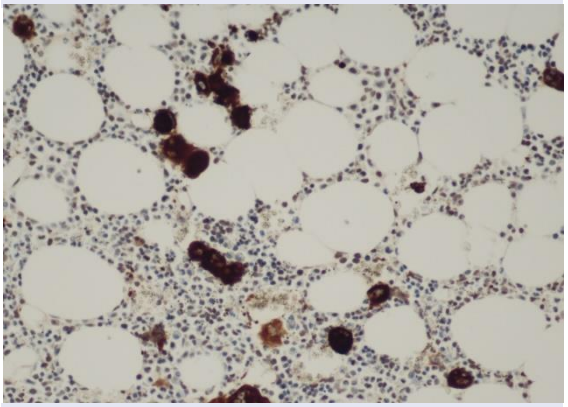

Figure 3. CD61 Immunohistochemical stain demonstrating clusters of megakaryocytes (bone marrow biopsy – magnification x200).

Since the discovery of the driver mutations in MPNs, genomic testing has become a critical component in diagnosis. Patients suspected of an MPN will have mutation analysis performed using next generation sequencing (NGS) methods. Myeloid gene sequencing panels can look for specific mutations in JAK2, CALR, and MPL genes.[13] Extended gene sequencing panels are used for identifying non-driver mutations assisting with prognostic information and therapeutic decisions. For example, PMF patients with mutations in serine and arginine rich splicing factor 2 (SRSF2), ASXL transcription regulator 1 (ASXL1), histone-lysine N-methyltransferase (EZH2), or isocitrate dehydrogenase 1/2 (IDH1/IDH2) have a shorter survival rate and increased risk of transformation to acute leukemia.[20] NGS allows for the identification of patients that are at high risk for disease progression and transformation, and factor into treatment decisions.[13,20]

Prognosis and Risk Stratification

Treatment decisions are often made based on overall survival and risk of transformation to acute leukemia. Thus, prognostic assessment of PMF (prognostic systems for pre-PMF have yet to be developed) has evolved over the years as scientific knowledge has advanced. Early prognostic scoring models were primarily based on clinical and hematology findings (Table 4).

One such system is the International Prognostic Scoring System (IPSS) developed in 2009 by the International Working Group for MPN Research and Treatment (IWG-MRT). The IPSS uses five risk factors (age > 65 years, hemoglobin < 10g/dl, WBC count > 25 x 10^9/L, percentage of circulating blasts ≥1%, and presence of constitutional symptoms), at diagnosis to predict survival.[4,5,14] The IPSS prognostic model places patients into one of four risk groups: low, intermediate-1, intermediate-2, and high based on the number of risk factors present.[5,14] The risk groups allow for better projections of median survival in years.

One year later the IWG-MRT developed the dynamic IPSS (DIPSS) that utilizes the same risk factors as the IPSS, however it is applicable throughout the course of the disease.[4] The DIPSS was further developed to include three additional risk factors (unfavorable karyotype, platelet count < 100 x 10^9/L, and the need for RBC transfusions) and is identified as the DIPSS-plus model.[5] Both the DIPSS and DIPSS-plus the

Table 4. Earlier Prognostic Models for PMF

	IPSS - estimates survival at time of diagnosis	**DIPSS** - can be applied anytime during clinical course	**DIPSS-plus** - can be applied anytime during clinical course
	RISK FACTORS		
	• Age >65 years (1 point)	• Age >65 years (1 point)	• Age >65 years (1 point)
	• Constitutional symptoms (1 point)	• Constitutional symptoms (1 point)	• Constitutional symptoms (1 point)
	• Hemoglobin <10 g/dL (1 point)	• Hemoglobin <10 g/dL (2 points)	• Hemoglobin <10 g/dL (2 points)
	• WBC Count >25 x 10^9/L (1 point)	• WBC Count >25 x 10^9/L (1 point)	• WBC Count >25 x 10^9/L (1 point)
	• Circulating (PB) blasts ≥ 1% (1 point)	• Circulating (PB) blasts ≥ 1% (1 point)	• Circulating (PB) blasts ≥ 1% (1 point)
			• RBC transfusion need (1 point)
			• Platelet count <100 x 10^9/L (1 point)
			• Unfavorable karyotype (1 point)
	RISK GROUPS		
Low	0 points (median survival 11.3 years)	0 points (median survival; not reached)	0 points (median survival 15.4 years)
Intermediate-1	1 point (7.9 years)	1-2 points (14.2 years)	1 point (6.5 years)
Intermediate-2	2 points (4.0 years)	3-4 points (4.0 years)	2-3 points (2.9 years)
High	≥3 points (2.3 years)	≥5 points (1.5 years)	≥4 points (1.3 years)

Key: IPSS, international prognostic scoring system; DIPSS, dynamic international prognostic scoring system; WBC, white blood cell count; PB, peripheral blood; RBC, red blood cell count; PMF, primary myelofibrosis.

model place patients into the same four risk groups as the IPSS.[5,14]

Newer prognostic scoring systems have further incorporated cytogenetic and molecular findings along with the hematological findings (Table 5). In 2018, the Mutation-Enhanced International Prognostic Score System (MIPSS-70) was developed to better select patients, less than 70 years old, as candidates for allogenic hematopoietic stem cell transplant (AHSCT).[9] MIPSS-70 included the classical hematology parameters but incorporated cytogenetic and molecular aberrations. The model places patients into one of three risk categories: low, intermediate or high. Building upon the MIPSS-70, the MIPSS-70 plus was developed and added a fourth risk category (very high) which allowed for better selection of patient candidates for AHSCT.[9] The MIPSS-70 plus version 2.0 incorporates more detailed anemia and cytogenetic information and added a fifth risk category (very low). Lastly, the Genetically-Inspired Scoring System (GIPSS) relies solely on cytogenetic and molecular findings and places patients into one of four risk categories.[5,14]

Any of the three Ph negative MPNs can transform into acute myeloid leukemia. However, the probability for leukemic transformation is the highest in PMF as estimates of incidence range from 11% to 30% with a poor prognosis.[3,21,22] This transformation is often termed blast-phase MPN or secondary acute myeloid leukemia. In PMF patients, the French-American-British (FAB) classification subtypes of M7 (acute megakaryocytic leukemia), M0 (acute myeloid leukemia, minimally differentiated), and M2 (acute myeloid leukemia with maturation) are common.[22] In general, risk factors useful in predicting transformation include: dependence on RBC transfusion, leukocytosis, thrombocytopenia, peripheral blood and bone marrow blasts, abnormal karyotypes, and triple negative mutational status.[22] Primary causes of death in PMF patients include leukemic transformation,

