

ACBI

Annual Conference 2012

Proceedings of the 35th Conference of the
Association of Clinical Biochemists in Ireland

5th and 6th October

Croke Park, Dublin

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A Message from the President of ACBI

On behalf of the Association of Clinical Biochemists in Ireland, I am delighted to welcome delegates to the ACBI Annual Conference 2012. Our venue this year is the historic Croke Park stadium which has particular relevance given the changes to our working lives following previous negotiations at this venue. This is a magnificent (carbon neutral) venue, home to the GAA, is well known for hosting sporting events for up to 83,000 people.

This year is a particularly good year for Science in Ireland as Dublin has been designated City of Science for 2012. Science is now recognised as an area at which Ireland excels and Clinical Biochemistry is one of the important applications of science to patient care in Irish hospitals.



We have an exciting and relevant scientific programme planned for the two days of the conference. Topics on the first day include Demand Management in Primary Care, Neuroendocrinology, Transplantation, Renal Disease. Topics for the second day include Diagnostic and Innovative Technologies and the Law and Toxicology. All are relevant to the practice on Clinical Biochemistry in Ireland today. In addition we continue to promote the Labs are Vital initiative. This important venture between the ACBI, the Faculty of Pathology and Abbott Diagnostics aims to raise awareness and understanding of the contribution of clinical laboratories to healthcare.

As well as the formal Scientific programme, I welcome the opportunity for delegates to submit their research in poster format. In our present circumstances it is hard to find the time for the research and audit that improves our service to patients and that can then be shared with our colleagues in poster format at the annual conference. The Geraldine Roberts medal will be awarded to the author of the best scientific poster.

I wish to congratulate Ger Collier and her team at Beaumont for all their work in putting together such an excellent programme. With the extended working day and many staff losses, it takes an enormous amount of dedication and hard work for those involved in the organisation of this conference.

I am sure that delegates will enjoy this conference and bring back new ideas to their workplace for the benefit of Clinical Biochemistry and ultimately for patients.

Ruth O'Kelly
President, ACBI

Welcome to ACBI 2012

On behalf of the ACBI and the organising committee I would like to welcome you to the 35th annual conference of the Association of Clinical Biochemists in Ireland. This year, we are hosting it in one of the most historic venues in Dublin, Croke Park, which recently has been a constant topic for discussion not only for the GAA finals but now for the infamous Croke Park agreement.

I would like to welcome all our speakers both from here and abroad and our delegates who travelled from near and far. Thank you for coming and I really hope you all enjoy the conference. The ACBI are also extremely grateful to our colleagues from the Corporate Sector, who have actively supported this conference for the last 35 years.

Finally, thanks to all the committee members who gave of their time freely and worked to make this conference happen. It is difficult times for all of us at present and the effort that everyone put in is really appreciated.

Geraldine Collier

Chair of ACBI Conference committee

Continuing Education

Royal College:

ACBI 2012 Conference has been approved for CPD by the Royal College of Pathologists.

The maximum credits awarded are 8 points for the two day conference.

In order to receive these credits, the participant must sign the RCPATH register for each day attended and is issued with a certificate of attendance by the conference organising committee.

Academy of Medical Laboratory Sciences:

ACBI 2012 has been approved by the Academy of Medical Laboratory Sciences for the award of 15 CPD points for the two day conference.

The password for attendees to gain these points is E0FB2D (E zeroFBtwoD).

Each member is issued a certificate of attendance by the conference organising committee.

Evaluation of ACBI 2012:

All conference participants are requested to complete the conference evaluation form located in the delegate bags. This form is to be returned to the conference registration desk.

Programme - Friday 5th October 2012

8.45am:

9.15am:

Breakfast sponsored by  Agilent Technologies and 

Message from the President of ACBI

**Official opening of ACBI Conference by
Ms. Laverne McGuinness,
HSE National Director, Integrated Services**

SESSION 1: FRIDAY AM

Management Issues & Neuroendocrinology

Chair: Mr. Roland FitzGerald, Beaumont Hospital

9.50am - 10.30am:

Demand Management in Primary Care

Dr. Tim Lang,

Consultant Clinical Scientist, University Hospital, North Durham

10.30am - 11.00am:

Poster Presentation and Tea/Coffee Break sponsored by Brennan and Company

11.00am - 11.40am:

Biochemical Diagnosis of Pheochromocytoma: from routine laboratory testing to disease stratification and personalised medicine

Professor Graeme Eisenhofer,

Professor and Chief in Clinical Neurochemistry, Dresden, Germany.

11.40am - 12.20pm:

The Management and Treatment of Neuroblastoma

Dr. Anne O' Meara,

Consultant Oncologist, Our Lady's Hospital for Sick Children, Crumlin.

12.20pm - 12.30pm:

Labs are Vital

12.30pm - 2.00pm:

Lunch & Poster Presentation

SESSION 2: FRIDAY PM

Transplantation & Renal Disease

Chair: Mr. Rowland Reece,

Department of Biochemistry, St. Vincent's University Hospital

2.00pm - 2.40pm

Generic substitution of Immunosuppressive drugs in Transplantation

Professor Teun Van Gelder,

Professor in Clinical Pharmacology,
Erasmus Medical Center, Rotterdam, The Netherlands

2.40pm - 3.20pm:

Kidney Transplantation in Ireland - recent observations

Professor Peter Conlon,

Consultant in Nephrology, Beaumont Hospital, Dublin

3.20pm - 4.00pm:

Acute Kidney Injury - How do we define it?

Dr. Andrew Lewington,

Consultant in Nephrology, St. James University Hospital, Leeds, UK.

4.00pm - 4.30pm

Coffee Break

4.30pm - 5.30pm

ACBI AGM or Tour of Croke Park Stadium

5.30pm - 9.30pm:

Drinks Reception, Dinner and Entertainment in Croke Park Hotel



Programme - Saturday 6th October 2012

9.15am - 10.30am:
10.00am - 10.30am:

Breakfast sponsored by  **Agilent Technologies** and  **Poster Presentations**

SESSION 3: SATURDAY AM **Diagnostics & Innovative Technologies**

Chair: Ms. Ellie Duly,
Clinical Chemistry Dept, Ulster Hospital, Belfast

10.30am - 11.10am:

The Biochemistry of Inflammation - how discoveries are leading to better medicine for Inflammatory Diseases

Professor Luke O'Neill,
Director of Biochemistry & Immunology, Trinity College, Dublin

11.10am - 11.50am:

Integrating platform technologies into point-of-care diagnostics

Professor Michael Berndt,
Director of Biomedical Diagnostic Institute, Dublin City University

11.50am - 12.30pm:

Genomics and Human Disease: Advances and Clinical Applications in the Post Genome Era.

Dr. Patrick Buckley,
Specialist Scientist, Department of Neuropathology, Beaumont Hospital, Dublin

Lunch & Posters:

12.30pm - 1.45pm

SESSION 4: SATURDAY PM **Science, The Law & Toxicology**

Chair: Mr. Neil O'Brien,
Department of Chemical Pathology, Beaumont Hospital.

1.45pm - 2.20pm

Role of Law in the Regulation of Science, Medicine & Technology

Mr. Asim A. Sheik,
Barrister-in Law, Lecturer in Legal Medicine, UCD

2.30pm - 3.00pm:

Judging of Posters

3.00pm - 3.40pm:

The Role of Toxicology in Emergency Medicine

Professor W. Tormey,
Consultant Chemical Pathologist, Beaumont Hospital, Dublin

3.40pm - 4.00pm:

Presentation of Geraldine Roberts medal and Close of Conference

SESSION 1

Management Issues & Neuroendocrinology

Chairperson

Mr. Roland FitzGerald,

Department of Chemical Pathology, Beaumont Hospital.



T E C H N O P A T H

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SESSION 1

Management Issues & Neuroendocrinology

Dr. Tim Lang

Consultant Clinical Scientist,
University Hospital,
North Durham

Dr Lang is a Consultant Clinical Scientist at the University Hospital of North Durham. He studied physiology at Newcastle University and then moved to Manchester University to study for a PhD. He then trained as a clinical biochemist in Nottingham before moving to senior posts at the John Radcliffe Hospital, Oxford and the Royal Victoria Hospital in Belfast. During these posts he developed his interest in paediatric biochemistry and inherited metabolic diseases. In Belfast he developed the regional paediatric biochemistry service establishing a strong clinical network across the province and a research interest in hypoglycaemia in infancy. In his current post he has had the opportunity to develop demand management solutions for primary and secondary care. He is the project lead for the National Minimum Re-testing Interval project.

Demand Management in Primary Care

Over the last 10 years, Pathology in the UK and Ireland has seen an average annual increase in workload of 10% accompanied by increasing costs and reduced revenues. It is also essential that the laboratory is able to identify appropriate and inappropriate requests to ensure the right test is done in the right patient at the right time. Demand management solutions continue to be developed to address this need using a variety of mediums and tools supported by appropriate evidence, where available and partnership working. The National Minimum Re-testing Interval Project has aimed to address the lack of consensus and evidence based guidance for use of minimum re-testing intervals in Primary and Secondary care, bringing together recommendations for common biochemistry tests in one document. Through a thorough review process these recommendations have been prepared to represent the best practice in the opinion of the authors and have been reviewed by a consensus approach. Implementing a demand management solution requires the provision of appropriate laboratory and information technology support. Computerised physician order entry software, originally developed for prescribing, provides a new tool which should encourage more laboratories to implement appropriate solutions. Feedback and the ability to override a solution by the clinician is important, in addition to recording the reason for override. By implementing such solutions the laboratory will be able to provide a better service to the patient and clinician within the limited resources available.

SESSION 1

Management Issues & Neuroendocrinology

Professor Graeme Eisenhofer

Institute of Clinical Chemistry and
Laboratory Medicine and
Department of Medicine III,
University of Dresden,
Dresden, Germany

Graeme Eisenhofer received his PhD in 1983 from the University of Otago, New Zealand, with thesis work involving clinical research on autonomic and neuroendocrine systems. He then moved to the National Institutes of Health (NIH) where he carried out basic and clinical studies mapping the pathways of catecholamine metabolism. After completing his postdoctoral studies, he moved in 1988 to the Baker Heart Research Institute (Melbourne, Australia), where he continued his research on sympathetic nerve function in health and disease. He returned to the NIH in 1991 as head of the Clinical Neurochemistry Unit before taking up a Professorship in the Institute of Clinical Chemistry and Laboratory Medicine and the Department of Medicine at the University Hospital in Dresden, Germany. Together with Dr. Jacques Lenders, he developed measurements of plasma free metanephrines as a biochemical test for diagnosis of pheochromocytoma. He was also responsible the first ever synthesis of ¹⁸F-fluorodopamine as a positron emission tomographic imaging agent for localizing catecholamine-producing tumors. These achievements rapidly led to the NIH becoming the leading clinical referral and research center in the US for patients with pheochromocytoma. Consequently, Eisenhofer's research increasingly focused on catecholamine-producing tumors, this ranging from strictly clinical studies on the biochemical diagnosis, genetics and localization of pheochromocytoma to basic studies on tumor cell biology. From 2004 until early 2009 Eisenhofer acted as a cochair of the Pheochromocytoma Research Support Organization, an International Consortium for facilitating research on pheochromocytoma. He has also been an organizer or co-organizer of five international meetings and several workshops focusing on catecholamine-producing tumors. To date, he has authored over 350 research articles, reviews and book chapters, a third of which have been directed at catecholamine-producing tumors. He has written or edited 3 books related to endocrine hypertension, including a recent monograph, "Pheochromocytoma: Diagnosis, Localization and Treatment".

Biochemical Diagnosis of Pheochromocytoma: From Routine Laboratory Testing to Disease Stratification and Personalized Medicine

The laboratory workup of patients with pheochromocytoma and extra-adrenal paraganglioma (PPGLs) has traditionally focused on biochemical measurements of tumour secretory products or their metabolites. To first think of the tumour remains the critical step for screening in patients with signs and symptoms of catecholamine excess. In these routine cases biochemical testing is usually straightforward and should always include measurements of either or both plasma free or urinary fractionated metanephrines. The most appropriate method of analysis is an important consideration, with mass spectrometric-based methods showing numerous advantages over other methods. Preanalytical factors that can impact test results and use of appropriate reference intervals represent other important considerations to reduce false-positive test results without negatively impacting tumour detection. While such considerations are of general importance for all patients with suspected PPGLs, the needs to distinguish potentially metastatic from benign tumours and to identify tumours with a hereditary basis have stimulated searches for additional means to stratify patients according to risk of metastasis or presence of a particular mutation. With increasing recognition of the heterogeneity of the tumours and identification of ten tumour susceptibility genes to date the choice of the most appropriate laboratory tests and interpretation of test results has become more complex. PPGLs can no longer be regarded as a uniform disease entity, but rather as a highly heterogeneous group of chromaffin cell neoplasms with different ages of onset, secretory profiles, locations, and potentials for malignancy according to underlying genetic mutations. These aspects all have to be considered when the tumour is encountered for optimal management of individual patients and appropriate selection of genes for testing. Those patients and other family members with identified mutations require an personalized approach to management, with focus according to the mutation on different biochemical test results and imaging studies during screening and tumour localization.

