# Clinical Biochemistry News

ACBI

ACB

May 2022

Newsletter of the Association of Clinical Biochemists in Ireland
and the Association for Clinical Biochemistry and Laboratory Medicine (Republic of Ireland Region)

**Professor Cedric Shackleton the 'MASSter of Steroid Mass Spectrometry' who recently celebrated his 80th birthday** Photo: Karen Heverin

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# A Message from the President of the Association of Clinical Biochemists in Ireland

## Dr. Jennifer Brady

We were delighted to be able to host our first in-person event in two years. We held our AGM on 6th April in the Mater Hospital, which was preceded by an excellent and well attended scientific session covering clinical and laboratory aspects of vitamin B12 delivered by Dr Aisling Fanning, SpR from CHI at Temple St and Ruth Cullen from the Mater Hospital. At the AGM we said farewell to Dr Marguerite MacMahon and acknowledged her for all her work as ACBI treasurer over the last 6 years. Karen Heverin, a Senior Biochemist from Beaumont Hospital, was nominated as her replacement and we look forward to working with Karen on Council. More recently we have said good-bye to Natividad Ricorios, who was an ordinary member on Council and Principal Clinical Biochemist in Cork. Along with her husband Antonio Reche Martinez, who worked as a Principal Clinical Biochemist in Limerick, they have returned to Spain. We thank them for their contribution to the ACBI during their time in Ireland. Micheál Ryan, Senior Clinical Biochemist in Limerick has been co-opted into the vacant ordinary member position on Council.

The National Working Group to inform the Strategic Direction of Laboratory Medicine, commissioned by the office of the Chief Clinical Officer is well underway. All the professional groups are represented, along with representatives of the HSE, Department of Health, and hospital groups. Myself and Dr Séan Costelloe are working on this wide-ranging and very welcome review. It is expected that this work will be completed in September.

As ever there are continued opportunities for our members to take up positions nationally and on EFLM and IFCC committees and working groups. These are great opportunities to expand your CV, learn from colleagues, and represent your national society. I want to congratulate Alison Bransfield, Principal Biochemist in Cork for her acceptance as a corresponding member of the IFCC committee on 'Reference Intervals and Decision Limits'. Congratulations also to Dr Brendan Byrne, Principal Biochemist at the Mater hospital, who has become the ACBI representative at the IEQAS Clinical Chemistry review group.

The EFLM Strategic Conference 2022 on the topic of Smart and Green Laboratories takes place virtually from 25th-27th May. Registration is free to members of the EFLM Academy. This is timely as the ACBI has recently signed up to the Irish Green labs initiative. This was launched on April 23rd, Earth Day, and is a collaborative network whose work aims to minimise the impact of Irish laboratories on the environment, delivered through four pillars: energy, waste, water and chemistry. Find out more at www.irishgreenlabs.org. Dr Séan Costelloe is representing the ACBI at this group as well as the EFLM Green-labs initiative. He is seeking volunteers to become involved in this very timely and important project which has an impact on us all. Please get in touch with him to find out more about this valuable work (directly or via secretary@acbi.ie)

The ACBI website is continuously being updated and I want to again thank Alison Bransfield for her work on this. If you have a meeting or other information to share, please contact Alison at webmaster@acbi.ie. The final module of the website that needs to be completed is the CPD module and this is currently undergoing active testing. Once this is live, I would encourage everyone to use this module to record your CPD activity which will become mandatory with CORU registration.

Council has been making plans for further evening educational meetings. Plans are also underway for Council members to hold a meeting specifically for younger and new members of the profession to provide a platform to discuss issues affecting this group. It is hoped this will take place in September so look out for further details.

If you would like to get in touch or ask a question, please email secretary@acbi.ie.

Dr Jennifer Brady, President ACBI.

# International Federation of Clinical Federation (IFCC) and European Federation of Clinical Chemistry (EFLM) Update

## IFCC/EFLM Committee opportunities

There are a number of EFLM committee opportunities open at the moment. Please see www.eflm.eu for more information.

**Please note that applications are to be made through the ACBI council. If you are interested in volunteering for a committee position please contact the ACBI President, Dr Jennifer Brady.**

## Clinical Chemistry and Laboratory Medicine (CCLM)

A new issue of CCLM is available from De Gruyter Online: Volume 60, Issue 5. It can be accessed through your EFLM Academy logon.

## IFCC/EFLM News

The current issue of "EuroLabNews", the bi-monthly EFLM newsletter, is available online: https://www.eflm.eu/upload/newsletters/2022-Mar-Apr-EuroLabNews.pdf

## EFLM Academy

All ACBI members should now have received their EFLM Academy login details. If you have not yet received yours please contact secretary@acbi.ie

Please note that EFLM Academy membership includes access to Clinical & Laboratory Standards Institute (CLSI) documents.

The new EFLM e-learning academy has now launched and is a comprehensive educational resource

## IFCC News

The current issue is available online:
https://www.ifcc.org/media/479375/ifccenewsapril2022.pdf

The current issue of "eJIFCC" ,Volume 33-no1, is available online:
https://www.ifcc.org/ifcc-communications-publications-division-cpd/ifcc-publications/ejifcc-journal/

## Upcoming Meetings/Events

### EFLM

EFLM has established a series of webinars covering different diseases and their diagnoses using biomarkers. The leaflet is available here https://www.eflm.eu/upload/docs/Leaflet-lessons-in-immunochemistry.pdf
EFLM live webinars are available here: https://www.eflm-elearning.eu/site/live-webinar
The next scheduled webinar is on 30th May at 1600 CET, and the topic is 'Vitamin D status: the role of the laboratory'.
On 21st June at 1800 CET a webinar will take place on 'Molecular Immunology:new tricks of the innate immune system'.