Table 5. Newer Prognostic Models for PMF

	MIPSS-70	MIPSS-70 plus version 2.0	GIPSS
RISK FACTORS			
	• Constitutional symptoms (1 point) • Hemoglobin <10 g/dL (1 point) • WBC Count >25 x 10^9/L (2 points) • Circulating (PB) blasts ≥ 2% (1 point) • Platelet count <100 x 10^9/L (2 points) • Bone marrow fibrosis ≥2 (1 point) • Presence of one HMR mutation (1 point) • Presence of ≥2 HMR mutations (2 points) • Absence of CALR type-1 mutation (1 point)	• Constitutional symptoms (2 points) • Severe anemia (2 points) • Moderate anemia (1 point) • Circulating (PB) blasts ≥ 2% (1 point) • VHR karyotype (4 points) • Unfavorable karyotype (3 points) • ≥2 HMR mutations (3 points) • One HMR mutation (2 points) • Absence of CALR type-1 mutation (1 point)	• VHR karyotype (2 points) • Unfavorable karyotype (1 point) • Absence of CALR type-1 mutation (1 point) • ASXL1 mutation (1 point) • SRSF2 mutation (1 point) • U2AF1Q157 mutation (1 point)
RISK GROUPS			
Very Low		0 points (not reached)	
Low	0-1 point (median survival 27.7 years)	1-2 points (16.4 years)	0 points (26.4 years)
Intermediate-1	2-4 points (7.1 years)	3-4 points (7.7 years)	1 point (8 years)
Intermediate-2			2 points (4.2 years)
High	≥5 points (2.3 years)	5-8 points (4.1 years)	≥3 points (2 years)
Very High		≥9 points (1.8 years)	

Key: MIPSS-70, Mutation-Enhanced International Prognostic Score System for transplant age patients (≤70 years old); GIPSS, Genetically-Inspired Scoring System; WBC, white blood cell count; PB, peripheral blood; HMR, high molecular risk; VHR, very high risk; PMF, primary myelofibrosis.

bleeding, hepatic failure (due to extra-medullary hematopoiesis), pulmonary embolism, and complications from AHSCT.[6]

Treatment

In PMF, the primary goal of treatment is to alleviate symptoms, reduce the degree of splenomegaly, reduce risk of complications, and ultimately increase overall survival and quality of life.[23] Specific treatment options are based on clinical findings and prognosis (prognostic scoring system risk group). As the goal is to relieve symptoms and improve quality of life, asymptomatic patients (very low, low, and possibly intermediate-1 risk groups) may be observed initially with treatment following as symptoms develop.[19] Symptomatic PMF patients will receive treatment for anemia and splenomegaly. Currently, pre-PMF patients are often treated similarly to those with ET as specific treatment guidelines have yet to be developed.[15]

Anemia is managed with transfusion therapy and conventional drug therapy. Drug options include erythropoiesis stimulating drugs, corticosteroids (e.g., prednisone), and androgens (e.g., testosterone enanthate).[4,5] Newer drugs, such as Luspatercept, currently used for beta-thalassemia and myelodysplastic syndrome are being investigated as a therapeutic option for PMF patients.[5] Luspatercept binds to transforming growth factor beta (TGF-β) superfamily thereby reducing the Smad-2/3 (transforming growth factor-beta superfamily) signaling pathway in hematopoiesis and ultimately enhancing late-stage erythropoiesis.[24] Luspatercept has shown modest response rates in PMF patients.[24] Two additional drugs, Sotatercept and Galunisertib, are also being investigated and have potential to become treatment options for PMF patients.[24]

Historically the treatment of splenomegaly, and its negative outcomes, was chemotherapy. One of the longstanding chemotherapeutics used to relieve the symptoms and reduce spleen size is hydroxyurea (HU).[6] HU is a chemotherapeutic agent used to reduce the number of cells by inhibiting DNA synthesis.[1] With the discovery of JAK2 mutations in PMF, newer therapeutic drugs focused on inhibiting activity of janus kinase enzymes. Currently, the only JAK2 inhibitor to be approved in Canada, Europe, and the United States (US) is Ruxolitinib. Ruxolitinib was approved by the US Food and Drug Administration (FDA) in 2011 and works by inhibiting the JAK pathway resulting in the initiation of apoptosis and reduced cellular proliferation.[23] Both HU and Ruxolitinib improve the symptoms of splenomegaly with varying degrees of effectiveness.

Symptomatic low risk patients are often treated with HU or Ruxolitinib. Whereas, intermediate-2 and high-risk patients are traditionally treated with Ruxolitinib if AHSCT is not an option (discussed in more detail below). A significant number of PMF patients, in the intermediate-2 or high-risk categories, may experience Ruxolitinib failure due to intolerance or resistance to the drug.[25,26] Regardless of the cause, these patients discontinue using Ruxolitinib and had limited treatment options until 2019. In 2019, a second-line JAK2 inhibitor Fedratinib, was approved by the US FDA.[25,27] Fedratinib is an option for initial therapy in intermediate-2 or high-risk patients, or an alternative for those who experience Ruxolitinib failure.[27] Fedratinib is a more selective inhibitor of JAK2 than Ruxolitinib (a dual inhibitor JAK1/JAK2 inhibitor).[25]

Other options for splenomegaly include splenectomy or splenic irradiation.[23] Splenectomy aids in controlling persistent anemia and thrombocytopenia while reducing constitutional symptoms and pain. Splenic irradiation also reduces spleen size and provides symptom relief, however response to this treatment is usually short-lived.[23]

For PMF patients that transition to secondary acute myeloid leukemia, treatment options are limited. Transformation to acute leukemia is associated with a poor response to therapy and shortened survival. Cytotoxic chemotherapy regimens are often used yet have limited efficacy.[28] Often supportive care, including RBC transfusions and HU, is used in conjunction with chemotherapy. Survival rates vary with 1-

3 months if supportive care is given and extended (6-9 months) if combined with chemotherapy.[28] If AHSCT is an option, survival improves with rates of 2-3 years.[28]

