APFCB News

The Newsletter of the Asia-Pacific Federation for Clinical Biochemistry and Laboratory Medicine for circulation among APFCB and IFCC members only

2021

Issue 2

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Submissions

The APFCB News welcomes suitable contributions for publication. These should be sent electronically to the Chief Editor. Statements of opinions are those of the contributors and are not to be construed as official statements, evaluations or endorsements by the APFCB or its official bodies.
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Cover page: "My Home at the Yellow-Leaves Mountain Village"
Contributed by Dr. Tan It Koon
Founding and Past President APFCB

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Contents

From the desk of Chief Editor

Dear friends and colleagues,

It is with great pleasure that we bring to you the second issue of the APFCB News 2021.

Despite the Covid-19 pandemic still raging in many countries in Asia Pacific, I am pleased that we have received many reports and articles form national society members and corporate members for this issue of the APFCB News. The positive response we received for educational articles is also very encouraging. This makes the APFCB News a useful platform for sharing the activities carried out in the Asia Pacific region as well as making it a useful resource for knowledge sharing. I take this opportunity to thank all contributors.

I would also like to thank Professor Khosrow Adeli and IFCC for sharing the report on the activities of the IFCC. A special thank you to Professor Bernard GOUGET; Chair-IFCC Committee on Mobile Health and Bioengineering in Laboratory Medicine (C-MHBLM) for sharing an interesting article entitled "A Summer with Delta variant" which I hope you will enjoy. My sincere gratitude also goes to Dr. Tan It Koon who has generously shared his painting for the cover of this issue.

On behalf of the C-CP, editorial and design teams, I would like to express my gratitude to everyone who had rendered their kind support and assistance towards making this publication a reality. I look forward to your continued support in the future release of the APFCB News.

My best wishes to all. Take care and stay safe!

Best wishes,
Dr. Raja Elina

Chief Editor, APFCB News

Message from APFCB President

Dearest APFCB colleagues,

Greetings and my best wishes for the rest of 2021 and for the coming year 2022!

These past eighteen months or so have been difficult and challenging for all of us. The Covid-19 pandemic has completely changed the way of life for many of us.   Both on personal social and professional levels, we no longer interact physically and depend heavily on electronic platforms like Zoom, Skype and Webex for all our interactions. Many of us are considered frontline workers in the healthcare environment and we play an important part in keeping our nations protected and safe.   I would like to commend everyone for their efforts and I pray that we will quickly recover and return to a safer and more stable lifestyle.

Allow me to take this opportunity to announce an important upcoming Conference in Seoul, Korea from 26-30 June 2022. This meeting is the IFCC 24TH International Congress of Clinical Chemistry and Laboratory Medicine and the 16TH APFCB Congress of Clinical Biochemistry. This meeting is less than a year away and everyone involved in the organizing committee is hopeful that we will be able to have a successful physical meeting at this period.   However, things are far from certain and there is also a backup plan for a virtual option for the meeting. Do keep these dates blocked in your busy schedules and do look out for further announcements in the coming months.

I have great pride and pleasure to write this forward to the APFCB Newsletter, produced by the capable team helmed by Dr. Raja Elina, Chair of the APFCB Communications and Publications Committee and Chief Editor of the APFCB Newsletter.

Thank you for taking the time to browse this offering of the APFCB Newsletter.

My best wishes, always.

SK Sethi

Assoc Prof. Sunil Sethi
President, APFCB

IFCC Activities: Latest Developments & Future Ahead

Report by Khosrow Adeli, President of IFCC, Shannon Steele and Silvia Colli-Lanzi

The IFCC has had a very successful start to 2021, including several scientific events executed and more planned for the near future. Alongside these events, the IFCC Task Forces have been making extensive progress towards IFCC's goals to become the largest provider of free distance learning in the field of laboratory medicine, contribute to global lab quality, aid in the fight against the COVID-19 pandemic, as well as impact healthcare delivery and patient outcomes.

As you are all aware, the COVID-19 pandemic has had an enormous impact on our laboratory practices and has engaged the laboratory community in test development, validation, and implementation to support patient care and public health initiatives. To highlight the critical role of clinical laboratories in the COVID-19 pandemic, the IFCC held their first-ever virtual conference: the IFCC Global Conference on COVID-19. This scientific event brought together leading experts to present and discuss the latest advances in COVID-19 diagnostics and therapeutics, with thousands of participants from 118 countries around the world. Now, the IFCC has several upcoming conferences planned, including the XXIV IFCC-EFLM EuroMedLab (November 28 to December 2, 2021) and the Joint WorldLab-APFCB Congress (June 26 to June 30, 2022). We are also currently planning the IFCC General Conference for all IFCC functional units to discuss ongoing activities and plan future actions, which will take place in Spring or Fall 2022. Importantly, these events are incredibly valuable in achieving our goal of advancing excellence in laboratory medicine for better healthcare worldwide. In conjunction with these large conferences, we are initiating IFCC Annual Town Halls. Starting this fall, the IFCC Executive, IFCC Board Members, and Chairs of IFCC Divisions will virtually meet with the IFCC community in different IFCC regions to significantly enhance internal communication within the organization.

Alongside these events, the IFCC Task Forces continue be very productive. Specifically, the IFCC Task Force on Global eLearning/eAcademy has been busy organizing monthly global webinars for the IFCC Webinars Live Series 2021, which is now co-sponsored by Siemens Healthineers and Boston Children's Hospital to support this important initiative over the next three years. Additionally, the IFCC Task Force on Global Lab Quality (TF-GLQ) has been planning a pilot program for internal quality control (iQC) and external quality assurance (EQA) in developing countries, including Malawi, Zambia, Bosnia, Georgia, Serbia, Sri Lanka, Indonesia, Bolivia, Columbia, and Peru. Further, the Task Force on COVID-19 has recently developed a new IFCC interim guidelines on rapid point-of-care antigen testing for SARS-CoV-2 detection in asymptomatic and symptomatic individuals (Clin Chem Lab Med, 2021) to aid in the successful implementation of rapid antigen testing protocols to assist global efforts in identifying and isolating SARS-CoV-2 cases earlier. Finally, the newly established Task Force on Global Newborn Screening (TF-NBS), a joint IFCC - International Society of Newborn Screening (ISNS),

Task Force, is identifying partner regions and analyzing the current state of newborn screening (through a recent global survey) so to launch a program aimed at the introduction of NBS screening in selected countries, in partnership with healthcare professionals and government local organizations.

In addition to these existing task forces, IFCC is currently establishing the Task Force on Outcome Studies in Laboratory Medicine (TF-OSLM) and the Task Force on Global Reference Interval Database (TF-GRID). The TF-OSLM will promote the value of laboratory medicine by gathering evidence to demonstrate the critical role of laboratory medicine in clinical decision making and healthcare delivery as well as communicating these findings to key stakeholders and the public. The TF-GRID will focus on the creation of a global reference interval database, which will act as a key resource on pediatric, adult, and geriatric reference intervals for healthcare and laboratory professionals both within and outside of the IFCC organization. Eventually, it will evolve into a searchable database that facilitate accurate test result interpretation as well as harmonization and comparison of reference intervals between regions around the world.

Finally, IFCC has recently enhanced its IFCC office resources by recruiting a new staff member. I would like to take this opportunity to welcome Mrs. Smeralda Skendaraj to the IFCC organization. She is working closely with Paola, Silvia C-L and Silvia C and will be supporting activities in several areas including some of the new projects recently initiated by IFCC.

As we reflect on the first half of 2021, it is exciting to see the great productivity of the IFCC organization, especially as it pertains to "advancing excellence in laboratory medicine for better healthcare worldwide". With exciting plans and opportunities ahead, we can all look forward to continuing this invaluable mission.

APFCB Committee for Communications and Publications (C-CP)

Raja Elina, Chair, C-CP

2021 has been a relatively busy year for the C-CP team. We held our first virtual meeting on 23 Jan 2021. As an outcome of this meeting, guidelines for the submission of reports, articles and advertisements to the APFCB News have been drawn and is now available on the APFCB website homepage. Document link is https://www.apfcb.org/Submission%20Guidelines%20APFCB%20News%20050721.pdf. Advertisement rates have also been revised to make it more attractive to our corporate members. The C-CP also held a meeting on 15 May 2021 with the team at Ubitech Solutions, the company which has been awarded to manage the APFCB website. The C-CP team agreed that the APFCB website needed upgrading and this is currently being looked into.

C-CP committee meeting with Ubitech team

In addition, the C-CP team also supported the activities of the APFCB by promoting its webinars and training courses. The use of the virtual platform for these educational programs as well as those under the auspices of the APFCB has given the opportunity for participation across countries and have received very good response. Latest announcements of upcoming events are now available on the homepage of the APFCB website. Currently also available on the homepage is the APFCB virtual workshop on Complete Guide on Laboratory Testing of COVID-19. To date, recordings and slides of the masterclass webinars on interpretative commenting have been uploaded on the webinars page of the APFCB website at https://www.apfcb.org/webinars.html and on APFCB social media. Also available on the webinars page are the IFCC webinars. Links to past webinars organised by the APFCB corporate members under the auspices of the APFCB are listed under the Congress and Conferences Committee page at https://www.apfcb.org/conferences.html and these are still accessible to members. Links to these events have also been made available on APFCB social media which are listed at the footnote on the APFCB website homepage. Members are encouraged to frequently visit the APFCB website at https://www.apfcb.org/index.html for updates on coming events.

APFCB Committee for Education and Laboratory Medicine

Tony Badrick, Chair, C-ELM

As we all grapple with the pandemic, both at home and in our workplace, the APFCB C-ELM has moved to support virtual educational activities that provide the needed ongoing professional development for members. We have been working with the corporate members to provide very relevant programs to laboratory staff. Examples include the Beckman Coulter Sepsis webinar which will soon join the other webinars currently hosted on the website (https://www.apfcb.org/webinars.html) and the BD Preanalytical Masterclass series that will go live in September. The C-ELM is also working with the IFCC and AACC to develop an App for laboratorians in the Asia-Pacific region which would contain material on QC/EQA/Validation. There is also the APFCB covid workshop which contains presentations on many laboratory aspects of the virus, its detection and monitoring of covid-19.

Members should look at the APFCB website to stay informed of these activities.

APFCB Scientific Committee - Report of 2021 Activities

Samuel Vasikaran, Chair APFCB Scientific Committee

I would like to highlight two of the activities of the APFCB Scientific Committee in the first half of 2021 for APFCB News

1.   WG on Diabetes Testing Harmonisation in APFCB Region

The Diabetes Testing Harmonisation Working Group chaired by Dr. Mithu Banerjee (India) and including Dr. Deepani Siriwardhana (Sri Lanka), Dr. Tan Jun Guan (Singapore) and Dr. Maria Ruth Pineda-Cortel (Philippines) has conducted surveys of diabetes testing and reporting practices in four countries in the Asia Pacific region. Results of the survey conducted in India was presented at the APFCB Congress in 2019 and has since been published.1 The results of the survey in the Philippines were presented previously at the PAMET conference in 2018. Survey results for Sri Lanka were presented recently at the Annual Academic Sessions of the College of Chemical Pathologists of Sri Lanka (July 2021). A survey has also been conducted in Singapore.

The survey uncovered several issues of lack of harmonization of testing practices and reporting that the laboratory profession as a whole and the Clinical Chemistry professional association in each country needs to address. For example, the units for reporting of blood glucose concentration are not uniform in most countries. Even though the recommended standard international units for reporting blood glucose is mmol/L, a significant proportion, in fact a majority, of laboratories in the AP region report blood glucose in mg/dL. Hence, this practice can lead to confusion amongst clinicians as well as patients when interpreting blood glucose results and monitoring over time especially when different laboratories are used for serial measurements.

Table 1. Reporting units for plasma glucose in laboratories from four countries in the Asia Pacific region

	Participant Number	mmol/L only	mg/dL only	Both units
India	312	1%	97%	2%
Philippines	79	53%	41%	6%
Sri Lanka	35	23%	63%	14%
Singapore	9	78%	11%	11%

The reporting of HbA1c units is similarly non uniform. The situation with HbA1c, however, is somewhat understandable in that the traditional % units are used by the vast majority of laboratories, with a significant proportion of laboratories reporting in mmol/mol [IFCC units] also. This is considered a transitional phase, and once clinicians (and patients) become familiar with the IFCC units, the latter would be used exclusively. However, the profession needs to work actively towards this goal together with educating our customers.

The survey identified a need to harmonize the provision of testing for gestational diabetes mellitus (GDM). Glucose challenge test is no longer recommended. Oral glucose tolerance test with appropriate GDM cut-offs for its diagnosis is now recommended, and should be followed by all laboratories.

Urine albumin testing should be performed on spot urine samples collected in the morning and reported as a ratio to creatinine. The use of 24-hour collection or timed overnight collection is not recommended. The variation in reporting units for creatinine was also found to lead to reporting of spot urine albumin as either mg/mol creatinine or mg/g creatinine by different laboratories, another potential area of confusion and needing harmonization.

Table 2. Type of sample used for urine albumin measurement in laboratories from four countries in the Asia Pacific region

	Participant number	Morning spot urine	24-hour Urine	Timed overnight	Random spot urine
India	305	59%	39%	2%	–
Philippines	65	69%	26%	5%	–
Sri Lanka	32	56%	16%	–	28%
Singapore	8	75%	12%	–	12%

Finally, we also encourage the exclusive use of certified methods in clinical laboratories and participation in proficiency testing (external Quality Assurance) programs for all tests offered.

Measures to harmonize practice according to recognized recommendations should be locally driven, led by each national professional body, but APFCB would strongly encourage and support national organisations to take forward plans to harmonise testing and reporting practices in every jurisdiction within the AP region.

Publication:

Trends in laboratory testing practice for diabetes mellitus. Banerjee M, Vasikaran S. eJIFCC 2020;31:(3):231-41.

Masterclass in Interpretative Commenting on Clinical Chemistry Reports - Webinars

Samuel Vasikaran, Chair APFCB Scientific Committee

Monthly webinars to discuss and analyse interpretative comments and to educate laboratory professionals on the addition of interpretative commenting has been ongoing for more than a year now. A report of the Webinar series is published separately, elsewhere in this issue and I would like to specially acknowledge the efficient organisational support of Dr. Pearline Teo of Siemens Healthcare Pte Ltd for this activity. The recordings of the webinars and resource materials are available on the APFCB website under the heading of **Webinars:** https://www.apfcb.org/webinars.html

I am grateful to my APFCB colleagues and to the corporate sector for their help and support to the activities of the Scientific Committee.

APFCB Masterclass on Interpretative Commenting Webinar series

Drs. Sam Vasikaran, Raja Elina and Pearline Teo

Since August 2020, the APFCB Scientific Committee has organized a monthly webinar series on Interpretative Commenting. So, in August 2021 we celebrate our First Anniversary! In this series, chemical pathology experts discuss the interpretation of laboratory test results and recommend comments that may be suitable to provide in the laboratory report. The format of the webinars is generally a discussion of case reports for 45 minutes followed by question and answer session for about 15 minutes.

The topics so far have been as follows:

Month	Topic	Speaker
August 2020	Thyroid Function Tests (basic)	Dr. Sam Vasikaran
September 2020	Thyroid Function Tests (advanced)	Dr. Sam Vasikaran
October 2020	Endocrine (Adrenal) Tests	Dr. Sam Vasikaran
December 2020	Fertility Tests	A/Prof. Ken Sikaris
January 2021	Calcium and Parathyroid	Dr. Sam Vasikaran
February 2021	Endocrine Dynamic Function tests	A/Prof. Cherie Chiang
March 2021	Lipids and Lipoproteins	A/Prof. Ken Sikaris
April 2021	Diabetes testing	Dr. Moh Sim Wong
May 2021	Cardiac Troponin	A/Prof. Chris Florkowski
June 2021	Dynamic Function Tests Part 2	A/Prof. Cherie Chiang
July 2021	Anti-Müllerian Hormone	Dr. Melissa Gillett

Registrations have fluctuated between 200 – 450 per event, and live attendance has been between 100–200 attendees per event. Depending on the topic, 60–70% of registrants are chemical pathologists, while 15–25% are scientific officers or lab technologists. The quality of the speakers and presentations have been excellent, and feedback has been overwhelmingly positive. In every session, >95% of responders agreed or strongly agreed that the session had been useful to them, and that they would recommend it to others. We thank the speakers for volunteering their time and effort to support this educational initiative. Their depth of knowledge and experience are clearly appreciated by our webinar participants.

We thank the participants for their attendance and lively discussion during the Q&A sessions. Many participants are consistent supporters of the series, and have provided valuable suggestions and feedback.
Last but not least, we thank the APFCB Communications team, for their support in publicizing each event, and making the slides and recordings available online.
We invite all interested laboratory professionals to participate in future webinars.
Recordings and slides of past webinars are available via the APFCB website and YouTube channel, while registration links for future webinars are posted on Eventbrite.
https://www.apfcb.org/webinars.html
https://www.youtube.com/channel/UCoiicTsnVX-COjklgZHQ54Q/videos
http://APFCB.eventbrite.com

Australasian Association for clinical biochemistry and laboratory medicine (AACB)

Dr. Fernando San Gil MSc PhD MAACB ARCPA
Chief Executive Officer, Australasian Association for clinical biochemistry and laboratory medicine

The presence of COVID-19 in Australia has meant a re-visioning of the way that scientific meetings are held in Australia. Throughout 2020 and 2021, AACB activities such as the branch meetings and the Annual AACB/RCPA Chemical Pathology Course (our major educational activity for the year) have been held virtually. This format for meetings has now become the "new" normal and has been readily embraced by members. Congratulations go to Dr. Samuel Vasikaran, who received the Geoffrey Kellerman Award (for commitment to education in the profession) at the 2021 Chemical Pathology Course.

The AACB is currently planning a hybrid annual scientific meeting. The theme of the meeting is "Get your head in the cloud" and is scheduled for 27-28 October, 2021 in Brisbane, Queensland. This is the premier meeting for the Association each year and brings together many colleagues and friends with an interest in Clinical Biochemistry and laboratory medicine. Moving to a hybrid platform brings new opportunities and we look forward to a successful event which will circumvent some of the logistical issues created by COVID-19. Registrants and participants will be able attend either in person or remotely (if COVID restrictions on travel are in place). Of course, we all look forward to a time in the not-too-distant future when we can meet again in person. Planning for the 2024 APFCB Congress in Sydney is continuing. We look forward to welcoming colleagues from around the region in 2024.

This year will also see a change in the AACB Executive, with Dr Tina Yen moving from the position of President Elect to be the new President. Mr. Peter Ward is the outgoing President, and will continue on the Executive Board for one year in the role of Past President. This year has also seen a change in the CEO of the AACB. Dr Kevin Carpenter has stepped down and has been replaced by Dr. Fernando San Gil. The AACB Council and members greatly appreciate the service given by both Peter and Kevin during their tenures.

It is with great sadness that we report an eminent scientist, colleague and friend, Dr. Ian Goodall, has passed away. Dr. Goodall was a longstanding member and Fellow of the AACB. He was highly active in the scientific community over many years, with a strong interest in the long-term monitoring of Diabetes. Over the years he gave many lectures and presentations, authored many papers on diabetes, glycated haemoglobins and fructosamine, and was a member of the IFCC Working Group for the standardisation of glycated haemoglobin.

Chinese Association for Clinical Biochemistry (CACB-Taiwan)

CACB held its Executive Board meeting on March 8th, 2021 and planned for the upcoming 35th Joint Annual Conference of Biomedical Science (JACBS). Unfortunately, the 35th JACBS was postponed from March 27th-28th to May 15th-16th and was finally cancelled one week before the event due to the COVID-19 situation in Taiwan. Nevertheless, CACB organized its annual meeting for the election of new board members and a scientific symposium entitled "Toward Next Generation Clinical Diagnostics and Therapeutics".

Four speakers had been invited to present the progress on identifying novel biomarkers and therapeutic targets for various diseases. Dr. Wen-Chien Chou, Director of the Department of Laboratory Medicine at National Taiwan University Hospital, will deliver a keynote lecture on "Precision Medicine- from blood cancers". Dr. Sui-Yuan Chang, Professor of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University, will present "Clinical diagnostics and therapeutics for emerging infectious diseases-experiences from COVID-19". Dr. Kuan-Ying Arthur Huang, Associate Professor of Pediatric Infectious Diseases from Chang Gung Memorial Hospital will present recent findings on "Potential of anti-spike human monoclonal antibodies against SARS-CoV-2". Dr. Wen-Hui Ku, CEO of Taipei Institute of Pathology, will share his experiences on "Precision Medicine: the utility of LC-MS". In addition to the scientific symposium, CACB will present 31 posters and 10 oral abstracts at the 35th JACBS.

Upcoming events for 2021:
Election of new Board Members and annual meeting.

Performance Report of Iranian Association of Clinical Laboratory Doctors (IACLD)

Dr. Alireza Lotfi Kian (DCLS)

Association International Secretary Kianlab3640@yahoo.com

With respect to fulfilling one of our main duties which is hosting annual congresses, the 12th International and the 17th National Congress on Quality Improvement in Clinical Laboratories was held in 2019.

However, the 2020 annual Congress was canceled due to the COVID-19 outbreak despite the association's readiness to hold it; fortunately, the 18th Congress will be held virtually in September 2021 while undergoing some delay.

Other activities of IACLD in recent years include conducting External Quality Assessment Program (EQAP) from 2020 to 2021 which has an important role in updating services and equipment, improving performance, and correcting errors of medical laboratories.
This also include the publication of the quarterly journal called " Laboratory & Diagnosis" and accrediting qualified laboratories.