Previous webinars available at https://elearning.eflm.eu/

EFLM has established the Task-Force "Green Labs". For more information click here

3rd EFLM Strategic Conference - SMART and GREEN LABORATORIES
How to implement IVDR, emerging technologies and sustainable practices in medical laboratories?
25-27 May 2022 (virtual)
http://www.eflm-strategic-conference.eu/
**Note: registration is free for EFLM Academy members**

6th EFLM Conference on Preanalytic Phase was held online from **Mar 15-18 2022** and registrants can now view all presentations online
https://www.preanalytical-phase.org/

XXIV IFCC –EFLM EUROMEDLAB Seoul 2022 XVI APFCB Congress Seoul 2022 **Jun 26th – June 30th 2022**

http://www.seoul2022.org/home

### IFCC

**Webinars**

Webinar '. Registration: https://www.eflm-elearning.eu/site/live-webinar
Webinar 'Laboratory diagnosis of thyroid disease' is available on demand:
Webinar 'Proficiency Testing/ External Quality Assessment: Program designs and practical approaches for addressing unexpected results' is available on demand here
Webinar 'Common laboratory measures of dysglycaemia; their strengths and weaknesses' is available on demand here
ICPLM 2021 Emerging Technologies in Paediatric Laboratory Medicine Conference 2021 - Watch all sessions on demand and download the Special Issue of Journal of Laboratory Medicine here

**Meetings**

XXIV IFCC WORLDLAB Seoul 2022 XVI APFCB Congress Seoul 2022 **Jun 26th – June 30th 2022**

http://www.seoul2022.org/home

XXV IFCC WORLDLAB Roma 2023
**May 21st – May 25th 2023**
http://2023roma.org

### IFCC/EFLM Websites

http://www.ifcc.org/

https://www.eflm.eu/

# A Selection of Members' Recent Publications

Hepburn S, Jankute M, Cornes MP, *Rios NR*, Stretton A, *Costelloe SJ*. Survey of patient perception of pre-analytical requirements for blood testing in the UK and RoI. Ann Clin Biochem. 2021 Mar;58 (2):132-140. doi: 10.1177/0004563220982325.

*Costelloe SJ*, *Rico Rios N*, Goulding N, Mistry H, Stretton A, De la Salle B, Hepburn S, Thomas A, Atherton J, Cornes M. A survey of practice in the management of haemolysis, icterus and lipaemia in blood specimens in the United Kingdom and Republic of Ireland. Ann Clin Biochem. 2021 Dec 13:45632211059755. doi: 10.1177/00045632211059755.

*Joyce CM*, Coulter J, Kenneally C, McCarthy TV, O'Donoghue K. Experience of women on the Irish National Gestational Trophoblastic Disease Registry. Eur J Obstet Gynecol Reprod Biol. 2022 Mar 28;272:206-212. doi: 10.1016/j.ejogrb.2022.03.039.

Vermeersch P, Frans G, von Meyer A, *Costelloe S*, Lippi G, Simundic AM. How to meet ISO15189:2012 pre-analytical requirements in clinical laboratories? A consensus document by the EFLM WG-PRE. Clin Chem Lab Med. 2021 Jan 15;59(6):1047-1061. doi: 10.1515/cclm-2020-1859.

Newman C, Kgosidialwa O, Dervan L, Bogdanet D, Egan AM, Biesty L, Devane D, *O'Shea PM*, Dunne FP. Quality of patient-reported outcome reporting in trials of diabetes in pregnancy: a systematic review. Diabetes Res Clin Pract. 2022 Apr 25:109879. doi: 10.1016/j.diabres.2022.109879.

Walsh JB, McCartney DM, Laird É, McCarroll K, Byrne DG, *Healy M*, *O'Shea PM*, Kenny RA, Faul JL. Title: Understanding a Low Vitamin D State in the Context of COVID-19. Front Pharmacol. 2022 Mar 4;13:835480. doi: 10.3389/fphar.2022.835480. eCollection 2022.

Curneen JMG, Rabbitt L, Browne D, O'Donoghue DF, Alansari Y, Harhen B, Ní Ghríofa A, Ferguson J, McEvoy JW, Lappin D, Finn DP, *O'Shea PM*, Dennedy MC. Major disparities in patient-reported adherence compared to objective assessment of adherence using mass spectrometry: A prospective study in a tertiary-referral hypertension clinic. Br J Clin Pharmacol. 2022 Feb 23. doi: 10.1111/bcp.15292.

Curtin L, Conway MC, *Kilbane MT*, McKenna MJ, McAuliffe FM. No effect of maternal calcium intake and bone resorption during pregnancy on offspring bone mineral density at age 5 years. Osteoporos Int. 2022 May;33(5):1165-1170. doi: 10.1007/s00198-021-06250-5.

Killeen SL, Byrne DF, Geraghty AA, *Kilbane MT*, Twomey PJ, McKenna MJ, Yelverton CA, Saldova R, Van Sinderen D, Cotter PD, Murphy EF, McAuliffe FM. Higher inflammation is associated with cardiometabolic phenotype and biochemical health in women with obesity. Ann Nutr Metab. 2022 Mar 18. doi: 10.1159/000522564.