SESSION 1

Management Issues & Neuroendocrinology

Dr. Anne O' Meara

Consultant Oncologist,
Our Lady's Hospital for Sick
Children, Crumlin.

Dr Anne O'Meara is a Paediatric
Oncologist in Our Lady's Hospital for
Sick Children with a special interest in
neuroblastoma and Haemopoietic Stem
Cell Transplant for metabolic disease.

The Management and Treatment of Neuroblastoma

SESSION 2

Transplantation & Renal Disease

Chairperson

Mr. Rowland Reece,

Department of Biochemistry, St. Vincent's University Hospital.



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SESSION 2

Transplantation & Renal Disease

Professor Teun Van Gelder

Professor in Clinical
Pharmacology, Erasmus
Medical Center, Rotterdam,
The Netherlands

Prof. Dr. Teun van Gelder is an internist-nephrologist and clinical pharmacologist in the Departments of Hospital Pharmacy and Internal Medicine at the Erasmus Medical Center in Rotterdam, the Netherlands. He was trained in internal medicine and nephrology at the Erasmus Medical Center, and completed his thesis in 1996 on the use of anti-interleukin-2 receptor monoclonal antibodies in solid organ transplantation. As a post-doctoral scientist, he worked in the Transplantation Immunology Laboratory of Dr. Randall E. Morris at Stanford University (1998-2000), and was awarded the Young Investigator Award from the American Society for Transplantation for his work during this time. Prof. Dr. van Gelder's current research at the Erasmus Medical Center is focused on clinical pharmacology and therapeutic drug monitoring. In 2010 he was appointed Professor in Clinical Pharmacology. He is the chairman of the Dutch Society for Clinical Pharmacology & Biopharmacy and the secretary of the Dutch Society for Transplantation.

Generic substitution of Immunosuppressive drugs in Transplantation

Switching transplanted patients who require life-long immunosuppressive therapy from brand name immunosuppressive drugs to generic formulations can lead to significant lower drug costs. As a society the European Society for Organ Transplantation is not opposed to the use of generic drugs. However, in order to safeguard the substitution process of generic drugs, we propose to regulate generic substitution of the immunosuppressive drugs in our vulnerable patient populations. This applies to the calcineurin inhibitors (ciclosporin and tacrolimus), mTOR inhibitors (sirolimus and everolimus) and to the mycophenolates (mycophenolate mofetil and mycophenolate sodium). In order to achieve safe and controlled generic substitution we propose the following guidelines:

1. Switching between the brand name drug and a generic formulation, and also between different generic formulations should only be initiated by the transplant physician.
2. Each switch needs to be followed closely to assure that the correct therapeutic window is established.
3. Repetitive consecutive substitutions to other generic formulations of the same drug should be avoided.
4. Patients should be informed about generic substitution, they should be educated how to identify the different formulations of the same drug, and they should alert the transplant physician if uncontrolled substitutions are made.
5. Further research is needed to fully explore the benefits and limitations of generic drug substitutions

Ref: European Society for Organ Transplantation advisory committee recommendations on generic substitution of immunosuppressive drugs. Transpl Int. 2011 Dec;24(12):1135-41.

SESSION 2

Transplantation & Renal Disease

Professor Peter Conlon

Consultant in Nephrology,
Beaumont Hospital, Dublin

Professor Peter J Conlon is consultant nephrologist and renal transplant physician at Beaumont Hospital and Professor of Nephrology at Royal College of Surgeons in Ireland and is clinical director of Transplantation Urology and Nephrology at Beaumont. He graduated from the Royal College of Surgeons and trained in Dublin and Duke University Medical center North Carolina USA. He has published more than 150 peer reviewed manuscripts and has a particular interest in genetics of kidney disease and transplantation outcomes.

Kidney Transplantation in Ireland - recent observations

The transplant unit at Beaumont Hospital has performed almost 4000 kidney transplants since it started transplantation in 1970. Professor Conlon will describe some recent studies undertaken with in the department looking at factors affecting the outcome of Kidney transplantation in Ireland.

SESSION 2

Transplantation & Renal Disease

Dr. Andrew Lewington

Consultant in Nephrology,
St. James University Hospital,
Leeds, UK

Dr Lewington studied renal medicine in Leicester and at Washington University in St Louis, where he investigated the molecular mechanisms underlying acute kidney injury. He is currently a full-time NHS consultant renal physician and Clinical Sub Dean for the Leeds Teaching Hospitals. Dr Lewington has provided a specialist renal opinion for critically ill patients on the intensive care units across Leeds. He was an expert adviser on the National Clinical Enquiry into Patient Outcomes and Death (NCEPOD) adding insult to injury acute kidney injury report in 2009. He was appointed to the Department of Health Acute Kidney Injury Delivery Board to address the recommendations proposed in the NCEPOD report.

He was a co-author of the British Consensus Guidelines on Intravenous Fluid Therapy in the Adult Surgical Patient (GIFTASUP). In 2011 he was appointed Chair of the Renal Association Clinical Practice Guidelines Committee. He is currently on the NICE AKI Guideline Development Group and Co-opted into the NICE IV Fluid Therapy Quality Standards Group. Dr Lewington's research is focused on biomarker discovery and development and he is Chief Investigator for the NIHR Kidney transplant biomarker study. He works in collaboration with the CRUK Proteomics group in Leeds.

Acute Kidney Injury - How do we define it?

Acute renal failure has traditionally been recognised as a rapid decline in kidney function over hours to days with failure to regulate fluid, electrolyte and acid-base balance. More than 35 different definitions of ARF have been used in the literature. This has hampered our ability to characterise the true incidence of the disease, assess its impact and make progress with clinical and scientific research. The proposals for a universal definition and classification system for AKI is the result of a collaborative effort by both nephrologists and intensive care specialists in the form of the Acute Dialysis Quality Initiative (ADQI), the Acute Kidney Injury Network (AKIN) and more recently the international guideline group Kidney Diseases: Improving Global Outcomes (KDIGO). The ADQI proposed the RIFLE staging system, which includes three levels of progressive kidney dysfunction, Risk, Injury and Failure and two outcomes, Loss of function and End stage kidney disease. Categorisation into a particular stage is dependent upon increases in serum creatinine concentration from baseline values within a one week time interval or reductions in urine output. The staging criteria (serum creatinine or urine output) selected is that which corresponds with the higher stage of AKI.

Subsequent studies have demonstrated that relatively small increments in serum creatinine concentration are associated with a significant increase in patient mortality. This prompted proposed further refinements to the definition and classification system by the AKIN in 2005. The Acute Kidney Injury Network modified the RIFLE staging system to produce an interim staging system to allow additional data to be gathered and research initiatives to be proposed. The modifications included using a rise in serum creatinine of $>26 \mu\text{mol/L}$ (0.3 mg/dL) within 48 hrs to define the presence of AKI thereby attempting to increase the sensitivity of the criteria. This change is based on work from the USA which demonstrated an increase in serum creatinine of just $26 \mu\text{mol/L}$ had a 70% higher multivariable adjusted odds of death compared to patients with little or no change in serum creatinine. Further changes included the introduction of a time constraint of 48 h between serum creatinine results and classifying any patient that received renal replacement therapy as stage 3 AKI. The 48 h time constraint was based upon data demonstrating that a poor outcome was associated with small increases in serum creatinine occurring within 24 to 48 h.

There are inherent difficulties with both of the newly proposed definitions as they rely on changes in either serum creatinine or urine output both of which are recognised as poor biomarkers. Serum creatinine concentration does not accurately reflect the true glomerular filtration rate (GFR) in a patient who is not in steady state. In the early stages of severe AKI serum creatinine may not be significantly increased despite a marked reduction in GFR due to there having been insufficient time for creatinine to accumulate in the blood pool. Once a patient has commenced on renal replacement therapy serum creatinine becomes less useful as a marker of kidney injury as it is removed. The accurate measurement of urine output is generally confined to patients with urinary catheters and is modified by the use of diuretics. It must also be remembered that AKI can be oliguric ($< 400 \text{ mL/24 h}$) or non-oliguric, so that increases in serum creatinine concentration can occur despite an adequate urine output. Another contentious issue is the deciding what constitutes the baseline serum creatinine concentration and what value to accept when calculating the stage of AKI.

The ADQI and AKIN definition and staging systems have now been harmonised by the KDIGO guidelines published in 2012. It is anticipated that there will be further modifications to the definition as further data becomes available. This may include the incorporation of novel biomarkers. There remains an obvious need for a universal definition to allow improved patient care and clinical research.

SESSION 3

Diagnostics & Innovative Technologies

Chairperson

Ms. Ellie Duly,

Clinical Chemistry Dept, Ulster Hospital, Belfast.



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SESSION 3

Diagnostics & Innovative Technologies

Professor Luke A.J. O'Neill

Director of Biochemistry &
Immunology,
Trinity College, Dublin

Professor Luke O'Neill was appointed to the Chair of Biochemistry at Trinity College Dublin in 2008, where he leads the Inflammation Research Group. He is also Academic Director of the Trinity Biomedical Sciences Institute. He has a PhD in Pharmacology from the University of London and carried out Post-Doctoral research at the Strangeways Laboratory in Cambridge. He has won numerous awards for his research, notably the Royal Irish Academy Medal for Biochemistry, The Irish Society for Immunology medal, the Royal Dublin Society/ Irish Times Boyle medal for Scientific Excellence and the Science Foundation Ireland Researcher of the Year Award 2009. He was elected a member of EMBO in 2005. He is a co-founder and director of Opsona Therapeutics. In 2008 he was appointed Chair of the Immunity and Infection panel of the European Research Council. His research is in the area of the molecular basis to inflammatory diseases, with a particular interest in pro-inflammatory cytokines and Toll-like receptors. He has published over 200 papers and reviews on his research, in journals such as Nature, Science, Cell, Nature Immunology, Nature Medicine, Nature Genetics and PNAS. He is on the editorial boards of 6 journals, including the Journal of Biological Chemistry and Trends in Immunology. He is also on the Board of Reviewing Editors for Science, covering Innate Immunity.

The Biochemistry of Inflammation - how discoveries are leading to better medicine for Inflammatory Diseases

In the field of inflammation research, the most important advances in the past 10 years has been in the uncovering of multiple pathways involved in innate immunity. There are now 7 distinct receptor families that sense microbial products and in some cases products of inflamed tissues, and trigger the innate response, which includes induction of pro-inflammatory mediators as well as effector mechanisms in host defence. The best characterised are the Toll-like receptors (TLRs), NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs). Genetic variation in several of these components has been linked to inflammatory diseases, notably in the TLR system and in the NLR protein Nlrp3 and associated proteins. Work on knockout mice and the use of inhibitors continues to validate some of these proteins in disease. From work on Nlrp3 there has also been a resurgence of interest in the IL1 system as a key driver of inflammation in diseases such as gout, diabetes (both Type I and Type II) and inflammatory joint diseases. Targetting of the TLRs is of interest in kidney transplant, myocardial infarct and in oncology. For investigators interested in signal transduction, the area has proved very fruitful in terms of the discovery of new signalling pathways and processes. Furthermore inhibitory mechanisms are also being revealed that are likely to be important for resolution of inflammation, notably miRNAs. It is also becoming evident that metabolic fluxes are triggered by innate immune receptors that are determining for inflammation. As we continue to unravel the molecular details of these processes, new therapeutic options will present themselves.

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SESSION 3

Diagnostics & Innovative Technologies

Professor Michael Berndt

Director of Biomedical
Diagnostics Institute,
Dublin City University,
Glasnevin, Dublin 9.

Prof. Michael Berndt is currently Director of the Biomedical Diagnostics Institute at Dublin City University and Professor of Experimental Medicine at the Royal College of Surgeons in Ireland.

His research interests include thrombosis, inflammation, vascular biology and biomedical diagnostics.

He has received numerous national and international research awards, including the Glaxo-Wellcome Medal in 1996 and a Distinguished Career Award from the International Society on Thrombosis and Haemostasis in 2003.