The IACLD team are the members of International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and Asian and Pacific Federation of Clinical Biochemistry (APFCB).
Meetings of board members have been held on a weekly basis, though sometimes virtually due to COVID-19 pandemic.

Training courses including 64 courses and webinars have been conducted by experienced professors in the education department of the Association, of which 56 courses have been held virtually (Webinars) and the rest in person, and more than 10,000 participants have attended them.

A Number of Conducted Training Courses:

Year 2021	
Training Courses (Webinars)	Time
Blood Sugar: Laboratory and Clinical Examinations	15 April
Lipids and Cardiovascular Diseases	16 April
Quality Control of Main Tools in Microbiology Department	30 April
Quality Control in Microbiology Center	1 May
Body Fluids: Urine	6 May
Body Fluids: CSF, Serous and Synovial Fluid	7 May

Laboratory Investigation of Superficial Fungal Infections of the Skin	20 May
Laboratory Mycological Diagnosis of Common Dermatophytes	21 May
Molecular Diagnosis of COVID-19	27 May
COVID-19 Serology Tests	28 May
Basic Concepts in Immunoassay	25 May
Requirements for the Internal Quality Control of the Immunoassay Department	26 May
Operations of Advanced Cell Counters	3 June
Interpretation of Advanced Cell Counter Graphs	4 June
Laboratory Aspects of Cortical Hormones of the Adrenal Glands	5 June
Laboratory Aspects of Gonad Hormones	6 June
Thyroid Histophysiology and Diseases	10 June
Interpretation and Clinical Applications of Thyroid Tests	11 June
Recent Findings in Quality Control of Hematology Department in Diagnosis of COVID-19	15 June
Laboratory Data Interpretation Regarding CUSUM Chart, Westgard Rules, and Youden Plots	16 June
Reference Range and Reference Values in Biochemical Experiments	15 July
Internal and External Quality Control in the Biochemistry Department of the Medical Diagnostic Laboratory	16 July
Quality Control in Antibiotic Susceptibility Testing (AST)	22 July
Year 2020	
Interpretation of Graphs and Advanced Cell Counter Results	6-7 August
Quality Management in Biochemistry Department	13-14 August 20-21 August
Principles, Interpretation, and Laboratory Challenges of Hepatitis	3-4 September
Quality Assurance of Hormone Analyses and Immunoassay	10-11 September
Quality Control in Hematology Department	17-18 September
Laboratory Diagnosis of HIV Infection and Interpretation of Results	1 October
Laboratory Aspects of Adrenal Cortex of the Adrenal Glands	2 October
Laboratory Aspects of Gonads	9 October
New Findings in Diagnosis of COVID-19	15-16 October
Body Fluids: Urine	5 November

Body Fluids: CSF, Serous and Synovial Fluid	2 October
Hematomorphology of Blood Cells and the Methods for Reporting and Standardization of the Results Based on New ICSH Guidelines	12 November
Hematomorphology and Platelets	13 November
Hematomorphology of White Blood Cells	19 November
Hematomorphology of Leukemia	20 November
Molecular Diagnosis of COVID-19	3 December
The Role of Serology Tests in COVID-19	4 December
Thyroid Histophysiology and Diseases	12 December
Interpretation and Clinical Applications of Thyroid Tests	11 December
New Findings in Diagnosis of COVID-19	17 December
The Role of Laboratory in Diagnosis and Control of COVID-19	18 December
Quality Control in Antimicrobial Susceptibility Testing	23 December
Laboratory Diagnosis of Haemoglobinopathies and A1C	4 December
Laboratory Diagnosis of Thalassemia	5 December
Testing Errors and Allowable Errors in Biochemistry Tests	7 January
Validity and Precision Bias in Biochemistry Tests	8 January
Reference Values and Reference Range in Biochemistry Tests	14 January
Internal and External Quality Control in Biochemistry Department of Medical Laboratories	15 January
Laboratory Aspects in Diagnosis, Primary Hemostasis, and Systemic Drugs	28 January
Laboratory Aspects of Thyroid Hormones	18 February
Laboratory Aspects of Growth Hormone and Prolactin	19 February
Basic Concepts in Immunoassay	27 February
Requirements for Internal Quality Control Programs in Immunoassay Department	28 February
PCR and COVID-19 Diagnosis	4 March
The Role of Antibody or Serology Tests in Diagnosis of COVID-19	5 March

Japan Society of Clinical Chemistry

Makoto Kurano, MD, PhD, Yutaka Yatomi, MD, PhD

A brief introduction to the Department of Clinical Laboratory, The University of Tokyo Hospital/ Department of Clinical Laboratory Medicine, Graduate School of Medicine.

Department of Clinical Laboratory, The University of Tokyo Hospital/ Department of Clinical Laboratory Medicine, Graduate School of Medicine, The University of Tokyo is the university-based Clinical Laboratory, the missions of which are clinical laboratory testing, education, and researches. Currently, there are more than 80 dedicated clinical laboratory technicians, 13 staff or members, 5 project researchers and 6 graduate students. We are accredited in 2007 by ISO 15189 "Medical laboratories — Requirements for quality and competence". In 2018, 255,698 urine samples, 5,454,903 serum enzyme tests (such as AST and ALT), and 579,702 immunological tests, 1,194,808 hematological tests (such as complete blood cell counts) were performed in the section of laboratory tests and 43,463 ECG, 26,613 pulmonary function tests, 11,179 EEG, 10,524 echocardiography tests, 14,274 abdominal echography tests, and 9,528 other ultrasonography tests were performed in the section of physiological tests. Our department is one of the biggest training facilities for clinical laboratory technicians, as well as for undergraduate and graduate students in Faculty of Medicine, The University of Tokyo.

In regard to the researches on the topics related to clinical biochemistry, we are especially interested in bioactive lipids, especially lysophospholipids. Lysophospholipids are lipids composed of one hydrophobic part and one hydrophilic base, represented by lysophosphatidic acid (LPA) and sphingosine 1-phosphate (Figure 1).
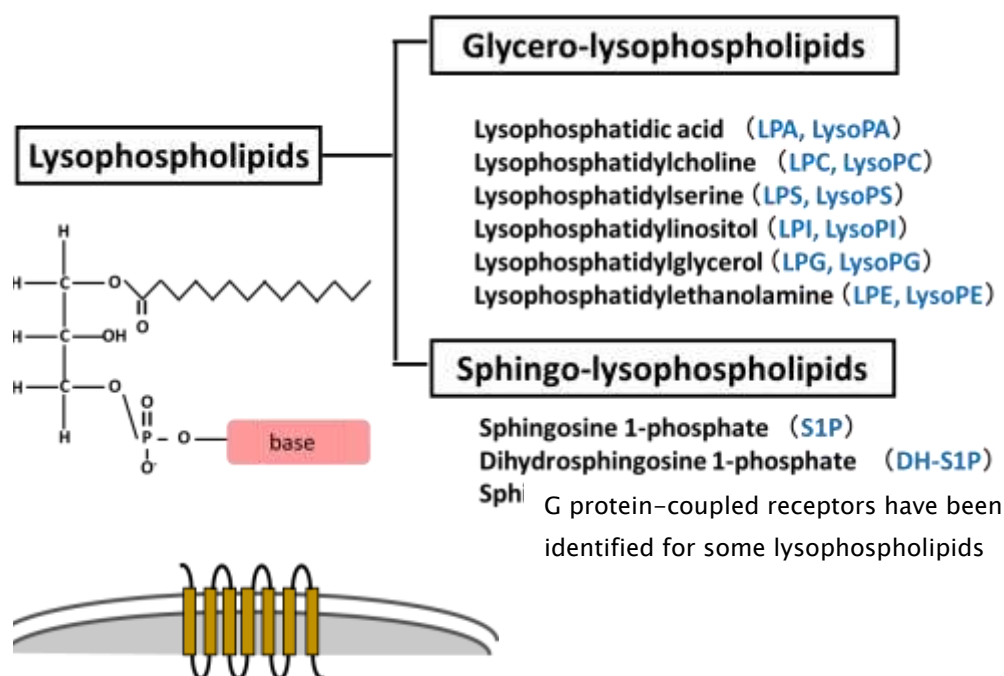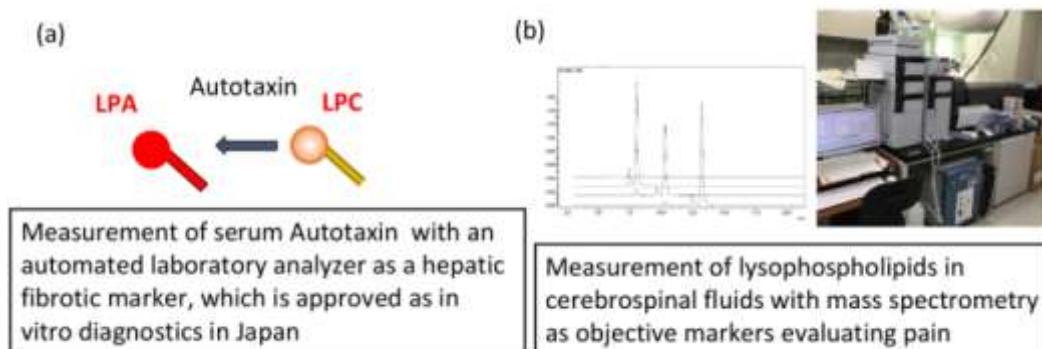

Figure 1. Lysophospholipids

Figure 2. Examples of our achievements

(a) Research conferences are held once a month.
(b) Some members are from overseas as a graduate student or a postdoctoral fellow.

Figure 3. Research conferences and Laboratory members

One of the achievements of our researches is the introduction of autotaxin, a producing enzyme for LPA from lysophosphatidylcholine (LPC), into laboratory medicine; recently, autotaxin was approved as in vitro diagnostics (IVD) for a novel hepatic fibrotic marker in Japan. And at present, we aim to introduce the measurement of LPA and LPC with mass-spectrometry as IVD for pain evaluation. (4) During the course of our lysophospholipid research, we got interested in the redox state of albumin, which may affect the biological activities of this new class of bioactive lipid. (5) Our laboratory is composed of diverse members. In addition to physicians (MD), many clinical laboratory technicians participate in both basic and clinical researches and several members are from overseas (Figure 3).

Please see our achievements at http://lab-tky.umin.jp/en/achievements/index.html

Malaysian Association of Clinical Biochemists (MACB)

Dr. Raja Elina, President, MACB

In Malaysia, the rate of Covid-19 positive cases has been steadily increasing since January 2021 and reached a peak in early August. With the high rate of vaccination in the country, the number of positive cases is maintained at a plateau; but to date, the country is still battling hard with the pandemic.

Due to the current situation, all MACB activities in 2021 have been conducted via the virtual platform.

Annual MACB Conference

The 31st MACB Conference 2021 was held on 21-22 June 2021. The conference which carried the theme "Empowering Laboratory Services in Keeping with Technology Advancement", highlighted the latest technology advancement in laboratory medicine and its possible future application in Malaysia. The conference also presented recent updates on laboratory quality improvement, updates in clinical advancements as well as the role of diagnostic laboratory in managing patient care.

The keynote address on the topic of "Variation in COVID19 vaccine: Tackling the Safety and Efficacy Issues" was delivered by Datuk Dr. Hishamshah Mohd Ibrahim, Deputy Director General of Health (Research & Technical Support), Ministry of Health, Malaysia.
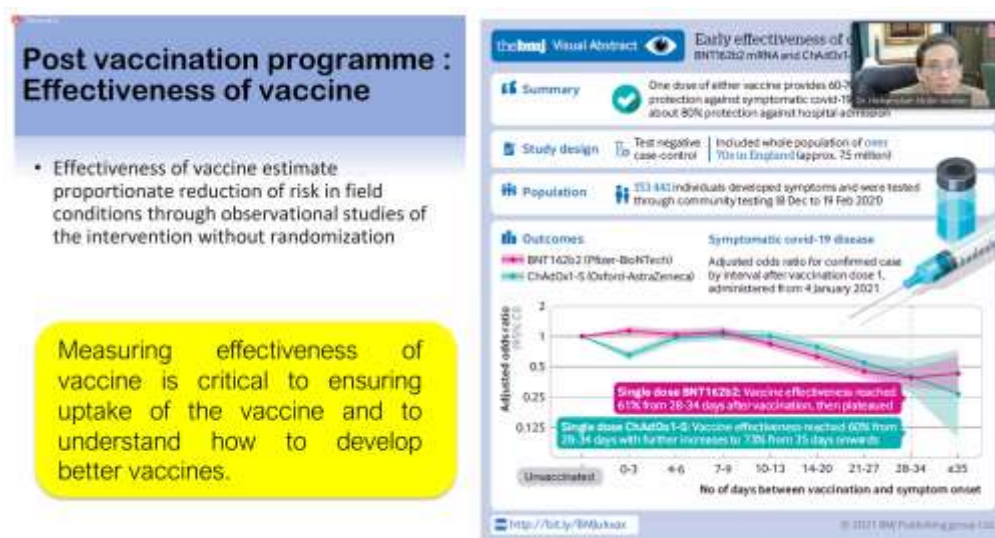
Keynote lecture by Datuk Dr. Hishamshah Mohd Ibrahim, Deputy Director General of Health (Research & Technical Support), MOH, Malaysia

In addition to the keynote address, eight additional lectures on various topics such as Lab 2.0, Requirement of a Newborn Screening Program, QC Approach Around Serum Indices, COVID-19 Updates: Variants, Vaccine and Serology, Clinical Utility of IL-6, Management Responsibility in GLP, Hs-troponin: updates of clinical utility in acute coronary syndrome and Informatics and Continuous Improvement were also presented. In addition to the lectures, the conference also included two forums on the topics of Method verification and Cybersecurity in Healthcare: Diagnostic Laboratory Perspective.

31st MACB Conference - Forum on Method Verification

The lectures were presented by local and international speakers from India, Australia, Singapore, Europe and USA. The conference was attended by 355 participants and there were a total of 4 oral and 25 poster presenters.

Asian Conference on Biomedical Research & Lab Medicine (ACBRLM) 2021

ACBRLM 2021 was jointly organised by three associations; the Malaysian Association of Clinical Biochemists (MACB), the Malaysian Biomedical Science Association (My Biomed) and the Association of Scientific Officers Ministry of Health (ASOMH). ACBRLM 2021 is the first joint collaboration among the three organisations. ACBRLM 2020 was initially scheduled for August 2020, but was postponed due to the COVID 19 global pandemic.

This conference was conducted on 24th-25th August 2021 with a post conference workshop on 26th August 2021. The conference and workshop were conducted virtually.

The conference which carried the official theme "Revolutionizing Laboratory Medicine Through Research and Innovation" covered topics which revolved around communicable and non-communicable diseases, genetics and molecular diseases, drugs and natural product discoveries, latest technology, methods and sharing of research findings. The conference was officially launched by the Director of Health, Malaysia.

Official launch of ACBRLM by Tan Sri Noor Hisham Abdullah, Director General of Health, Malaysia.

The two-day conference featured one keynote speech, two plenary lectures and 16 symposia lectures. In the post-conference workshop seven interesting topic in laboratory medicine were presented.
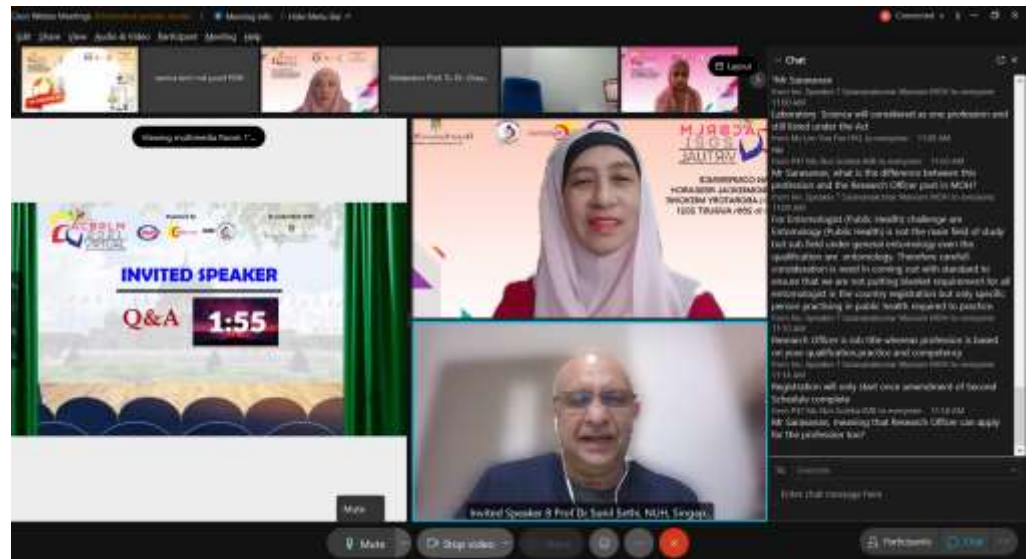
ACBLRM lecture on the topic of "Innovations in Diabetes and its Impact on Disease Monitoring" by Assoc. Prof. Dr. Sunil Sethi, President of APFCB.
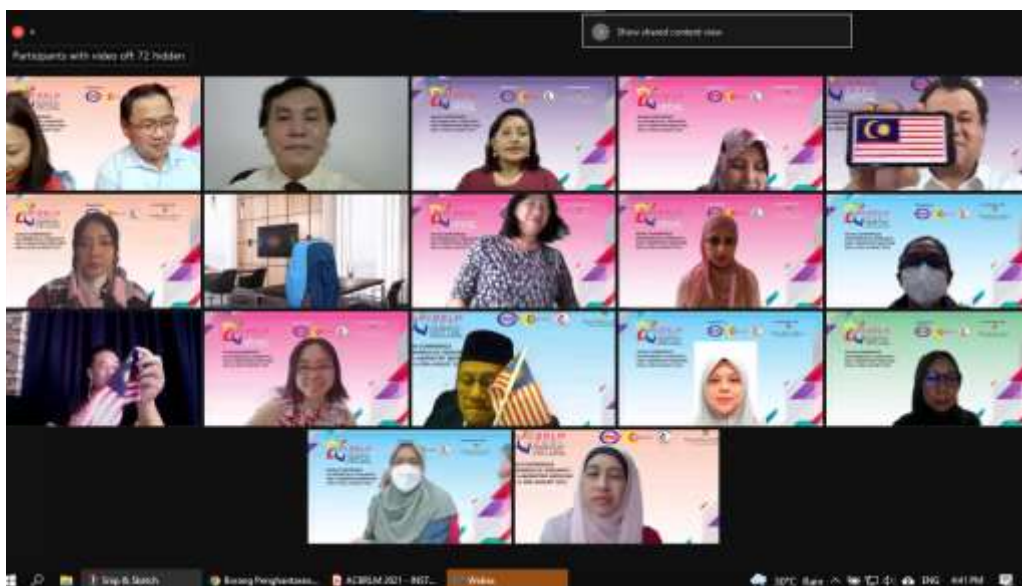
ACBRLM Organising Committee at the ACBRLM closing ceremony

The participants of ACBRLM were from continent of Asia and comprise of laboratory scientists, pathologists and various other categories of laboratory professionals. More than 400 participants attended the conference and among them there were 70 posters and 22 oral contributors who shared their experiences and knowledge in their respective fields.

MACB Webinars

In addition to the two conferences, the MACB held four webinars on the following topics:

1) Method Verification on 5 May 2021

The lecture was delivered by Dr. Joshua Hayden, PhD DABCC FAACC, Chief of Chemistry for Norton Healthcare. This is a very useful webinar which discussed the practical approaches that laboratories can take to ensure adequate assessment of new methods. Particular emphasis was given on examples of problems that laboratories might encounter during method validation and how to address them. The webinar was supported by Medicine. A total of 631 participants registered for the webinar of which 350 attended the live event. Of the total participants, 64% were Malaysians. Other participants who registered were from Philippines, Macau, Singapore, United States, Sri Lanka, Pakistan, Oman, Indonesia, India and Bangladesh. Recording and webinar presentation slides for the lecture are available at the following link: https://www.webinar.macb.org.my/

2) Breathing Not Properly: Heart Failure – Insights on Natriuretic Peptides on 6 May 2021.

The lecture was delivered by Dr. Pearline Teo, the Clinical Marketing Manager for Laboratory Diagnostics at Siemens Healthineers. The webinar presented the pathophysiology, diagnosis and classification of heart failure and the importance of Natriuretic Peptides (BNP and NT-proBNP) in the diagnosis, prognosis and management of Heart Failure. The best practice sharing from AP region on the clinical utility of Natriuretic Peptide assays in Asia Pacific was also discussed.

This webinar was supported by Siemens Healthineers and received 175 registrations and 69 attendees. Recording on the webinar is available on this link:https://www.siemens-healthineers.com/en-my/news-and-events/events-malaysia/macb-heart-failure

3) **A Practical and Standardized Approach to tackle Reagent Lot-to-Lot using CLSI's EP26 on 5 Aug 2021.**

This lecture was delivered by Mr. Norizhar bin Mohamed Zakaria, the Clinical Quality Manager at Siemens Healthineers. This lecture presented the utilization of a standardized and practical approach to document and evaluate reagent lot to lot difference with CLSI's EP26. It discussed the possible scenarios that may arise when evaluating or performing lot to lot differences using EP26's protocol and actions that can be taken. The webinar was supported by Siemens Healthineers and had 364 registered participants with 202 attendees for the live event.