*Reeve JLV*, Davis M, Twomey PJ. Observations from a teaching hospital in Ireland: changing from MDRD to CKD-EPI eGFR in routine practice. J Clin Pathol. 2021 Sep;74(9):608-611. doi: 10.1136/jclinpath-2020-206713.

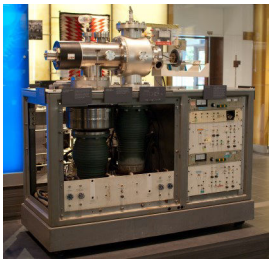
Hughes D, Crowley J, *O'Shea P*, McEvoy JW, Griffin DG. Lipid reference values in an Irish population. Ir J Med Sci. 2021 Feb;190(1):117-127. doi: 10.1007/s11845-020-02309-0.

Scully H, Laird E, *Healy M*, Crowley V, Walsh JB, McCarroll K. Vitamin D retesting by general practitioners: a factor and cost analysis. Clin Chem Lab Med. 2021 Jul 19;59(11):1790-1799. doi: 10.1515/cclm-2021-0607.

*Eagleton M*, *Stokes S*, Fenton F, Keenan E. Therapeutic potential of long-acting opioids and opioid antagonists for SARS-CoV-2 infection. Br J Anaesth. 2021 Dec;127(6):e212-e214. doi: 10.1016/j.bja.2021.08.022.

Fenton F, *Stokes S*, *Eagleton M*. A cross-section observational study on the seroprevalence of antibodies to COVID-19 in patients receiving opiate agonist treatment. Ir J Med Sci. 2021 Jul 9:1-6. doi: 10.1007/s11845-021-02660-w.

*Eagleton M*, *Stokes S*, Fenton F, Keenan E. Does opioid substitution treatment have a protective effect on the clinical manifestations of COVID-19? Comment on Br J Anaesth 2020; 125: e382-3. Br J Anaesth. 2021 Mar;126(3):e114-e116. doi: 10.1016/j.bja.2020.11.027.



The single quadrupole mass spectrometer and ion source used for Prof. John Fenn's Nobel Prize winning discovery of electrospray ionization.

# Steroids, Mass Spectrometry and Endocrinology – Past, Present and Future Symposium

## University of Birmingham 22-23rd April 2022

**Dr. Karen Heverin, Senior Clinical Biochemist, Beaumont Hospital, Dublin**

I recently attended the Scientific Symposium "Steroids, Mass Spectrometry and Endocrinology – Past, Present and Future". It was my first live, in-person event since before the COVID-19 pandemic and, what a meeting to make a comeback for! It was so lovely to network with colleagues once again in the impressive setting of the Great Hall of the Aston -Webb Building in the University Of Birmingham.

This wonderful meeting was organised by Prof Wiebke Arlt with her local and scientific organising committees all of whom have associations with the Institute of Metabolism and Systems Research (IMSR) in the University of Birmingham. It was organised to celebrate the 80th birthday of Prof. Cedric Shackleton, widely acclaimed as the 'MASSter of steroid mass spectrometry'.

Prof. Shackleton's career spans more than five decades and includes the development of several ground-breaking approaches and techniques that have advanced the field in a major way. Dr. Cedric Shackleton has been a fundamental contributor to steroid metabolome analysis by mass spectrometry and has pioneered the technology and its application to the diagnosis of inborn disorders of sterol and steroidogenesis, with major translational impact. His work over five decades has defined the steroid metabolome of many congenital steroid disorders from synthetic to metabolic and receptor defects, pioneering the use of GC-MS in steroid profiling over 30 years ago. He has authored more than 350 PubMed-listed research publications and remains highly active, working at the Institute of Metabolism and Systems Research (IMSR) of the University of Birmingham, UK, helping to set up their specialist steroid methods. Furthermore, Prof. Shackleton has had great influence on those he has mentored over the years and was widely commended for his willingness to share his vast expanse of knowledge.

The meeting kicked off on Friday afternoon with the jam packed 'Steroid Analysis – Past and Present' session with some fantastic speakers such as Dr. Norman Taylor, retired Consultant Clinical Biochemist of King's College Hospital, who I had the pleasure of meeting at the evening reception. Dr. Taylor relayed that his first encounter of an inborn error of steroid metabolism in a newborn was the day he first met Prof Shackleton for an informal interview! He was subsequently involved in the identification of many new steroids and disorders over his illustrious career. He spoke in particular about the difficulties identifying inborn errors of steroid metabolism in the newborn and distinguishing the metabolome of the baby from the remnant maternal metabolome otherwise known as 'day 1 metabolites'. He highlighted that it was this focus on the newborn that was the cornerstone for all that came after in the elucidation of etiology and pathogenesis of steroid disorders.

We were then treated to an engaging talk by Dr. Bill Griffiths about his research in the field of cholesterol biosynthesis and metabolism with the talk focussed on oxysterols, the intermediates between cholesterol and bile acids and steroids. Dr Griffiths relayed how oxysterols are biologically active and can cross membranes easier than cholesterol, mediating their effects. He relayed this work and its role in Huntington's disease (HD), which is an autosomal dominant disease of the neurological system. It is the disordered cholesterol metabolism that in fact produces the HD phenotype and as neurons die off the levels of 24-S-hydroxycholesterol (24OHC) decrease. Thus 24OHC has been used as a marker of HD progression.