He serves on several editorial boards including the journals, *Blood* and *Journal of Thrombosis and Haemostasis*. He has published >290 papers including major international journals such as *Science*, *Journal of Experimental Medicine* and *Blood*.

Integrating platform technologies into point-of-care diagnostics

This talk will focus on recent work at the Biomedical Diagnostics Institute (BDI) in the development of Point-of-Care (POC) diagnostic systems. The production of portable, low-cost systems that, nonetheless, perform on a par with their high-end counterparts in the central laboratory constitutes a significant technological challenge. This requires the integration of multiple cutting-edge technologies and scientific know-how in fields such as biomolecular recognition, polymer micro- / nanofabrication, microfluidics, nanotechnology-enabled enhanced detection strategies, and nanoscale surface functionalisation. This talk will provide an overview of these capabilities with an emphasis on three recent developments:

1. A novel, degas-driven flow-based polymer chip with integrated filtering capabilities for immunoassay implementation;
2. Coagulation assay development on an Open Lateral Flow (OLF)-based sensor chip, and
3. Glassy surfaces produced by Plasma-Enhanced Chemical Vapour Deposition (PECVD) for the functionalisation of polymer microfluidic chips.

These examples illustrate underpinning scientific and technical expertise in addition to their effective integration, both of which are required in order to meet the challenge of robust, sensitive diagnostic performance with inherently low-cost, mass producible platforms.

SESSION 3

Diagnostics & Innovative Technologies

Dr. Patrick Buckley

Specialist Scientist,
Department of Neuropathology,
Beaumont Hospital, Dublin

In 2005, Patrick Buckley completed a PhD in Clinical Genetics at the Department of Genetics and Pathology, Uppsala University, Sweden.

He was later awarded a Post Doctoral fellowship between Uppsala University and the Karolinska Institute in Stockholm.

Moving back to Ireland, Patrick spent two years in the in-vitro diagnostics unit of the Medical Devices Department within the Irish Medicines Board working with all major IVD manufacturers and other European competent authorities on various regulatory aspects of in-vitro diagnostics. He then spent 4 years to initially set up the Department of Cancer Genetics with Professor Ray Stallings and then lecture at RCSI, before moving to Beaumont Hospital to establish a Molecular Pathology Laboratory. He is an organising member of the RCSI Research Summer School and lectures in Trinity College and DIT.

He has a keen interest in translational biomedical research with a focus on peripheral and central nervous system tumours.

Genomics and Human Disease: Advances and Clinical Applications in the Post Genome Era

Since the landmark draft human genome sequence publications in 2001, our understanding of the genetics of health and disease has deepened significantly. With the combination of faster and cheaper methods for genetic analysis, coupled with advances in bioinformatics, a mass of data in relation to the physical complexity of the genome and genomic interactions is finally being deciphered. This talk will focus on the application of current genetic methods for the diagnosis and prognosis of human diseases such as cancer, the practical benefits of translational biomedical research and an outline of the molecular facilities recently developed in Beaumont Hospital to meet these exciting new advances in biomedical science.

SESSION 4

Science, The Law & Toxicology

Chairperson

Mr. Neil O'Brien,

Department of Chemical Pathology, Beaumont Hospital.

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SESSION 4

Science, The Law & Toxicology

Mr. Asim A. Sheik

Barrister-in Law,
Lecturer in Legal Medicine,
UCD

Asim A. Sheikh is a practising Barrister, specialising in clinical negligence and healthcare law.

He is also a CEDR Accredited Mediator.

Graduated from the University of Limerick where he obtained B.A. in European Studies with Law and Spanish

Undertook and completed an LL.M. thesis by research, the topic of which was *"Human Genetics: The Ramifications for Law & Ethics"*

Organiser of Training Course in Courtroom skills for Medical Experts in UCD.

Specialist interest in the area of genetics & law. He has published in this area on the topics of human cloning, gene patents and genetic research.

Currently - Member of:

National Advisory Committee on Bioethics.

European Association of Healthcare Law.

Member of the Ethical, Legal and Societal Issues Working group for Gene Library Ireland.

World Association of Medical Law.

Research Ethics Committee (REC) of the Health Research Board (until 2007).

He is currently Editor-in-Chief of the Medico-legal Journal of Ireland.

Role of Law in the Regulation of Science, Medicine & Technology

The discussion will involve an examination of the law of negligence and the role that professional guidelines play in deciding standards of care in medicine and science and how this interaction plays out in the Courtroom or Professional Regulatory Body.

SESSION 4

Science, The Law & Toxicology

Professor W. Tormey

Consultant Chemical
Pathologist,
Beaumont Hospital, Dublin

Professor William Tormey is Consultant Chemical Pathologist at Beaumont and Connolly Hospitals, Dublin and visiting Professor at the University of Ulster at Coleraine. He graduated in Medicine and Pharmacology at UCD where he also holds a Diploma in Child Health. He holds Fellowship in Medicine at RCPI and in Chemical Pathology at RCPI and RCPATH. Previously, he was Clinical Tutor in Medicine at TCD, Lecturer at Leeds University and Reader at RCSI. He holds a PhD from TCD and has recently completed an MD in Forensic Toxicology at UU. He is Chairman of the HSE Forum for Dublin and the Northeast and is a member of the Economics and Finance Strategic Policy Committees of Dublin City Council. He has >125 publications listed in PUBMED and is a regular media panellist on Public Affairs. He is listed in Specialist Register of the Medical Council in Chemical Pathology and General Internal Medicine.

The Role of Toxicology in Emergency Medicine

Appropriate toxicology analyses for the Emergency Department has generic and site specific aspects. The Core Curriculum for Medical Toxicology Training in the US and in UK is explored to examine the fit with the reality of poisoning in these islands as demonstrated by the National Poisons Information Services and the National Drugs Related Death Index as collated by the Health Research Board in Ireland. The compounds usually involved in poisoning as presented to Accident Departments are discussed in conjunction with analytical methods and turnaround times. The NPIS/ACB recommendations are discussed in detail as are the complimentary US menus. Yale/New Haven and San Francisco Hospital menus are presented for comparison. Common toxicological syndromes are described and the shortcomings in current services highlighted.

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Poster 1

Removing the 'middleman' in a clinical laboratory environment

Maura Kehoe, Louise Lawlor, Paul Murray, Siobhan Stokes

The Drug Treatment Centre Board Laboratory, 30-31 Pearse Street, Dublin 2.

Introduction:

Historically routine Laboratory sample processing in the DTCB laboratory required two different information systems - LabWare-LIMS to enter, handle, monitor and store sample details and results and a commonly used commercial middleware interface system to provide a communication link between LabWare-LIMS and the chemical analysers (Beckman Coulter/Olympus AU2700). The middleware system functioned as a temporary store for sample details and results leading to duplication in the data processing cycle. The Laboratory carries out in excess of one million immunoassay screening tests per annum thus requiring automated systems coupled with efficient data processing and data storage compliant with the Data Protection Acts (1988 & 2003).

Aims:

Develop a novel solution with Labware-LIMS to transfer data directly between Labware-LIMS and AU2700 chemical analysers.

Key objectives:

- Create more streamlined processing system removing duplication
- Reduce the complexity of laboratory testing
- Improve turnaround times
- Reduce costs
- Improve Business Contingency - remove unsupported hardware, reduce risk of errors and points of system failure

Methods:

The chemical analysers provide serial data transfer using standard RS-232 communication ports, enabling network communication directly to LabWare-LIMS. The Lantronix UDS1100 Universal Device Server allows serial communication devices to connect and communicate over Ethernet networks using Transport Control Protocol – Internet Protocol (TCP-IP). Reconfiguration of LabWare-LIMS provided the validation functionality previously carried out in middleware.

The new system takes only six steps compared to eleven required previously saving time, reducing complexity, risk of data transfer errors through various systems and potential for system failure. The solution implemented also eliminated the unsupported concentrator previously required. Cumulative cost savings achieved include hardware and software support.

Conclusion:

We successfully implemented direct interfacing of AU2700 chemical analysers to Labware-LIMS providing real time bidirectional sample data transfer to and from the hosting system, reducing risk of error, saving time and costs within the laboratory. The novel application pioneered on our site has been put into production by Labware. Currently, over 50 analysers are now directly interfaced to LabWare-LIMS throughout Europe.



Poster 2

Diagnosis of Diabetes Mellitus by Oral Glucose Tolerance Test or HbA1c – the impact of stressed conditions

Gavin C, McGing P, Murphy E, Davis M, Fitzgibbon M

Mater Misericordiae University Hospital, Eccles St, Dublin 7

Background:

Diabetes mellitus (DM) is diagnosed by fasting plasma glucose (FPG), 2-hour plasma glucose (2hPG) post 75g oral glucose tolerance test (OGTT) or glycated haemoglobin (HbA1c). All criteria correlate well with risk of diabetic retinopathy.

Methods:

We looked at the performance of HbA1c to similarly diagnose DM in those having an OGTT. Diagnostic thresholds: FPG ≥ 7.0 mmol/L, 2hPG ≥ 11.1 mmol/L, HbA1C ≥ 48 mmol/L. Acute (in-patient) versus non-acute (out-patient) groups were assessed separately to address the potential impact of stress hyperglycaemia.

Results:

2694 OGTTs were analysed from July 2010 to June 2012. 837/2694 (31.1%) had a paired HbA1c in a 3-day period pre/post OGTT; 318/837 (38%) in acute conditions, 519/837 (62%) non-acute. In the acute group, 118/318 (37.1%) were diagnosed with DM by at least one test as follows: 3/118 (2.5%) by FPG only, 55/118 (46.7%) by 2hPG only, 4/118 (3.4%) by HbA1c only, and 32/118 (27.1%) by all three. 7/118 (5.9%) reached DM thresholds in both FPG and 2hPG, 1/118 (0.8%) in FPG and HbA1c and 16/118 (13.6%) in 2hPG and HbA1c. In the non-acute group, 308/519 (59.3%) had DM, 107/308 (34.7%) were diagnosed by FPG only, 48/308 (15.6%) by 2hPG only, 39/308 (12.7%) by HbA1c only, and none by all three. 19/308 (6.2%) reached DM thresholds in both FPG and 2hPG, 5/308 (1.6%) in FPG and HbA1c and 90/308 (29.2%) in 2hPG and HbA1c.

Conclusion:

In patients with at least one positive diagnostic test for DM, HbA1c was diagnostic in the same percentage of acute/non-acute patients, 45%/44% respectively, compared with OGTT - 97%/87% respectively. 2hPG was diagnostic in 93% of acute patients but just 51% of non-acute. FPG was diagnostic in a similar percentage - 36% acute/43% non-acute. 84% of non-acute and 53% of acute DM diagnoses were made using FPG or HbA1c.

Poster 3

A Review of Hyperamylasaemia in Clinical Practice in an Irish Academic Teaching Hospital

E Rasheed, R Abdul-Wahab, L Carleton, VEF Crowley

Biochemistry Department, St James's Hospital, Dublin 8

Introduction:

Serum amylase is a commonly requested clinical investigation particularly in the context of acute abdominal pain. However, while a raised serum amylase may be suggestive of acute pancreatitis there are many other potential explanations for hyperamylasaemia (serum amylase > 100 IU/L) in this clinical setting. This audit set out to review the characteristics associated with hyperamylasaemia encountered in routine clinical practice in a major academic teaching hospital in Ireland

Methods:

Using available IT resources, including the LIS and EPR systems in St James's Hospital, all requests for serum amylase and the associated results over a 3-month period were collated and reviewed by laboratory medical staff. In total 1500 requests for serum amylase were received and 226 (15%) of these were considered to have hyperamylasaemia (Range: 101 – 4508 IU/L). This represented a total of 150 individual patients. In relation to hyperamylasaemic results, 51% emanated from Emergency Dept. while 45% were from the in-patient cohort. In the majority (70%) GI-related symptoms prompted the amylase request. In 44 patients (29%) a diagnosis of pancreatitis was clinically established with 84% of these classified as acute pancreatitis (Range: 104 - 4508 IU/L) and the remainder chronic pancreatitis (Range: 109 - 371 IU/L). Among the patients with serum amylase >300 IU/L, 72% had acute pancreatitis. Considering the whole cohort of hyperamylasaemic patients this suggested a clinical sensitivity of 70% and specificity of 91% at the cut-off level >300IU/L.

Conclusion:

Overall this audit has provided some useful insights into the nature and value of serum amylase requesting in clinical medical practice in Ireland. Based on this data set, acute pancreatitis is only present in a minority of patients (25%) with hyperamylasaemia. While the diagnostic value of this test increases, to some extent, with the degree of hyperamylasaemia, the possibility of an alternative aetiopathogenesis for hyperamylasaemia must also be considered.