4) **Chemical Screening of Urine by Reagent Strip on 10 Aug 2021.**

The lecture was delivered by Associate Prof. Dr. Pavai Sthaneshwar, Chemical Pathologist in Pathology Department, University Malaya. The lecture emphasised on the correct urinalysis testing procedure and guidelines to ensure correct patient results. This webinar was supported by Siemens Healthineers. A total of 512 participants registered for the event with 198 live attendees.

For updates on the upcoming MACB events and recording of previous events, please visit the official MACB website at https://www.macb.org.my/.

National Society Report of The Philippine Council for Quality Assurance In Clinical Laboratories (PCQACL)

Bryan Emil Garcia, MD, FPSP

Over a year now, we have seen the pandemic of SARS-COV-2 disrupting our country and the whole world. The unprecedented health crisis has paralyzed much of our lives and we continue to see its ravaging effects. We initially had our fears, concerns and uncertainties but the advent and promise of the vaccines being developed and rolled out keeps us looking forward to an improved new normal. We in the clinical laboratory are at the forefront of this battle. Now, more than ever, we needed to provide and sustain the quality services to our clients.

Philippine Council for Quality Assurance in Clinical Laboratories (PCQACL) is not an exception on the negative impact of the COVID-19 pandemic but we can only be true to our mission of being a dynamic, highly professional organization committed to the promotion of the provision of quality services by the clinical laboratories whatever the circumstances are and despite the difficult times. The officers and trustees had to meet virtually to discuss the needed adjustments to the national implementation of health protocols and held regular monthly meetings online since May 2020.

When everyone else in our country was preoccupied with their own responses to the pandemic, PCQACL provided the following relevant online trainings and educational activities:

2020:

1. "Laboratory Empowerment Against CoviD-19 (L.E.A.D)" Online Course – July 3, 2020 – Number of Registered Participants: 1,489

Online course on "Laboratory Empowerment Against CoviD-19 (L.E.A.D)"

2. "Addressing Current Challenges in Global Healthcare" Webinar – July 22, 2020 – Number of Registered Participants: 2298

Webinar on "Addressing Current Challenges in Global Healthcare"

3. "Specimen packaging, transport and shipment" – September 1, 2020 – Number of Registered Participants: 493

Webinar on "Specimen packaging, transport and shipment"

4. "Beyond QC Metrics"– September 17, 2020 – Number of Registered Participants: 500

Webinar on "Beyond QC Metrics"

5. PCQACL Virtual 17th Annual Convention with the theme: "Growing Laboratory Quality in the Midst of Pandemic" - September22-24, 2020
– Number of registered participants: 1,394

2021 "PCQACL Online Webinar SeRies" (P.O.W.E.R):

1. POWER 1: "Blood Gas Analysis in POCT and Its Challenges" – March 26, 2021
– Number of Registered Participants: 324
2. POWER 2: "Beyond the Usual CBC Analysis: Digging Deeper into Histograms"– June 11, 2021
– Number of Registered Participants: 831
3. POWER 3: "The Purpose, Practicality and Current Situation of Body Fluids Testing"– July 2, 2021
– Number of Registered Participants: 1,018

2021 "PCQACL Online Webinar SeRies" (P.O.W.E.R)

National Meeting: PCQACL held its first-ever virtual business meeting during its Annual Convention on Sept. 23, 2020.

Other Educational Activities:
Another milestone for the organization for 2021 is the launching of PCQACL Academy online Certificate Training program or P.A.C.T. The first-ever online certificate course related to laboratory medicine in the country, where five (5) of the top hematopathologists in the Philippines were gathered together as faculty of the course. It started accepting applicants since April 27, 2021.

PCQACL Academy online Certificate Training program

List of Upcoming Events:
Virtual Annual Convention "MOVING ON, MOVING FORWARD…. BEYOND COVID-19" will be held on September 22-24, 2021.

PCQACL board meetings

For 2021, a total of six (6) regular monthly board meetings have already been conducted by the officers and board of trustees.

Vietnam Association Clinical Biochemists

1. Recent activities of VACB.

Last year, due to the complicated situation of the Covid-19 epidemic with Hanoi and some southern states of Vietnam under blockade, the direct activities of VACB were mostly postponed. The main activities of VACB were carried out online. Some of the main activities are as listed below:

- On June 3th, 2021 in collaboration with ABBOTT company, VACB organized a workshop on the topic; "Super sensitive generation HBsAg test, Optimize clinical management of hepatitis B" at Media Hotel, 44 Ly Thuong Kiet. Tran Hung Dao, Hoan Kiem, Hanoi.

- On March 26th, 2021 in collaboration with SNIBE, VACB organized an online seminar on the topic: "Laboratory quality assurance and management and some commonly used cancer biomarkers and their clinical applications".

Fig 1. Webinar online "laboratory quality assurance and management and some commonly used cancer biomarkers and their clinical applications".
– On July 23th, 2021 in collaboration with SNIBE, VACB organized an online seminar on the topic: "SARS-CoV-2 antibodies neutralizing activity".

Fig 2. VACB hands over medical supplies to support the two provinces of Bac Giang and Bac Ninh the fight against covid 19.

- Coordinate with Roche Company to organize the program "Don't give up hope" to improve understanding about treatment and prevention of incurable diseases and difficult diseases for members and the community.

Fig 3. Webinar online with content: " SARS- CoV-2 antibodies neutralizing activity".

- With the serious situation of Covid 19 epidemic in Bac Giang and Bac Ninh provinces in May 2021, VACB organized a campaign to raise money and donate medical supplies and equipment with the slogan "Sharing hands together in the fight against covid 19 in the two provinces of Bac Ninh and Bac Giang"

Fig 4. The program "don't give up hope"

- Currently, there are VACB members who are involved in anti-epidemic work in the epidemic center of Ho Chi Minh City, Dong Nai province.
- VACB is also participates in Covid-19 testing in complicated epidemic areas.
- Regular online Executive Committee meetings were conducted.

Planned activities in the near future.

- Further strengthening the organization of classes on expertise and laboratory quality management organization by online learning.
- Actively participate in the health sector and the government activities in the prevention and control of the Covid-19 epidemic.
- Organize the 12th Congress and the 26th Annual Scientific Conference of VACB

Hanoi, August 2th, 2021

Hoang Thi Bich Ngoc, President of VACB

A Summer with Delta variant

Bernard GOUGET; Chair–IFCC Committee on Mobile Health and Bioengineering in Laboratory Medicine (C–MHBLM), co–Chair IFCC–TF on History, SFBC–International Committee, President–Human Health Care Committee–Cofrac, President–National Committee for selection of the French Reference Laboratories, Ministry of Health.

We thought the summer would be quieter, but new epidemic flares are clearly still with us. Delta variant has snuffed out the optimism we had in the spring and disturbed the summer reveries. COVID–19 will not be going away so quickly and the pandemic situation could get worse. At the same time, disastrous environmental events are converging like never before: extreme heat, out–of–control wildfires, droughts, flood. The climate catastrophes are just part of the weather now. The crisis is becoming routine. Finding happiness under fraught circumstances can be challenging and ruminating over what might have been and what might happen just deliver unhappiness.

COVID–19 deaths are on the rise once again. The number of cases is rising quickly due to the Delta variant. These variant exhibits two characteristics: a higher infectiousness and mutations of some of the antibody binding sites on the spike protein, which can be associated with immune escape. Some projections are cold–sweat inducing and impel a new turn of the screw in the face of the variant in the most affected areas. A continued arms race against the virus is inevitable.

Governments are passing legislation to adapt their tools to the evolution of the health crisis. Nonessential sectors are operating remotely and new travel restrictions are reappearing. Health control measures are imposed with the implementation of the "pass sanitaire" as in France and vaccine requirements for some occupations, with potential sanctions for those who refuse. The engagement of everyone is a vital component to be able to live as normally as possible after experiencing the trials of curfew and lockdown. The pandemic continues to shake up our lives, our relationship to freedom, our conception of civic duty. If the epidemic becomes out of control again, all economic and social activity will be disrupted again.

A more vaccinated world creates a more hostile global environment for SARS–CoV–2.

Mutations will still occur, but fewer of them will be of consequence. Globally, it is a race between vaccine delivery and virus transmission. These two sides are interconnected. The untrammeled spread of COVID-19 through large, vulnerable populations worldwide increases the risk that new variants will emerge. Every new variant carries with it the possibility of a devastating turn in the pandemic, a mutation that further weakens the efficacy of the vaccines, or that causes the disease to be more severe in children and young adults. Vaccines are still beating the variants, but the unvaccinated world is being pummeled. While several measures have boosted vaccination, we must not forget the hesitant minority not yet convinced by this almost civic obligation to get vaccinated, while there is a strong demand in low-income countries where populations have not been vaccinated much. Given a more contagious virus, pharmaceutical companies and medical laboratories are mobilizing. The idea of a third dose would certainly have a positive effect regardless of the vaccine, insofar as it would strengthen the protection of individuals who have already been vaccinated by cross immunity. It has become difficult to say if herd immunity can be attained. It has become a very ambitious challenge; vaccination in the name of the community remains completely relevant.

In the meantime, even highly vaccinated countries should continue investing in other measures that can control COVID 19 but have been inadequately used: improved ventilation, widespread rapid tests, smarter contact tracing, better masks, places in which sick people can isolate, and policies like paid sick leave. Such measures will reduce the spread of the virus among unvaccinated communities, creating fewer opportunities for an immune-escape variant to arise. Vaccines remains our most powerful tools. Immunization against diseases is among the most successful global health efforts of the modern era, and substantial gains in vaccination coverage rates have been achieved worldwide.

The COVID-19 vaccine is one of the most spectacular embodiments of this scientific, technical and political progress thanks to which we have a better quality of life today than ever before in human history. Even as many countries do not yet have sufficient access to the vaccines, antivaccine crusades strangely resemble medieval witch hunts with the same references to absolute evil, the same fear of hybrid beings seeking to alter nature. It is characteristic of great crises to accelerate the march of progress while mixing hope and horror. The success of the messenger RNA vaccines reminds us that catastrophes stimulate human ingenuity, and necessity dissolves the most deeply-entrenched beliefs. How can we not be amazed by the discoveries of genetics, a very young discipline! The RNA currently dominating the news is called messenger RNA but is also a messenger of hope! This amazing molecule deserves recognition of its potential, and scientists have been thoroughly inspired. The superiority of mRNA is due to its ability to rapidly adjust to virus mutations. The work around RNA is a saga populated with anonymous researchers who have ploughed forward come what may. A chance meeting in front of a photocopier, a beautiful analogy for RNA, which copies DNA sequences, allowed Katalin Kariko to meet her partner, Drew Weissman. Together and with their team, they succeeded in removing the obstacles that prevented messenger RNAs from triggering adequate immune responses.

While the race for vaccines, the main weapon against COVID-19, already has its champions, the race for treatment is still looking for its winners. There is no shortage of candidates. Researchers, biotech companies and major pharmaceutical companies have all been mobilized. There are still more than 1600 clinical trials underway worldwide. One of the difficulties is that, due to the number of clinical trials in progress, the chance of finding patients lengthens the time for developing treatments. The therapeutic arsenal is still meager in the treatment market that directly addresses the virus. However, research is progressing and we are hopeful that several drugs will arrive in the coming months. Treatments are an additional tool that can serve to anticipate the next crises. The stakes are not trivial, because while vaccines have so far provided an effective shield, nothing excludes new, more dangerous variants from escaping their protective net in the future. It is also a matter of protecting immunocompromised patients for whom vaccination is less effective and who are at greater risk of developing a severe form of the disease. The competition is vigorous on the monoclonal antibody and antiviral market, in which big pharma is well positioned.

We can see that the magic of the precious ribonucleic acid molecule is not limited to COVID. This technique makes it possible to hope that our cells will learn how to make effective shields against other serious diseases themselves. An extraordinary leap has taken place over the past two years. It has been demonstrated that knowledge of living organisms at the level of molecules and DNA-RNA relationships could prove to be fundamental in the fight against certain diseases. However, RNA should not be seen as a miracle drug; in biology nothing is won in advance and treatments do not always work. mRNA technology could become an additional weapon in the field of cancer treatment. One of the projects operates in a way very close to the method for COVID vaccines. It consists of introducing mRNA into cancer cells and having them produce a protein that will be recognized very efficiently by the immune system.

By injecting mRNA that codes for tumour neoantigens, cells are able to produce them. In reaction, the immune system specifically attacks tumour cells that produce these neoantigens, and therefore the tumour mass. The strength of these new technologies is their potential action against all types of cancer. In addition, they allow personalized care. Mutated proteins are actually specific to each tumour and each patient. By means of a biopsy it is possible to sequence its genome, identify the mutations present and rapidly produce the corresponding mRNA. The potential of mRNA in oncology is not limited to immunotherapy. These new technologies could also make it possible to induce cells to produce the drug proteins they need themselves.

It is vital to in still trust in innovation. Progress needs us as much as we need it. It must be supported by science education, appropriate communication and refined legal devices. Saving lives is urgent. The world has a moral obligation to do so and solidarity is needed more than ever. No one is safe until everyone is.

The publication first appeared in IFCC News September issue 2021

Evidence-Based Medicine and Clinical Practice Guidelines in Sepsis Detection

On 26 July 2021, 490 attendees across Asia Pacific and beyond gathered virtually to learn about evidence-based medicine and clinical practice guidelines in sepsis detection from experts in the field. The program was hosted by Prof Tony Badrick under the auspices of APFCB. Prof Simon Finfer discussed the current guidelines and scoring systems in defining and detecting sepsis that could be combined with artificial intelligence and electronic health record warnings to provide the optimal combination of sensitivity and specificity. While there is no magic bullet, the ideal assay for sepsis detection would be affordable, easy to interpret and universally available. Prof Carlo Tascini gave a talk on the current and future biomarkers in sepsis. In addition to some of the more common markers like lactate, procalcitonin and CRP, Prof Tascini also highlighted the utility of pro-adrenomedullin as used in his practice and the potential utility of monocyte distribution width. Prof Francesco Curcio discussed the clinical data for monocyte distribution width in early sepsis detection and shared the data from his study. Prof Sang-Bum Hong shared case studies on monocyte distribution width from his clinical study and highlighted that a combination of markers and monocyte distribution width together with SOFA score, would provide optimal clinical utility in early sepsis detection. The audience was asked many questions throughout, ranging from further clarifications on the novel technologies, tests and biomarkers available to detect sepsis, to the ideal cut offs that could be used. The speakers also discussed the accessibility of monocyte distribution width, affordability and high sensitivity that could aid in early sepsis detection, especially when combined with other markers and scores. Feedback from the audience post symposium was highly appreciative of the comprehensive content shared by the experts across different clinical practices and many looked forward to future sessions. As with many virtual meetings conducted during this pandemic, we experienced technical issues and it was thanks to the patience and good humor of Prof Badrick, the speakers and the audience that we were able to conclude a successful symposium. For those who missed the meeting, the on demand link is available at: https://attendee.gotowebinar.com/register/6199847418474257164

Many thanks to APFCB for granting us auspices to host this

Report on webinar series organized by Thermo Fisher Scientific

Romina C. De Leon (romina.deleon@thermofisher.com)

With the COVID 19 pandemic still very much within the Asia Pacific region, opportunities for face-to-face meetings and conferences are still very limited or even non-existent. After more than a year of varying lockdowns in majority of the countries in the region, webinars still prove to be a useful tool and an effective way of sharing updates to our healthcare professionals. This has prompted Thermo Fisher Scientific to organize a series of educational webinars last July, that were graced by esteemed speakers from APFCB as Dr. Raja Elina, Dr. Pavai Sthaneshwar and Dr. Tony Badrick.

The first webinar was held last July 7th, with the topic "Drugs of Abuse Automated Screening vs Manual Testing: What are the Benefits?". The lecture was delivered by Dr. Raja Elina, who has a wealth of knowledge on this topic. She covered the basic information on what drug testing is about and why we do drug testing. She also went thru the different methods that are currently used in the labs and the benefits and limitations of such test, ending the lecture with how to interpret results correctly. There were 579 registered participants across the Asia Pacific region including those in the Middle East.

Region	Country	Registrants
APAC	Malaysia	246
	Indonesia	103
	Sri Lanka	36
	India	33
	Taiwan	28
	Thailand	21
	Vietnam	14
	Singapore	10
	Bangladesh	5
	Philippines	5
	Macao	3
	Australia	2
	Hong Kong	2
	China	2
	Myanmar	1
	Korea, South	1
EMEA		66
Grand Total		578

The second webinar was held on July 22nd, revisiting a topic that is still very much relevant to us that is "Biochemical Markers in COVID 19".   We are very grateful for Dr. Pavai Sthaneshwar who have accommodated this lecture despite her very full schedule. Dr. Pavai took us back to when this pandemic started, the pathogenesis of the virus, and the important role that the laboratory plays during the pandemic. After, she gave an extensive run thru on the recommended biomarkers that are used for COVID 19 management, the recommendations for test selection and interpretation, as well as the considerations and limitations of such biochemical tests. As there is still a lot more to learn from this virus, Dr Pavai concluded that no single test is specific to SARS-CoV-2 infection nor its disease progression and these tests must be used in the context of the patient's clinical presentation. The different countries in the region as well as the Middle east also participated in this webinar that had 581 total registrants.

Region	Country	Registrants
APAC	Malaysia	146
	Indonesia	102
	India	87
	Philippines	75
	Thailand	28
	Sri Lanka	25
	Vietnam	14
	Singapore	7
	Korea, South	4
	China	3
	Australia	3
	Bangladesh	2
	Taiwan	1
	Myanmar	1
EMEA		83
Grand Total		581

The third webinar, focused on quality control, was completed last July 28th with none other than Dr. Tony Badrick who is an expert in the field.   The lecture was entitled "Clinical Application of Measurement Uncertainty". Measurement of Uncertainty was a concept introduced a couple of years back and majority of the clinical labs are required to report this as part of their accreditation. The approach of the lecture of Dr. Tony was very practical, simplifying the concept as he provided case examples prompting the audience to apply their understanding of Measurement of Uncertainty. Dr. Tony highlighted the different publications on this topic and its applicability in real world practice clarifying any open questions the audience might have in their minds. The webinar, similar to the first two, was also very well attended as we had 730 registered participants globally.

Region	Country	Registrants
APAC	Australia	150
	Malaysia	143
	India	139
	Taiwan	70
	Hong Kong	50
	Vietnam	32
	Philippines	24
	Thailand	19
	Korea, South	12
	China	11
	New Zealand	9
	Japan	7
Rest of APAC		44
United States		20
Grand Total		730
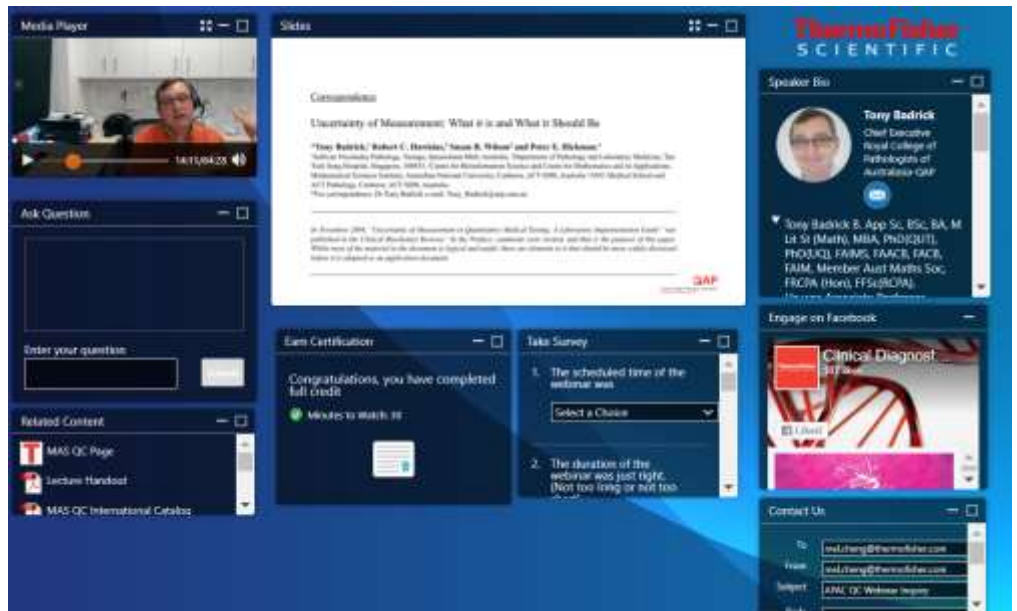
Overall, the participants were very satisfied with the lectures giving an average of > 98% rating on the relevance of the topics and the length of the webinar. Participants were also very comfortable and eager to raise their questions during the Q&A part that was gladly addressed by our esteemed speakers. There was a unanimous clamor for such webinar series to continue as such events provide an opportunity for them to get their updates specially during this pandemic.

The webinars are still available on-demand until July 2022 for those who have missed it or for those who would want to go back to get a refresher.

We are very grateful to Dr. Raja Elina, Dr. Pavai Sthaneshwar and Dr. Tony Badrick for sharing their expertise in this webinar series and to APFCB, for granting auspices for these webinars as we look forward to the continued collaboration in advocating the continued education in the region.