The only treatment that is potentially a cure for PMF patients is an AHSCT.[1,23] Deciding to pursue AHSCT depends on numerous factors: age, medical comorbidities, clinical and genetic risk factors (high molecular risk mutations such as ASXL1 and SRSF2), and donor availability.[23] When successful, transplantation results in normalization of bone marrow and reduction in splenomegaly. This treatment option has a high rate of mortality and morbidity due to disease relapse and graft-versus-host disease. (Long-term survival occurring in roughly one third of patients.)[4] Therefore AHSCT is only recommended for those in high risk groups based on prognostic models such as the DIPPS and DIPSS-plus. [1,19]

NGS can significantly aid in therapeutic decision-making for PMF patients. Long-term use of HU can result in additional mutations and detection of SRSF2 at diagnosis. This is associated with a higher risk of developing additional mutations.[20] The efficacy of Ruxolitinib is another example, as mutations in ASXL1 often have a shorter time to treatment failure.[20] NGS is useful in determining candidates for AHSCT as those with adverse mutations (high-molecular risk) are ideal candidates for transplantation. However, those with multiple mutations have a higher incidence of post-AHSCT relapse.[20]

Several other JAK inhibitors are currently in clinical trials and include Pacritinib, Momelotinib, and Itacitinib. Pacritinib is a JAK2 inhibitor that shows promising results in reducing spleen size and reduction in symptoms, yet it was placed on clinical hold in 2016 due to concerns of hemorrhagic risk.[29] Momelotinib is a JAK1/JAK2 inhibitor that decreases transfusion dependency.[29] Itacitinib is a JAK1 inhibitor that reduces symptoms but is less effective at reducing spleen size than Ruxolitinib.[29] Most patients with PMF will be treated with a JAK inhibitor. As more inhibitors are approved, combinations of inhibitors might prove to be beneficial as a treatment option.[21,29]

Conclusion

Recent advances in molecular and genomic studies have contributed to the understanding of the pathogenesis and pathophysiology of PMF. Due to these advances, the diagnosis of, and prognostic models and therapeutic options for PMF have evolved to incorporate molecular and genetic findings. Despite the progress, the pathophysiology is complex, and diagnosis can be challenging especially in differentiating pre-fibrotic PMF from ET. In addition, multiple scoring systems have resulted in a variety of risk stratification groups to consider, and an AHSCT is the only curative option for a limited number of patients. As researchers continue to discover additional mutations associated with PMF and novel therapeutic agents are developed, the next decade will hopefully lead to better outcomes for PMF patients.

Acknowledgement

Images used in figures were provided by Nichola H Lawrence BSc MSc CSci FIBMS, taken at University Hospitals of North Midlands NHS Trust.

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Literature Review

Polycythemia Vera

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Polycythemia vera is a rare, acquired clonal disorder of the hematopoietic stem cells resulting in unregulated proliferation of erythropoiesis leading to an accumulation of red cells. The main complications of the disorder arise from hyperviscosity and an increased risk of thrombosis. In recent years, advances in next generation sequencing (NGS) techniques have identified promising targets to enable more accurate risk stratification of patients as well as targets for new classes of therapeutic drugs. This article aims to review the current research in this field to provide an overview of the key features of this disease.

Key words: Polycythemia vera, myeloproliferative neoplasms, erythrocytosis

Introduction

Polycythemia vera (PV) is an acquired clonal disorder of the hematopoietic stem cells.[1] It is the most common of the Philadelphia negative myeloproliferative neoplasms (MPNs), arising due to a loss of the regulatory mechanisms for erythropoiesis, resulting in the overproduction of red blood cells (RBCs).[2,3] First described in 1892, the discovery of janus kinase 2 (JAK2V617F) in 2005 has revolutionized the diagnosis of this condition. This, paired with technological advances in NGS and the therapeutic use of monoclonal antibody-based drugs, has stimulated an explosion of active research in recent years.

Epidemiology

PV has an incidence of 0.4-2.8 per 100,000 persons/year, occurring in 1/3300 people worldwide.[1] It can occur at any age but is rare in those under 60 years of age.[1,2] Under the age of 60 it is more commonly diagnosed in women, but this equalizes in the over 60 age bracket.[3] However, there is some controversy regarding the gender ratio, with some reporting a lower incidence in women overall.[4] This discrepancy in the reported gender ratio may be due to rapid changes in diagnostic criteria.[2] For most patients, PV will present as an indolent disease and if treated people may live with the disease for more than 40 years. However, PV is thought to be underdiagnosed, as it is frequently mistaken for essential thrombocythemia (ET) and for this class of patients, the lack of appropriate treatment may lead to a worse prognosis.[3,5]

Pathogenesis

The pathogenesis of thrombosis as seen in PV is multifactorial and complex, arising from the interplay between different cell lineages. In red blood cells (RBCs), the constitutive activation of the JAK-STAT (janus tyrosine kinase signal transducer and activator of transcription) pathway due to the JAK2V617 mutation (found in 95% of PV patients) leads to phosphorylation of Lu/BCAM (Lutheran blood group and basal cell adhesion molecule), resulting in abnormal adhesion to the subendothelial protein laminin.[6-8] Meanwhile, the increased neutrophil count results in increased proteolytic enzyme

Accepted: August 24, 2021

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(elastase and cathepsin G) activity as well as an increase in reactive oxygen species. This is accompanied by an increase in the expression of CD11b (pan-macrophage marker), leading to increased platelet activation and endothelial damage, with the subsequent increased release of tissue factor.[8] At the same time, there is often an increased platelet count with increased platelet activation leading to an increase in platelet induced thrombin production. This is worse in immature platelets. The increase in immature platelets correlates with the JAK2V617F allele burden.[3] The activated platelets also show increased expression of P selectin (cell adhesion protein) on their surface.[8] When paired with the reduced blood flow and endothelial damage as a result of hyperviscosity, these events combine to produce a perfect storm of increased procoagulant and proteolytic properties, with increased secretion of inflammatory cytokines and increased expression of adhesion molecules.[8] In contrast to the procoagulant effects of PV, extreme thrombocytosis (platelet count >1500 x10 ^{9}/L) may be associated with an increased risk of hemorrhage due to an acquired von Willebrand syndrome (VWS).[5] The exact mechanisms underlying fibrotic and leukemic transformation of PV are poorly understood, but evidence suggests that aging is associated with the accumulation of potentially harmful somatic mutations.[6]