Poster 4

Serum Resistin Predicts Weight Change Response in Severe Obesity

Fidelma Lennon FAMLS SCH

Contributors:

Prof. Donal O'Shea SCH, Dr. Gerard Boran AMNCH, Dr. Tomás Ahern SCH, Dr. Gerard O'Connor, AMNCH

Context:

Serum resistin is a biomarker that could predict weight change response in severely obese people, participating in a weight management intervention programme. To date a biochemical explanation for the three weight change responses, weight loss, weight gain and no weight change, has not been documented. This paper illustrates the difference in serum resistin concentrations between the three possible weight change responses during the weight management intervention program.

Objective:

To determine if there is a statistically significant difference in serum resistin concentrations between the three weight change responses of obese participants, attending the obesity intervention weight management programme.

Participants:

The study cohort consisted of 63 severely obese participants and 12 non obese participants. The obese participants in the study were volunteers and were attending the Weight Management Clinic in St. Colmcille's hospital (SCH) at Loughlinstown, Co. Dublin.

Methods:

At the start of each participant's programme, baseline sera were collected. Participants' weight change response was recorded and each participant was grouped accordingly to weight gain, weight loss or weight static. Serum resistin concentrations were determined with a manual DuoSet ELISA resistin assay from R&D Systems. This cell supernatant assay was validated for serum estimation according to ACBI guidelines prior to participant testing, during the study. Statistically significant differences between obese subgroups were determined using non-parametric statistics with GraphPad Prism statistical software.

Results:

25 of the obese participants lost weight, 18 remained at the same weight and 20 participants gained weight. A significant association was found between weight change and serum resistin concentrations. Using the Kruskal Wallis test, the serum resistin concentrations were 10.35 ± 7.07 ng/ml for weight loss, 4.45 ± 4.17 ng/ml for weight static and 17.50 ± 4.22 ng/ml for weight gain ($p < 0.0001$). A pre-intervention serum resistin concentration of 4.45 ± 4.17 ng/ml could predict that no weight change will occur during a weight management intervention program.

Conclusions:

There is a statistically significant difference in serum resistin concentrations between those obese participants who lost weight, gained weight and those whose weight remained the same during the obesity intervention weight management programme. It may be possible to predict weight change response to diet and exercise by measuring a pre-intervention biomarker, serum resistin. In the future, it could be possible to offer surgical treatment to patients whose resistin concentrations predict that no weight loss will occur on a weight management intervention programme of diet and exercise.

Further Study:

I am proposing to verify this study on a larger number of severely obese participants as well as to expand the study cohort to include participants with other aberrant eating behaviours.

Evaluation of the Abbott Anti-Thyroglobulin Assay: Does it Detect Interference in Thyroglobulin Assays?

Jennifer J Brady, Gemma O'Brien, Darina O'Sullivan, Michael Davis, Maria C Fitzgibbon

Department of Biochemistry and Diagnostic Endocrinology, Mater Misericordiae University Hospital, Dublin 7

Introduction:

Thyroglobulin is a marker of thyroid cancer used to monitor patients post treatment. However immunoassays to measure thyroglobulin are subject to positive or negative interferences from thyroglobulin antibodies (Anti-TG), which are present in up to 30% of patients. Thyroid cancer guidelines recommend concurrent measurement of Anti-TG to avoid reporting false-negative thyroglobulin results. UK NEQAS for Thyroglobulin surveys have shown considerable variability among Anti-TG assays and between the cut-offs at which they are considered positive.

Aim:

To evaluate the performance of the Abbott Anti-TG assay at the cut-off quoted in the kit insert (4.1 IU/L), in correctly identifying samples with potentially interfering antibodies.

Methods:

Over a seven month period, samples received for thyroglobulin were divided into three aliquots. Thyroglobulin was analysed on the Immulite (prone to negative interference), and by radioimmunoassay (RIA, prone to positive interference). Anti-TG was analysed on the Abbott Architect.

xResults:

Eighty-two samples were analysed for all 3 parameters. Fifty-six (68%) had Anti-TG <4.1 IU/L (negative), 48 of these gave concordant thyroglobulin results between the Immulite and RIA. Six were discordant due to numerically different cut-offs used by the two methods (< 2ng/ml on the Immulite and < 5ng/ml by RIA). Two had a higher result on the Immulite than by RIA and therefore were not clinically significant. Anti-TG was positive (>4.1 IU/L) in 27 samples. Ten of these showed discordant thyroglobulin results between the two methods (RIA higher than Immulite), indicating the presence of interference. The lowest Anti-TG titre at which interference was noted was 5.4 IU/L, indicating that the cut-off quoted in the kit insert is appropriate to detect interference.

Conclusions:

The Abbott Anti-TG kit is fit for purpose at the cut-off quoted for predicting interference in thyroglobulin assays. We have modified our practice so that only Anti-TG positive samples require confirmation by RIA.

Poster 6

Impact of CF newborn bloodspot screening on sweat testing

Philip Mayne^{1,2}, John Brady², Deirdre Cooney¹, Geraldine Roche¹

¹*National Newborn Bloodspot Screening Laboratory, Children's University Hospital, Temple Street and*

²*Department of Pathology and Laboratory Medicine, Our Lady's Children's Hospital, Crumlin, Dublin*

One of the identifiable cost benefits for the introduction of CF newborn bloodspot screening is the reduction in the number of sweat tests performed on Infants and children. We reviewed the performance of the sweat test, considered to be the gold standard for the confirmation or diagnosis of CF during the first year of CF newborn screening and compared the findings with those of the previous year.

From the 1st July 2011 to the 30th June 2012, samples from 74,028 newborn babies were screened for CF; of these 775, representing the top 1.05% with a raised bloodspot immunoreactive trypsinogen, were referred for a 38 panel CFTR mutation screen. Eighty two (10.6%) samples were identified with either one or two mutations; these babies were referred to one of six HSE designated CF centres for a sweat test.

Insufficient sweat was obtained on 21 (25.6%) necessitating repeat testing; eight babies had a borderline result and 67 had either an initial positive or negative result. On review of the number of sweat tests performed by two of the CF centres which covered approximately 38% of the births, there was a significant reduction in the number of sweat tests performed on infants born during the first year of the programme compared to the previous year (168 versus 215), excluding those arising from the CF newborn screening programme. However, the numbers performed on infants aged between one year and five years increased compared to the previous year (408 versus 332). The combined failure rate for infants less than one year was 5.7% and for those aged between 1 and 5 years of age was 5.1%.

The sweat test failure rate on infants referred by the newborn screening programme was significantly in excess of the target of 10%. This probably reflects the lack of experience of performing the test on very small infants as the overall failure rate on older infants is well within the target. The reduction in the number of sweat tests in infants born following the introduction of screening in two of the six centres is encouraging. However, the increase in the number of tests on infants and children between one and 5 years may reflect the heightened awareness of CF following the promotion of CF newborn screening.

Poster 7

Vitamin D status as a predictor of bone health in older Irish adults (>60yrs)

E Laird¹, MJ Healy², A Molloy¹, M Ward³, H McNulty³, JMW Wallace³, G Cox² and JJ Strain³.

¹Trinity College, Dublin, ²Department of Biochemistry, St James's Hospital, Dublin, ³Northern Ireland Centre for Food and Health (NICHE), University of Ulster, Coleraine

Background and Aims:

Chronic vitamin D inadequacy is associated with increased bone loss and osteoporosis, particularly in the older adult population. High rates of vitamin D insufficiency are reported within far latitude countries due to the seasonality of synthesis. The aims of this study were to assess vitamin D status within free-living Irish elderly adults (aged>60yrs) and to evaluate the role of vitamin D as a predictor of bone health.

Methods:

This observational study was conducted in a sub-set of participants (n 1936) from the Trinity Ulster Department of Agriculture (TUDA) cohort study. Participants provided blood samples for serum vitamin D (25(OH)D) and bone biomarker analysis. BMD was measured by dual-energy X-ray absorptiometry at the total hip, femoral neck and the lumbar spine.

Results:

31% of participants were identified as osteopaenic and 18% osteoporotic. Osteoporosis was more prevalent in females (25%) compared to males (10%; $P<0.05$). 16% of participants were vitamin D deficient ($<25\text{nmol/l}$) while 42% were reported to be insufficient ($25\text{-}50\text{nmol/l}$). Females had significantly lower vitamin D levels and higher concentrations of bone turnover markers compared to males ($P<0.001$). A significant seasonality effect was observed with higher concentrations of vitamin D in summer compared with spring ($P<0.05$). In both sexes, serum vitamin D concentration was found to correlate positively with BMD at the hip ($P<0.05$) and femoral neck ($P<0.05$) and inversely with PTH ($P<0.001$) and bone turnover markers ($P<0.05$) (after adjustment for age, BMI, season sampled and bone treatment). Individuals with vitamin D deficiency or insufficiency were significantly more likely to be osteoporotic than those who were sufficient ($P=0.01$; $P=0.013$ respectively) after adjustment for covariates.

Conclusion:

These results indicate that low serum vitamin D concentrations and high rates of inadequate bone health (especially within females) are prevalent within this population group. Consideration should be given to vitamin D fortification within the UK and Ireland as a potential cheap and effective measure of maintaining vitamin D status throughout the year to reduce the risk of osteoporosis development.



Poster 8

Vorinostat: A potential approach for liver cancer

Gray SG, Mc Lafferty K, Taggart CC, Lawless MW

Experimental Medicine Research Group, UCD School of Medicine and Medical Science, Mater Misericordiae University Hospital, Dublin, Ireland

Hepatocellular carcinoma (HCC) is one of the most common causes of death from cancer worldwide. With no effective pharmacological therapy for liver cancer it is paramount to discover new approaches to subvert its pathogenesis. Histone deacetylases (HDAC) inhibitors are reported to induce growth arrest, differentiation and inhibit angiogenesis of cancerous cells. Vorinostat/suberoylanilide hydroxamic acid (SAHA) is a member of a large class of compounds that inhibit HDACs reported in clinical trials of patients with other solid tumours.

This study demonstrates that SAHA causes an elevated level of Secretory Leukocyte Protease Inhibitor (SLPI) in a panel of liver cancer cell lines (Hep3B, HepG2 and Huh7). This compared with an altered expression in members of the CXC family and microenvironment regulators. SAHA markedly suppressed CXCL8 (IL-8) expression an effect that was hepatic specific. This was associated with reduced expression of both CXCL1 and -2 which are reported to have a clear effect on metastasis potential. Interestingly, rSLPI itself was found to induce an antiproliferative effect that was associated with a marked translocation of the transcription factor p65 into the nucleus of the liver cancer cell. In conclusion: HDACi have shown great potential for their anti-cancer effects on tumours. This study identifies that SAHA treatment on a panel of liver cancer cell lines results in specific immunomodulatory activity of potential benefit. The delineation of these events on the protumorigenic microenvironment may lead to better treatments and delayed progression for HCC patients.

Poster 9

Evaluation of the Immunoturbidimetric Microalbumin Assay on the Beckman Coulter AU5800® Clinical Chemistry System

R.J. FitzGerald¹, L. Murphy¹, C.C. Adley², B. Godber¹

¹Beckman Coulter Ireland Inc., Lismeehan, O'Callaghans Mills, Co. Clare, Ireland

²Department of Chemical and Environmental Sciences, University of Limerick, Ireland

Background:

Mildly increased urinary albumin (UA) excretion (30-300 mg/L) is considered a clinically important indicator of progressive renal disease, atherosclerotic disease and cardiovascular mortality¹. It is used to predict the development of diabetic nephropathy, as this protein tends to appear ahead of other serum proteins in urine during the course of renal glomerular damage². Screening for UA is therefore recommended by the American Diabetes Association for all diabetic patients³. The AU5800® is the latest series of ultra-high throughput clinical chemistry systems from Beckman Coulter, it can complete up to 8000 photometric tests/hour in addition to electrolytes.

Objective:

This study is to evaluate the performance of the Beckman Coulter Microalbumin assay on the AU5800® Clinical Chemistry System.

Assay Principle:

This assay measures albumin by a turbidimetric method. In the reaction, albumin reacts specifically with antibody to form insoluble antigen-antibody complexes. The absorbance of these complexes is proportional to the albumin concentration in the sample.