EliA SymphonyS - An integral part of your diagnostic screening algorithm for connective tissue diseases

Gerben Zuiderveld

Global Marketing Autoimmunity, Phadia GmbH, Freiburg, Germany

Connective Tissue Diseases (CTD) represent classical models of systemic autoimmune diseases. They are a heterogeneous group of diseases characterised by abnormal structure or function of one or more of the elements of connective tissue, i.e., collagen, elastin or the mucopolysaccharides. Differential diagnosis of CTD is mainly based on clinical findings but is complicated because of the similarity of their symptoms. Therefore, autoantibodies are useful markers to support the diagnosis or exclusion of CTD. The most prominent CTD are systemic lupus erythematosus (SLE; potentially affecting all organs), Sjögren's syndrome (SS; characterised by diminished lacrimal and salivary gland secretion), scleroderma (systemic sclerosis, SSc; a chronic, progressive dermatosis), limited systemic sclerosis (a scleroderma formerly known as CREST syndrome, with a more benign disease course), polymyositis/dermatomyositis (PM/DM; an acute or chronic inflammatory disease of muscle and skin), and mixed connective tissue disease (MCTD; a syndrome with features of scleroderma, rheumatoid arthritis, SLE and PM/DM).

Why a new EliA Symphony test?

With our mission "We enable our customers to make the world healthier, cleaner, and safer" we want to offer the best and most reliable test, in order to provide the physician / requester with the right test results which will help them to make the correct diagnosis and start appropriate treatment.

Table 1: Performance data of EliA SmDP-S compared with three automated tests for anti-Sm antibodies from other suppliers using 97 sera from SLE patients and 536 disease controls (table 2).

Cohort n=633	EliA SmD^{P}-	Supplier 1	Supplier 2	Supplier 3
Sensitivity	14.4%	19.6%	19.6%	16.5%
Specificity	98.3%	95.9%	96.1%	95.5%
Sensitivity at stratified specificity of 98%	14.4%	13.4%	11.3%	7.2%
Positive Likelihood Ratio	8.5%	4.8%	5.0%	3.7%
Positive Predictive Value	60.9%	46.3%	47.3%	40.0%

Background - Most innovative Sm test

Sm antibodies against SmD protein are a highly specific marker for SLE and are included in the ACR 1997 and SLICC 2012 criteria for systemic lupus erythematosus (SLE). While most extractable nuclear antigens can be produced recombinantly (preferentially in eukaryotic cells like Sf9 insect cells), this is not possible in the case of SmD. Compared to native SmD, recombinant SmD3 lacks the antigenicity for Sm autoantibodies to bind.

Therefore, most tests for Sm antibodies use native Sm purified from animal material. However, SmD is part of the larger multi-subunit U1-snRNP complex, and native Sm preparations can contain not only SmD but also other subunits that can interact with other autoantibodies and in consequence to lower test specificities.

To avoid these false positive test results, we identified an SmD3 peptide as antigen for Sm antibodies that met all the requirements for an antigen to be used in a high-quality diagnostic test [1,2]. This peptide is used in EliATM SmDP-S that replaces EliA Sm using native Sm. When comparing with tests using native Sm from other manufacturers (table 1), EliA SmDP-S showed a lower sensitivity, but the highest specificity, positive likelihood ratio and positive predictive value. When comparing sensitivity at a stratified specificity of 98% (the specificity of EliA SmDP-S), EliA SmDP-S had the highest sensitivity (table 1).

Table 2: Serum panel used for the development of EliA SmDP-S 8

Disease group	Amount
SLE	97
Disease Controls:	
Scleroderma	87
Sjogrens' Syndrome	96
Rheumatoid arthritis	85
Poly-/Dermatomyositis	78
MCTD	46
Infections (bacterial & viral)	119
Tumor	25
Total	536

Need for specificity

Systemic lupus erythematosus (SLE), like all connective tissue diseases, is a rare disease. Still, diagnostic markers for SLE are often ordered in the immunology laboratory as doctors want to rule out SLE when patients present with unspecific symptoms such as fatigue, fever, pain, skin irritations, joint pain or others. Sm antibodies are present only in about a fifth of SLE patients 9,10, 11, which makes them unsuitable for ruling out SLE. On the other hand, they are highly specific for SLE. Most clinicians assume that a positive Sm antibody is a clear sign for SLE. However, different tests have different clinical specificity for SLE. Some tests include not only SmD but also SmBB'. Since SmBB' and the U1snRNP antigens A and C share a cross-reactive epitope, antibodies against SmBB' are considered less specific for SLE (table 3).1,3,4 Therefore, it is of utmost importance to use the right antigen in an Sm test, to avoid false positives and provide high clinical usefulness.

Table 3: Frequency of U1snRNP and Sm antibodies in SLE, scleroderma and mixed connective tissue disease1,3,4

	SLE	MCTD	others
U1RNP(A,C,70)	30-40%	>95%	RA, PM/DM, SSc
SmD	20-30%		

Benefits of EliA SmDP-S

High confidence in the identification of SLE patients

Background – Highly sensitive Scl-70 test

In 2013, a joint committee of the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) developed new classification criteria for systemic sclerosis (SSc).5 One of these eight new criteria was SSc-specific autoantibodies, namely anti-Scl-70 (anti-topoisomerase I), anti-centromere, and anti-RNA polymerase III. Scl-70 antibodies are an indication for progressive systemic sclerosis.5 In the same year, we launched an improved Scl-70 test on the EliA system applying an innovative way of coating the antigen to the well. This resulted in improved antigen presentation, better accessibility of epitopes and, therefore, a higher sensitivity (table 4). The new EliA Scl-70S Well (14-5637-01) was evaluated with 336 clinically defined samples.

Table 4: Performance of EliA™ Scl-70S vs EliA™ Scl-70 and Scl-70 tests from other suppliers8

	EliA Scl-70^S	EliA Scl-70	Scl-70	Scl-70
Sensitivity	30.7%	26.7%	28.7%	28.7%
Specificity	99.5%	99.5%	98.0%	99.5%
PPV	96.9%	96.4%	87.9%	96.7%
NPV	74.2%	73.1%	73.3%	73.6%
LR (+)	61.4	53.4	14.4	57.4
LR (-)	0.7	0.7	0.7	0.7

Benefits of EliA Scl-70S

Aids in clear differentiation between systemic sclerosis and other connective tissue diseases

Supports early diagnostic guidance

The EliA SmDP-S and EliA Scl-70S tests have an excellent clinical performance indicated by a high sensitivity and specificity. Both antigens are important members of the antigen-specific screening test EliA SymphonyS test. A screening test should not only be aligned with the corresponding single antigen tests but should also have as high a sensitivity as possible without losing specificity. Therefore, the alignment of EliA Symphony with the EliA SmDP-S and EliA Scl-70S test was a logical and necessary consequence.

EliA SymphonyS

EliA SymphonyS is the first ENA screen to use only human recombinant antigens in combination with a synthetic peptide. Therefore, the test has all the advantages of recombinant antigens – pure antigens with no contamination, leading to a high specificity; controlled production of all test ingredients, leading to a high consistency over time; antigen lots which last over several years, leading to low lot-to-lot variation. The result is a clinically relevant, sensitive and highly specific screening assay.

This makes it an excellent aid for clinical decisions and, therefore, maximizes the usefulness in a diagnostic setting. As an intact three-dimensional structure of the antigens (conformation) is crucial for recognition by antibodies, most of our human recombinant antigens are produced in the eukaryotic baculovirus/insect cell system. This system, in contrast to bacterial systems, can express the antigens in the correct conformation and performing the complex posttranslational modifications necessary to ensure that the protein is antigenically identical to the human native form. The natural SmD protein consists of three parts: SmD1, D2 and D3. Mahler et al. demonstrated that one particular peptide of SmD3 represents the relevant epitopes for Sm and is a more sensitive and more reliable substrate for the detection of anti-Sm antibodies.[1,2] Both EliA SymphonyS and EliA SmDP-S use SmD3 peptide, as it was shown to be the most specific and sensitive antigen for SLE.[2]

Clinical performance

The use of antigens and antigen coating methods as described above should be matched by an improvement of the diagnostic performance. Therefore, the diagnostic performance of EliA SymphonyS was not only compared to EliA Symphony but also to other screening assays (EliA™ CTD Screen and 3 ANA Screening tests from different suppliers). All six screening tests include U1RNP, SS-A/Ro, SS-B/La, Scl-70, Jo-1 and Sm (purified Sm or SmD3 peptide in the case of EliA SymphonyS. All but one (supplier 1) include Centromere protein B. EliA CTD Screen as well as the tests from supplier 2 and 3 include dsDNA, and EliA CTD Screen and the test from supplier 3 include further markers for connective tissue diseases (see box, according to the suppliers' websites).

The assays were compared by using 404 clinically defined samples from patients with different connective tissue diseases, as well as 229 patients with different non-autoimmune diseases as controls. Here it should be mentioned that, at a ratio of 404:229, the proportion of CTD patients versus non-CTD patients in this cohort is much higher than in any routine situation. A proportion of 0.5-5% - depending on the patient background present in the laboratory - of connective tissue disease patients is more realistic in a routine diagnostic cohort. The more non-CTD patients are included, the more obvious is the relevance of specificity, even when used as first line testing.

The data show that EliA SymphonyS has a slightly increased sensitivity compared to EliA Symphony, due to the use of an improved coating method which resulted in better antigen presentation and better accessibility of epitopes. As expected, the main improvements in sensitivity were observed in the Systemic Lupus Erythematosus and Scleroderma cohort (table 5).

Table 5: Sensitivity of EliA SymphonyS and EliA Symphony in an SLE cohort (n=97) and a Scleroderma cohort (n=87).

	EliA Symphony[S]	EliA Symphony
Sensitivity in SLE	59.8%	58.8%
Sensitivity in Scleroderma	67.8%	64.4%

Improved sensitivity for SmD antibodies

In our cohort of 404 samples from patients with connective tissue diseases, there were only three samples which were monospecific positive for SmD antibodies. Most SmD samples also contain other antibodies like Ro52, Ro60, U1RNP or La. However, these three samples were negative in the current EliA Symphony but clearly positive for EliA SymphonyS due to the improved sensitivity for these antibodies (see table 6).

Table 6: 3 samples with SmD antibodies, positive in EliA SymphonyS but negative in EliA Symphony.

Sample	EliA Symphony^S ratio cut-off 1.0	EliA Symphony ratio cut-off 1.0	EliA SmDP in U/ml cut-off 10
1	6.73	0.45	137.7
2	1.47	0.41	14.1
3	1.06	0.23	11.8

EliA SymphonyS is used in combination with EliA dsDNA

Of course, the three tests which do not include dsDNA (EliA SymphonyS, EliA Symphony and the test from supplier 1) have a lower sensitivity than the three tests including dsDNA (EliA CTD Screen and the tests from suppliers 2 and 3), but on the other hand, all non-dsDNA tests are clearly superior in terms of specificity, with EliA SymphonyS having the highest specificity (93%). Of the dsDNA-containing screening tests, only EliA CTD Screen has a good specificity of almost 90% (figure 1).

Bearing in mind the routine approach of an immunology laboratory, the results of EliA SymphonyS and EliA Symphony were combined with a specific dsDNA test (see figure 2). Unfortunately, the results of the screening test from supplier 2 could not be combined with a specific anti-dsDNA test as the sera were not available in sufficient volume. This study reflects the expected improvement in the performance of EliA SymphonyS in routine. Both, EliA CTD Screen and the combination of EliA SymphonyS with EliA™ dsDNA showed the highest specificity and highest positive likelihood ratio (figure 2). EliA Scl-70S test was a logical and necessary consequence.
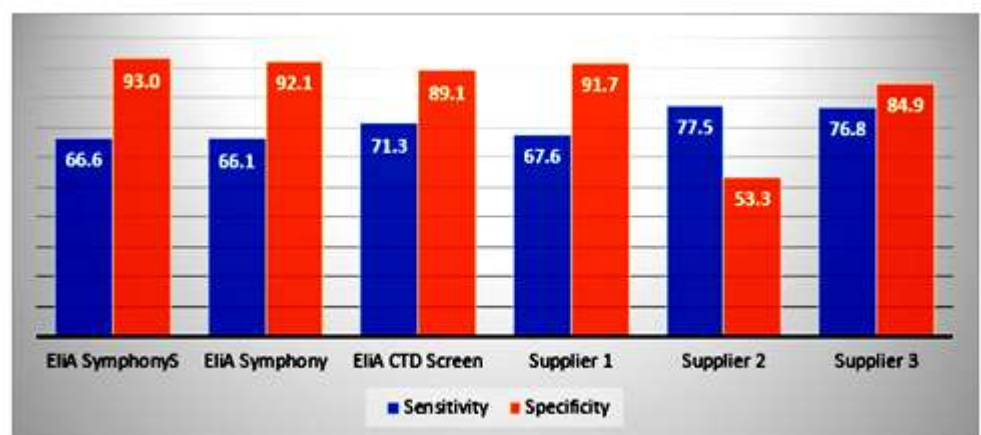

Figure 1: Sensitivity & specificity of EliA SymphonyS, EliA Symphony, EliA CTD Screen and ENA screen tests of 3 other suppliers[8].
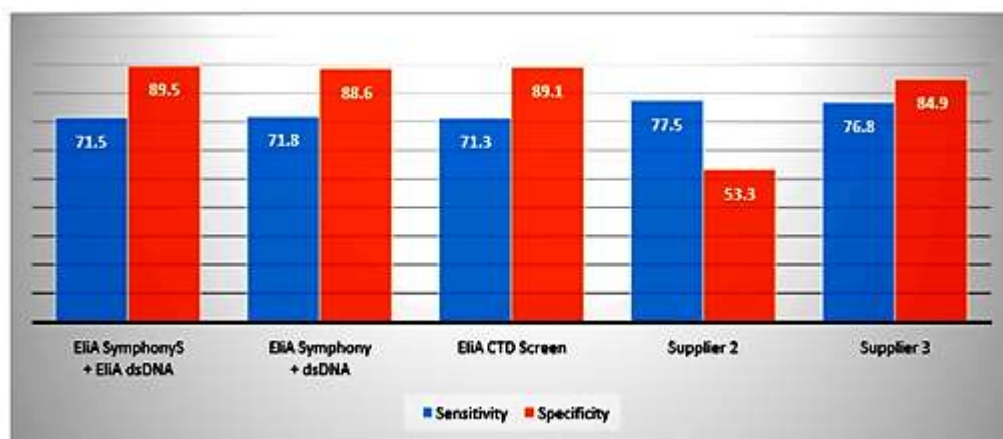
Figure 2: Sensitivity and specificity of EliA SymphonyS plus EliA dsDNA, EliA Symphony plus EliA dsDNA, and of three different dsDNA–containing screening tests[8].

Is specificity important for a screening test?

In the diagnosis of connective tissue diseases, screening tests are used to rule out autoimmune diseases. Therefore, doctors expect high sensitivity from a screening test in order not to miss any patient with connective tissue diseases, while the specificity is usually seen as unimportant. However, this approach is risky, particularly in rare diseases such as the connective tissue diseases. As the pre–test probability is often less than 1%, a non–specific screening test is far more often falsely positive than correctly positive (low positive predictive value). Although a screening test is not meant to be decisive for the diagnosis of any disease, it is often used as such, which leads to a high number of false diagnoses. Up to 50% of patients diagnosed with SLE because of ANA–IIF positivity do not have SLE.6, 7. In addition to the 633 clinically defined samples listed above, 400 healthy blood donors were tested with EliA SymphonyS. Seven out of the 400 samples gave a positive result. In further analysis, all these samples contained specific antibodies, as shown in table 7. Therefore, the results were technically correctly positive, as the blood donors really did have these autoantibodies. However, without clinical symptoms, a single positivity of antinuclear antibodies is not diagnostically significant. It remains to be studied, whether individuals with (high titre and persistent) antinuclear antibodies will develop a connective tissue disease in the long–term follow–up.

Table 7: Results of seven samples from apparently healthy blood donors positive in EliA SymphonyS[8].

7 samples positive	EliA Symphony^S [Ratio]	Result
1	1.2	EliA Ro52 positive
2	2.4	EliA U1RNP positive
3	33.7	EliA Ro52 and Ro60 positive
4	11.4	EliA Ro60 positive
5	1.1	EliA U1RNP positive
6	23.0	EliA U1RNP and Ro60 positive
7	25.9	EliA CENP positive

EliA SymphonyS Conclusions

Well-known EliA quality, first fully recombinant ENA screening test

High specificity and therefore high clinical accuracy

EliA SymphonyS and single EliA ENA perfectly aligned

Increased sensitivity at maintained specificity

Fully automated. Can be run on:
- PhadiaTM 100 instrument
- PhadiaTM 250 instrument
- PhadiaTM 2500 instrument
- PhadiaTM 5000 instrument

References

1. Mahler M, Stinton LM, Fritzler MJ. Improved serological differentiation between systemic lupus erythematosus and mixed connective tissue disease by use of an SmD3 peptide-based immunoassay. Clin Diag Lab Immunol. 2005; 12:107-113.
2. Mahler M, Fritzler MJ, Blüthner M. Identification of a SmD3 epitope with a single symmetrical dimethylation of an arginine residue as a specific target of a subpopulation of anti-Sm antibodies. Arthritis Res Ther. 2005;7: R19-R29 (DOI 10.1186/ar1455).
3. Van den Hoogen FHJ, van de Putte LBA. Anti-U1snRNP antibodies and clinical associations. In: vanVenrooij WJ, Maini RN (eds), Manual of Biological Markers of Disease. Kluwer Academic Publishers, Dordrecht. 1996; pp C3.1, 1-8.
4. Peng SL, Craft JE. Spliceosomal snRNPs autoantibodies. In: Peter JB, Shoenfeld Y (eds), Autoantibodies. Elsevier, Amsterdam. 1996; pp 774-782.
5. Van den Hoogen F, Khanna D, Fransen J, et al. Classification Criteria for Systemic Sclerosis. Arthritis Rheum. 2013; 65:2737-2747.
6. Rasmussen A, Radfar L, Lewis D, et al. Previous diagnosis of Sjögren's Syndrome as rheumatoid arthritis or systemic lupus erythematosus. Rheumatology (Oxford). 2016;1195-1201.
7. Narain S, Richards HB, Satoh M, et al. Diagnostic accuracy for lupus and other systemic autoimmune diseases in the community setting. Arch Intern Med. 2004;164:2435-2441.
8. Unpublished, internal study by our Research and Development department in Freiburg.
9. Wenzel J, et al. Antibodies targeting extractable nuclear antigens. Br J Dermatol 2001;145(6):859-67
10. Peng SL, Craft JE. Sm antibodies. In: Peter JB, Shoenfeld Y (eds), Autoantibodies 1996; pp 774-782, Elsevier, Amsterdam
11. Benito-Garcia E, et al Guidelines for Immunologic laboratory testing in the rheumatic diseases: anti-Sm and anti-RNP antibody tests. Athritis Rheum 2004;51:1030-1044

The role of IL-6 in inflammation and disease

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IL-6 in Signaling and Inflammation

IL-6 is a pleiotropic pro-inflammatory multifunctional cytokine synthesized by both hematopoietic (T cells, monocytes and macrophages) and non-hematopoietic (fibroblasts and endothelial cells). The IL-6 gene was first cloned in 1986 by Hirano, et al.. [1] and subsequent research progressively uncovered its signaling pathways. IL-6 signal transduction occurs through a hexameric high- affinity complex of IL-6, IL-6 receptor (IL-6R) and glycoprotein 130, represented as gp130 [2]. IL-6 exists in both soluble and insoluble forms and its signal transduction pathways comprises both classical and trans-signaling pathways. The downstream effects of IL-6 signaling involve phosphorylation and activation of Janus kinase 1 (JAK1), JAK2, tyrosine kinase 2, and STAT3, which is then followed by transcription of genes involved in mediating local and systemic inflammatory responses. The relevance of IL-6 in a variety of cell types has led to discovery of its roles in diverse physiological processes such as T-cell activation; induction of acute phase proteins; stimulation of hematopoietic precursor cell growth and differentiation; proliferation of hepatic, dermal, and neural cells; bone metabolism; lipid metabolism; atherosclerosis; hepatoprotection; and fibrosis [3]. Circulating IL-6 is found in the blood of healthy humans at low concentration (≤ 1 pg/mL), and significantly increases during inflammatory conditions, reaching concentrations in the range of μg/mL in disease states (for e.g. sepsis).

IL-6 has been shown to be generated in response to environmental stress factors such as infections and tissue injuries [4]. It is an inducer of the acute phase response where it is known to stimulate the production of acute phase proteins such as C-reactive protein (CRP), serum amyloid A, fibrinogen, haptoglobin, and α1-antichymotrypsin [5]. Expression of the acute phase proteins are presumed to be part of the physiological response to infections and inflammation, thus the ability of IL-6 to induce expression of these proteins would suggest that it is an early indicator of an insult or injury. Time course analyses of IL-6 in relation to other biomarkers show that IL-6 levels elevate earlier than CRP or PCT in response to a bacterial infection, although its half-life is shorter than that of CRP or PCT. In fact, serum levels of IL-6 reflected the severity of organ dysfunction in critically ill patients most accurately compared to PCT and CRP [6]. In this regard, IL-6 was seen to elevate soonest from the insult and reached its peak earlier than the Sequential Organ Failure Assessment (SOFA) score used for diagnosis of sepsis [6]. It has been shown that if the free serum concentration of tocilizumab, a humanized antibody to IL-6R that inhibits IL-6 signaling, is maintained at more than 1 μg/ml, CRP remains negative [7]. These data indicate that IL-6 plays a major role in the induction of CRP expression and may be involved in the pathological development of almost all chronic inflammatory diseases with CRP elevation. However, the exact trigger and mechanisms behind such pathological conversion to chronic inflammation is still not fully understood.