Clinical and Diagnostic Findings

The classical presentation of PV (Table 1) is erythrocytosis in isolation or combination with leukocytosis and/or thrombocytosis.[3,5,8,9] PV occurs in both indolent and aggressive forms, with the more indolent forms often being identified only as an incidental finding.[5] In contrast, more aggressive forms may lead to symptoms which significantly reduce quality of life.[5] Females tend to have fewer deregulated genes and thus may present with a less severe clinical picture than males.[4]

An increased hematocrit may be the first indication of PV, with the hematocrit thought to be more important in the diagnosis than hemoglobin, because hyperviscosity results from increased RBC numbers rather than individual RBC content.[3,9] Therefore, patients with a persistently increased hematocrit (above 0.52 in males and 0.48 in females) will warrant further investigation.[9] Early detection of PV presents challenges as the increase in hematocrit will often be matched by an increase in plasma volume, thus masking the overall increase in red cell mass. In cases where the hematocrit is less than 0.590, it will be difficult to differentiate between PV and other causes of erythrocytosis (Table 2).[3] This plasma expansion means that, in contrast to the World Health Organization's 2016 guidelines (Table 3) PV cannot be excluded based on a normal hematocrit alone.[5-9] The situation is further complicated by conditions such as pregnancy or

Table 1. Complications and symptoms of Polycythaemia vera

Complications	Symptoms
• Splenomegaly due to extramedullary hematopoiesis	• Gastric discomfort • Early satiety
• Increased secretion of proinflammatory cytokines	• Pruritus
• Hyperviscosity	• Occipital migraines • Dizziness • Erythromelalgia • Amaurosis fugax • Transient Ischaemic Attacks (TIA)
• Arterial and venous thrombotic events	• Pain, swelling, warmth, redness, and cramps particularly in the legs (DVT) • Increased incidence of miscarriage • Chest pain, shortness of breath (pulmonary embolism, myocardial infarction) • Increased incidence of strokes/TIA
• Hemorrhage	• Epistaxis
• Hyperuricemia	• Severe pain and stiffness of the joints • Red, swollen and or misshapen joints

Key: DVT- deep vein thrombosis.

Table 2. Exclusion of causes of secondary Erythrocytosis

Cause	Diagnostic Test
Drugs, smoking, alcohol	• Comprehensive patient history with systematic questioning
Renal tumours and hepatic disease	• Urea and Electrolytes, • Liver Function Tests including serum calcium • Erythropoietin levels (raised) • Ultrasound
Tissue hypoxia	• Arterial oxygen saturation (May be misleading in cases of carbon monoxide poisoning, high affinity hemoglobins and sleep apnoea) • Erythropoietin levels (raised)
Dehydration	• Red cell mass
Congenital causes such as mutations of the erythropoietin receptor genes	• Family history • Gene sequencing
High affinity hemoglobins	• HPLC • Mass spectrometry • Gene sequencing

Key: HPLC- High pressure liquid chromatography.

Table 3. Diagnostic criteria for Polycythemia vera

• RBC mass 25% above the mean normal predicted value
• Hemoglobin > 165 g/L in men and >160 g/L in women
• Hematocrit >0.490 in men and >0.480 in women
• Trilineage hypercellularity in the bone marrow
• JAK2v617F or JAK2 exon 12 mutation present
• Subnormal serum erythropoietin

Key: RBC- Red blood cell.

splenomegaly, which will also cause plasma expansion. The hematocrit may also be normal in cases of iron deficient PV, as the body defends the mean cell hemoglobin concentration (MCHC) by reducing the mean cell volume (MCV). Therefore, an isolated increased red blood cell count may be the only suspicious finding .[3] This presents a cautionary tale against the practice of offering a trial course of iron supplementation for unexplained microcytosis. It should be noted that ferritin levels are often low in iron deficient polycythemia as the diminished iron stores limit erythropoiesis.[9] However, the additional presence of leukocytosis with or without splenomegaly may help to secure the diagnosis.[3] Ultrasound may be useful to detect splenomegaly in the absence of a palpable spleen and can also be useful for the detection of renal and/or hepatic pathologies. The diagnosis is further complicated by the fact that MPNs are not mutually exclusive. The systematic exclusion of secondary causes of erythrocytosis (Table 2) is of paramount importance.[3]

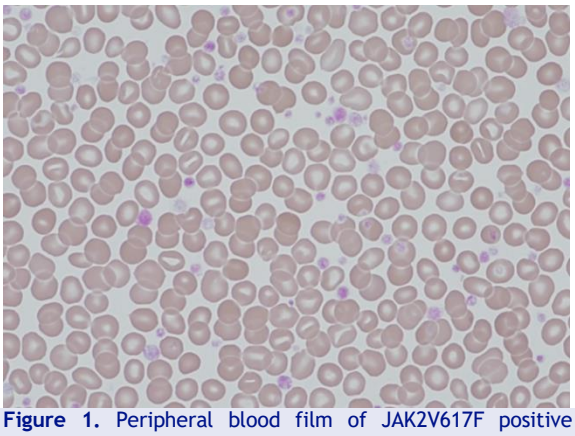

Figure 1. Peripheral blood film of JAK2V617F positive polycythemia vera at x600 magnification stained with May Grünwald Giemsa. Note the thrombocytosis and platelet anisocytosis that may result in a misdiagnosis of essential thrombocythemia.

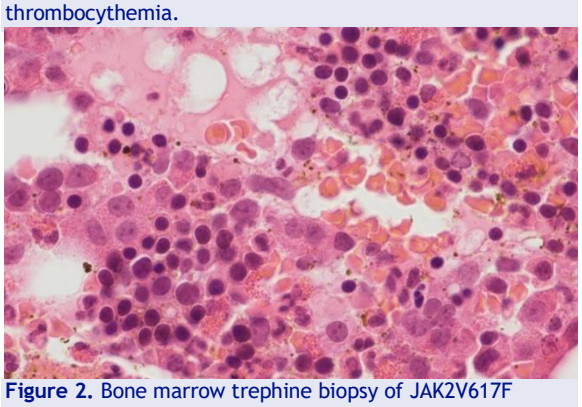

Figure 2. Bone marrow trephine biopsy of JAK2V617F positive polycythemia vera at x600 magnification stained with hematoxylin and eosin. Note the expanded erythroid islands