Results:

Precision studies were carried out following the CLSI guideline EP5-A2. Repeatability was 2.2%, 0.9%, 0.7% and Within Laboratory Precision was 3.9%, 1.1%, 1.0% at concentrations of 7.2, 146, 278 mg/L respectively. Method comparison studies were carried out based on CLSI guideline EP9-A2. Results were calculated using Deming regression analysis. The following data is from a method comparison of an AU5800 versus an AU2700: number of samples (n) = 101; Slope = 1.001; Intercept = 0.73 mg/L; R = 0.9998. The assay was demonstrated to be linear over the analytical range 5-300 mg/L following CLSI guideline EP6-A. No high-dose hook effect was seen for albumin concentrations up to 6000 mg/L.

Conclusion:

The results of this study demonstrate that the Beckman Coulter Microalbumin assay on the AU5800® Clinical Chemistry System met or exceeded the performance specifications.

References:

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2. Mogensen, C.E. (1987) Microalbuminuria as a predictor of clinic diabetic nephropathy. *Kidney International*, 31, 6173-6689
3. American Diabetes Association, (2002) Diabetic Nephropathy, *Diabetes Care*, 25, (1), 85-89

Validation of a liquid chromatography-tandem mass spectrometry method for the measurement of urinary free cortisol

MR Cullen, KJ Mulready, MF Fitzgibbon and MM MacMahon

Department of Clinical Biochemistry and Diagnostic Endocrinology, Mater Misericordiae University Hospital, Dublin 7

Introduction:

The Endocrine Society recommends the measurement of urinary free cortisol (UFC) as one of the first line investigations for the diagnosis of Cushing's syndrome. Historically, UFC has been routinely analysed by immunoassay, however it is well documented that this method is prone to cross-reactivity from cortisol metabolites and synthetic glucocorticoids. Analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) offers improved specificity and sensitivity. Our aim was to develop and validate a LC-MS/MS method for the measurement of urinary free cortisol.

Method:

Sample, calibrator and quality control were prepared using a protein precipitation and dilution procedure. Following mixing and centrifugation, the supernatant was analyzed by LC-MS/MS using an Acquity UPLC linked to a TQD (Waters) and detected in positive electrospray ionization mode with multiple reaction monitoring. The retention time for cortisol was 1.59 minutes with a total injection-to-injection time of 4 minutes. Quantifier & qualifier transitions for both cortisol and cortisol-d4 (m/z 363.4-120.9 and m/z 363.4-97.0) were monitored.

Results:

The assay was found to be linear up to 1612 nmol/L with a LOQ of 7.0 nmol/L. Intra- and inter-assay imprecision at concentrations of 27, 169 and 442 nmol/L were 5.2, 4.1, 3.1% and 6.9, 4.2, 4.3% respectively. Analysis of EQA samples demonstrated good agreement with UKNEQAS LC-MS/MS method mean ($r^2=0.99$; LC-MS/MS=1.08X UKNEQAS - 3.3) as did patient sample comparisons with another LC-MS/MS method (LC-MS/MS=0.97X other LC-MS/MS method -0.4). No ion suppression was observed. Several compounds were examined for potential interference with the method but none were detected. Samples were found to be stable for up to 7 days when stored at either room temperature, 4°C or -20°C.

Conclusion:

We have developed a robust, high throughput, and rapid method for the quantitation of UFC. This cost effective method benefits from increased specificity, sensitivity and accuracy when compared to immunoassay.

Evaluation of the Role of Novel High Sensitive Troponin I in Heart Failure

B. Guest, A. Roy, M.F. Fitzgibbon

Introduction:

Heart Failure (HF) is the end stage of most diseases of the heart and a major cause of morbidity and mortality. The clinical utility of high sensitive troponin I (hsTnI) assays in the investigation of HF has been at the centre of many recent studies. It has been proposed as a robust predictor of outcome in HF patients. A biomarker and clinical correlation between a prototype hsTnI biomarker in patients with New York Heart Association (NYHA) classified stages of HF is reported.

Methods:

49 patients with varying degrees of HF were analysed using the prototype ARCHITECT STAT high sensitive Troponin-I from Abbott™ with a view to establishing the correlation between NYHA stages and biomarker.

Results:

The trend towards higher hsTnI with more severe HF did not reach statistical significance in our HF patient cohort ($P=0.42$). BNP correlation with NYHA stage was significant ($P=0.014$).

Conclusion:

BNP provided better correlation than hsTnI in patients with HF. This study provided the basis for on-going studies at this centre to include a larger cohort of patients which would allow better stratification of sub-types of patients with HF.

Poster 12

Measurement of Blood Spot 17 Hydroxyprogesterone in Congenital Adrenal Hyperplasia (CAH) Patients

Ms. Ruth Cooney¹, Mr. John Brady¹, Dr. Declan Cody²

¹Biochemistry Dept, Our Lady's Children's Hospital, Crumlin, Dublin 12

²Endocrinology Department, Our Lady's Children's Hospital, Crumlin, Dublin 12

Introduction:

Congenital Adrenal Hyperplasia (CAH) is a group of diseases whose common feature is an enzymatic defect in the pathway leading to the biosynthesis of cortisol. In the most common form a deficiency of the enzyme 21hydroxylase results in a build up of the steroid 17hydroxyprogesterone which is measured both for diagnosis and to monitor treatment.

Known patients on treatment collect four saliva samples at timed intervals throughout the day which are submitted to a reference lab in the UK for analysis. This is costly and it can take several months to obtain results causing potential delays in treatment changes. It was decided to investigate the possibility of replacing the saliva assay with a blood spot assay performed in-house.

Methods:

Following ethical approval and patient/parent training, patients attending the CAH clinic were asked to collect four blood spot samples corresponding to the four saliva samples normally collected.

A commercial plasma 17hydroxyprogesterone radioimmunoassay procedure was adapted to measure the dried blood spots. In addition various techniques for extracting the 17hydroxyprogesterone from the dried blood spots were evaluated. The results of the blood spot assay were compared to the saliva results.

Results:

Following a series of experiments a successful technique for extracting 17hydroxyprogesterone from blood spots and analyzing it by radioimmunoassay was established. The procedure was extensively evaluated and found to give good analytical performance. The results on the blood spots gave good correlation with the saliva results.

The results were evaluated by the endocrine team and the decision was made to discontinue saliva testing and replace it with the blood spot technique.

Conclusion:

The introduction of the blood spot 17hydroxyprogesterone assay has resulted in an improved test turnaround time allowing clinical decisions on treatment changes to be made faster.

Blood spot collection is quicker for the patients than saliva collection and less prone to contamination.

There is good patient compliance once they have been trained in the collection technique.

In addition the change has resulted in savings to the hospital.



Poster 13

Prevalence of Macroprolactinemia: High vs. Low Reacting Prolactin Immunoassays

S. Kelly¹, S. K. Cunningham², M.N. Fahie-Wilson³ and T.P. Smith¹

¹Department of Endocrinology, St. Vincent's University Hospital, Elm Park, Dublin 4

²Department of Clinical Biochemistry, St. Vincent's University Hospital, Elm Park, Dublin 4

³Department of Clinical Chemistry, Southend Hospital, Westcliff-on-Sea, Essex, UK

Background:

Macroprolactinemia is characterised by hyperprolactinemia due to the presence of circulating but biologically inactive high molecular mass prolactin-IgG complexes. Laboratories need to be able to correctly differentiate patients with elevated levels of biologically active monomeric prolactin (true hyperprolactinemia) from those with macroprolactinemia. Currently, accurate data on the prevalence of true hyperprolactinemia and clinically irrelevant macroprolactinemia in unselected sera using a given prolactin immunoassay is not available. We undertook a comparative study to determine the prevalence of true hyperprolactinemia and macroprolactinemia using immunoassays which are reported to react strongly (Tosoh) or weakly (Roche) with macroprolactin.

Methods:

Based on an assessment of all routine requests for prolactin measurement (n=3,101) received at the Endocrine Laboratory, St. Vincent's University Hospital over a one year period, we identified 670 sera with elevated prolactin levels (21.6%) using the Tosoh immunoassay. Polyethylene glycol (PEG) precipitation was carried out on the hyperprolactinemic sera to distinguish true hyperprolactinemia from macroprolactinemia as previously described (1). The 670 sera were subsequently re-analysed using the Roche prolactin immunoassay.

Results:

Of the 670 hyperprolactinemic sera identified, 548 originated from females and 122 from males. PEG screening revealed that 505 sera had elevated levels of monomeric prolactin, consistent with true hyperprolactinemia, while hyperprolactinemia due to macroprolactin was present in 165 sera (24.6%). Prolactin levels were significantly lower ($p < 0.005$) in the macroprolactinemic cohort using the Roche immunoassay (mean \pm SD; 473 ± 132 mU/L) vs. the Tosoh immunoassay (697 ± 252 mU/L) and correlated poorly; $r = 0.35$. Using the Tosoh immunoassay, misdiagnosis of hyperprolactinemia occurred with 24.6% of the sera tested. The incidence fell to 11.8% with the Roche immunoassay. Where hyperprolactinemia due to macroprolactin was identified using the Roche immunoassay it was of a more modest nature than that seen with the Tosoh immunoassay, with prolactin levels in the 500 to 700 mU/L range in 90% of cases.

Conclusions:

The prevalence of macroprolactinemia identified using the Roche immunoassay was lower than that obtained with the Tosoh immunoassay but nevertheless significant at 11.8%. To prevent biochemical misdiagnosis of hyperprolactinemia PEG screening is still necessary with the Roche immunoassay.

(1) Beltran L, Fahie-Wilson MN, McKenna TJ, Kavanagh L, Smith TP. Clin Chem. 2008; 54: 1673-81.

Biochemical Estimation of Phosphate Status in Congenital Renal Tubular Defects and in Parathyroid Disorders

O'Keane MP, Kilbane M, Morrin M, McKenna MJ, Cunningham SK

Metabolism , Clinical Chemistry Group of Laboratories, St Vincent's University Hospital, Dublin 4

Introduction:

The kidneys regulate phosphate homeostasis. The tubular maximum reabsorption of phosphate $TmP_{0.4}$ is mainly regulated by PTH. Other factors affecting phosphate reabsorption include phosphate intake, drugs, activated vitamin D, acid/base status and phosphatonins, of which FGF23 is the most extensively studied.

The ratio of the maximum rate of tubular phosphate reabsorption to the glomerular filtration rate TmP/GFR is considered the most convenient way to evaluate renal phosphate transport. It is the theoretical lower limit of plasma phosphate below which all filtered phosphate would be reabsorbed. Historically, TmP/GFR has been used as a test for the differential diagnosis of hypercalcaemia; although, superseded in the modern context by intact PTH assays, it is still useful in the investigation of inherited disorders of tubular phosphate handling and evaluating hypophosphataemia.

Aim:

To review TmP/GFR results in specific clinical groups.

Methods:

Renal phosphate handling was assessed by calculating TmP/GFR (normal range 0.8-1.48 mmol/L) in 2 groups: (i) those with primary hypoparathyroidism ($n=4$), and secondary hyperparathyroidism ($n=5$) and (ii) those with congenital renal phosphate wasting disorders. Group (ii) consisted of X-linked hypophosphataemic rickets (XLH) ($n=8$), renal tubular acidosis (RTA) ($n=1$), and tumor-induced osteomalacia (TIO) ($n=2$). The associations between TmP/GFR , serum phosphate ($P_{0.4}$), PTH, ionized calcium and 25-hydroxyvitaminD (25OHD) were studied.

Results:

A reduced TmP/GFR was evident in 11/11 of group (ii) subjects with known disorders of tubular phosphate reabsorption. The median (Interquartile Range) of TmP/GFR for this group was 0.49 (0.38-0.55). Hypophosphataemia ($P_{0.4} < 0.8$ mmol/L) was most strongly associated with reduced TmP/GFR in this group; 9/11 subjects had hypophosphataemia. In comparison 3/4 hypoparathyroid subjects had a raised TmP/GFR in conjunction with hyperphosphataemia ($P_{0.4} > 1.40$). The median of TmP/GFR for this group was 2.01 (1.34-2.06). This group was profoundly hypocalcaemic; median ionized calcium and PTH was 0.84 mmol/L (0.82-0.86) and 9.9 ng/L (6.0-17.2) respectively. Secondary hyperparathyroid subjects demonstrated low, normal and high TmP/GFR in association with normophosphataemia in 5/5 subjects studied.

Summary/Conclusions:

Generally, a reduced TmP/GFR in the presence of hypophosphataemia is indicative of a renal defect in phosphate reabsorption.

Generating method-specific reference ranges - A harmonious outcome?

Lee GR, Griffin A, Halton K, Fitzgibbon M

Department of Clinical Biochemistry and Diagnostic Endocrinology, Mater Misericordiae University Hospital (MMUH), Dublin

Introduction:

Laboratory reference ranges are intended to guide clinicians on the interpretation of test results. When ranges do not reflect analytical methodology or patient demographics, interpretative accuracy is reduced. We report currently on the generation of method-specific reference ranges from a cohort of healthy individuals to promote accurate result interpretation and appropriate patient management.