As the IL-6 signaling pathway contributes to a variety of homeostatic and pathogenic roles in physiology disease, there has been extensive debate over when and how to target IL-6 signaling in disease [8]. Pharmacological inhibitors of IL-6 signaling prevent IL-6 from binding to IL-6R by targeting either the cytokine itself or its receptor. Il-6 blockade is effective for some disease states but not others. This review will focus on the role of IL-6 in mediating inflammation in a few critical diseases where IL-6 blockade is currently used as a therapeutic modality, such as SARS-CoV-2 infection and rheumatoid arthritis, and diseases where IL-6 signaling has been implicated but not used as a therapeutic modality, such as inflammatory bowel disease and cancer.

IL-6 in the pathogenesis of diseases

The ability of IL-6 to induce acute phase proteins, as well as its role in mediating inflammatory response, implicates dysregulated IL-6 signaling in the pathogenesis of multiple, diverse inflammatory diseases from rheumatic conditions to manifestations of cytokine storms in infections and the hyperplasia and neoplastic processes associated with malignancy. One example of an inflammatory disease where IL-6 is a key player is systemic juvenile idiopathic arthritis (sJIA). Studies on sJIA have shown that over production of IL-6 explains most, if not all of the clinical and laboratory features of the disease, including fever spikes, anemia, growth impairment and systemic osteoporosis [2]. As proof-of-principle, a phase III clinical trial recently demonstrated that IL-6 inhibition with tocilizumab resulted in improvements in sJIA with improved growth velocity leading to catch-up growth in patients [9] and tocilizumab is currently approved for use in Japan for sJIA. Beyond autoimmune diseases, another example of IL-6 driving inflammatory signaling towards pathogenic outcomes would be Castleman disease. Castleman disease is a lymphoproliferative disorder where there is over-production of IL-6 from the germinal centers of hyperplastic lymph nodes, and serum IL-6 concentrations have been shown to correlate with clinical abnormalities that may lead to malignancies like lymphoma [10]. Siltuximab (EU and USA), a chimeric monoclonal antibody to IL-6, and tocilizumab (Japan) have been approved for the treatment of Castleman disease [2].
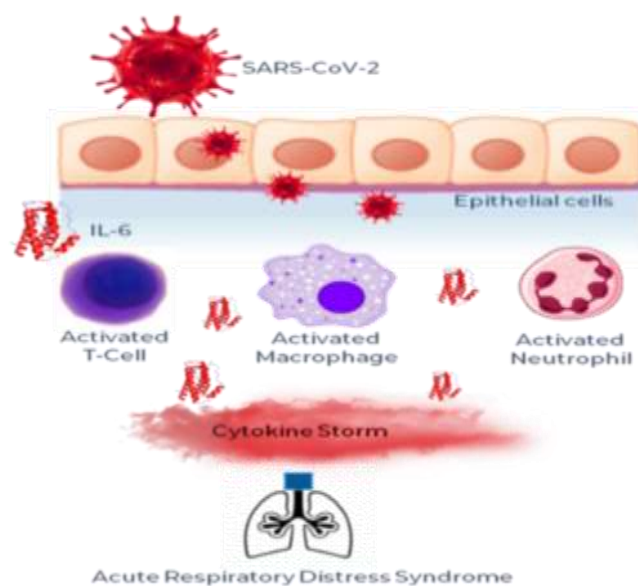

Figure 1. Figure depicting the players in the cytokine storm syndrome that can lead to acute respiratory distress syndrome in SARS-CoV-2 infection

The role of IL-6 in SARS-COV-2 infection and sepsis

Cytokine storm is a general term applied to maladaptive cytokine release in response to infection and other stimuli [11]. Although a rapid and well-coordinated immune response is the first line of defense against any infection, in a cytokine storm situation, cells are triggered to secrete high levels of pro-inflammatory cytokines as a result of loss of regulatory control at local and systemic levels, through a process that is complex and still not completely understood. This phenomenon has most recently been reported in SARS-CoV-2 respiratory infections where respiratory cells secrete pro inflammatory cytokines such as interleukin (IL)-1β and IL-6, propagating dysregulated and excessive immune responses that sustain a vicious cycle of immune attack, which may lead to or exacerbate the acute respiratory distress syndrome.

High SARS-CoV-2 viral load has been associated with elevated levels of pro inflammatory cytokines contributing to the hyper-inflammatory state characteristic of a severe infection. While it is acknowledged that there is wide heterogeneity in manifestation and phasing of disease, the trend of inflammatory cytokines like TNF-· , IFN-· , IL-2, IL-4 and IL-6 have been observed in such patients presenting with hyper-inflammation [12].

In a study of 63 SARS-COV-2 pneumonia patients, the IL-6 level in patients upon hospital admission was important in predicting disease severity and was associated with the length of hospitalization [13]. In a separate study of 53 patients from a long-term care facility, the concentration of IL-6 > 24 pg/mL at initial assessment predicted the development of hypoxemia requiring hospitalization with excellent sensitivity (100%) and good specificity (88.9%). Positive and negative predictive values were 76.9% and 100% respectively [14]. In addition, IL-6 demonstrated potential as a prognostic marker as SARS-CoV-2 patients in IIb stage (characterized by cough, high fever, dyspnea, abnormal thoracic imaging, lymphopenia, and increased levels of inflammatory markers with hypoxemia) were observed with very high IL-6 levels just before entering stage III (clinical manifestations of a severe systemic inflammatory syndrome, culminating in severe respiratory failure with an unfavorable prognosis), 1 or 2 days later [15]. Such patterns were not observed with CRP levels, despite the positive correlation between IL-6 and CRP as discussed earlier in this review.

In a separate cohort of 50 patients diagnosed with SARS-COV-2 pneumonia with different degrees of disease severity [16], higher levels of IL-6 were also found in patients with more severe pneumonia according to CURB-65 scale (p = 0.001), with ICU mechanical ventilation requirements (p = 0.02), and who subsequently died (p = 0.003). Of the clinical and analytical parameters analyzed in the current study, the serum levels of IL-6 were the most effective predictor of disease severity. From the data obtained in ROC curve analysis, a cut-off point for serum IL-6 levels of 35 pg/mL was defined, above which both the risk of mortality (OR = 20.00, 95 % CI 4.214-94·912, p = 0.0001) and ICU admission (OR = 12.750, 95 % CI 2,159-75,3,3, p = 0.005) were increased.

Table 1. Clinical evaluation statistics for performance of Beckman Coulter Access IL-6 identifying patients who were at increased risk of mechanical ventilation (PaO2/FiO2 ratio < 150 mmHg). Statistics shown were calculated based on a cutoff of 35 pg/mL and patients who had PaO2/FiO2 ratio < 150 mmHg, which is indicative of the risk for intubation with mechanical ventilation. The score approach was used to calculate the 95% confidence intervals.

Estimate (95% C.I)	
Sensitivity	85.4% (71.6 to 93.1%)
Specificity	64.7% (47.9 to 78.5%)
PPV	74.5 % (60.5 to 84.8%)
NPV	78.6% (60.5 to 89.8%

These data parallel the observations made in our own study of 75 RT-PCR confirmed SARS-CoV-2 patients who presented to the Emergency Department at the University Hospital Germans Trias I Pujol, a public research center in Spain [17]. The study enrolled adults who presented to the Emergency Department between March 18th and May 4th, 2020 with symptoms suggestive of SARS-CoV-2 infection and whose standard of care testing involved IL-6 and RT-PCR COVID-19 testing. Based on analysis of the data, an IL-6 level above 35 pg/mL accurately identified 85.4% of patients who had PaO2/FiO2 ratio < 150 mmHg (Table 1), which is indicative of the risk for mechanical ventilation [18]. The prevalence of the PaO2/FiO2 ratio <150 mmHg was 55% (41/75) in this cohort (Table 2) and the analysis is based on the first Beckman Coulter Access IL-6 value obtained at presentation to the ED. The data showed that PCR confirmed SARS-CoV-2 patients that have Beckman Coulter Access IL-6 concentration > 35 pg/mL at ED presentation are at increased risk for mechanical ventilation during their hospitalization. Nevertheless, IL-6 values should be used in conjunction with clinical findings and the results of other laboratory parameters. IL-6 values alone are not indicative of the need for intubation or mechanical ventilation.

Table 2. Number of patients with Beckman Coulter Access IL- 6 > or ≤ 35pg/ml and Pa02/Fi02 Ratio ≥ or < 150mmHg

	PaO2/FiO2 Ratio < 150 mmHg	PaO2/FiO2 Ratio ≥150 mmHg	Total patients
IL-6 > 35pg/mL	35	12	47
IL-6 ≤ 35 pg/mL	6	12	28

Extremely high levels of IL-6 were also strongly associated with the presence of septic shock or sepsis in SARS-CoV-2 patients. Maximal plasma IL-6 levels were significantly elevated in SARS-C0V-2 patients with high PCT levels compared to patients with low PCT levels [19].

In a separate study investigating the clinical value of IL-6, pentraxin 3 and PCT in patients with sepsis and septic shock [20], serum IL-6 levels could discriminate sepsis (area under the curve [AUC], 0.83-0.94, P< 0.001; cut-off value, 52.60 pg/mL, 80.4% sensitivity, 88.9% specificity) from controls and could distinguish septic shock (AUC, 0.71-0.89; cut-off value, 348.92 pg/mL, 76.1% sensitivity, 78.4% specificity) from sepsis. In comparison to pentraxin and PCT, IL-6 displayed superior diagnostic and prognostic value for sepsis and septic shock in this study [20]. Clinical trials evaluating the efficacy of blocking IL-6 signaling in SARS-CoV-2 have so far delivered mixed results. Optimal use of tocilizumab is likely to be in combination with glucocorticoids [21], as recent clinical trials showed clinical benefit in 15 – 20% of patients if IL-6 blockade was administered early after hospitalization and used in combination with dexamethasone [22]. However, there are scenarios where blockade of IL-6 may have limited utility. In a recent phase III trial which included patients with hospitalized with severe SARS-CoV-2 pneumonia, the use of tocilizumab did not result in significantly better clinical status or lower mortality than placebo at 28 days [23]. IL-6 blockade too early in the disease course may also be unsuitable, due to its role as a regulator of immune signaling. Inhibition of IL-6 in the early stages of disease may disrupt the development of robust anti-viral T cell responses [21]. Nevertheless, current guidance from the WHO and CDC recommends usage of IL-6R blockages under specific guidelines although this may be subject to change with evolving clinical evidence [24, 25].

The role of IL-6 in the pathogenesis of joint damage and extra-articular manifestations in rheumatoid arthritis (RA)

IL-6 is essential for CD4+ T-lymphocyte differentiation to T helper 17 cells, and it inhibits the development of TGF-β-induced regulatory T-cells [26] in RA, an auto immune disease characterized by chronic inflammation and progressive joint destruction. The imbalance of this ratio of T helper 17 cells and regulatory T cells, with increased levels of T helper 17 cells, is thought to play a major role in RA development. IL-6 induces B-cell differentiation and is associated with increased auto-antibody secretion and B cell activation in RA patients [27]. Through these mechanisms, IL-6 was shown to be a potential key player in osteoporosis, cartilage destruction and synovial inflammation associated with RA [28, 29]. RA patients show elevated IL-6 levels in synovial fluid and blood, which correlate with disease activity and structural damage progression [30], where associations were observed between IL-6 and C reactive protein and between the Ritchie articular index and duration of morning stiffness [31].

In addition, the observed increase in levels of IL-6 in patients with RA, was shown to have a significant inverse correlation with bone mineral density measurement in a study by Meguid et al. in 2013 [32], suggesting an important role of IL-6 in the pathogenesis of pre-mature osteoporosis, systemic bone loss, and structural joints' damage . In this study, the role of IL-6 in developing pre-mature osteoporosis in RA patients was seen to be independent of age, duration of the disease, body mass index, and the drugs used. IL-6 was significantly positively correlated with pain, erythrocyte sedimentation rate, platelet counts, and anti-CCP level, which are markers of disease activity in RA.

Besides promoting joint inflammation and damage through effects on chondrocytes, osteoclasts, macrophages and fibroblasts, IL-6 also mediates systemic inflammation in RA leading to extra-articular manifestations with accompanying symptoms of fatigue, pain, morning stiffness and anemia. As IL-6 has multiple roles in the dysfunction of the immune and inflammatory systems, anti- IL-6R therapy has been shown to be able to relieve the above-mentioned symptoms and improve overall quality of life [2, 33]. In addition, there are common co-morbidities associated with extra-articular manifestations of RA which include cardiovascular disease, diabetes, infection, malignances, mood and mental disorders [33]. The reason for these co-morbidities in RA are complicated and not fully understood, but genetic associations affecting the expression of IL-6 R [34] may offer one explanation for the common pathogenic inflammatory processes that connect RA and co-morbidities such as CVD and diabetes. Currently, tocilizumab and sarilumab, both of which are antibodies against IL-6R, are approved for the treatment of rheumatoid arthritis.

The role of IL-6 in inflammatory bowel disease (IBD)

IBD is an idiopathic disease of gut inflammation that comprises Crohn's disease and ulcerative colitis. These conditions are characterized by local inflammation in the gut as well as extra gastrointestinal manifestations with a variety of symptoms that appear to be patient dependent. Serum IL-6 and soluble IL-6R levels were previously observed to be elevated and significantly correlate with CRP in both ulcerative colitis and Crohn's disease [35]. Evaluation of tocilizumab in a phase II randomized controlled trial for Crohn's disease achieved its primary end point of reduction in disease activity however, rare reports of adverse events of gastrointestinal perforations in concurrent trials for arthritis halted further development of tocilizumab for Crohn's disease [36] as the role of IL-6 in maintaining gut homeostasis required critical consideration prior to targeting IL-6 for IBD patients. While other cytokine inhibitors have since been approved by regulatory authorities for treatment of IBD, IL-6 remains a topic of discussion as a key cytokine to monitor and target as IBD patients progressively develop resistance to anti-tumor necrosis factor treatment.

The role of IL-6 in cancer

It is unsurprising that IL-6 is upregulated in hematological malignancies and solid tumors, as cancer has long been deemed to be an inflammatory disease [37]. Elevated IL-6 serum levels in Hodgkin lymphoma has been shown to correlate with symptoms, response rate and survival in adult patients [38]. Similarly, serum IL-6 levels have been shown to be elevated in patients with untreated metastatic or castration-resistant prostate cancer, suggesting that IL-6 can play a major role in the transition from hormone-dependent to castration-resistant prostate cancer. This occurs most notably through accessory activation of the androgen receptor and levels of IL-6 in such patients correlate negatively with tumor survival and response to chemotherapy [39]. The role of IL-6 in oncogenesis has also been well established in colorectal cancer, which is the third most common cancer globally [40], where activation of the JAK/STAT3 pathway by IL-6 is associated with the neoplastic phenotype of colorectal cancer cells [41].

Elevated levels of IL-6 are found both in serum and tumor tissue and closely correlates with tumor stage, size, metastasis and patient survival [42]. While it is generally acknowledged that elevated levels of IL-6 can correlate with tumor burden as discussed in this section, to date, there has been no approved therapies utilizing IL-6 blockade for cancer therapy.

Conclusion

Substantial advances have been made in translating the biology of IL-6 to disease treatment and management. Nevertheless, targeting IL-6 remains the subject of much debate as it can pivot from extremely effective therapy to causing adverse events due to its pleiotropic role in regular physiology and disease. Furthermore, as discussed earlier in this review, clinical trials with tocilizumab for SARS-C0V-2 patients display mixed results. However, the role of IL-6 as a marker of inflammation across different inflammatory diseases remains undisputed and the World Health Organization and the NIH have updated their patient care guidelines for SARS-CoV-2 to include IL-6R blockers[24, 25].

With the advent of the SARS-CoV-2 pandemic, some clinical laboratories have started to adopt high throughput automated testing of serum IL-6 levels in SARS-CoV-2 patients. As data is continually emerging on the ability of IL-6 to assess worsening clinical features and disease progression, there have been suggestions by investigators to perform immediate evaluation of IL-6 levels upon hospital admission [43] and kinetic IL-6 quantification in order to predict patient outcomes [15].

It has been 40 years since IL-6 was discovered and it has been a long, rich history of translational research linking IL-6 biology to management and treatment of a diverse range of inflammatory diseases [2]. However, much work remains to be done, to further understand why some diseases like rheumatoid arthritis respond well to IL-6 blockade and some do not. Increased understanding of how to therapeutically target IL-6 across different diseases and how this will affect its pleiotropic downstream signaling pathways will further improve the treatment and management of inflammatory diseases while maintaining functional physiology.

References

1. Hirano, T., et al.., Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature, 1986. 324(6092): p. 73-6.
2. Choy, E.H., et al.., Translating IL-6 biology into effective treatments. Nat Rev Rheumatol, 2020. 16(6): p. 335-345.
3. Schmidt-Arras, D. and S. Rose-John, IL-6 pathway in the liver: From physiopathology to therapy. J Hepatol, 2016. 64(6): p. 1403-15.
4. Tanaka, T., M. Narazaki, and T. Kishimoto, IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol, 2014. 6(10): p. a016295.
5. Heinrich, P.C., J.V. Castell, and T. Andus, Interleukin-6 and the acute phase response. Biochem J, 1990. 265(3): p. 621-36.
6. Shimazui, T., et al.., Serum levels of interleukin-6 may predict organ dysfunction earlier than SOFA score. Acute Med Surg, 2017. 4(3): p. 255-261.

7.   Nishimoto, N., et al.., Mechanisms and pathologic significances in increase in serum interleukin-6 (IL-6) and soluble IL-6 receptor after administration of an anti-IL-6 receptor antibody, tocilizumab, in patients with rheumatoid arthritis and Castleman disease. Blood, 2008. 112(10): p. 3959-64.

8.   Garbers, C., et al.., Interleukin-6: designing specific therapeutics for a complex cytokine. Nat Rev Drug Discov, 2018. 17(6): p. 395-412.

9.   De Benedetti, F., et al.., Catch-up growth during tocilizumab therapy for systemic juvenile idiopathic arthritis: results from a phase III trial. Arthritis Rheumatol, 2015. 67(3): p. 840-8.

10. Dispenzieri, A. and D.C. Fajgenbaum, Overview of Castleman disease. Blood, 2020. 135(16): p. 1353-1364.

11. Ye, Q., B. Wang, and J. Mao, The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. J Infect, 2020. 80(6): p. 607-613.

12. Shenoy, S., SARS-CoV-2 (COVID-19), viral load and clinical outcomes; lessons learned one year into the pandemic: A systematic review. World J Crit Care Med, 2021. 10(4): p. 132-150.

13. Hong, W., et al.., Critically Ill vs. Non-Critically Ill Patients With COVID-19 Pneumonia: Clinical Features, Laboratory Findings, and Prediction. Front Cell Infect Microbiol, 2021. 11: p. 550456.

14. Sabaka, P., et al.., Role of interleukin 6 as a predictive factor for a severe course of Covid-19: retrospective data analysis of patients from a long-term care facility during Covid-19 outbreak. BMC Infect Dis, 2021. 21(1): p. 308.

15. Santa Cruz, A., et al.., Interleukin-6 Is a Biomarker for the Development of Fatal Severe Acute Respiratory Syndrome Coronavirus 2 Pneumonia. Front Immunol, 2021. 12: p. 613422.

16. Guirao, J.J., et al.., High serum IL-6 values increase the risk of mortality and the severity of pneumonia in patients diagnosed with COVID-19. Mol Immunol, 2020. 128: p. 64-68.

17. In Instructions for Use for Access IL-6 (Part Number C72294) Data on file Beckman Coulter, Inc.

18. Herold, T., et al.., Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. J Allergy Clin Immunol, 2020. 146(1): p. 128-136 e4.

19. Huang, L., et al.., Sepsis-associated severe interleukin-6 storm in critical coronavirus disease 2019. Cell Mol Immunol, 2020. 17(10): p. 1092-1094.

20. Song, J., et al.., Diagnostic and prognostic value of interleukin-6, pentraxin 3, and procalcitonin levels among sepsis and septic shock patients: a prospective controlled study according to the Sepsis-3 definitions. BMC Infect Dis, 2019. 19(1): p. 968.

21. Ascierto, P.A., B. Fu, and H. Wei, IL-6 modulation for COVID-19: the right patients at the right time? J Immunother Cancer, 2021. 9(4).

22. Jones, S.A. and C.A. Hunter, Is IL-6 a key cytokine target for therapy in COVID-19? Nat Rev Immunol, 2021. 21(6): p. 337-339.

23. Rosas, I.O., et al.., Tocilizumab in Hospitalized Patients with Severe Covid-19 Pneumonia. N Engl J Med, 2021. 384(16): p. 1503-1516.

24. NIH. Interleukin-6 Inhibitors. 2021 [cited 7 Aug 2021].

25. WHO. WHO recommends life-saving interleukin-6 receptor blockers for COVID-19 and urges producers to join efforts to rapidly increase access. 6 July 2021[cited 2021 7 Aug].

26. Lazzerini, P.E., et al.., Spotlight on sirukumab for the treatment of rheumatoid arthritis: the evidence to date. Drug Des Devel Ther, 2016. 10: p. 3083-3098.

27. Gottenberg, J.E., et al.., Serum IL-6 and IL-21 are associated with markers of B cell activation and structural progression in early rheumatoid arthritis: results from the ESPOIR cohort. Ann Rheum Dis, 2012. 71(7): p. 1243-8.

28. Guerne, P.A., et al.., Synovium as a source of interleukin 6 in vitro. Contribution to local and systemic manifestations of arthritis. J Clin Invest, 1989. 83(2): p. 585-92.

29. Jilka, R.L., et al.., Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science, 1992. 257(5066): p. 88-91.

30. Burska, A., M. Boissinot, and F. Ponchel, Cytokines as biomarkers in rheumatoid arthritis. Mediators Inflamm, 2014. 2014: p. 545493.