Dr. Karl Storbeck provided the background on 11-oxygenated sex steroids and 11-ketotestosterone specifically, a potent androgen of which there was much discussion of its applicability to several disease states such as PCOS, castration-resistant prostate cancer, Congenital Adrenal Hyperplasia (CAH) and premature adrenarche.

This talk linked in with that of Beaumont Hospital's Prof. Michael O'Reilly, Consultant Endocrinologist and Clinical Associate Professor at the RCSI, who focussed on the topic of 'PCOS – the female andro-metabolic syndrome'. Prof. O'Reilly discussed the role played by androgens in mediating metabolic dysfunction in women with PCOS, which affects up to 10% of all women. Prof. O'Reilly explained the pathogenesis of PCOS and the interplay between androgen excess and insulin resistance, work which he completed during his PhD and post-doctoral post in the IMSR. He then discussed his ongoing research at the RCSI in the field of skeletal muscle and the 11-oxygenated an-

drogen subclass, which is an exciting body of work we can look forward to.

Dr Hans Hofland discussed the role of 11-ketotestosterone in castration-resistant prostate cancer. Prostate cancer is an androgen dependent disease that affects 1 in 8 males and castration is an effective treatment as it depletes androgens. However, castration removes testicular testosterone but adrenal androgens drive the androgen receptor and 11-ketotestosterone has a key role as the most abundant androgen in this cohort.

In a similar vein but in the context of CAH, Dr Nicole Reisch spoke about the positive correlation of 11-ketotestosterone and testosterone in females with CAH. She also outlined how 11-OH androgens and 11-ketotestosterone are higher in CAH in patients with amenorrhoea when compared with those with normal cycles. This is mirrored in males with CAH that experience hypogonadism also. She also presented data on a retrospective analysis of laboratory data of CAH patients published in 2021 that showed that 17% of laboratory assessments of androstenedione and 17-hydroxyprogesterone (17OHP) showed discrepancies, with elevated 17OHP found in cases where androstenedione was within reference intervals. This highlighted the role of 11-oxyandrogens, especially 11-hydroxytestosterone in the management of CAH when conventional biomarkers are inconclusive.

Finlay MacKenzie, a very familiar face to all Clinical Biochemists, opened the second session entitled 'Steroid Analysis – Present and Future' discussing the state of play measuring steroids in hospital laboratories from the UKNEQAS perspective. Following a very insightful talk discussing the advantages and disadvantages of the different steroid hormone platforms available for various tests, he then discussed some of the frequently used methods employed by UKNEQAS to challenge the methodologies in use such as spiking experiments e.g. prednisolone in Cortisol distributions which was of huge interest to the clinicians.

Prof. Brian Keevil presented his work from Wythenshawe on the use of salivary steroids in the context of adrenal insufficiency and mild autonomous cortisol excretion. He presented data on the use of waking salivary cortisone in the context of adrenal insufficiency which is estimated to reduce the need for Short Synacthen Testing by 70% with implications for cost reduction. The group has also examined the role of salivary cortisone sampling post-dexamethasone suppression. This was an incredibly insightful talk highlighting the need to respond to the pandemic and life thereafter, by keeping vulnerable patients out of the hospital unnecessarily.

I would like to sincerely thank the organisers and sponsors for a fantastic 2 day event, with highly engaging and inspiring scientific content and a fabulous evening reception with great opportunities for networking with Clinicians, Researchers and Clinical Biochemists.

It was inspiring to see the close working relationship of Clinical Biochemists and Clinician-Scientists is still as strong today as it ever was and that the symbiosis of research and development can produce an enormous body of work, highlighted by the long and influential work of Prof Cedric Shackleton.

Let's all take a moment to appreciate the GC-MS birthday cake, with the MASSter himself, Prof. Cedric Shackleton!

## Journal Review

Have you seen an interesting paper in a journal that not everyone might see? Would you consider writing a short review of the paper to bring it to the attention of our readers? Clinical Biochemistry News would welcome such submissions — please send your review to me at secretary@acbi.ie. There is no requirement on length, which can be as short or long as suits the topic.

To kick off the process here's one I prepared earlier. As I am an older retired member I have chosen a 2021 paper re-looking at a clinical biochemistry scenario from over a century ago. **Dr. Peadar McGing, Principal Clinical Biochemist (ret)**

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# *The signs and symptoms of Ernest Shackleton*

[J Med Biogr;. 2021 Apr 24; Online ahead of print.
DOI: 10.1177/09677720211002205;
Authors: P G Firth, O J Benavidez, L Fiechtner. Massachusetts General Hospital, Boston, MA, USA.]

The discovery this year of Sir Ernest Shackleton's ship, *The Endurance,* frozen in time three kilometres down in the freezing Antarctic waters, together with an RTE programme this month on a re-creation of the cabin Shackleton lived in, and died in, on board the *Quest,* has put this famous Kildare man firmly back in the public eye. As one who since childhood has had an interest in Shackleton and Crean and their great adventures, I was intrigued when I saw a paper entitled *The signs and symptoms of Ernest Shackleton* in the Journal of Medical Biography.