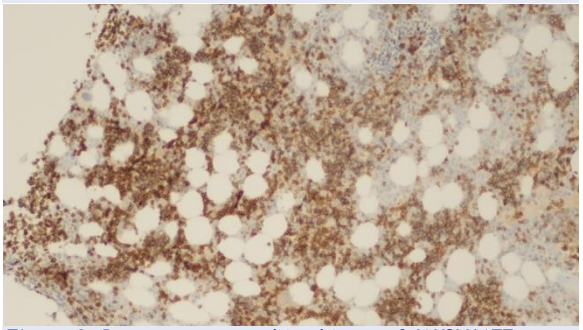

Figure 3. Bone marrow trephine biopsy of JAK2V617F positive polycythemia vera using Glycophorin-C immunohistochemical staining at x100 magnification. Note erythroid hyperplasia and expanded erythroid islands

The complete blood count (CBC) can confirm a persistently raised hematocrit along with the presence of leukocytosis and/or thrombocytosis (Table 3).[5-9] The subsequent peripheral blood film (Figure 1) may then identify circulating blasts or leuko-erythroblastic features, which may be indicative of bone marrow impairment, triggering a bone marrow biopsy.[9] The bone marrow biopsy can be useful for distinguishing between PV and ET (Table 4), demonstrating significantly increased erythroid precursors relative to the other myeloid precursors in the case of PV (Figures 2 and 3).[7-9] The other myeloid precursors may be moderately increased and left shifted and there may be anisocytosis of the megakaryocytes with larger forms seen more frequently, which may display uneven or reduced lobulation. However, several studies have reported a failure to reach a diagnostic consensus based on morphological findings alone.

Table 4. Differential diagnosis of Polycythemia vera relative to Essential Thrombocythemia

• Lower platelet count
• Lower MCV
• Lower ferritin
• Lower erythropoietin levels
• Increased splenomegaly
• Increased pruritus

Key: MCV- Mean cell volume.

A direct measurement of RBC mass and plasma volume affords a more concrete diagnosis and alongside erythropoietin levels and arterial blood gas analysis, this was the most used diagnostic test for PV in 2002.[1,3] However, in the post JAK2V617F era, this is rarely performed due to the human and financial resources required, which are often prohibitive.[1,9] The detection of JAK2V617F remains the cornerstone of diagnosis for PV and will usually be used in conjunction with hemoglobin as a surrogate for RBC mass.[9] It is important to note that the mutation will frequently also be seen in the other MPNs (Table 4) and although the neutrophil JAK2 allele burden tends to be higher in PV than in ET, a valid threshold has not been established.[3,7-9] It should also be noted that this mutation has been identified at low levels in normal patients, with the allele burden increasing with age.[3,9]

Prognosis and Risk Stratification

The median age of PV patients at diagnosis is 61 with a median survival of 18.9 years, ranging from 10.9 to 27.8 years depending on the risk group.[1] Potentially fatal complications for PV include fibrotic (15% of patients) and leukemic transformations (1.5% of patients), both of which are associated with a significant exacerbation of symptoms and reduced life expectancy, with a median survival of 1.5-2.5 months untreated.[3,8,10] Therefore, transformation carries a poor prognosis and treatment is challenging.[10] However, it should be noted that post PV myelofibrosis has a more favorable prognosis than de-novo primary myelofibrosis.[3] Post PV acute myeloid leukemia typically has a French American British Classification (FAB) M6 or M7 phenotype and is relatively resistant to chemotherapy, with an allogeneic stem cell transplant being the only curative option, as most drugs are ineffective at securing a long-term remission.[11] Transformations, if they occur, will usually occur within 12 years of the initial diagnosis. Transformation from ET to PV also occurs in 20-30% of JAK2V617F positive cases and is more commonly seen in women.[3] The most common cause of morbidity and mortality in PV patients is thrombotic events. Traditional risk factors for thrombosis such as smoking, diabetes mellitus

Table 5. International working group for MPN research and treatment (IWGMRT) prognostic scoring system

Risk Factor	Weighed Hazard Ratio Points	Points	Risk Stratification	Median Survival (years)
Age		0	Low	26
• ≥ 67 years	5			
• 57-66 years	2			
Leukocytosis		1-2	Intermediate	15
• WBC > 15 x10^9/L	1			
Previous thrombotic event	1	≥ 3	High	8.3

Key: WBC-White blood cell count.

and hypertension, which are not currently included in available prognostic systems, should also be considered in any prognostic evaluation of patients.[11,13] Based on this consideration, some low risk patients may be regarded as high risk due to pre-existing cardiovascular disease or cardiovascular risk factors.[8,9]

Existing tools for the risk stratification of patients with PV include the International Working Group for Myeloproliferative Neoplasms Research and Treatment (IWGMRT) (Table 5), the Mutation Enhanced Prognostic Scoring System (MEPSS) (Table 6), and Dynamic Prognostic Model. [7-12] The IWGMRT prognostic score has not yet been validated by prospective studies. However, the Dynamic Prognostic Model can be useful in monitoring the impact of treatment and adjusting care plans accordingly. Risk factors used by this model are associated with a 4.2-fold increase in the risk of death and are defined as a hemoglobin less than 100 g/L, a platelet count less than 100 x10^9/L and a white blood cell count (WBC) greater than 30 x10^9/L.[9] The low frequency of PV combined with an even lower frequency of abnormal karyotypes has provided little data on which to evaluate the prognostic potential of cytogenetic abnormalities, but it may be useful to obtain cytogenetic information at diagnosis and following changes in the clinical course of the disease.[8,12] More recent candidates for prognostic evaluation include both driver mutations (directly responsible for dysregulated cellular proliferation) and non-driver mutations (which exacerbate the complications of the disease but do not directly affect proliferation). Active research continues in an attempt to establish the role of the mutations in the pathophysiology of PV and thus their prognostic value (Tables 7, 8a and 8b). [7-9,14,15]

Table 6. Mutation enhanced prognostic scoring system (MEPSS)

Risk factor	Fibrotic transformation	Leukemic transformation
≥60 years of age	✓	✓
WBC 15-30 x10^9/L	✓	✓
Homozygous JAK2 mutation	✓	
Exposure to alkylating agents	✓	✓
Previous radiotherapy		✓
Non-driver mutations of myeloid genes including ASXL1, SRSF2, RUNX1, SF3B1, IDH1/2	✓	✓
TP53 mutations		✓
Reticulin fibrosis and raised LDH	✓	
Splenomegaly	✓	✓
Abnormal Karyotype (+1q, +8, +9, 20q- most associated with transformation)		✓

Key: LDH – Lactate dehydrogenase, WBC-White blood cell count.