31. Madhok, R., et al.., Serum interleukin 6 levels in rheumatoid arthritis: correlations with clinical and laboratory indices of disease activity. Ann Rheum Dis, 1993. 52(3): p. 232-4.

32. Abdel Meguid, M.H., et al.., Relation of interleukin-6 in rheumatoid arthritis patients to systemic bone loss and structural bone damage. Rheumatol Int, 2013. 33(3): p. 697-703.

33. Favalli, E.G., Understanding the Role of Interleukin-6 (IL-6) in the Joint and Beyond: A Comprehensive Review of IL-6 Inhibition for the Management of Rheumatoid Arthritis. Rheumatol Ther, 2020. 7(3): p. 473-516.

34. Ferreira, R.C., et al.., Functional IL6R 358Ala allele impairs classical IL-6 receptor signaling and influences risk of diverse inflammatory diseases. PLoS Genet, 2013. 9(4): p. e1003444.

35. Mitsuyama, K., et al.., Soluble interleukin-6 receptors in inflammatory bowel disease: relation to circulating interleukin-6. Gut, 1995. 36(1): p. 45-9.

36. Monemi, S., et al.., Incidence of Gastrointestinal Perforations in Patients with Rheumatoid Arthritis Treated with Tocilizumab from Clinical Trial, Postmarketing, and Real-World Data Sources. Rheumatol Ther, 2016. 3(2): p. 337-352.

37. Coussens, L.M. and Z. Werb, Inflammation and cancer. Nature, 2002. 420(6917): p. 860-7.

38. Bhethanabhotla, S., et al.., Prognostic Significance of IL-6 in Hodgkin Lymphoma. Indian J Pediatr, 2019. 86(6): p. 551-554.

39. Nguyen, D.P., J. Li, and A.K. Tewari, Inflammation and prostate cancer: the role of interleukin 6 (IL-6). BJU Int, 2014. 113(6): p. 986-92.

40. WHO. Cancer. 2021 3 March 2021 [cited 2021 7 Aug].

41. Gordziel, C., et al.., Both STAT1 and STAT3 are favourable prognostic determinants in colorectal carcinoma. Br J Cancer, 2013. 109(1): p. 138-46.

42. Knupfer, H. and R. Preiss, Serum interleukin-6 levels in colorectal cancer patients-- a summary of published results. Int J Colorectal Dis, 2010. 25(2): p. 135-40.

43. Ulhaq, Z.S. and G.V. Soraya, Interleukin-6 as a potential biomarker of COVID-19 progression. Med Mal Infect, 2020. 50(4): p. 382-383.

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Practical Implementation of a Quality Approach for Serum Indices

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INTRODUCTION

The pre-analytical phase is an important phase where potential errors can have a significant impact on test results. This article outlines the background of serum indices interference. Most importantly, it emphasizes the unmet need for a strong quality control (QC) approach utilizing automated analyzer assessments. A practical QC approach is shared with examples on how to set up a Serum Indices QC program in your laboratory.

BACKGROUND

Hemolysis, icterus, and lipemia (HIL) are interference factors in biological samples that can contribute to inaccurate test results.

Hemolysis is the most well- known and described interference factor. It occurs when the red blood cells rupture and release their contents into surrounding fluid. As a result, hemolysis causes a reddish hue in the plasma and serum. Hemolysis is often an artefact of the blood collection, transportation, or storage issues.

Hemolysis can affect the photometric measurement results of specific analytes significantly, even at very low concentrations of interference.

Icterus is related to the increased concentration of bilirubin often associated with liver disease. As a result, a yellowish to brownish hue is present in the plasma and serum. High serum and plasma bilirubin concentrations cause interference with assays near the bilirubin absorbance peak.

Lipemia is characterized by an increased concentration of lipoprotein particles. It creates a milky or turbid appearance that interferes with multiple biochemical tests.

The three interferences described above, depending on the amount, concentration, and specific analyte, will bias the final analytical result.

Of note, even very low concentrations of interference can cause clinically relevant deviations from "real" values, and these cannot be detected visually. Undetected interference can be harmful to the patients because correct results are crucial for the accurate diagnosis and treatment of patients. It is therefore imperative to identify interfered samples upfront and to flag the result without any delay when communicating with the clinician.

In the past, HIL detection has mainly been performed through visual checks. It has been established that visual checks are unreliable. (1) For many currently utilized automated platforms, HIL detection is performed by the instrument.

"HIL indices" are the automated detection of the three interfering substances described above. HIL indices are commonly measured on automated instruments like chemistry or hemostasis analyzers. The majority of analyzers now have this functional capability.

NEED FOR SI "QC APPROACH"

Automatic HIL assessment to detect HIL in samples is crucial. As demonstrated by von Meyer et. al., HIL can significantly impact diagnostic results and serum indices should be subject to regular internal and external quality control procedures." (2)

There are several steps that are needed to implement QC for HIL in the laboratory. First, laboratory personnel need to define the number of levels and QC volume needed for the instruments. Additionally, they need to establish the frequency at which they need to run internal and external QC procedures and then define the statistical parameters to accurately evaluate the results. According to the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for Preanalytical Phase (WG-PRE) (3), at least two different levels for each of the three interfering substances should be used. Each material used should be as close as possible to the patient sample and specific for each of the three substances.

The initial target means and standard deviation (SD) or coefficient of variation (CV) for a new QC material can be established by collecting 20 QC results over a timeframe of 20 days. During these 20 days, the instrument should be in a stable, control state and mimic routine operation as close as possible. It is also recommended to include calibration and maintenance events during this initial study phase to include as much variation as expected during the normal instrument routine. If the lab does not have 20 days to perform this study, a shorter time frame can be used.

The EFLM working group recommends running 20 replicates for each interfering substance in one run on the same day. Once these targets are established, laboratory personnel should continue to monitor these values. It is important to update the SD when more data has been collected over time because the SD can have a lower reliability due to the short time interval when estimating this value. This will include more long-term sources of variation such as reagent lot changes, maintenance, calibration cycles, and other environmental influences. When the long-term SD is available, a new estimation of the mean (when starting a new lot number of QC material) can be established by collecting 10 QC results over a 10-day timeframe. The historical long-term SD or CV from the current lot number can then be copied as target for the new QC lot number (4).

The EFLM working group recommends running this control at least 2 times a day in a single run (same as patient samples), ideally before starting the morning analytical session,8 to 12 hours after the initial QC, or at the end of the analytical session. Moreover, each QC result should be evaluated using the 1-3s QC rule, this rule will reject any QC results with a deviation higher or lower than 3 SD from the mean. Additionally, other QC rules can also be applied for continued assurance and timely detection of deteriorating performance such as the 2-2s, R4s, or 4-1s QC rules. HIL QC should be integrated in the laboratory QC data management system which will provide the same QC evaluation and monitoring tools already used for other clinical chemistry analytes.

These data management solutions can provide automated data import with QC rule evaluation, overview tables, QC reports, QC charts (e.g., Levey-Jennings charts and Bar charts), and many other features which allow laboratories to review their QC data. The use of these software solutions is highly recommended as they streamline and automate the QC review process and therefore reduce the possibility for human error or incorrect evaluation.

Fig 1: Example of a Levey-Jennings chart used in Unity Real Time QC data management software (Bio-Rad Laboratories). The LJ chart visualizes the QC results graphed against the mean and SD.
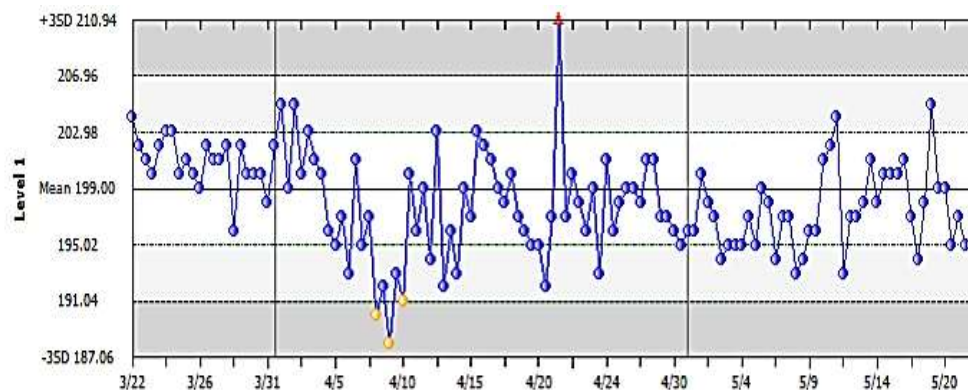
QC troubleshooting should closely follow the regular clinical chemistry analytical processes. When a QC result falls outside the acceptable ranges, the first action is to identify whether this is due to a QC product failure or an instrument failure. This can be done by taking a new QC aliquot and repeating the QC measurement. If that result passes within the acceptability limits, it can be assumed that the instrument performs within specifications and deterioration of the QC material might have been the cause of the first failure. If the new aliquot confirms the interfering substance(s), all reporting of results should be stopped, and further troubleshooting started. The system can be corrected by replacing dilution solutions, performing blank calibrations, performing maintenance, or other instrument specific actions. If these actions do not provide an acceptable solution, the instrument manufacturer might need to intervene. During this period, laboratories can temporarily fall back on a basic visual inspection of the samples (5).

SUMMARY

It is important to monitor an instrument's ability to continuously and correctly detect HIL interferences. Due to the impact of HIL on test results and ultimately patient care, this necessitates the need for monitoring HIL indices. Guidance has been developed that applies a practical, QC approach for the use of materials for assessing the performance of automated HIL detection systems.

REFERENCES

1. Unreliable visual estimation of the incidence and amount of turbidity, hemolysis, and icterus in serum from hospitalized patients. Glick MR, Ryder KW, Glick SJ, Woods JR. Clin Chem 1989;35:837-9.
2. For more transparency in manufacturers declarations on serum indices: On behalf of the Working Group for Preanalytical Phase (WG-PRE), European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) von Meyer A, Cadamuro J ;Lippi G, Simundic AM   Clinica Chimica Acta Volume 484, September 2018, Pages 328-332 https://doi.org/10.1016/j.cca.2018.03.043
3. Local quality assurance of serum or plasma (HIL) indices. Lippi G, Cadamuro J, von Meyer A, Simundic AM, on behalf of the EFLM Working Group for Preanalytical Phase (WG-PRE) Clin Biochem 2018;54:112-8 https://www.sciencedirect.com/science/article/pii/S000991201830095X
4. C24 A4: Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions, 4th Edition. CLSI., Wayne, PA, 2016.
5. C56-A: Hemolysis, Icterus, and Lipemia/Turbidity Indices as Indicators of Interference in Clinical Laboratory Analysis. Approved Guideline. CLSI., Wayne, PA, 2012.

Reticulocyte Hemoglobin Concentration (Chr) and Hypochromic Erythrocytes Percentage (%Hypo) in Screening Test for Iron Deficiency Anemia in Cancer Patients

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ABSTRACT

Introduction: The early detection of iron deficiency anemia (IDA) can significantly enhance the treatment effectiveness as well as improve the living standards of cancer patients. The aim of this study was to evaluate the feasibility of using %HYPO in screening IDA in cancer patients.

Objectives: The Receiver Operating Curve (ROC) and the Area under the Curve (AUC) were utilized to determine the value of CHr and %HYPO as a screening test for IDA in cancer patients with anemia. The Youden index was used to determine the optimal threshold value.

Method: A retrospective cross-sectional descriptive study of 347 participants, comprising healthy people (178), patients with IDA (39) and cancer patients with anemia (130), was conducted at Cho Ray Hospital.

Results: Screening of IDA in cancer patients was performed using both CHr and %HYPO. Combining both CHr and %HYPO resulted in a better differentiation between IDA and non-IDA amongst cancer patients (p<0.05), and AUC = 0.686; 95% CI: 0.592 – 0.780 (p<0.001); corresponding to predicted values of CHr $\leq$ 29 pg (Sensitivity: 46%; Specificity: 74%) and %HYPO $\geq$ 8 (Sensitivity: 50%; Specificity: 76%). These predicted values are optimized for the screening of IDA in cancer patients. AUC of %HYPO on its own was observed to be a poor predictor of IDA in cancer patients. However, when combined with CHr, there was increased reliability in differentiating between IDA and non-IDA in cancer patients (p<0.003).

Conclusion: CHr and %HYPO in combination, both of which are cheap tests, appear to be useful in screening for IDA in cancer patients with anemia.

Key words: CHr: Concentration of Hemoglobin in Reticulocyte, HYPO: Hypochromic

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Introduction

Anemia is a common complication in patients with malignant tumors, especially in patients on chemotherapy. The main mechanisms causing anemia are diverse and complicated. The causes of anemia may be due to tumors or postoperative blood loss, iron deficiency, malnutrition, and chronic kidney disease. The gold standard for assessing iron status is bone marrow biopsy, an invasive procedure that is seldom used in normal practice. Therefore, to assess the patient's iron status, it is necessary to rely on indirect indicators such as Ferritin and %Transferrin saturation (TSAT). However, these tests do not reflect the correlation between bone marrow's ability to produce erythrocytes and iron reserves. Reticulocytes are the youngest erythrocytes that are 3 – 4 days old in the bone marrow and 1 – 2 days in peripheral blood before becoming adult erythrocytes. Reticulocytes can provide information about the bone marrow red blood cell production status and response to iron therapy. The percentage of asthenic erythrocytes shows the proportion of red blood cells with low hemoglobin, iron-deficient erythrocytes. The percentage of asthenic erythrocytes also reflects iron status several months prior to clinical manifestation. Our study of reticulocyte concentrations and percentage of erythrocytes, aimed at providing a low-cost, early screening solution for iron-deficiency anemia in cancer patients with anemia using basic tests, namely, concentration of hemoglobin in reticulocytes (CHr) and percentage of hypochromic red cells (%HYPO), has not been performed in Viet Nam.

Objectives

The ROC curve and Area Under the Curve (AUC) were utilized to determine the value of CHr and %HYPO in screening for IDA in cancer patients with anemia. The Youden index was used to determine the optimal threshold value.

Subjects And Methods

Study design: A retrospective cross-sectional descriptive study of 347 people was conducted at Cho Ray Hospital, from September 2019 to January 1, 2020. According to the procedure, 2 ml of EDTA anticoagulant blood were collected from participants to perform routine hematological tests, including: RBC, Hb, BC, PLT, MCV, MCH, MCHC, CHr, %HYPO (Complete Blood Count and Reticulocytes). Ferritin and Transferrin saturation (TSAT) were measured in patients with anemia. All hematological tests were performed on the ADVIA 2120i hematology analyzer (Siemens) in the Department of Hematology at Cho Ray Hospital.

Study subjects: Medical records in the hospital database were studied and the study subjects were divided into four specific groups for analysis.

Reference group: Subjects who had regular health check-ups, who did not have anemia and chronic diseases, attending the general clinics of Cho Ray Hospital.

Group of patients:

Non-cancer patients with iron deficiency anemia, selected as the control group, iron deficiency was diagnosed based on ferritin: female $\leq$ 15 ng/ml, male $\leq$ 20 ng/ml (according to WHO standards). Anemia cancer patients (according to ESMO anemia standard 2018)

• *Solid organ cancer patients with iron-deficiency anemia* with ferritin < 100 ng/ml and/or TSAT < 20% (standard iron deficiency according to ESMO 2018). Absolute iron deficiency (AID) is present when the ferritin concentration is < 20 ng/ml and/or TSAT < 20%; functional iron deficiency (FID) is present when the ferritin < 100 ng/ml and TSAT < 20%.

• *Solid organ cancer patients with anemia and without iron deficiency* with ferritin > 100 ng/ml and TSAT > 20%.

Exclusion criteria: Cancer patients who were diagnosed with anemia due to acute blood loss, metastatic bone marrow cancer or other causes.

Data processing: By using the software SPSS 20, data are shown as the average value ± standard deviation (SD) or median with inter-quartile range. The result is considered significant when $p<0.05$. The ROC and the AUC of the model were used to evaluate the potential of CHr and %HYPO and their combination as a screening tool for IDA in cancer patients with anemia. The optimal threshold value was determined by using the Youden index.

Research team characteristics: Of the 347 study participants, we obtained 178 reference samples, 39 non-cancer patients with iron-deficiency anemia and 130 patients with solid organ cancers with anemia. Of the 130 patients with solid organ cancers and anemia, 89 had IDA, accounting for 68.4%, with 27 patients having AID (20.8%) and 62 patients with FID (47.6%). Forty-one (41) of the 130 patients (32.6%) had iron-unrelated anemia. The 130 cancer patients with anemia were divided into cancer groups: The gastrointestinal group had 41 patients, the female genital cancer group had 19 patients, the male genital group had 1 patient, the hepatitis group had 25 patients, the lung group had 22 patients, the group with biliary, pancreatic and peritoneal cancers had 5 patients, the urinary group had 6 patients, the thymus and adrenal group had 1 patient, the oral and pharyngeal group had 4 patients, the metastatic cancer had 6 patients.

Table 1. Characteristic of the studied groups

Characteristic	Group	Reference (n = 178)	IDA (n = 39)	K[5] with anemia, non-ID (n = 41)	K[5] with IDA (n = 89)	
Ratio in K patient population (%)				31.6	68.4	
Gender	Female (%) (patients)	50 (89)	94.9 (37)	56 (23)	48 (43)	
	Male (%) (patients)	50 (89)	5.1 (02)	44 (18)	52 (46)	
Age	< 50 (%) (patients)	88.8 (158)	76.9 (30)	2 (01)	28 (25)	
	≥ 50 (%) (patients)	11.2 (20)	23.1 (09)	98 (40)	72 (64)	
Parameters (tests)	Hb (g/L) 2	144 (134–155)	93 (83–107)	96 (86–104)	95 (88–102)	
	MCV (fL) 2	91 (89–93)	71 (65–77)	94 (88–100)	89 (79–95)	
	CHr (pg) 1	31.2 (±1.2)	23 (±3.2)	32 (±3.7)	29 (±3.9)	
	%HYPO 1,2	2.5 (±1.7)	34.7 (17–59)	5.5 (2–7.6)	7.8 (4.1–19.5)	
	Ferritin 2 (ng/ml)	200–400	6.3 (4–16)	708 (381–1453)	AID 3 25.8 (13–48)	FID 4 760 (359–1362)
	TSAT 1 (%)	≥ 20	10.8 (±9)	37 (±21)	11.9 (±6)	

(1) In average (± Standard deviation)
(2) Median (inter-quartile range)
(3) Absolute Iron Deficiency
(4) Functional Iron Deficiency
(5) K is the abbreviation for cancer patients

The Mann-Whitney U non-parametric test was used to assess the differences amongst the study groups. Significance was achieved when p<0.05. In particular, for CHr test, we found the difference with p<0.05(p=0.028) between data samples control group and non-iron deficiency anemia cancer group. The remaining difference between the other pairs had p<0.001 as: control group and IDA group; the control group and iron deficiency anemia cancer group; the non-iron deficiency anemia cancer group and IDA group; iron deficiency anemia cancer group and the non-iron deficiency anemia cancer group. Similarly, the difference amongst the study groups for %HYPO test was significant with p<0.001.
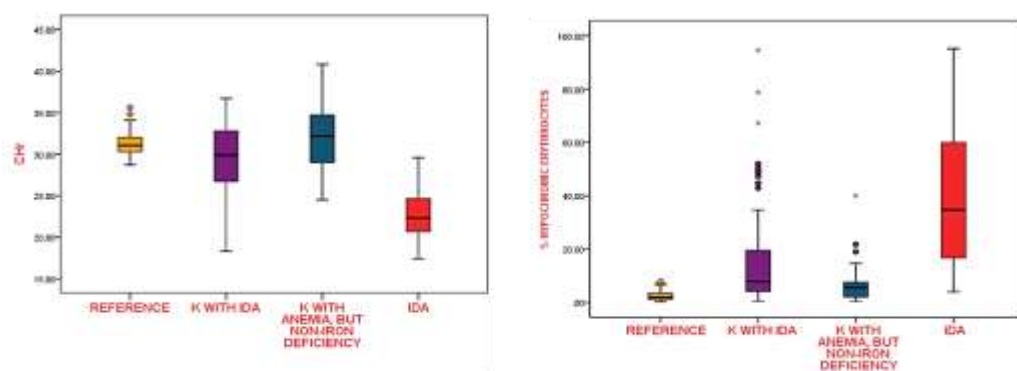