Ernest Shackleton was born on February 15th, 1874 at Kilkea House, near Athy, and as the paper states 'was one of the giants of the heroic era of Antarctic exploration'. His family moved to England when he was 10, and at age 16 having been bored by school he went to sea, where he was to spend the bulk of his life. He died of a heart attack aged 47 on board the *Quest* in Grytvken harbour, South Georgia, at 3:30 a.m. on January 5th, 1922.

This paper focusses on the general medical problems which beset Shackleton on his four Antarctic expeditions. 'On [these] trips, he experienced significant signs and symptoms that his contemporaries and subsequent historians attributed to scurvy or underlying heart disease.' We are reminded that in the early 1900s vitamins were unknown and the biological mechanisms of related diseases were not established. Scurvy is the clinical manifestation of severe vitamin C deficiency, and though rarely seen in our modern western society it was the scourge of seafarers for many centuries and also a feature of the Irish famine. For the Antarctic explorers having fresh food was very difficult and cooking meat destroyed what small amounts of vitamins the raw meat contained. There seems little doubt that Shackleton and his teams suffered from scurvy, but what the paper questions is whether this was the main / only cause of his symptoms.

Like any good 'Case Report' this paper explores the evidence for and against scurvy as the cause of his 'life-threatening illness during the *Discovery* Antarctic expedition of 1901-4', linking what medical records exist from the time with what we know nowadays (thanks to that most powerful of diagnostic tools – the retrospectroscope). Scurvy, as mentioned above, is the result of severe vitamin C deficiency. Since Vitamin C is necessary for the production of collagen which is an essential component of capillary walls, one finds clinical symptoms such as bleeding gums, loose teeth, and subcutaneous bleeds. While Shackleton and his men did show these signs at some stages, the diagnosis did not fit neatly with the clinical picture. There were two doctors on board the ship, Reginald Koettlitz and Edward Wilson, so there are good clinical records available.

In early November 1902 a three-man team of Robert Scott (expedition leader), Shackleton, and Wilson (the doctor, but also an accomplished naturalist) headed south from their base at McMurdo Sound to explore the territory towards the South Pole. On Christmas Eve, over a month into the journey, Wilson diagnosed initial signs of scurvy in all three, but the next day they enjoyed the most southerly Christmas Day ever, celebrated with plum pudding as the star attraction. On January 1st, 1903, and over 500 miles from their ship, the decision was made to turn for home. The men's symptoms were all worsening including swollen and purple gums, and symmetrical haemorrhages under each big toe nail. Though all had symptoms of scurvy, Shackleton had additional problems, being very short of breath and unable to pull the sled with his colleagues. When they eventually made it back to the *Discovery,* and 'a more varied diet', the three recovered. Though Shackleton was invalided back to London he was deemed 'fit for Antarctic duty'.

The question of Shackleton's symptoms which were not clearly scurvy has engaged attention over many years, and particularly in this century. There was a suggestion that Shackleton may have had a congenital cardiac condition, but this seems unlikely if you consider his whole career rather than just his episodes of illness. The paper discusses aspects of clinical disease and how certain cardiac conditions would

be expected to affect an Antarctic explorer but his ascent of glaciers and walking 'hundreds of miles at high altitude' effectively rule this out. Also it was not just Shackleton exhibiting cardiac symptoms as these were found in many of his travelling companions. A paper in 2012 in the journal *Polar Record* (not on most clinical biochemists' reading lists) had the title ''Polar anaemia': cardiac failure during the heroic age of Antarctic exploration'.

So what was the likely diagnosis, as per the 2021 review? The expedition doctor, Wilson, had recorded he was puzzled – "I re-read all our literature on scurvy to remind me of the details…Spent the afternoon reading medicine, beriberi.' Beriberi is the clinical manifestation of severe thiamine (Vitamin B1) deficiency. Although 'in 1900, beriberi was considered to be a pathology of South-East and Southern Asia' a modern view would certainly implicate it. The Polar Anaemia paper mentioned above, and discussed by Firth et al, attributes the cardiac and related symptoms of the Antarctic explorers to beriberi. The Shackleton paper states that 'many of the signs of these early explorers – peripheral neuropathy, extensive edema, breathlessness and effort intolerance, presenting in a setting of prolonged reliance on preserved provisions – could be attributable to beriberi. Symptoms typically develop after three months of thiamine deficiency. The onset of symptoms would be consistent with the months of a thiamine-deficient diet during the winter or subsequent sled journeys.'

The biographies of these three authors indicates they cover the relevant expertise to make appropriate conclusions, being an anaesthetist with expertise in altitude physiology, a Division Chief in Pediatric / Congenital Cardiology, and a Director of Nutrition, all at the Massachusetts General Hospitals. While rightly adding the caveat that 'Almost a hundred years from the close of the heroic age of Antarctic exploration, it is difficult to determine the exact nature of Shackleton's disease', they do offer a clear opinion (which, after all, is the point of the paper). 'With the benefits of later insights into nutritional diseases, we suggest that Wilson's passing consideration – beriberi – was the correct diagnosis. Thiamine deficiency with cardiomyopathy, either alone or subsequently exacerbated by advance scurvy, was a prominent cause of Shackleton's condition.'