In addition to the association with increased risks of fibrotic and leukemic transformation of PV, DNA-methyl transferase 3 alpha (DNMT3A), tet-methylcytosine dioxygenase 2 (TET2) and ASXL transcription regulator 1 (ASXL1) mutations, collectively referred to as DTA mutations, have also been associated with increased thrombotic risks.[13-15] DNMT3A, TET2 and ASXLI are epigenetic regulators. Mutation of these genes leads to altered DNA methylation, resulting in increased transcription of proinflammatory genes. This results in increased secretion of inflammatory cytokines, such as interleukin 1ß. The increase in inflammatory cytokines predisposes patients to inflammatory conditions such as atherosclerosis, resulting in an increased risk of thrombotic and thrombo-occlusive events. [13]

NGS for somatic mutations is not yet standard practice, but it may become so as technological advances make this option more accessible, as inclusion of this information has been shown to improve the accuracy of prognostic assessment.[9] Improved knowledge and application of DNA analysis may improve risk stratification of patients for both transformation and thrombotic events.[7] Therefore, if the predictive value of these mutations can be verified, it may enable the identification of patients requiring closer monitoring or a more proactive treatment plan, including anti-inflammatory agents for example.[13,14]

However, JAK2V617F neutrophil allele burden remains the main stay for staging of the disease.[3] This has the advantage of being able

Table 7: The prognostic impact of somatic driver mutations

Driver mutations	Function	Prognostic impact of mutation
JAK2V617F	• Constitutively active tyrosine kinase	• Homozygosity is linked to reduced platelet count, increased splenomegaly, increased need for cytoreductive therapy and increased pruritus. • An allele burden over 50% is associated with increased risk of fibrotic transformation but not leukemic transformation. • An allele burden of less than 50% is indicative of the indolent form of the disease,
JAK2V625F	• Tyrosine kinase with enhanced function	• Under investigation
JAK2 F556V	• Tyrosine kinase with enhanced function	• Under investigation
CALR	• Multifunctional Ca^{2+} binding endoplasmic reticulum chaperone protein. • Binds and activates MPL in a TPO dependent manner.	• More commonly seen in males and in younger patients. • Associated with a reduced risk of thrombosis relative to JAK2V167F positive patients
CXCL4/PF4	• Inhibition of cell death due to downregulation of TGFB signalling	• Under investigation

Key: JAK- Janus Kinase, MPL- Myeloproliferative leukemia, also known as thrombopoietin receptor gene, TPO-Thrombopoietin, TGFB – Transforming Growth Factor Beta, CXCL4/PF4 - Platelet factor 4.

Table 8a: The prognostic impact of somatic non-driver mutations

Non-Driver mutations	Function	Prognostic impact of mutations
TP53	• Encodes p53 tumour suppressor protein essential for DNA repair. • Inactivation leads to increased hematopoietic stem cell self-renewal and resistance to cellular stress	• Associated with cytopenia following hydroxyurea therapy. • Increased risk of leukemic transformation • Poor prognosis for overall survival
RUNX1	• Encodes a transcription factor involved in hematopoiesis. • Inactivation leads to reduced myeloid differentiation and increased hematopoietic stem cell self-renewal	• Genetic instability • Increased risk of leukemic transformation. • Reduced overall survival
SRSF2, ZRSF2, U2AF1, SF3B1	• Splicing factors which play an important role in DNA stability	• Increased risk of acquiring further mutations. • Associated with cytopenia following hydroxyurea therapy • Increased risk of fibrotic and leukemic transformation. • Reduced overall survival rate
TET2	• Encodes enzyme that catalyses conversion of 5-methyl cytosine to 5 hydroxy methyl cytosine resulting in DNA methylation • Inhibition of TET2 leads to decreased DNA methylation and impaired hematopoietic differentiation • Epigenetic repression of tumor suppressor genes • Increased expression of hematopoietic stem cell self-renewal genes	• Increased risk of fibrotic and leukemic transformation. • Associated with increased risk of vascular events. • Reduced overall survival rate

Table 8b. The prognostic impact of somatic non-driver mutations (continued)

Non-Driver mutations	Function	Prognostic impact of mutations
IDH1/2	• Encode enzymes that catalyse conversion of isocitrate to ketoglutarate which acts on TET2. • Important for protection from oxidative stress	• Associated with cytopenia following hydroxyurea therapy. • Increased risk of fibrotic and leukemic transformation • Reduced overall survival rate
ASXL1	• Encodes a nuclear polycomb protein that affects regulation of transcription and RAR mediated signalling. • Interacts with chromatin modifying proteins including PCRC2	• Associated with increased WBC and platelet counts. • Increased risk of fibrotic and leukemic transformation • Reduced overall survival rate. • More likely to be transfusion dependent.
LNK/SH2B2	• Inhibits signalling through tyrosine kinase receptors such as the erythropoietin receptor. LNK mutations disrupt negative feedback loops affecting proliferation.	• Associated with increased extramedullary hematopoiesis and enhanced growth of JAK2V167F positive cells in clonal assays and mouse models.

Key: WBC – White blood cell count

to be performed on peripheral blood samples. In terms of monitoring, there are no recommendations to monitor the allele burden sequentially, as the clinical impact of directly lowering the allele burden has yet to be confirmed.[3,9,14] Similarly, there are no indications for serial bone marrow biopsies to monitor morphology or fibrosis, but a repeat bone marrow biopsy may prove useful if transformation is suspected.[9]

Treatment

A typical treatment pathway for PV usually begins with phlebotomy and low dose aspirin, prior to risk stratification. High risk patients will then go on to receive cytoreductive therapies such as hydroxyurea (HU) or interferon as a first line therapy.[16] Ruxolitinib may then be used as second line therapy, with subsequent leukemic transformations treated with chemotherapy and/or allogeneic stem cell transplants for suitable candidates.[16]

Treatment of PV focuses on minimising the risk of thrombosis, reducing myeloproliferation, alleviating the symptoms of the disease and managing the complications (Table 1).[7,16] Treatment may yield a symptomatic, hematological, or molecular response which may be complete or partial. In contrast if there is no response, the disease will be progressive. The impact of a complete hematological response on long term survival, including thrombotic risk and disease progression, has yet to be fully established.[7]