Figure 2.The average CHr and %HYPO of the study groups

The optimal threshold value of the group in IDA has CHr of 28 pg with a sensitivity of 95% and a specificity of 100%; AUC = 0.997; 95% CI: 0.993 – 1.000; and the %HYPO ≥ 8 with a sensitivity of 98% and a specificity of 100%; and AUC = 0.994; 95% CI: 0.983 – 1.000); p<0.001. The combination of the two tests resulted in an increase in AUC = 0.998, 95% CI: 0.993 – 1.000; p<0.001. Similarly, it is possible to differentiate between the iron-deficient anemic cancer patient and the reference group with CHr and %HYPO that have AUC = 0.611; 95% CI: 0.522 – 0.701; p<0.003 and AUC = 0.866; 95% CI: 0.818 –0.914; p<0.001, respectively. The combination of the two tests resulted in an increase in AUC = 0.870; 95% CI: 0.821 – 0.918; p<0.001, corresponding to the optimal threshold. A value of CHr ≤ 30 pg has a sensitivity of 53% and a specificity of 80%, and %HYPO > 4 has a sensitivity of 75% and a specificity of 81%. The combination of the two tests resulted in an increase in AUC = 0.870, 95% CI: 0.821 – 0.918; p<0.001. It is possible to between a non-iron deficient anemia cancer group and the reference group with AUC = 0.589; 95% CI: 0.452 – 0.726; p = 0.029 and AUC = 0.718; 95% CI: 0.609 – 0.826; p<0.001, respectively, corresponding to an optimal threshold value of CHr > 31.5 pg that has a sensitivity of 63% and a specificity of 66%, and %HYPO < 4 has a sensitivity of 75% and a specificity of 81%. The combination of the two tests resulted in an increase in AUC = 0.774, 95% CI: 0.677 – 0.871. Among the anemic cancer patients, CHr and %HYPO can differentiate between IDA and non-IDA corresponding to AUC for CHr = 0.639; 95% CI: 0.536 – 0.741 and AUC for %HYPO = 0.675; 95% CI: 0.579 – 0.771. The combination of the two tests resulted in an increase in AUC = 0.686, 95% CI: 0.592 –0.780; p=0.011, with a proposed threshold value of CHr ≤ 29 pg that has a sensitivity of 46% and a specificity of 74%, and the %HYPO ≥ 8 has a sensitivity of 50% and a specificity of 76%.
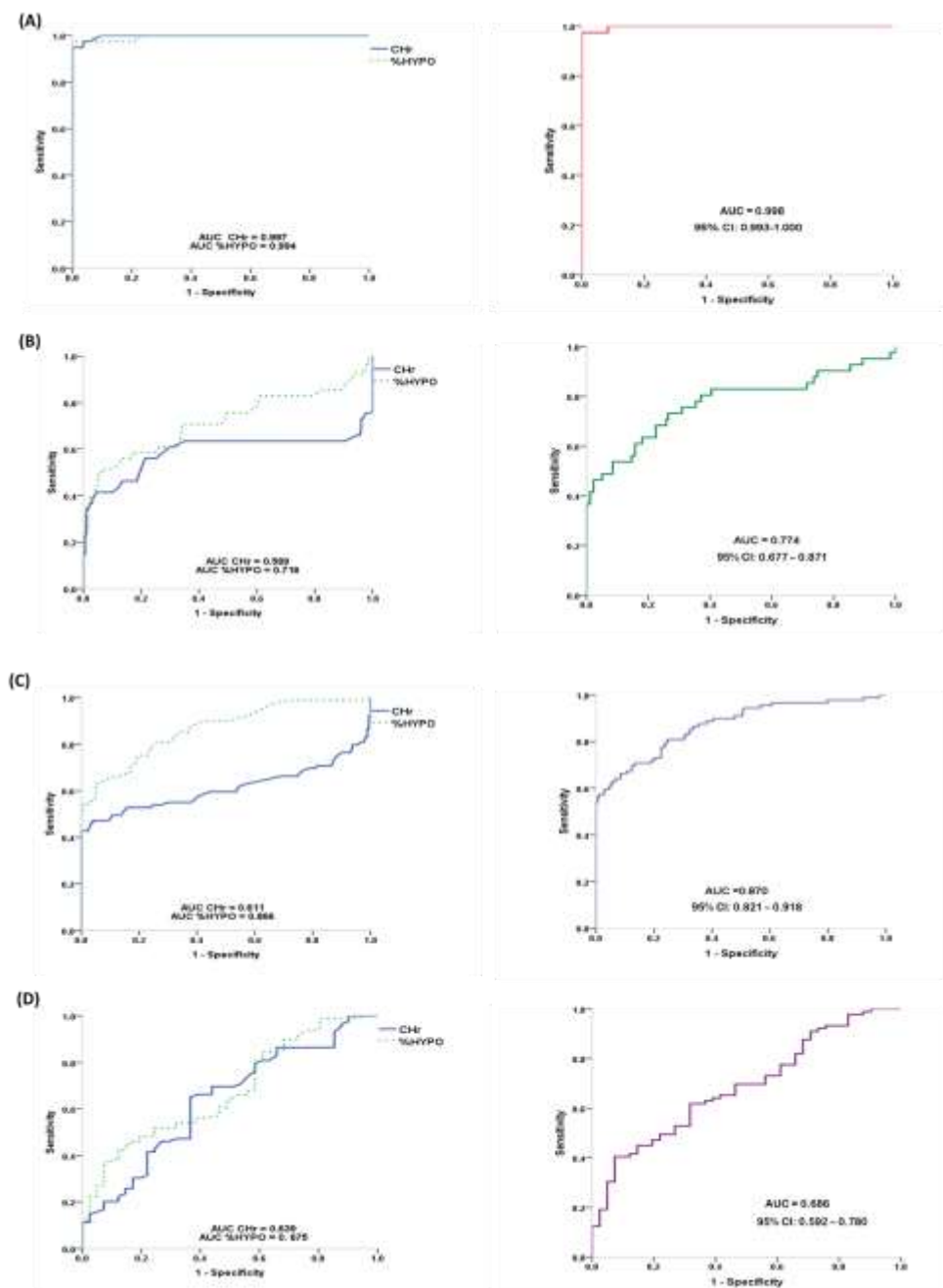
(A): distinguish the IDA group from the reference group, (B): distinguish the non-iron deficient anemia K group from the reference group, (C): distinguish the iron deficient anemia K group from the reference group, (D): distinguish the iron deficient anemia K group from the non-iron deficient anemia K group.

Figure 3. The ROC of CHr and %HYPO of the study groups

DISCUSSIONS

From the 347 samples, we found that the percentage of cancer patients with iron deficiency anemia $\geq$ 50 years of age was higher than those < 50 years of age, and the percentage of male patients were higher than female cancer patients with iron deficiency anemia (Table 1). The cancer patients with iron deficiency anemia accounted for 68.4% of the total number of cancer patients with anemia compared to the Ludwig H's study [5] where the cancer patients with iron deficiency anemia accounted for 42% of the total number of cancer patients with anemia.

The CHr and %HYPO have been proposed as promising indices to differentiate IDA in several studies. However, it is not common to use these parameters in cancer patients in Vietnam. In this study, our team investigated the usefulness of CHr and %HYPO in differentiating between IDA patients and non-IDA patients in the cancer group with anemia. The differences in the CHr and %HYPO between the reference group of healthy people (group 1) and the IDA control group (group 2) were significant. Our findings showed that these two parameters are better than the AUC = 0.998; 95% CI: 0.993 – 1.000 in distinguishing the IDA group (Figure 2(A)), corresponding to a threshold value for CHr of $\leq$ 28 pg with a sensitivity of 95% and a specificity of 100%; and %HYPO $\geq$ 8 has a sensitivity of 98% and a specificity of 100%. The threshold value for CHr of 29 pg has a sensitivity of 90.6% and a specificity of 60.7% and is better than that of the study by Mustafa Karagülle, Eren Gündüz [3]. Distinguishing IDA in cancer patients is more complicated due to the effects of latent inflammation, tumor inhibition, nutritional regime, chemotherapy, radiation therapy, ferritin, etc. Our findings indicate that AUC of CHr in predicting IDA in the cancer patients with anemia is only 0.639; 95% CI: 0.536 – 0.741, not like the control group (IDA) (Figure 2(D)). However, the %HYPO on its own was able to differentiate between the cancer patients with IDA and the healthy reference group with AUC = 0.870; 95% CI: 0.821 – 0.918 (Figure 2(C)). In Europe, the utilization of CHr and %HYPO or TSAT is recommended for the screening for functional IDA in ESMO 2018 [7]. However, it should be remembered, when using %HYPO, that the erythrocyte hemoglobin concentration may decrease with time due to erythrocyte swelling in vitro. Therefore, it is required that blood samples should not be stored for a long time so that the accuracy of the results with not be adversely affected. As the AUCs for CHr and %HYPO in predicting non-iron deficiency anemia in cancer patients are not very good, these two parameters are better at determining iron deficiency anemia than other causes of anemia. Among the cancer patients with anemia, CHr and %HYPO can distinguish IDA from non-IDA cases, corresponding to AUC = 0.639; 95% CI: 0.536 – 0.741 (Figure 2 (D)). Once both of these parameters are used, an increase in the threshold value is observed: CHr $\leq$ 29 pg with a sensitivity of 46% and a specificity of 74%, and %HYPO $\geq$ 8 with a sensitivity of 50.6% and a specificity of 76%. Our findings showed that the combination of these two parameters, namely, CHr and %HYPO tended to improve the accuracy of diagnosing IDA in cancer patients with anemia compared with using only a single parameter, but the this did not achieve significance.

With regard to CHr $\leq$ 29 pg and %HYPO $\geq$ 8, our study findings are similar to the ESMO 2018 guidelines. In our opinion, the combined results of these two parameters can be used for the purpose of IDA screening in cancer patients with anemia.

CONCLUSION

The combination of CHr and %HYPO as a tool for IDA screening will reduce the economic burden on the cancer patients with anemia, compared to ferritin and TSAT tests in diagnosing IDA among cancer patients with anemia using the recommended threshold values for CHr of $\leq$ 29 pg and %HYPO of $\geq$ 8.

REFERENCES

1. Anemia of chronic disease. Weiss G, Goodnough LT. N Engl J Med. 2005 Mar 10; 352(10):1011-23.
2. Clinical experience with ferric carboxymaltose in the treatment of cancer- and chemotherapy-associated anaemia. Steinmetz T, Tschechne B, Harlin O, Klement B, Franzem M, Wamhoff J, Tesch H, Rohrberg R, Marschner N. Ann Oncol. 2013 Feb; 24(2):475-82.

3. Clinical significance of reticulocyte hemoglobin content in the diagnosis of iron deficiency anemia. Karagulle M, Gunduz E, Sabin Mutlu F, Olga Akay M (2013) 30(2),153–6

4. Iron and cancer: recent insights. Manz DH, Blanchette NL, Paul BT, Torti FM, Torti SV. Ann N Y Acad Sci. 2016 Mar; 1368(1):149–61.

5. Iron metabolism and iron supplementation in cancer patients. Ludwig H, Evstatiev R, Kornek G, Aapro M, Bauernhofer T, Buxhofer-Ausch V, Fridrik M, Geissler D, Geissler K, Gisslinger H, Koller E, Kopetzky G, Lang A, Rumpold H, Steurer M, Kamali H, Link H. Wien Klin Wochenschr. 2015 Dec; 127(23–24):907–19.

6. Jie Cai, al Meng Wu and (2017), "Evaluation of the Efficiency of the Reticulocyte Hemoglobin Content on Diagnosis for Iron Deficiency Anemia in Chinese Adults". Pubmed.

7. Management of anaemia and iron deficiency in patients with cancer: ESMO Clinical Practice Guidelines. Aapro M, Beguin Y, Bokemeyer C, Dicato M, Gascón P, Glaspy J, Hofmann A, Link H, Littlewood T, Ludwig H, Österborg A, Pronzato P, Santini V, Schrijvers D, Stauder R, Jordan K, Herrstedt J. ESMO Guidelines Committee. Ann Oncol. 2018 Oct 1; 29(Suppl 4):iv96–iv110.

8. National Comprehensive Cancer Network. [(accessed on 27 August 2018)]; Available online.

9. Concentration hemoglobin reticulocyte in peripheral blood of patients with iron deficiency anemia. Trần Thị Ánh Loan, Suzanne Monivong Cheanh Beaupha, Hoàng Thị Thúy Hà, Phạm Thị Thúy An, Phạm Ngọc Diễm, Võ Trúc My, Phạm Thị Bích Tuyền, Nguyễn Ngọc Mai, Hồ Trọng Toàn, Trần Thanh Tùng, Nguyễn Trường Sơn. (2018). Tạp chí y học 467 tr.486.

10. Prevalence of iron deficiency across different tumors and its association with poor performance status, disease status and anemia. Ludwig H, Müldür E, Endler G, Hübl W. Ann Oncol. 2013 Jul; 24(7):1886–92.

11. Schapkaitz Elise, Suvarna Buldeo Johnny Ndoni Mahlangu (2016), "Diagnosis of iron deficiency anaemia in hospital patients: Use of the reticulocyte haemoglobin content to differentiate iron deficiency anaemia from anaemia of chronic disease". SAMJ.

12. The validity of reticulocyte hemoglobin content and percentage of hypochromic red blood cells for screening iron deficiency anemia among patients with end-stage renal disease: a retrospective analysis. Nhan Hieu Dinh, Suzanne Monivong Cheanh Beaupha, Loan Thi Anh Tran (2020) 21:142 BMC Nephology.

Cut-off values of red blood cell indices in silent carrier state and α-thalassemia trait

Dung Nguyen Ngoc, Yen Pham Hai, Thanh Nguyen Ha, Khanh Bach Quoc

1. Introduction

Hemoglobinopathies are the most common genetic disorders among the people living in Southeast Asia. The gene frequencies of α-thalassemia reach 30-40 percent in Northern Thailand and Laos, 4.5 percent in Malaysia whereas β-thalassemia varies between 1 and 9 percent (Suthat Fucharoen et al., 2011). In Vietnam, there is no published rate of alpha thalassemia in the community. Statistics in some regions also record a high rate of thalassemia. Viet Nam Thalassemia Association was established in 2011. The association has had many public and community campaigns about thalassemia and conducted screening projects in the community. Our study is based on community-screened data.

Each person has a pair of α-globin genes, α1 and α2, on chromosome 16. The different phenotypes in α-thalassemia are primarily attributed to whether one or both α-globin genes are deleted in each of the two loci. Silent carrier state: The presence of a single α-globin gene deletion or deletional α+-thalassemia results. Heterozygotes of one missing α-globin gene are not anemic and have normal or mildly hypochromic and microcytic red blood cell indices. The most common mutations are 3.7 and 4.2 kb-deletions. α-thalassemia trait: Subjects with two residual functional α-genes either by deletions that remove two linked α-globin genes from the same chromosome or α0- (--/αα) or combination of deletional α+-thalassemia (-α/-α), have mild hypochromia and microcytosis. In addition, Mediterranean countries, Southeast Asia and China often encounter mutations that produce abnormal hemoglobin such as: Hb Constant Spring (Hb CS - TAA → CAA mutation at codon 142), Hb Quong Sze (HbQs - CTG → CCG mutation at codon 152) (Ali Taher et al., 2017: X M Xu et al., 2004: Rahimah Ahmad et al., 2013). α-Thalassemia trait and silent carrier state have no clinical symptoms, hemoglobin electrophoresis is normal, red blood cell indices may have mild hypochromia and microcytosis. In addition, genetic analysis is expensive. So, red blood cell indices are important in screening oriented diagnosis.

2. Materials and Methods

Based on community screening data from January 2017 to December 2017 with the following criteria: Age greater than or equal to 15; Without iron deficiency anemia (Ferritin > 30 ng/ml); Normal hemoglobin electrophoresis (HbA2 < 3.5% and HbF < 2%); Screening for common mutations in Vietnam by multiplex PCR, including: SEA, 3.7, THAI, 4.2, FIL, HbCS, HbQs, C2del. Silent carrier state (-α/α) include mutations: -α3.7α, -αCsα, -α4.2α ,-αQsα, -αC2delα. α-thalassemia trait includes mutations: - -/α thalassemia (-SEA, - -SEA) and -α/-α thalassemia (-α3.7/-α3.7, -α3.7/-αCs, -α4.2/-αCs, -αCs/-αCs). Retrospective and observational study of 1863 cases (1172 silent carrier state (-α/αα), 215 (-α/-α) thalassemia, 476 (- -/αα) thalassemia) was conducted. Full blood count was performed on hematology analyzers: ADVIA 2120i (Siemens) and Unicel DxH800 (Beckman Coulter), in the department of cytology and histology. Multiplex PCR was performed on a Mastercycler nexus GX2, Eppendorf in the Department of Molecular Genetics.

Hemoglobin electrophoresis was performed on the Ultra 2 (Trinity Biotech). Serum iron was measured on an AU 5800 (Beckman Coulter). Ferritin was measured on an ADVIA Centaur XPT (Siemens) in the Department of Biochemistry. All laboratories were in the National Institute of Hematology and Blood Transfusion, Ha Noi, Vietnam.

3. Results

Based on genetic analyses, we recorded 1863 cases α-thalassemia trait and silent carrier state, in which, men accounted for 35.4% and women accounted for 64.6%, with an average age of 25.4 years.

Table 2. Parameters of red blood cells of the 3 groups

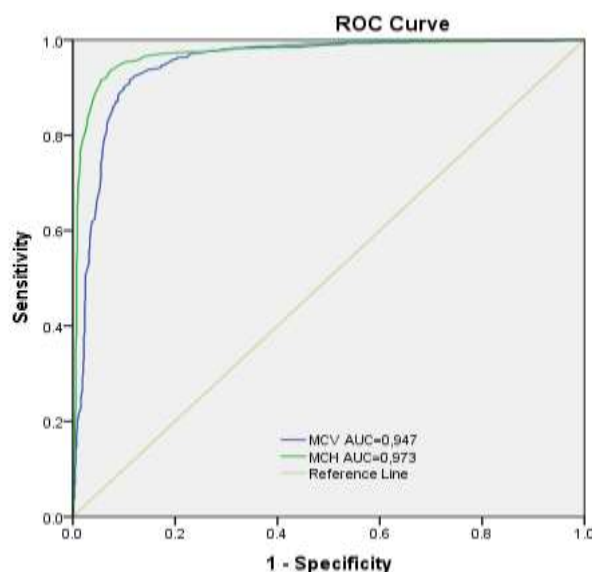| Parameters		$-\alpha/\alpha\alpha$ (n = 1172)	$-\alpha/-\alpha$ (n = 215)	$--/\alpha\alpha$ (n = 476)
RBC	Male	5.5 ± 0.5	5.8 ± 0.5	6.4 ± 0.5
	Female	5 ± 0.4	5.3 ± 0.5	5.7 ± 0.4
Hb	Male	142.8 ± 12.9	134 ± 9.9	119.4 ± 8.4
	Female	128.6 ± 10.9	120.2 ± 9.5	133.3 ± 9.6
MCV	Male	82.6 ± 4.2	76.8 ± 5.6	69.3 ± 4
	Female	83.1 ± 4.1	75.6 ± 4	69.4 ± 4.3
MCH	Male	25.9 ± 1.4	23.1 ± 1.4	21 ± 1.1
	Female	25.9 ± 1.4	22.6 ± 1.1	20.9 ± 1.4
MCHC	Male	313.5 ± 11.5	300.7 ± 11.8	303.1 ± 10.9
	Female	311.6 ± 12.3	298.7 ± 11.2	301.4 ± 12.7
RDW-CV	Male	14.3 ± 1.3	15.3 ± 1.4	16.4 ± 1.6
	Female	13.9 ± 1.3	15.3 ± 1.4	15.8 ± 1.4

Figure 1. Comparison of $[(-\alpha/-\alpha), (--/\alpha\alpha)]$ and $(-\alpha/\alpha\alpha)$ thalassemia

Comparison of subjects with deletion of 2 alpha genes $[(-\alpha/-\alpha), (--/\alpha\alpha)]$ and $(-\alpha/\alpha\alpha)$ revealed significantly lower MCV (71.4 fL vs. 82.9 fL, p<0.001), lower MCH (21.5 pg vs. 25.9 pg, p<0.001) and lower MCHC (301.2g/l vs. 312.3g/l, p<0.001) in $[(-\alpha/-\alpha), (--/\alpha\alpha)]$ subjects. MCV showed an AUC of 0.947 and a cut-off point of 77.45 fL provided a sensitivity of 91.6% and a specificity of 88.9%. MCH showed an AUC of 0.973 and a cut-off point of 23.65 pg provided a sensitivity of 93.6% and a specificity of 92.6% (Figure 1).
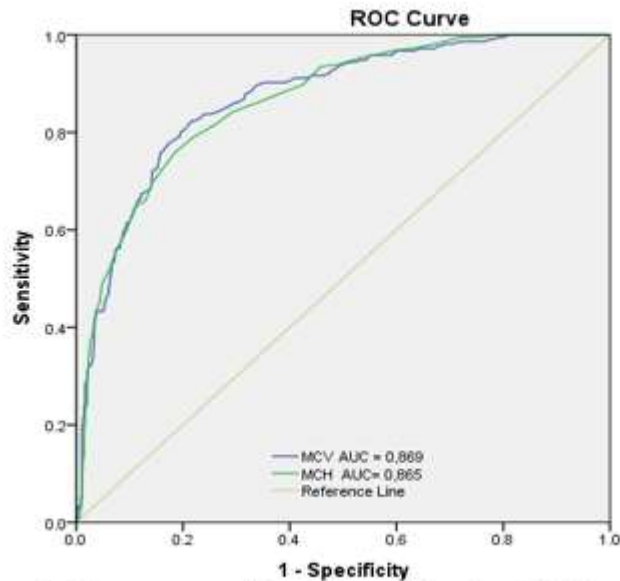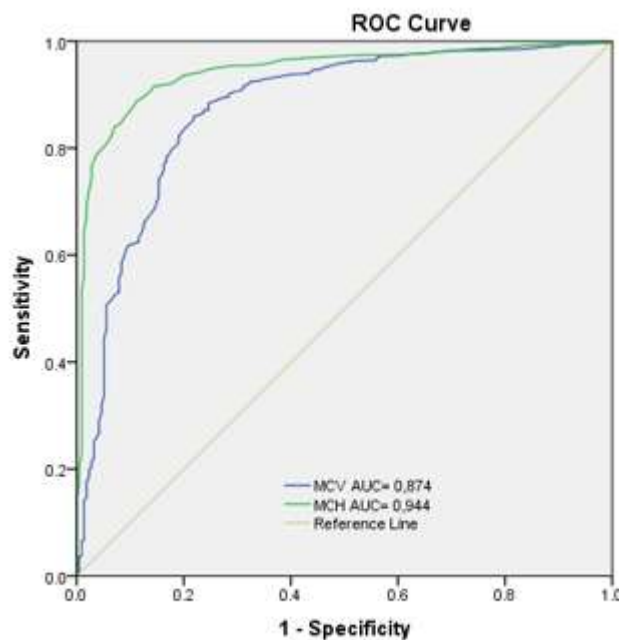
Figure 2. Comparison of (–α/–α) and (– –/αα) thalassemia

Comparison of (–α/–α) and (– –/αα) thalassemia revealed significantly lower MCV (69.4 fL vs. 76 fL, p<0.001), lower MCH (20.9 pg vs. 22.7 pg, p<0.001) and higher MCHC (302.1g/l vs. 299.3g/l, p<0.005) in (– –/αα) subjects. MCV showed an AUC of 0.869 and the cut–off point of 72.15 fL provided a sensitivity of 82.3% and a specificity of 78.4%. MCH showed an AUC of 0.865 and a cut–off point of 21.85 pg provided a sensitivity of 75.8% and a specificity of 81.5% (Figure 2).