I'm sure you'll believe me when I say it's hard for me to remember back to much of my reading from my early teens. However, I do remember first reading about Shackleton and Crean and their heroic crossing of the ocean in a small boat to South Georgia, followed by an even more heroic crossing of the island's glacier to reach Stromness whaling station. From there they organised the safe rescue of the entire crew of the *Endurance*. In this year of the *Endurance* being found after more than a century beneath the Antarctic waters, it is interesting that as a clinical biochemist I can now appreciate even more the courage they and other explorers showed in achieving the feats they did while coping with illnesses arising from their exploits. And I can appreciate how our modern knowledge of clinical biochemistry helps solve an age-old mystery. Finally, a 'case' like this is certainly a different way to learn about nutritional deficiencies, so I'll note that in my CPD log.

Sub-Lieut. Ernest Henry Shackleton, R.N.R, aged 27.

# ACBI Spring Scientific Meeting

## Mater Hospital, April 6th 2022

Report by Ruth Cullen, Senior Clinical Biochemist, Mater Hospital, Dublin

The ACBI scientific meeting held in April in the Mater Hospital, Dublin and via zoom marked the return of in-person meetings. Discussions focused on the "Highs and Lows of Vitamin B12". Diagnostic challenges of Total B12 (TB12) deficiency or raised levels, from both a clinical and laboratory perspective, were presented.

Dr. Niamh Fanning, Paediatric Registrar, CHI Crumlin, presented an interesting case of TB12 deficiency in a newborn. Lack of TB12 harmonisation across laboratories was discussed as well as the treatment and management of B12 deficiency in a paediatric cohort.

The clinical presentations of infantile B12 deficiency were reviewed and a practical approach to investigation was summarised. The causes of infantile B12 deficiency are heterogeneous and can be further divided into causes by age, ranging from dietary deficiency in a breastfeeding mother (as in the presented case) to inborn errors of metabolism.

The next half of the talk focused on TB12 from a laboratory perspective. Standardisation remains a problem, with between method differences for commercially available assays namely, TB12 and Active B12. Active B12 Reference Intervals and diagnostic cut-offs in paediatrics are not well described. Dr. Fanning explained that experts suggest models that combine multiple markers of B12 status into a single diagnostic indicator is the most sophisticated way of assessing B12 deficiency. However, the cost associated with the latter would be significant. Therefore, sequential analysis is the preferred option in most laboratories today. In the absence of a Gold standard test to define TB12 deficiency, it is the clinical picture that remains the most important factor. Standard B12 replacement in paediatrics is intramuscular B12 with requirements of repeating levels of homocysteine and MMA 1 – 2 weeks after commencement of treatment. TB12 deficiency due to metabolic causes requires an individualised, specialised follow-up.

The talk concluded with learning points that stressed the importance of identifying B12 deficiency in the paediatric setting and how it may be an under recognised cause of neurological morbidity in infants. Dr. Fanning stressed that maternal dietary history taking in suspected infantile TB12 deficiency is paramount, even in cases where there is subclinical maternal B12 deficiency.

Ms. Ruth Cullen, Senior Clinical Biochemist, MMUH, followed with a talk on raised B12. A MMUH study, supervised by Dr. Graham Lee, which investigated Macro-B12 interference, and the proposed implementation of a PEG precipitation procedure was introduced.

Paradoxically, investigation of patients with symptoms of B12 deficiency may reveal raised B12 levels. TB12 elevations are a frequent and underestimated anomaly, reportedly more common than deficient levels. Increased B12 may commonly reflect patients on supplementation but may also be indicative of myeloproliferative disorders, liver, idney, intestinal disease, or disorders with a related inflammatory activity. In these cases, raised B12 levels may be caused by; up-regulation and synthesis of transport proteins, increased release of cellular B12 from the liver and decreased clearance from plasma. Analytical interference that may result in erroneously reported high B12 levels should always be considered. We are aware that anti-intrinsic antibodies pose a diagnostic challenge in pernicious anaemia.

Ms. Cullen emphasised that reported cases of immune complexes formed between vitamin B12 binding proteins and immunoglobulins in the blood are becoming more frequent. Furthermore, that this interference was not analyser dependent, with reported cases across all major platforms.

Large-scale studies are needed to access the actual incidence and prevalence of macro-B12 in the general population. More recent studies report a prevalence of 25%, this value increasing with markedly elevated B12 values.

The MMUH study included establishment of a post-PEG TB12 reference range (RR). Macro-B12 prevalence was determined by treating consecutive samples that had a raised B12 with a PEG procedure. Samples were then analysed on the Abbott Architect. The presence of macro-B12 was evaluated by comparing the B12 following PEG treatment against the newly established post-PEG RR. Recovery % was determined using the following formula; (B12 + PEG result/B12 + PBS result) x 100. Discussions concluded with a proposed algorithm for the investigation of elevated B12 which incorporated macro-B12 screening. The estimated macro-B12 prevalence in the MMUH (7%) was lower than previously reported in a Roche study (18%). Differences in study design, methods and interpretative criteria may have accounted for the notable variation and might make comparisons less relevant to data from the MMUH.

Closing remarks followed including comments that PEG precipitation may be a crude method for removing interfering molecules, but it appears effective. It shows potential for mitigating against misdiagnosis and mismanagement of patients with unexplained or clinically discordant raised TB12.