Methods:

Serum was obtained from volunteers (Male + Female, n>120) attending a hospital health-check session. Aliquots were pseudo-anonymised (coded) and stored (-70oC) prior to analysis on Abbott ARCHITECT c16000 chemistry and i2000SR immunoassay analysers. Data were stratified for sex when appropriate and outliers were detected statistically (Tukey method). Reference ranges (2.5th + 97.5th percentiles) were thereby determined for a comprehensive list of clinical chemistry and endocrine tests and in accordance with IFCC/CLLS guidance¹. Reference ranges were also compared to other published sources (Abbott diagnostics and Pathology Harmonization). For a cohort of tests (Ca, Mg, PO₄), our method-specific reference ranges were verified against those determined from retrospective analysis of primary care data.

Results:

Reference ranges were generated for 28 chemistry and 25 immunoassay tests, including sex-specific ranges for CK, Creatinine, Iron, GGT, Transferrin, Ferritin and Prolactin. Ranges (mmol/L) for Na (138-144), K (3.9-4.9) and Cl (102-110) were considerably narrower than the consensus ranges reported by Pathology Harmonization (133-146, 3.3-5.0 and 95-108, respectively). The possible effect of gender on ferritin reference ranges (M: 30-432, F: 8-247 ng/ml) was more pronounced than that reported by the manufacturer (M: 22-275, F: 5-204 ng/ml). Reference ranges for Ca (2.17-2.49), PO₄ (0.82-1.39) and Mg (0.79-1.00) were also similar (P>0.05) to respective ranges generated from a separate primary care cohort (2.12-2.53, 0.79-1.43 and 0.79-1.01).

Conclusion:

The establishment of method-specific reference ranges is a more resource intensive but potentially more accurate approach to verifying published ranges and is most essential for analytes which currently are not easily harmonized.

¹International Federation of Clinical Chemistry and the Clinical and Laboratory Standards, Third Edition. Horowitz et al., 2008; 28(30).

The National Alpha-1 Antitrypsin Deficiency Targeted Detection Programme

L. Fee, T.P. Carroll, C. O'Connor, G. O'Brien, O. Floyd, R. Costello, I. Ferrarotti*, S. Ottaviani*, M. Luisetti*, S. J. O'Neill and N. G. McElvaney

Respiratory Research, Department of Medicine, RCSI Education and Research Centre, Beaumont Hospital, Dublin.

**Department of Biochemistry and Clinical Genetics, University of Pavia, Italy*

AAT deficiency (AATD) is a hereditary disorder characterised by low levels of the antiprotease alpha-1 antitrypsin (AAT). It is associated with the development of chronic obstructive pulmonary disease (COPD), generally by the third or fourth decade as well as liver disease. AATD deficiency results from mutations in the SERPINA1 gene; the most common mutation causing AATD is the Z mutation with the S mutation weakly associated with lung disease. AAT deficiency is under-diagnosed and prolonged delays in diagnosis are common. ATS/ERS guidelines advocate screening all COPD, poorly-controlled asthma, and cryptogenic liver disease patients, as well as first degree relatives of known AATD patients.

Over 8,500 individuals have been screened to date following ATS/ERS guidelines in the national targeted detection programme. AAT levels are measured by nephelometry and qualitative detection and characterisation of phenotype is carried out by isoelectric focusing. Sequencing of the SERPINA1 gene is performed to identify rare mutations.

To date we have identified 117 ZZ, 123 SZ, 42 SS, 1249 MZ, 876 MS, and over 30 individuals with clinically significant rare phenotypes (e.g. IZ, FZ, IS). This yields gene frequencies of 0.064 and 0.095 for S and Z respectively in a symptomatic population. A number of rare and novel SERPINA1 mutations have also been identified in the Irish population.

Our results underline the need for increased awareness and early detection of AATD. The advantages of early and accurate diagnosis of AATD are manifold, particularly regarding pulmonary and liver surveillance, family member testing, aggressive smoking cessation efforts as well as consideration of occupational and environmental exposures. All COPD patients should be tested for AATD as per ATS/ERS guidelines. Overall, our data demonstrates that AATD in Ireland is not a rare disease but a disease that is rarely diagnosed.

Poster 17

Changeover to the candidate IFCC Alkaline Phosphatase method- validation of published harmonised reference ranges for neonates and adults

Carroll L., Fagan D., Culliton M and Maguire OC

Department of Biochemistry, National Maternity Hospital, Holles Street, Dublin 2

Introduction:

A decision was taken to changeover to the IFCC ALP method in our laboratory. With the introduction of any new method, the establishment of a reliable reference range is required to enable the correct interpretation of the laboratory result. In the UK, the Pathology Harmonisation (PH) initiative has identified analytes for which there is no identifiable reason for a difference in quoted reference ranges and has published consensus ranges for these analytes.

Aim:

To validate the performance of the IFCC ALP method and to investigate the suitability of the published PH reference ranges for ALP, in both neonates and adults, for use in our laboratory.

Methods:

Current ALP method pNPP/ DEA buffer, IFCC method pNPP/AMP buffer performed on the Beckman Coulter AU680 analyser. The imprecision and bias of the IFCC method was assessed. Plasma samples from women (n=349) and neonates (n=62) were analysed by the two ALP methods with results being determined to be abnormal or normal for each method by comparison with the appropriate reference range (current quoted laboratory range and PH published reference ranges for IFCC method).

Results:

At ALP levels of 32 and 179 U/L the within run CV/ between run CV was 0.7 and 0.4% / 2.4 and 1.1% respectively. Comparison with our peer group in an EQA scheme showed a mean z- score – 0.86. Comparison with another laboratory using the IFCC method showed a mean negative bias of 4.2%. Assessment of the normality/ abnormality of results showed that, for adult patients, 9/ 349 (2.6%) were allocated differently; 7 were abnormal with current method and normal with IFCC method, and 2 vice versa. In the case of neonates, 5/62 (8%) were allocated differently; 4 were abnormal with IFCC method and normal with current method, 1 vice versa. In the discordant cases, the ALP values in both methods were very close to the relevant cut offs demonstrating a minimal degree of difference.

Conclusion:

The IFCC ALP method on the AU 680 was found to have acceptable imprecision and bias and performs within the manufacturers specifications. Based on the results of our present study, we have chosen to quote the published Pathology Harmonisation reference ranges on our reports after the changeover to the IFCC method. The process of the introduction of harmonized common references ranges will lead to more evidence based laboratory practice and as a result to an improvement in patient safety.

Investigating the difference between measured free phenytoin levels and estimated adjusted total phenytoin levels in hypoalbuminemic patients

GP. O'Brien, P McGing and MF Fitzgibbon

Department of Clinical Chemistry and Diagnostic Endocrinology, Mater Misericordiae University Hospital, Dublin 7

Introduction:

Phenytoin is one of the most commonly prescribed anti-epileptic drugs. Phenytoin exists in human blood in a free form and in a form bound to plasma proteins, principally albumin. Only the free fraction is biologically active.

The majority of routine clinical laboratories measure phenytoin as total phenytoin concentration. Various formulae have been derived to estimate the free phenytoin concentration using the laboratory total phenytoin and serum albumin concentrations. The most widely adopted equation is the Sheiner-Tozer equation (ST Eqn), which adjusts to an albumin of 44 g/L as normal. A modified equation uses an albumin level of 40 g/L.

The objective of this study is to investigate these different equations.

Methods:

We compared the measured and adjusted free phenytoin levels in a total of 62 patients taking phenytoin, using the ST and modified formulae. Total phenytoin was measured on our c16000 Abbott Architect platform. Free phenytoin was measured using LC-MS/MS in Chalfont Centre for Epilepsy, Buckinghamshire, UK. Serum albumin was measured on the c16000 by two dye-binding methods, BCP and BCG.

Results:

The average difference between albumin measurements using BCP and BCG methods is 4 g/L. The modified equation for adjusting phenytoin is based on albumin BCP measurements; hence an albumin of 40g/L is best used in this instance.

Within the total study group of 62 patients, there was a moderate linear correlation ($r^2=0.71$) between the measured free phenytoin and the free equivalent of the total, $y = 0.71x + 0.23$.

The linear correlations between the measured free phenytoin and the corrected total phenytoin using the ST and modified equations were the same ($r^2=0.83$). A Deming regression analysis of the ST and modified equation showed $y = 1.14x + 0.16$ and $y = 1.04x + 0.16$ respectively.

Of the total study group, 26 patients (42%) were hypoalbuminemic. Of this cohort, 14 (54%) had phenytoin levels which were re-characterised from the sub-therapeutic to therapeutic range or therapeutic to toxic range when the modified equation was applied.

Conclusion:

A modified version of the ST equation has been shown to best correlate with the free phenytoin measurements, particularly in hypoalbuminemic patients. However, in more complex situations such as pregnancy and patients on interfering medications it will still be advised to assay free phenytoin directly.

Serum vitamin D, physical activity, body composition and the obstructive sleep apnea syndrome

C. Kerley¹, K. Hutchinson², K. Bolger¹, K Fennell¹, A O'Brien¹, A McGowan¹, JL. Faul¹ and LJ. Cormican¹

¹Asthma Research Centre, Connolly Hospital, Blanchardstown, Dublin 15, Ireland

²Department of Clinical Chemistry, Biomnis Ireland, Sandyford Ind. Est., Dublin 18, Ireland

Obstructive sleep apnea syndrome (OSAS) and vitamin D deficiency (VDD) are common conditions associated with similar adverse effects. Additionally, OSAS seems to exhibit racial and seasonal variation¹, which may be influenced by variation in vitamin D status. Data on OSAS and VDD is lacking. We hypothesized that OSAS cases would have low vitamin D levels, which may be related to physical activity and body composition.

Untreated subjects recently diagnosed with OSAS by polysomnography were recruited. Body mass index (BMI) was calculated using standard protocols. Body composition was determined by bio-electric impedance analysis (Tanita BC-418). The Diasorin assay was used to quantify total 25-hydroxy-vitamin D (25(OH)D) levels. Physical activity was objectively estimated by SenseWear armband® (SWA). Here we use the 2011 Endocrine Society guidelines to define serum 25(OH)D status ².

55 participants (36 male) participated - mean age = 54y (30-75); mean BMI = 35kg/m² (22-51). SWA data was included if average wear time was >90%, therefore 226 days were included. No participant had sufficient vitamin D (>75nmol/L), 71% were deficient (<50nmol/L), 29% were insufficient (50-75nmol/L). Severe OSAS had significantly lower 25(OH)D levels compared to mild (P=0.049). 25(OH)D was associated with walking ($R^2 = 0.1$; P=0.001) but not physical activity duration. Additionally, 18 non-OSAS subjects (PSG confirmed) followed the same study protocol. These controls were matched for vitamin D determinants, including: age, skin type, season, gender, and BMI. Mean 25(OH)D differed significantly between OSAS cases and matched controls (34.2 vs. 50.5 nmol/L; P=0.011).

Among this sample body fat and OSAS severity were inversely associated with 25(OH)D, while walking activity was positively associated. Regardless of age, sex, body size, season or physical activity, all OSAS cases had insufficient vitamin D. Consideration of hypovitaminosis D among OSAS cases is warranted, though its clinical significance is as of yet unclear.

References:

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2. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011 Jul;96(7):1911-30.

An Audit of Point of Care Drugs testing in Northern Ireland

Hamilton JS, McClean L

Regional Toxicology Laboratory, Belfast Health & Social Care Trust, Northern Ireland

Introduction:

Northern Ireland (NI) has 5 Health Trusts. Each has a Point of Care Policy/Committee. Wards performing point of care testing (POCT) must assess clinical need, outcomes and cost. Approval is granted with assurances that training, documentation, and competency procedures are developed. However, many wards used POCT for drugs of abuse (DoA) before such Committees evolved.

Aims:

Using RCPATH 2004 POCT guidelines, this audit surveys how widely DoA assays are used, assesses procedures in place, and determines accuracy of performance and interpretation.

Methods:

Questionnaires were sent to Pharmacy in each Trust asking which kits were used by which wards, and provision of information and training with kits. Questionnaires were sent to these wards asking frequency of assays, staff grades performing tests, SOP/training availability, and results reporting procedures. Two samples spiked with DoA were sent with questionnaires, requesting analysis and interpretation.