Figure 3. Comparison of (–α/αα) and (–α/–α) thalassemia

Comparison of (–α/αα) and (–α/–α) thalassemia revealed significantly lower MCV (76 fL vs. 82.9 fL, p<0.001), lower MCH (22.7 pg vs. 25.9 pg, p<0.001) and lower MCHC (299.3 g/l vs. 321.3 g/l, p<0.001) in (–α/–α) subjects. MCV showed an AUC of 0.874 and a cut–off point of 78.45 fL provided a sensitivity of 88.4% and a specificity of 75.3%. MCH showed an AUC of 0.944 and a cut–off point of 23.95 pg provided a sensitivity of 91.6% and a specificity of 85.6% (Figure 3).

4. Discussion

Similar to other studies, group (–α/αα) thalassemia accounted for the highest proportion in α thalassemia trait or carriers (62.9%). Deletions –α3.7α accounted for the highest proportion of 37.6%, followed by – –SEA (24.9%), similar to those recorded in Malaysia (Rahimah Ahmad et al., 2013), Taiwan (Tyen–Po Chen et al., 2002).

For the group with (–α/αα) thalassemia, the average MCV was 82.9 fL. In particular, MCV values from 80 fL to 85 fL accounted for 32.7% of cases, MCV > 85 fL accounted for 19.1% of cases, which means that MCV are not low in many cases. All cases in this study had normal hemoglobin electrophoresis using HPLC, so screening for thalassemia with the threshold of 80 fL may miss 51.8% of cases of α thalassemia trait or carrier. The average MCH was 25.9 pg which is higher than studies in Malaysia (Rahimah Ahmad et al., 2013), Iran (Haleh Akhavan–Niaki et al., 2012). In particular, MCH values ≥ 27 pg accounted for 23.1% of cases. Therefore, the selection of thalassemia screening threshold of MCH < 27 pg may also miss 23.1% of cases. The average MCHC is 312.3 g/L which is lower than in other studies. With the Receiver Operating Characteristic Curve (ROC), thresholds for MCV and MCH had a better ability to differentiate between groups. Specifically, thresholds for MCV of 77.45 fL and MCH of 23.65 pg have the ability to distinguish between (–α/αα) thalassemia and group [(–α/–α), (– –/αα)] thalassemia with sensitivities of 91.6 % & 93.6%, and specificities of 88.9% & 92.6%. Thresholds for MCV of 78,45 fL and MCH of 23.95 pg were capable of highly differentiating between (–α/αα) thalassemia and (–α/–α) thalassemia with sensitivities of 88.4% & 91.6% and specificities of 75.3% & 85.6%.

For the group (– –/αα) thalassemia, – –SEA was the most common. This was similar to reports from north eastern Thailand, Laos and Cambodia in the Chinese community (Wittaya Jomoui et al., 2017). 33% of cases had mild anemia, but hemoglobin electrophoresis was normal so it was easy to miss these cases. It is important to diagnose (– –/αα) thalassemia cases because parents with (– –/αα) and (–α/αα) thalassemia may have a child with HbH or Hb Bart disease. Genetic counseling in this case is essential. The (– –/αα) of thalassemia group had average MCV of 69.4 fL and MCH of 20.9 pg. These are significantly lower than the other 2 groups. Compared with the (–α/–α) thalassemia group, thresholds for MCV of 72.15 fL and MCH of 21.85 pg had sensitivities of 82.3% & 75.8% and specificities of 78.4% & 81.5%.

In our study, all cases were ≥ 15 years old. Therefore, the change of MCV and MCH with age is excluded. MCV and MCH can help to differential orientate types of thalassemia mutation, even with (–α/–α) or (– –/αα) thalassemia. MCH has higher sensitivity and specificity than MCV. MCH values are more stable than MCV, especially with blood sample storage time of > 24 hours. Therefore, some authors use MCH values as the only tool in thalassemia screening (Kate Ryan et al., 2012). Our research showed that MCH values are a better screening tool than MCV values. However, using an MCH threshold of < 27 pg 275 cases will be missed, accounting for 14.8% of cases. Using an MCH threshold of < 28 pg also missed 8 cases, accounting for 0.4%. Moreover, the study was limited to the common deletions in Vietnam. Therefore, our recommendation to clinicians is to coordinate the use of both MCH < 28 pg and/or MCV < 85 fL.

References

1. Suthat Fucharoen, Pranee Winichagoon, Hemoglobinopathies in Southeast Asia, Indian J Med Res 134, October 2011, pp 498-506.

2. D.J. Weatherall, J.B. Clegg, Inherited haemoglobin disorders: an increasing global health problem, Bulletin of the World Health Organization, 2001, 79.

3. Ali Taher, Khaled Musallam, Maria Domenica Cappellini, Guidelines for the management of non-transfusion dependent thalassemia,2nd Edition, 2017.

4. Goonapa Fucharoen. Kanokwan Sanchaisuriya, Nattaya Sae-ung, A simplified screening strategy for thalassemia and haemoglobin E in rual communities in south-east Asia, Bulletin of the world health organization, May 2004, 364-372.

5. Rahimah Ahmad ,Mohamed Saleem,Nisha Sabrina Aloysious, Distribution of Alpha Thalassaemia Gene Variants in Diverse Ethnic Populations in Malaysia: Data from the Institute for Medical Research, Int. J. Mol. Sci. 2013, 14(9), 18599-18614.

6. X M Xu, Y Q Zhou, G X Luo, C Liao, The prevalence and spectrum of α and β thalassaemia in Guangdong Province: implications for the future health burden and population screening, J Clin Pathol 2004;57:517-522.

7. Haleh Akhavan-Niaki, Reza Youssefi Kamangari, Ali Banihashemi, Hematologic Features of Alpha Thalassemia Carriers, Int J Mo1 Cell Med summer 2012; Vol 1 No3, 163-167.

8. Anavillegas, Ameliaporres, Jesussasanchez, Red blood cell phenotypes in a-thalassemias in the Spanish population, Haematologica1998; 83:99-103.

9. Tyen-Po Chen, Ta-Chih Liu, Chao-Sung Chang, PCR-Based Analysis of α-Thalassemia in Southern Taiwan, International Journal of Hematology April 2002, Volume 75, Issue 3, pp 277-280

10. Diego Velasco-Rodríguez, Carlos Blas, Juan-Manuel Alonso-Domínguez, Cut-off Values of Hematologic Parameters to Predict the Number of Alpha Genes Deleted in Subjects with Deletional Alpha Thalassemia, Int. J. Mol. Sci. 2017, 18, 2707.

11. Wittaya Jomoui, Goonnapa Fucharoen, Kanokwan Sanchaisuriya, Genetic origin of $\alpha 0$-thalassemia (SEA deletion) in Southeast Asian populations and application to accurate prenatal diagnosis of Hb Bart's hydrops fetalis syndrome, J Hum Genet. 2017 Aug; 62(8): 747-754.

12. Kate Ryan, Barbara J. Bain, David Worthington, Significant haemoglobinopathies: guidelines for screening and diagnosis, British Journal of Haematology 2010, 149, 35-49.

Leveraging Patient Moving Averages into the Auto verification and QC Process

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It is a well-recognized fact that, Standard quality control (QC) procedure in the medical laboratory is pivotal in the delivery of high-quality patient results. Quality control is a statistical process used to monitor and evaluate the analytical process that produces patient results.

QC results are used to validate whether an instrument is operating within pre-defined specifications, inferring that patient test results are reliable. Complementing this with Patient Moving Averages as patient- based QC can help to fine-tune the QC process to reduce turnaround time (TAT) and improve efficiency. While routinely used QC practices and statistical tool in Labs have their benefits, they also have limitations under certain situations and scenarios.

Limitations of current QC practices

1. Provides only snapshot of assay performance at that point in time

2. Systemic error (SE) can develop anytime between QC events, insufficient to rapidly detect SE

3. SE may go undetected for hours affecting many patient results Increasing QC frequency may improve SE detection but will add huge cost to the lab. Thus, it becomes extremely important to use additional statistical levers to reinforce the process. This tutorial reviews the use of patient moving averages (PMA) as a component of the QC program and how it can be applied in day- to-day operations to detect problems and proactively address issues.

What is patient moving averages (PMA)?

PMA are running averages of patient results for a specific assay over a preset number of data points (batch size). PMA evaluation is based on actual patient results and complement scheduled analysis of control materials. Historically, PMA has been commonly used in Hematology laboratory. Now, with the computing power made available through Data Management Systems, PMA is increasingly used in Chemistry and Immunoassay as an adjunct to the routine QC process.

What are the benefits of PMA and how is it utilized?

1. Early Detection of Systemic error as monitoring is continuous, shifts and changes can be detected before errors are detected by QC events. PMA can detect SE in assay performance attributable to specific reagent lot or issues with specific reagent container.

2. Saves cost and effort as no additional QC material and reagent needed, there is no need to perform additional tests.

3. The use of PMA also qualifies the regulatory requirement for Auto verification (CLSI guideline Auto 10 A).

Integrating PMA into your QC process

The data management system plays an important role in integrating PMA seamlessly into day-to-day laboratory operations. Atellica Data Manager (ADM, the Data Management System) can be configured to define acceptable mean, limits and rejection rules. ADM notifies the user when a possible shift in performance/QC failure occurs and holds patients result for review (Figure-3). The notification, including quality severity (QS), is displayed on the Patient Review screen. The QC screen can readily be assessed from the navigation screen to provide additional information for troubleshooting.

Probable causes may be a change in patient population, a calibration issue, an instrument issue, or a reagent issue. Once on the QC screen, the user has the ability to view the assay across all instruments on the network (local or remote) to further troubleshoot the problem. When integrated with a Siemens Aptio Automation System, the Atellica Data Manager (ADM) system reroutes and reruns the assay automatically, if indicated during review and troubleshooting.

It should also be noted that the ADM system uses a moving average method based on a variation of Bull's algorithm. The PMA is an exponentially weighted average of the previous N-1 patient results. The previous average contributes to the calculation of the current batch average.

Requisites for a Laboratory to set up Moving averages include

1. Establishing mean of the identified assay

2. Selection of control limits

3. Number of patient results to average (N) (Batch size)

4. Concentration at which truncation limits are placed to minimize the effects of outliers

Determining batch size

The batch size or number of patient results used to compute the averages should be large enough so that it is representative of the patient population, in order to avoid false rejections. Conversely, an overly large batch may take too long to accumulate and result in delayed detection of issues that arise during the longer intervals. Determining the optimal batch size will require a process of trial and error. The batch size may vary from assay to assay. The rationale holds true for defining the acceptable range.

Types of PMA
Audit or Undefined PMA

Audit or undefined PMA (Figure-1) is passive, continuous monitoring. Batch size and truncation limits need to be defined. PMA are calculated from patient results and presented in QC population statistics. No rejection rules or limits are defined and no alerts are triggered. It is essentially the first step in using PMA in order to develop understanding of how PMA trend.

Figure 1: IL-6 Audit in use - Undefined PMA: Over a period of few days, mean and deviation calculated from patient results

Defined PMA

In defined PMA (Figure-2), QC alerts are triggered and results held based on defined limits and rules. Many labroratories use a combination of audit and defined PMA, depending on the assay. PMA also helps in troubleshooting of SE in QC data, for example, if there is shift in QC data when a new reagent lot/reagent bottle is used then the shift may be either related to QC material matrix or to new reagent lot/bottle.

However, to verify this it is important to look at patient results. When a QC flag is triggered, instrument-specific PMA audit data can be retrieved from data management system to review when the problem began and which samples may be affected.

If PMA shows no shift in patient results, then the reagent lot is performing acceptably and the shift in QC data may be a QC material matrix issue. In such cases lab may need to update QC targets and limits. If PMA also shows comparable shift as in QC data then there may be a reagent lot problem and the manufacturer should be contacted.
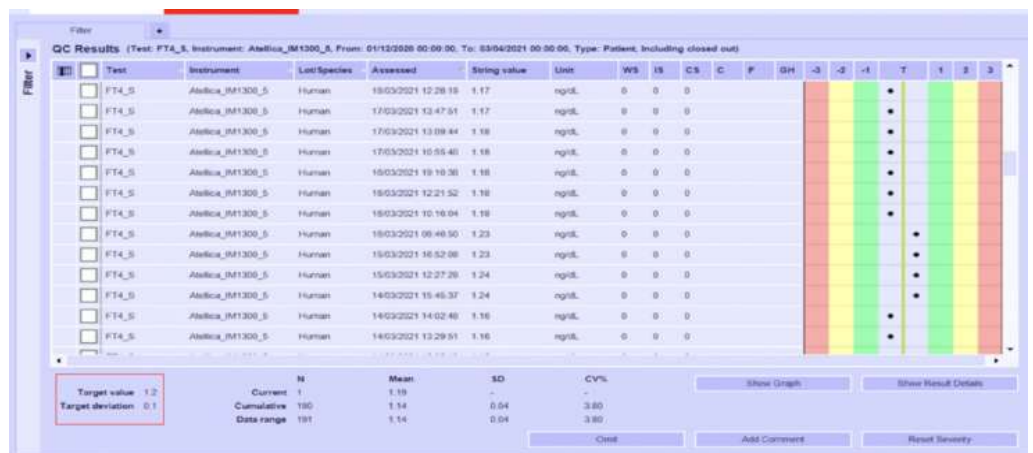

Figure 2: FT4, Defined PMA

Which tests should be monitored?

Not all assays benefit from PMA monitoring. Table 1 summarizes attributes to consider when deciding when deciding when to use PMA

Table 1: Assays vs. suitability for monitoring

Attribute	Rationale
Stable assays: day-to-day for a single patient, over time for a patient population	Inherent instability can make it difficult to isolate problems
Reasonable analytical range	Inherent broad range (e.g., CA 125, CK, or ALT) may challenge data collection
Significant volume	Sufficient data can be gathered in a reasonable amount of time to detect shifts and trends
Target value and deviation can be established for a patient population	Once target value (typically the average of the PMA values obtained from weeks or months of auditing can be used) and acceptable deviation are set, the system can be configured to flag "out of control" batches

Patients results on one analyzer getting held for review due to PMA drift in Total T4

Figure 3: QC Alert, Home screen of ADM

Figure 4: T4 having outlier
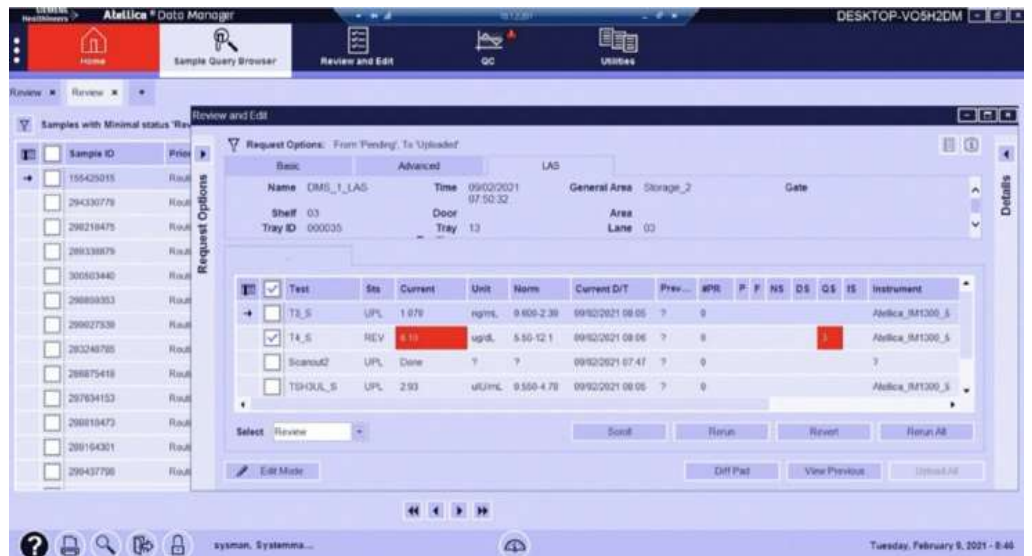
Figure 5: Total T4 result held for review

Figure 6: PMA chart - Before taking corrective action

Figure 7: PMA chart - After corrective action

Summary:

Patient moving averages (PMA) is a valuable adjunct to the QC process, allowing tighter control of assay performance, faster and better responses to issues, and cost savings on QC material and tech time. A clear vision of goals and methodical planning will help guide proper use of PMA to focus on issues that need to be addressed and avoid false alarms. PMA has helped National Reference Lab, Dr. Lal Path Labs to achieve a post– automation Auto–verification of 70% from previous 49% pre - automation.

References:

1. Westgard JO, Smith FA, Mountain PJ, Boss S. Clin Chem 1996; 42:1683–1688.

2. 140962–GC1_CentraByteIssue7Final_1800000001781237

3. Patient–based real–time quality control: review and recommendations". Tony Badrick, Andreas Bietenbeck, Mark A Cervinski, Alex Katayev, Huub H van Rossum, Tze Ping Loh. Clin Chem 65(8); 972–981: 2019

4. Recommendation for performance verification of patient–based real time quality control". Tze Ping Loh, Andreas Bietenbeck, Mark A Cervinski, Alex Katayev, Huub H van Rossum, Tony Badrick. CCLM 58(8); 1205–1213: 2020; DOI: 10.1515/cclm-2019-1024

5. Understanding Patient–Based Real–Time Quality Control using Simulation Modeling." Bietenbeck A, Cervinski MA, Katayev A, Loh TP, van Rossum HH, Badrick T. Clin Chem 66(8); 1072–1083: 2020.

My Home at the Yellow-Leaves Mountain Village

《During my frequent trips overseas, I was mesmerised by the beautiful scenes of autumn when the leaves of most trees turned a bright golden yellow. Mountain-top villages surrounded by golden yellow trees appear especially beautiful and attractive when viewed from a sailing boat. Inspired by such unforgettable sights, I turned my impressions into a painting. An appropriate title was selected from a sentence in the poem by a famous poet of the Northern Sung Dynasty in China: 《My Home at the Yellow-Leaves Mountain Village》.

Dr. Tan It Koon
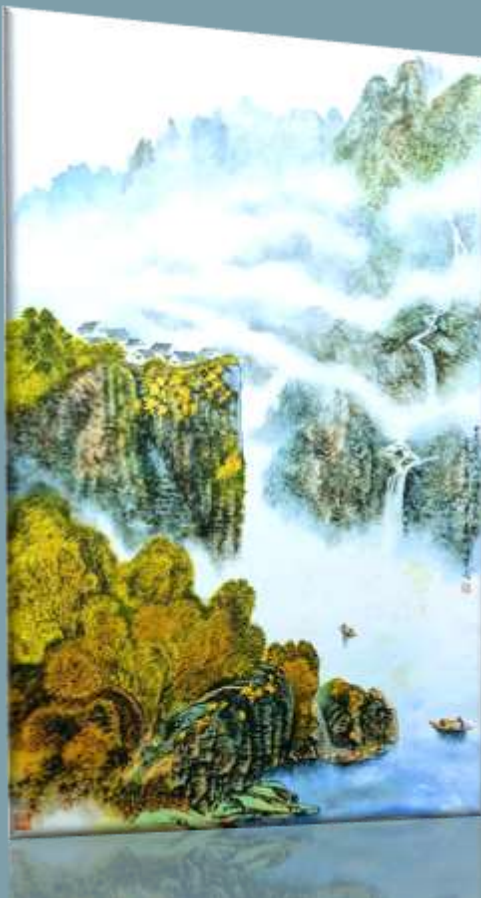
The poet is Su Shi, also known as Su Dongpo, a well-known scholar, calligrapher, painter, and historical water control celebrity official in the Northern Song Dynasty. Su Shi was the leader of the literary world in the mid-Northern Song Dynasty. His poems are wide-ranging and unrestrained; the poems are broad in subject matter, fresh and vigorous, using exaggerated metaphors, and have a unique style. The original poem in Chinese is copied below:

野水参差落涨痕，

疏林欹倒出霜根。

扁舟一棹归何处，

家在江南黄叶村。

This may be translated as follows:

The water level of the river rises and falls leaving marks on the exposed winding river beds during low tide, while the sparse forest trees that have fallen expose their roots which are as white as frost. A boat is seen being paddled hurriedly in the river. Where is it heading for? It must be returning to the Yellow-Leaves Village in Jiangnan, an area in the south of the river where the boatman and his passengers have their homes.

The title of the painting 《 My Home at the Yellow-Leaves Mountain Village 》 is written in running script on the right side of the painting.