# EFLM Lessons in Immunohistochemistry — Hypertension

**Reported by Dr. Ciara Cunning, Clinical Biochemist, Mater Misericordiae University Hospital**

At the beginning of the year, the European Federation of Clinical Medicine (EFLM) Executive Board announced a new educational activity for EFLM academy members. The 2022-2023 "Lessons in Immunohistochemistry" are presented by clinicians and laboratory medicine specialists, and discuss specific diseases and their diagnostic challenges from both a clinical and laboratory perspective. The first of the lessons was titled "Hypertension: the role of laboratory in differential diagnosis". Prof. Damien Gruson (Belgium) opened the lesson with facts on the prevalence, morbidity and mortality of HTN; which is the leading risk factor for death worldwide. Prof. Gruson discussed the many aetiologies of HTN focusing mainly on primary hyperaldosteronism. The lesson covered the importance of the aldosterone-renin ratio as a screening test in hypertensive patients and furthermore discussed confounding factors such as medications, potassium status, dietary sodium and age.

Prof. Ah Jan Danser (The Netherlands) followed up with an overview of the renin-angiotensin-aldosterone system (RAAS) and the consequences/effects of RAAS inhibition with ACE inhibitors, Angiotensin II receptor blockers and direct renin inhibitors. This talk covered analytical issues associated with renin, aldosterone, and angiotensin assays and highlighted the fact that many (non-validated) kits exist with different standards and in some cases are known to yield unreliable data.

The last leg of the lesson was presented by Prof. Alexandre Persu (Belgium) who gave us a clinician's perspective on HTN and discussed antihypertensive treatments and their importance in reducing complications such as myocardial infarction, stroke, heart failure and renal insufficiency. The lesson detailed some characteristic signs and symptoms patients may exhibit that should raise suspicion of secondary HTN, as well as informing us of the common causes. Prof. Persu also covered how to recognise patients that may have a phaeochromocytoma, and discussed the tests involved in screening for this (urinary normetanephphrines and metanephrines) and some potential sources of unreliable results (e.g. the risk of false positives due to pharmacodynamic interference).

To conclude, I found the lesson met my key learning objective to gain knowledge about the laboratory and clinical aspects of HTN and the diagnostic challenges. The session was highly beneficial; a credit to the quality of speakers invited, and I hope to be able to attend more EFLM lessons in the future to continue to gain knowledge in my "thrilling" journey towards undertaking the FRCPath exams.

Next up… EFLM Lesson 2: Vitamin D status: the role of laboratory (30 May 2022).

EFLM Lesson 3: Heart Failure and NT-ProBNP (13 September)

## Retired Members' Section for ACBI

All registered retired members of ACBI should have received an email canvassing opinion on forming a Retired Members' Section of ACBI. The focus of this new subgroup would be social. Retired members are already fully entitled to attend all ACBI scientific meetings and engage in all academic activities. A good number of responses to that email have been received, almost all being interested in participating in such a group. If you have yet to reply, please do get back to me (now is a good time). I will be emailing interested members very soon to take this initiative forward.

Any thoughts members may have on this, and particularly any offers of help with organising, please drop me a line at secretary@acbi.ie (or contact me personally if you prefer).

Peadar McGing, ACBI Secretary.

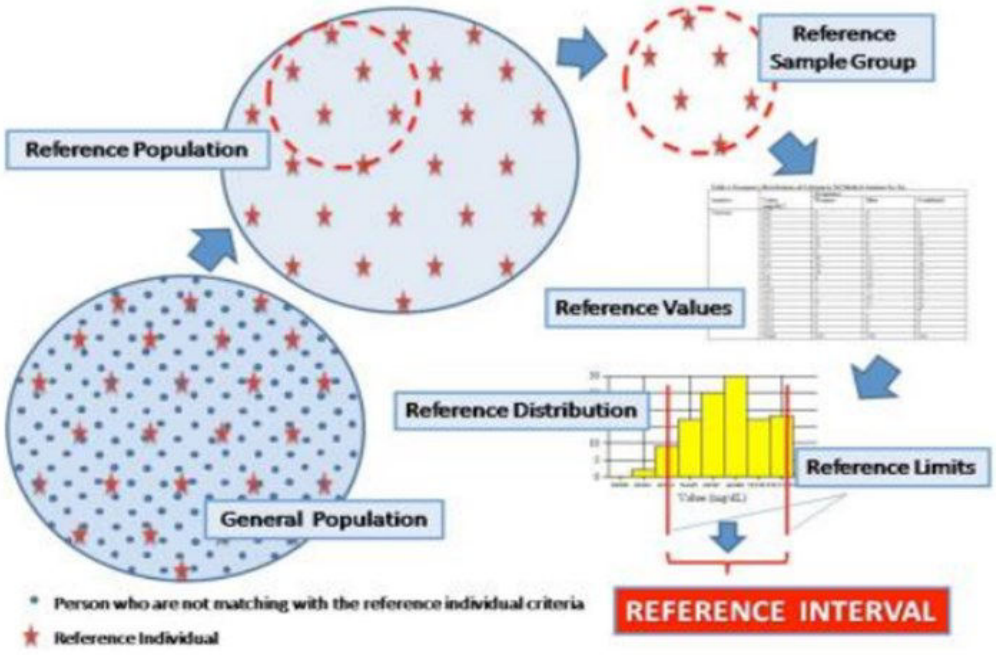
# EFLM Webinar — Reference Interval Verification

Natividad Ricorios, Principal Clinical Biochemist, Cork University Hospital

The European Federation of Clinical Chemistry (EFLM) offers fantastic and useful free e-learning material on their website. There are live webinars with a monthly or bimonthly frequency on a variety of topics, covering not only scientific, quality and clinical aspects of laboratory medicine but also management and interpersonal skills aspects.