A monthly schedule of phlebotomy is the cornerstone of treatment in low risk cases.[5,17] It reduces the red blood cell mass and expands the plasma volume.[5] The link between reducing the hematocrit and reducing the thrombotic risk was established by the CYTO-PV trial.[16] However, the same study failed to establish a link between reducing leukocytosis and thrombotic risk.[16] Phlebotomy is generally combined with low dose aspirin, although recent studies have cast doubts on the benefits of the prophylactic use of aspirin in asymptomatic patients without additional risk factors for cardiovascular disease.[3,10]

A consensus target of a hematocrit of 0.45 independent of gender has been established, based on the association with increased cerebral blood flow and reduced risk of vascular occlusive episodes. However, there have been no trials which confirm this effect with phlebotomy in isolation, with most study cohorts including a significant proportion of patients who were also being treated with HU.[17] There are also recommendations for a target below 0.42 for patients with persistent or recurrent symptoms.[1,2] In addition, despite a lack of evidence to support gender specific targets, such targets are applied by almost half of clinicians. In such cases a lower threshold of 0.42 is generally applied for women.[9] There are conflicting opinions on the application of lower thresholds during pregnancy, with some

advocating a target below 0.33.[5,8,17] This may be more appropriate in high risk pregnancies.[17] Phlebotomy may alleviate symptoms associated with hyperviscosity but is not effective at treating severe headaches or pruritis.[5]

Apheresis is an additional option for reducing the haematocrit and has the advantage of being able to achieve the target hematocrit in one session.[17] However possible side effects include dizziness, twitching, muscle cramps, fainting, arrhythmias and fever. It is also very expensive when compared to phlebotomy. It may, however, be an appropriate option where a rapid reduction in the hematocrit is necessary, e.g. to reduce the risk of thrombosis prior to emergency surgery.[17]

Cytoreductive therapies such as HU may be indicated for higher risk patients. A platelet count greater than 1000 $x10^9$/L may be used as a trigger for the implementation of this therapy, but many clinicians will only treat symptomatic thrombocytosis, as concerns that post phlebotomy thrombocytosis may increase thrombotic risk.[1,17] Reducing thrombocytosis may relieve migraines and reduce transient ischemic attacks. As previously discussed, extreme thrombosis may be associated with von Willebrand like syndrome, which occurs due to excess platelets exhausting the supply of von Willebrand factor.[5] This does not tend to cause spontaneous bleeding and, tranexamic acid can be used to prevent bleeding during minor procedures. In contrast, major surgery will require platelet counts to be normalized and a normal ristocetin cofactor activity should be confirmed before proceeding.[5] Cytoreductive therapy (Tables 9a and 9b) is also recommended for patients who are resistant or intolerant to phlebotomy.[7] While a definition for resistance

Table 9a. Conventional medical intervention for Polycythemia vera

Drug	Mode of action	Advantages	Limitations
Hydroxyurea	• Cytoreductive agent • Ribonucleotide reductase inhibitor that reduces intracellular deoxynucleotide triphosphate pools, thus inhibiting DNA synthesis resulting in cytotoxicity.	• Reduces thrombotic risk relative to phlebotomy alone	• Optimal dose must be determined through individual titration. • Associated with secondary cancers including squamous cell and basal cell carcinoma and breast cancer due to inhibition of TP53. • Myelosuppressive • Side effects include ulceration, skin lesions and gastrointestinal toxicities. • Not safe in pregnancy due to teratogenic properties • May not be well tolerated in the long term. • Does not cause molecular remission
Anagrelide	• Suppression of transcription factors required for proliferation of megakaryocytes.	• Effective platelet reduction • Non-Leukemogenic	• The most common side effects include: Bloating or swelling of the face, arms, hands, lower legs, or feet; body aches or pain; burning, itching, numbness; chest pain; congestion, cough, difficult or labored breathing, dryness, or soreness of the throat; heart palpitations; fever; rapid weight gain; lymphadenopathy

Table 9b: Conventional medical intervention for Polycythemia vera (continued)

Drug	Mode of action	Advantages	Limitations
Pegylated Interferon α 2a	• Cytoreductive agent • Immune regulation via binding to interferon α receptors 1 and 2	• Effective at reducing the neutrophil JAK2V167F allele burden. • Complete molecular remission can be achieved. • Effective cytoreduction. • Reduces symptoms of pruritus. • Reduced splenomegaly, but not back to normal size • Non Leukemogenic • Less toxic than Ruxolitinib • Safe in pregnancy	• Not always effective at reducing erythrocytosis as it can activate erythroid gene expression. • Immunosuppressive • Thyroid and liver toxicity • Side effects include flu like symptoms, atrial fibrillation/arrhythmias, neuropathy and depression. • Hypothyroidism • Autoimmune and endocrine disorders may occur in a minority of patients • Stimulates hematopoiesis so phlebotomy may remain necessary.
Ruxolitinib	• JAK1/2 inhibitor	• Effective and durable reduction of symptoms • Reduced splenomegaly relative to hydroxyurea • Phlebotomy is often no longer necessary • Molecular remission rates similar to interferon. • Normalisation of severe iron deficiency. • Reduces risk of thrombosis • Effective cytoreduction. • May increase the effectiveness and tolerability of pegylated interferon α -2b (phase II trials) • Non-myelotoxic • Non-Leukemogenic	• Immunosuppressive • Thrombocytopenia • Anemia • Increased rates of non-melanoma skin cancers

to phlebotomy has not been standardized, some have recommended criteria of more than three venesections a year, as this has been linked to an increased risk of thrombosis, and/or severe symptomatic iron deficiency.[5] Criteria for intolerance includes fainting episodes or a blood phobia. Iron supplementation may be cautiously recommended for patients suffering from severe symptoms of iron deficiency due to phlebotomy, with the resulting increase in hematocrit being treated with cytoreductive therapies.[17]

HU is the most common first line treatment for high risk patients.[16] However, some clinicians avoid the use of HU in those under 40 years of age, particularly women, in favor of interferon (Tables 9a and 9b).[1] Where HU is used in women of childbearing age, it is recommended that patients receive appropriate contraception and that treatment with HU is stopped three months before any intended conception.[9] The use of HU for the treatment of PV is not strictly evidence based, as much of the evidence has been extrapolated from the treatment of ET. However there have been small studies that have demonstrated a benefit in terms of a reduction in thrombotic risk.[7]