Results:

21 (70%) questionnaires were returned; 10 with all results for both samples correct. Of these, 6 offered correct interpretation. 3 users gave no interpretation, 1 interpreted positive amphetamine result incorrectly as Ecstasy. 1 ignored positive amphetamines and methamphetamines in the samples. 11 correctly interpreted results recorded, although some of those were incorrect. Pharmacy distributes kits with no responsibility for training, IQC, SOPs or kit choice. Drug panel range is historical with Pharmacy unaware of cut-offs. Few wards have SOPs, some have package inserts. Training is cascade, with many wards having none, and any grade of staff performing analyses. Few wards keep competency records. Despite many results being wrong, all are recorded in patients' notes. Most wards know to send positive or unexpected results to the laboratory for confirmation.

Conclusion:

One Trust met the standards set. Four Trusts were noncompliant with standards, particularly regarding training and SOPs. Much work is required before DoA POCT in NI can become CPA accredited.



Analysis of 25-hydroxy vitamin D automated solid phase extraction and LC-MS/MS

MacMahon MM, Mulready KM and Fitzgibbon M

Department of Biochemistry & Diagnostic Endocrinology, Mater Misericordiae University Hospital, Dublin

Introduction:

In addition to the role of vitamin D in bone metabolism, studies have shown a link between vitamin D deficiency and conditions such as heart disease, multiple sclerosis and certain cancers. In serum the accepted indicator of vitamin D status is measurement of 25-hydroxy vitamin D (25OHD) concentration. LC-MS/MS allows separation and quantitation of both distinct forms, 25OHD3 and 25OHD2, which can facilitate monitoring of supplementation therapy with either form as well as diagnosis of hypovitaminosis D. Analysis of 25OHD by LC/MS/MS requires sample pre-treatment to release 25OHD from vitamin D binding protein and to minimise matrix effects. This method describes a semi-automated sample pre-treatment protocol in combination with UPLC/MS/MS for the analysis of 25OHD.

Methods:

Serum samples, calibrators & QC samples were placed on a Tecan Freedom Evo 100, identified by bar code and tracked throughout the extraction procedure. Internal standard was added to the dispensed samples prior to protein precipitation. Following centrifugation (off-line), the supernatant was transferred to a conditioned Oasis μ Elution solid phase extraction (SPE) plate, washed & the retained analytes eluted. Preparation time for 96 samples is 1.75 hours. The eluent was injected onto an ACQUITY C18 BEH Phenyl column (2.1x50mm) using a water/methanol/ammonium acetate gradient. A Waters TQD tandem mass spectrometer was used to quantify 25OHD2 and 25OHD3.

Results:

Total run time was 4.5 minutes. The assay was linear up to 321 nmol/L ($r^2=0.99$) and 371 nmol/L ($r^2=0.99$) with lower limits of detection of 3 and 2 nmol/L for 25OHD2 and D3 respectively. No evidence of ion suppression or carryover was observed. Average intra- and inter assay imprecision were <6.9% and <7.4% for 25OHD2 and 25OHD3 over the concentration ranges 24-149 nmol/L and 31-186 nmol/L respectively. Comparison with the DiaSorin RIA ($n=112$) demonstrated an expected negative bias ($r^2=0.96$). Recovery was within accepted limits, 100% (98-103%) for 25OHD2 and 97% (95-98%) for 25OHD3.

Conclusion:

Use of Oasis μ Elution SPE eliminated the need for solvent evaporation and reconstitution preparation while the Tecan Freedom EVO 100 reduced manual steps, operator variability allowing analysis of at least 260 samples per working day.

Biochemical investigation and clinical management of obstetric cholestasis (OC): How useful is bile acid quantification?

Dr G McKeown¹, Dr TF Lang², Dr A Hunter¹

¹*Department of Obstetrics, Royal Jubilee Maternity Service, Belfast*

²*Department of Clinical Biochemistry, Belfast Health and Social Care Trust, Belfast*

Introduction:

Alkaline phosphatase activity is used to biochemically assess cholestasis in non -pregnant subjects. However, due to the production of a placental isoform, it is not useful in pregnancy. Measurements of bile acids (TBA) have been shown to be a sensitive marker in the diagnosis of suspected OC cases.

Methods:

Retrospective audits were performed against the standards of care outlined in the RCOG green top guideline in patients who attended the Royal Jubilee Maternity Hospital (RJMH) during 2005 and 2009. In 2005 requests for TBA were referred to West Midlands. A regional service was introduced in 2009.

Results:

In 2009, 43 patients were identified with OC compared to 23 in 2005, an increase of 200%. The prevalence is similar to the estimated incidence of 0.6% in the UK.

The sensitivity and specificity of TBA was 54% and 92% respectively. The sensitivity and specificity of ALT was 79% and 74% respectively.

Conclusion:

Since the introduction of a locally available TBA analytical service and improved awareness of OC following the first audit, there has been an increase in the identification of this cohort attending the RJMH.

TBA and ALT are both useful in the recognition of pregnancy associated liver pathologies. ALT remains a useful test in identifying liver pathology in pregnancy including OC. However TBA in this audit performed better as a rule out test having a specificity of 92%.

Poster 23

Digital Image Analysis of Immunohistochemical Profiling in High Grade Gliomas

Howley, R.¹, Kinsella, P.², Brett, F.¹, Amberger-Murphy, V.², Farrell, M.¹

¹RCSI, ²NICB, DCU

Manual scoring of immunohistochemistry (IHC) is subject to inter-observer and intra-observer variability. Variations in tissue architecture from patient to patient, as seen in Glioblastoma Multiforme (GBM), lead to further difficulties in standardizing manual scoring. The production of high through-put tissue microarrays (TMA) combined with the advent of virtual slides has led to the need for faster, more standardized screening of IHC markers (1). In this study, we have established a novel method of screening GBM for the expression of Epidermal and Platelet-Derived Growth Factor Receptors (EGFR / PDGFR) along with their downstream targets PTEN, AKT, P70S6K, c-Abl, c-KIT.

Consent was obtained for patients (n=37) pathologically confirmed as having GBM. Digital images of all IHC stained tissue microarrays were captured using the NDP System (Hamamatsu). Image Analysis positive-pixel algorithms were developed to determine the 'concentration' of DAB staining using Digital Slide Server (Slidepath, Ireland). Manual scoring (based on intensity and percentage of staining) was performed independently by two experienced reviewers.

Inter-observer and intra-observer variability was determined to have 82.6% and 83.3% concordance respectively. Comparison of manual scores with automated image analysis results using Spearman's Rank Correlation statistics showed Rs=0.948, (where Rs=1.0 demonstrates perfect correlation).

We describe a standardized method of high through-put screening that can be applied to any IHC marker eliminating observer variability and laborious manual interpretation.

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False Negative PEG Precipitation Test in a Case of Hyperprolactinemia due to an IgA Macroprolactin

T.P. Smith¹, F. Kilvington², M.N. Fahie-Wilson³

¹Department of Endocrinology, St. Vincent's University Hospital, Elm Park, Dublin 4

²Department of Clinical Biochemistry, Birmingham Heartlands Hospital, Birmingham, UK

³Department of Clinical Chemistry, Southend Hospital, Westcliff-on-Sea, Essex, UK

Background:

Macroprolactinemia is characterised by hyperprolactinemia due to the presence of a high molecular mass prolactin immunoglobulin complex, generally IgG in nature. Detection of macroprolactin and quantitation of the bioactive monomeric prolactin component in sera is most frequently carried out following precipitation of the macroprolactin complex with polyethylene glycol (PEG). We report a case in which the results of PEG precipitation were misleading.

Methods:

Serum prolactin levels were measured using the Roche prolactin immunoassay. Polyethylene glycol precipitation and gel filtration chromatography were carried out as previously described (1) as was adsorption with protein G-Sepharose, anti-human IgG-agarose and anti-human IgA-agarose (2).

Results:

A 37 year old female with a 10 year history of hyperprolactinemia was referred for endocrine review when her serum prolactin increased from 2,073mU/L to 3,389mU/L over a 12 month period while not on dopamine agonist treatment. An MRI of her pituitary appeared normal and the patient exhibited no clinical symptoms of hyperprolactinemia. Her most recent sample, with a total prolactin of 4,062mU/L, was subjected to detailed investigation. Following PEG treatment the monomeric prolactin concentration appeared significantly elevated at 2,680mU/L suggesting true hyperprolactinemia. However, gel filtration chromatography revealed the predominant presence of an unusually high molecular mass (210kDa) macroprolactin, together with normal monomeric prolactin levels. Gel filtration chromatography of the redissolved PEG precipitate confirmed that macroprolactin in the sample was incompletely precipitated by PEG. Serum prolactin was not absorbed by protein G-Sepharose or anti-human IgG-agarose indicating that it was not IgG in nature. In contrast 73% of the serum prolactin was absorbed by anti-human IgA-agarose confirming the presence of an IgA macroprolactin complex.

Conclusions:

The PEG precipitation test may lead to misdiagnosis in rare cases where the hyperprolactinemia is due to the presence of an IgA macroprolactin.

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Is vitamin D deficiency associated with inflammation in free living older adults?

E Laird¹, A Molloy¹, M Ward², H McNulty², JMW Wallace², E McSorley², MJ Healy³ and JJ Strain²

¹Trinity College, Dublin, ²Northern Ireland Centre for Food and Health (NICHE) University of Ulster, Coleraine

³Department of Biochemistry, St James's Hospital, Dublin

Background and Aim:

Inadequate vitamin D status may be implicated in the aetiology of autoimmune disease and immune dysfunction. In-vitro and in-vivo studies have reported vitamin D to be a powerful immune-modulator although the evidence is not entirely consistent. Few studies have investigated the modulatory effect of vitamin D in humans and particularly, in the older adult population who are more vulnerable to age-related immune system dysfunction. Therefore, the aim of this study was to investigate the association between vitamin D status, immune markers of inflammation and the ratio of TH-1:TH-2 cytokines in large sample of older adults.

Methods:

This observational study was conducted as part of a larger investigation of Irish older adults (aged >60yrs), namely the Trinity Ulster Department of Agriculture (TUDA) cohort study. Participants (n 998) provided blood samples and plasma concentrations of interleukin-6 (IL-6), tumor necrosis factor alpha (TNF- α), C-reactive protein (CRP) and interleukin-10 (IL-10) were measured along with serum concentrations of vitamin D (25(OH)D).

Results:

The concentrations of TNF- α , IL-6, CRP and the ratio of IL6:IL-10 were significantly lower in individuals with a sufficient vitamin D status (>75nmol/l) compared with a deficient status (<25nmol/l) following adjustment for age and BMI (P<0.05). Vitamin D was a significant predictor for the IL-6:IL-10 cytokine ratio with vitamin D deficient (<25 nmol/l) participants significantly more likely to have an IL-6:IL-10 ratio >2:1 than those with sufficient status.

Conclusion:

This is the first study to demonstrate an association between vitamin D status, markers of inflammation and the IL-6:IL-10 ratio within a free-living, older adult population. These findings demonstrate the in-vivo capability of vitamin D to significantly modulate inflammatory markers toward an anti-inflammatory profile and highlight the need for sufficient vitamin D status particularly within the older adult population, for optimal immune function.

Benzodiazepines: A survey of prevalence in methadone maintenance patients using LC/MS.

Sinead Mc Namara, Paul O' Byrne, Siobhan Stokes, Ross Kilduff, Nicola Coleman, Emma Burke, Seibh Conroy, Grainne Smith

The Drug Treatment Centre Board (DTCB) Laboratory, 30-31 Pearse Street, Dublin 2

Abstract:

Benzodiazepine positive samples tested in the DTCB Laboratory have increased from 51% of samples testing positive in 2005 to 59% positive in 2012.

Benzodiazepines play a major role in poly-substance poisoning deaths. As well as being frequently prescribed for methadone maintenance patients, Benzodiazepines and other sedative drugs are widely sold in the illicit drug market. Under legislation being drawn up by the Department of Health it is to become an offence to possess tranquillisers such as Valium, Xanax or Zimovane without a prescription.

Aim:

To determine the profile of benzodiazepine drugs currently used in the addiction population.

Method:

The LC/MS method developed in-house uses an ABSciex 3200 QTrap with Electrospray positive ionisation and multi-reaction monitoring (2 MRM transitions per compound tested) coupled with Enhanced Product Ion monitoring (EPI). This gives full scan data to screen qualitatively for 18 benzodiazepine drugs or metabolites separated in an 11 min gradient. Samples were hydrolysed with β -glucuronidase, centrifuged and diluted prior to analysis. Samples above the limit of detection (7.8ng/ml) and meeting MRM/EPI matching criteria were deemed positive.