These lectures are scheduled at 18:00 CET time and they can be watched upon registration in real time through the EFLM e-learning platform (https://www.eflm-elearning.eu/site/ ) with the opportunity of asking questions to the speakers in real time. The EFLM issues a certificate of attendance for CPD records that can be found in the member area. If unable to attend live, all webinars are recorded, and video and slides are available in the "on demand-webinars section" using EFLM Academy log in.

One webinar that I thought was very interesting as all the laboratories should have a clear procedure in place was "Reference intervals (RIs) verification by indirect methods" by Matteo Vidali (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano). Dr Vidali divided the talk in three sections: direct methods, indirect methods and alternative methods of RIs verification.




https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4975208/ Aytekin and Emerke, JIFCC 2008

During the first section, the direct methods, he described all variables to consider beforehand: non-statistics procedures such as exclusion and partitioning criteria, pre-analytical and analytical aspects (biological and methodological) and the statistic aspects such as outliers' detection (to obtain a homogeneous set of data), partitioning (to increase sample size), RI calculation using parametric, nonparametric, percentile calculation and %CI calculation. He highlighted some pros like the well-defined reference population, control over pre-analytical and analytical variables and simpler statistical methods; and cons such as the pre-analytical conditions are generally not matching routine conditions, requirement of a bigger sample size (and bigger still if partitioning is needed), samples from extreme age subjects or uncommon sample types are difficult to obtain, extra cost and ethical issues.

On the second section, the indirect methods, Dr Vidali highlighted the key elements of the indirect methods: the data is already available in the laboratory database but it could require high number of subjects, the data reflect routine lab operating conditions and the majority of data in the laboratory database should come from healthy subjects. Then he described the steps to follow. First, data sets are obtained from the laboratory databases and this should be cleaned following a pre-selection or exclusion strategy where healthy individuals' results should only be considered. These strategies can be based on request form information, drug or clinical details information, other laboratory results obtained, machine learning approach, etc. Next steps include the study of the data distribution (Gaussian, skewed) and transformation of it (Log, Box-Cox); review of method and population stability (e.g. population median or percentiles over time, IQC or EQA trends assessment); and use of algorithms. Dr

Vitali explained in detail the different algorithms available to use: Hoffmann and modifications, Bhattacharya and modifications, Arzideh and updates; and the softwares developed based on these algorithms: Bhattacharya - Bellview (Douglas Chesher), Bhattacharya Spreadsheet (Graham Jones); Arzideh and DGKL - Reference Limit Estimator; Zierk- Kosmic and implementation in R (packages already available https://labrtorian.com/)
Dr Vidali finished this second part with pros and cons of indirect methods where the pros are the large amount of available data including data for uncommon sample types and from extreme age subjects readily available, the possibility to derive continuous reference intervals, the pre-analytical and analytical conditions match routine conditions, reduced costs, easy to reproduce and no ethical issues, while the cons include the requirement of more statistical knowledge, poor knowledge of population characteristics, poor control over pre-analytical conditions and no gold standard procedure.

The third part of the talk summarised alternative approaches to derive RIs:

1) Transference of RI
a) RI established previously in the same lab by method comparison study
b) RI established elsewhere or Multicenter/Common RIs (e.g. IFCC protocol) where lab should verify similar pre analytical processes, method comparability and if relevant population differences exist. Local validation is recommended in both scenarios.
2) Verifying/Validating RIs. Statistical test on a small sample of reference individuals (20 samples) on a bigger sample of reference individuals (but <120).
This is just an example, but these webinars are excellent to all laboratory medicine professionals.

## Miscellaneous thoughts from Peadar

***A Thought on Committees:***

Is it coincidence that of the nine letters in the word 'committee', two-thirds are duplicate letters?

***Celebrity Health Endorsements:***

You might think that endorsement of health messages by stars of stage and screen is a relatively modern phenomenon but not so. The Irish Times of September 21, 1906 carried a report under the heading 'Miss Olga Nethersole on Tuberculosis'. Olga Nethersole was a very famous English actress (later CBE) and she was in Dublin on a special mission. The Times reported 'Yesterday afternoon a public meeting was held in the Mansion House for the purpose of hearing an address from Miss Olga Nethersole in reference to the formation of a Universal Women's League for assisting in the prevention and cure of tubercular disease.' TB was the biggest medical problem in Ireland at the time but the plans outlined may have been too ambitious and required considerable private donation, which I don't believe came about. However, the meeting probably helped ongoing efforts by many doctors to highlight education on the disease. Sir Charles Cameron MD, Superintendent Medical Officer of Health, in proposing a vote of thanks to Miss Nethersole used the occasion to outline the high death rate from TB in Dublin plus discuss the causes of the disease.

***Dipstick invention in elite company:***

In the March edition of Clinical Biochemistry News I wrote a piece on Helen Free and her achievement in developing the first effective dip-sticks for glucose. Information I saw later shows just how important to humanity that simple clinical biochemistry test was, as seen by the judges for the 2009 National Medal of Technology and Innovation, the USA's highest honour for technological achievement. As well as Helen's award for 'development of dip-and-read urinalysis' the other three awards were for the invention of cyanoacrylates ("super glues"), the invention of the digital camera, and the invention, design, and application of the first microprocessor. Elite company indeed for a simple biochemistry test we probably take so much for granted.

Olga Nethersole, stage actress (SAYRE 1645) [public domain]