Resistance or intolerance to HU will develop in a quarter of patients.[16,17] Resistance is associated with a poor prognosis and may manifest as continued pruritus, progressive or symptomatic splenomegaly or a requirement for an increased frequency of phlebotomy and/or uncontrolled myeloproliferation.[8,9,18] Intolerance to HU usually manifests as mucosal or cutaneous lesions or genital and leg ulcers. For older patients, actinic keratosis or non-melanoma skin cancers may occur. It is recommended that patients on this treatment avoid sun exposure.[9,16] Links between HU and secondary leukemia remain unproven and are

unsupported by long term follow up studies.[7,9] In contrast the use of alkylating agents and radiotherapy are associated with increased rates of leukemia.[7]

Ruxolitinib has been approved as a second line drug for patients who become resistant or intolerant to HU or interferon therapy.[19,20] There is an estimated response duration of three years.[18,20] However, the immunesuppressive properties have been shown to cause an increased rate of infection, with a 4.6% 10-year mortality risk rate in PV patients.[18] Ruxolitinib affects both the innate and adaptive immune system, impacting natural killer cells, dendritic cells, and regulatory T lymphocytes in terms of both activation and proliferation. While the risk of infection is lower in PV than for the other MPNs, (according to epidemiological studies based on data from Swedish registries) an in-depth risk assessment is recommended for infection prior to commencing treatment.[18] Patients should be screened for hepatitis B and those who test negative should be offered vaccination. Antiviral prophylaxis should be considered for those who test positive. Herpes zoster and pneumococcal vaccination should also be considered.[18] Additional risk factors would include tuberculosis or travel to tuberculosis endemic areas. It is also important to educate patients on the signs of infection and encourage them to seek early medical attention.

New therapies continue to be added to the arsenal. Additional therapies, currently under investigation include Ropeginterferon α 2b, which has a longer half-life than the 2a form and appears to be better tolerated.[21] It was approved for use as a monotherapy in patients without splenomegaly by the European Medicines Agency in 2019.[22] In addition, histone deacetylase (HDAC) inhibitors such as Givinostat and murine double minute 2 (MDM2) inhibitors such as Idasanutlin have also entered clinical trials.[8] Histone deacetylase catalyzes the removal of acetyl groups from lysine residues on histones, leading to down regulation of tumor suppressor genes. Inhibitors are being investigated for use as anticancer drugs. Givinostat has been shown to suppress clonogenic activity of JAK2V617F positive cell lines in *in vitro* models. It has demonstrated a hematological and molecular response in phase II trials and appears to be well tolerated.[22] MDM2 has been shown to be over expressed in JAK2617F positive cells, leading to a reduced the tumor repressor protein, p53, response and thus DNA damage. Idasanutlin is a selective small molecule MDM2 antagonist, shown to reactivate p53, stimulating apoptosis of JAK2617F CD34 (hematopoietic marker) positive cells.[23] It is also in phase II trials and appears to be well tolerated.[6,23]

Conclusion

Although PV is a relatively rare disorder, the discovery of the JAK2V167F mutation has revolutionised the diagnosis and prognostic evaluation of the disease, making it the subject of intense research. The underlying mechanisms for fibrotic and leukemic transformation of PV are not yet fully understood. They are likely to be multifactorial, but improved knowledge and application of DNA analysis may be beneficial for stratification of patients for risks of both transformation and thrombosis.[5] As technological advances increase the identification of genes associated with transformation, future research may yield new targets for therapy that may prevent transformation, thus improving the prognosis for patients with this disease.[1] Further research is also needed to investigate how newer therapies can be integrated into existing treatment plans for optimal results, as well as their impact on disease progression, allowing targeted and individualised treatment for patients.[4,5,18]

Acknowledgement

Images were provided by Nichola H Lawrence BSc MSc CSci FIBMS, University Hospitals of North Midlands NHS Trust.

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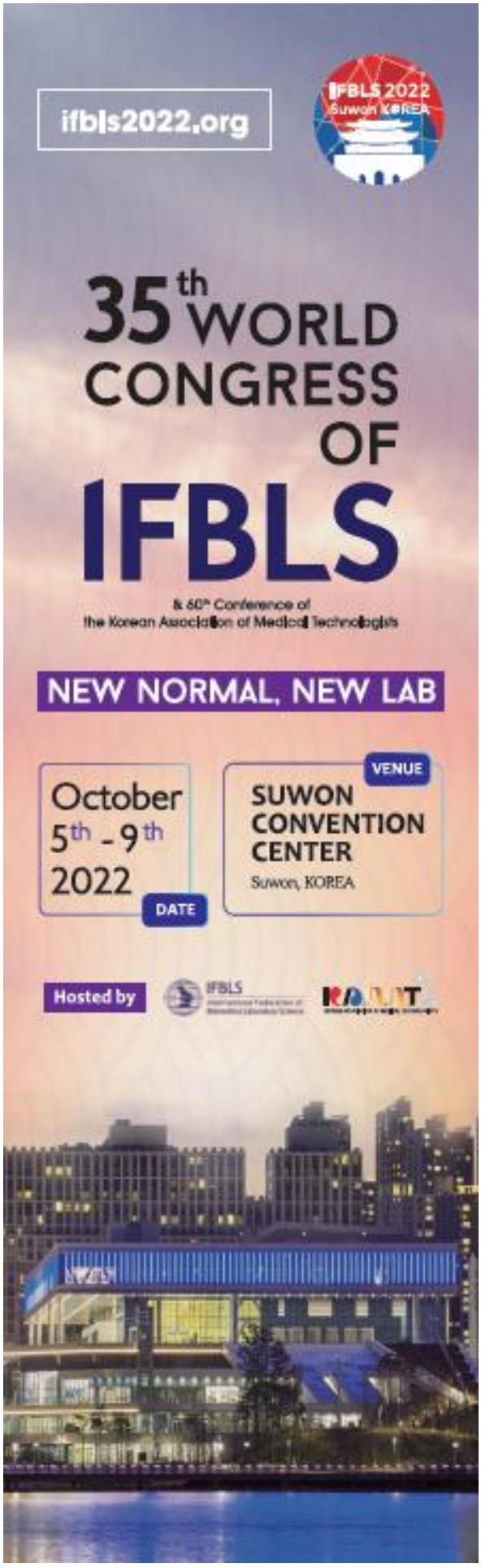

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