Results:

247 urine samples were analysed from DTCB methadone maintenance patient samples which had screened positive for benzodiazepines. Positives were as follows: 234 (94.7%) oxazepam, 218 (88.3%) temazepam, 183 (74.1%) nordiazepam, 66 (26.7%) 2-hydroxyethylflurazepam, 24 (9.7%) alprazolam, 21 (8.5%) γ -hydroxyalprazolam, 1 (0.4%) 7-aminoflunitrazepam and 1 (0.4%) lorazepam.

Conclusion:

Benzodiazepines being used are diazepam (Valium), chlordiazepoxide (Librium), flurazepam (Dalmane) and alprazolam (Xanax). Other possibilities are oxazepam (Serax), prazepam (Centrax), temazepam (Insomniger). Notably alprazolam (found in 9.7% of samples) is not prescribed at the DTCB. The profile of the usage in the DTCB mirrors 2011 Gardaí seizures with diazepam (208,196), alprazolam (38,829) and flurazepam (3,338) reportedly being seized.

Carnitine-acylcarnitine translocase (CACT) deficiency: A Case Study with Severe Hyperammonaemia

Fitzsimons PE¹, Urbano Blanco G¹, Murphy AA², Phillips R² and Mayne PD¹

¹*Department of Clinical Biochemistry, Children's University Hospital, Temple Street, Dublin.*

²*Department of Paediatrics, University Hospital Limerick*

Introduction:

Hyperammonaemia is associated with a number of inherited disorders. If not recognised and treated urgently it may cause severe permanent damage to the central nervous system. In order to provide optimal care for patients, it is essential that health professionals involved in the diagnosis and management of inherited metabolic disease have a thorough understanding of this condition.

Case Report:

A 6 week old infant girl (born prematurely 34/40) was brought into A&E of an external hospital with a short history of poor feeding, pallor and decreased respiratory effort. She was well up to the morning prior to admission. On examination the infant was hypothermic, hypotonic and had severe hepatomegaly and collapsed soon after, requiring resuscitation and ventilation. Urgent laboratory investigations were requested and results showed very raised plasma ammonia (1100 µmol/L and 1500 µmol/L; neonatal ref. range <150 µmol/L) and a finger-prick blood glucose of <1.3 mmol/L. The initial clinical diagnosis was a urea cycle defect (UCD) and plasma for amino acids and urinary organic acids were urgently sent to CUH, Temple St.

Plasma amino acids showed no evidence of a UCD in two samples. Urinary organic acids were grossly abnormal with a very marked increase in excretion of dicarboxylic acids in the absence of ketones. In view of the organic acid profile a fatty acid oxidation defect was suspected and acylcarnitine analysis was performed on the newborn screening card which showed increased long-chain acylcarnitines with decreased acetyl-carnitine, and free carnitine at the lower end of the reference range.

Overall, the pattern was suggestive of a long-chain fatty acid oxidation defect, possibly a deficiency of either carnitine:acylcarnitine translocase (CACT) or carnitine-palmitoyl transferase II deficiency (CPTII). Despite prompt intervention the infant died of ventricular tachycardia within 12 h of admission and was transferred to CUH, Temple St for post-mortem including biopsies for fatty acid oxidation studies.

Enzyme analysis of CPTII was performed showing normal activity. Diagnosis of CACT deficiency was confirmed by molecular genetics of the SLC25A20 gene and the patient was homozygous for a novel mutation c.[326+1delG].

Conclusion:

This case highlights 1. The importance of prompt accurate confirmation of hyperammonemia and determination of urgent metabolic tests, 2. Awareness of the differential diagnosis of hyperammonaemia and, 3. In patients with a fatal outcome performance of a post-mortem involving biopsies for full metabolic work-up and obtaining blood for DNA isolation/mutation analysis is recommended.

Determination of α -Klotho Levels in Serum of Patients with Chronic Kidney Disease

Abdul Wahab R¹, Collier G¹, Tormey WP¹, Denton M²

¹Department of Chemical Pathology, Beaumont Hospital, Dublin 9

²Department of Nephrology, Beaumont Hospital, Dublin 9

Background:

Klotho is a putative aging suppressor gene linked with chronic kidney disease-associated mineral and bone disorder (CKD-MBD)-like phenotype in experimental models. Klotho is expressed predominantly in the kidney, mainly in the distal convoluted tubule and to a lesser extent in the proximal tubule 1. Recent studies showed that CKD is a state of systemic Klotho deficiency and a decreased Klotho expression is the initiator of CKD-MBD 2.

The aims of the study were (1) to verify the analytical performance characteristics of the Immuno-Biological Laboratories (IBL) Human soluble α -Klotho (saKI) assay. (2) To measure saKI levels in healthy volunteers and compare these levels in patients with Stage 5 kidney disease and (3) to correlate saKI with parameters such as calcium, phosphate, PTH, vitamin D, creatinine, age and gender.

Methods:

Samples were collected from healthy volunteers (n=39), peritoneal dialysis patients (n=31) and patients on haemodialysis (n=118). Verification of the assay performance characteristics was carried out in line with the recommended guidelines.

Results:

The within-run imprecision values of 5572 pg/mL, 1156 pg/mL and 916 pg/mL gave CVs of 3.9%, 6% and 3.7% respectively. The inter-assay imprecision was 17%, 11% and 10% at a mean concentration of 4819 pg/mL, 738pg/mL and 514 pg/mL respectively.

Serum saKI levels in the healthy volunteers were statistically higher than the levels in HD and PD patients (p=0.001). In all subjects, serum saKI was negatively correlated with age ($p = -0.43$, p=0.001), serum creatinine ($p = -0.35$, p=0.001), serum phosphate ($p = -0.24$, p=0.001) and serum PTH ($p = -0.401$, p=0.001). Serum saKI was positively correlated with corrected calcium ($p = 0.19$, p=0.01).

Conclusions:

In conclusion, we found that the performance characteristics of Human soluble α -Klotho assay kit by IBL met with its manufacturer's claim. Furthermore, most of our clinical findings were comparable to previous Klotho studies in dialysis patients and healthy volunteers using serum saKI as the primary measurement.

References:

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A Comparison of Formulae for Serum Corrected Calcium and its Impact on assigning Calcium Status in Clinical Practice

R. Abdul Wahab¹, E. Rasheed¹, L. Carleton¹, D.Griffin², VEF Crowley¹

¹Department of Chemical Pathology, St. James Hospital, Dublin 8

²Department of Chemical Pathology, Galway University Hospital, Galway

In clinical practice, serum total calcium (TCa) measurement can be misinterpreted and lead to inappropriate treatment of patients due to a failure to take account of protein binding effects. Formulae integrating albumin concentration have been constructed in order to calculate the active fraction of TCa concentration. The objective of this study is to compare serum corrected calcium (CCa) levels derived from three different formulae and to examine the impact that this would have on the clinical classification of calcium status.

Serum TCa levels was classified into four groups 1) moderate to severe hypocalcaemia, 2) mild hypocalcaemia, 3) high normocalcaemia and 4) mild hypercalcaemia. Data from 352 patients (including hospital and GP) were collected prospectively for five days. Patients with albumin <20 g/L were excluded. CCa was calculated using the conventional [Corrected Ca (mmol/L) = TCa (mmol/L) + 0.020(40 - albumin (g/L))], Payne's formula and from a locally derived equation, [Corrected calcium (mmol/l)=total calcium (mmol/l)-[0.0144 x albumin (g/L)]+0.64]. Statistical analysis was performed using analysis of variance (ANOVA) and Chi-square test.

CCa levels (mean±SD) were significantly higher in Payne's formula (2.33±0.17) compared to local (2.30±0.14, p=0.035) and conventional equation (2.28±0.15, p<0.001). However, the clinical significance of these differences was marginal as evident in the small effect size obtained (eta squared=0.01). Following application of the conventional formula, analysis showed that 95% (n=19/20) moderate to severe hypocalcaemic patients were reclassified as mild hypocalcaemic, 74% (n=238/321) of mild hypocalcaemic patients became normocalcaemic and 33% (n=3/9) of high normocalcaemic patients became mildly hypercalcaemic after correction.

Overall, we found that there was no clinically significant difference in CCa levels between the three formulae and in a significant proportion of the patient groups examined, TCa levels did not reflect the appropriate calcium status. Estimating 'true calcium' levels is vital to enable physicians to make an appropriate clinical decision.

Uncertainty: Method Evaluation and Pre analytical Effects

Dervla Murphy

Clinical Chemistry Laboratory, Bon Secours Hospital, Glasnevin

Abstract:

Uncertainty of measurement (UM) is an essential part of the quality system in today's clinical laboratory. UM provides a quantitative value that represents the quality of the result that is released to the clinician.

Generally pre-analytical variables are not accounted in classic uncertainty formulae. They are widely considered to be of no clinical significance.

The aims of this experiment were twofold:

1. To determine a clinically relevant method for calculating uncertainty of measurement.
2. To determine if pre-analytical factors influence uncertainty of measurement results.

Two methods for calculating uncertainty were compared, although a statistically significant difference was not observed, Method 2, was more user friendly, and was more cost effective. It was therefore decided to be the method of choice, on which Method 3 was based.

Method 3 had the same standard uncertainty as Method 2; it also incorporated pre analytical factors as standard uncertainties.

Ten volunteers donated blood for this project. These samples were subjected to different methods of transport to the laboratory and varying degrees of time delay before analysis. This was carried out to determine whether any of these pre-analytical factors affected the uncertainty result.

Results showed that analysis of samples within one hour produced minimal effects on uncertainty.

It was also concluded that modes of transport to the laboratory only significantly affected LDH. It is therefore recommended that all samples requested for LDH be delivered to laboratory and this mode of transport be included in the uncertainty calculation for LDH.

Poster 31

Rare Alpha-1 Antitrypsin Mutations in the Irish Population

T.P. Carroll, L. Fee, G. O'Brien, C. O'Connor, I. Ferrarotti*, S. Ottaviani*, M. Luisetti*, S. J. O'Neill and N. G. McElvaney.

**RCSI Education and Research Centre, Beaumont Hospital, Dublin, Ireland
Department of Biochemistry and Clinical Genetics, University of Pavia, Italy*

AAT deficiency (AATD) results from mutations in the SERPINA1 gene, classically presenting with COPD and liver disease. The most common mutation causing AATD is the Z mutation, with the S mutation weakly associated with lung disease. AAT deficiency is under-diagnosed and prolonged delays in diagnosis are common. ATS/ERS guidelines advocate screening all COPD, poorly-controlled asthma, and cryptogenic liver disease patients, as well as first degree relatives of known AATD patients.

Over 8,500 individuals were screened following ATS/ERS guidelines as part of the Irish national targeted detection programme for AATD. Suspected rare and novel mutations were identified by DNA sequencing of the SERPINA1 gene.

A number of rare SERPINA1 mutations including I, F, Xchristchurch, Zbristol, and Mmalton were identified. The I mutation (Arg39Cys) was present at a relatively high frequency (0.0043) with over 60 cases identified. The F mutation (Arg223Cys) was also found in 20 cases. In addition, two novel Null mutations were identified, Q0dublin and Q0cork, as well as Q0bolton.

Current testing of suspected AATD cases is often limited and can miss rare and novel clinically significant SERPINA1 mutations. The rare mutations described in this study would not be detected by a commonly used genotyping assay. However, the low AAT levels prompted their correct identification using more detailed genetic analysis. Our findings underline the need for a comprehensive diagnostic work up of all patients with low AAT levels including phenotyping, genotyping and if necessary, DNA sequencing of the SERPINA1 gene.

Investigation of Developmental Delay reveals a Urea Cycle Defect in Two Siblings

Deverell D¹, Trench C¹, Hughes J², Mayne PD¹

¹Department of Biochemistry, ²National Centre for Inherited Metabolic Disorders, Children's University Hospital, Temple Street, Dublin 1

Introduction:

The urea cycle is a series of enzymatic steps responsible for the elimination of waste nitrogen from the body. The ammonia produced from the catabolism of amino acids is toxic and this is converted by the urea cycle in the liver to the harmless compound urea which is then excreted in urine. Defects in this process result in the accumulation of toxic substances which, among other physiological effects, cause neurotoxicity in the brain. Neonatal presentation is severe and rapidly progressive with encephalopathy leading to coma and even death, whereas later presentation is milder with symptoms including feeding problems, vomiting and developmental delay.

Patient A:

This female infant presented at 16 months of age with global developmental delay. Initial clinical investigations were suggestive of cerebral palsy. She had a history of significant gastroesophageal reflux, with early infantile vomiting persisting for the first year of life. Further investigation was requested including MRI, chromosome studies, extensive biochemical, haematological and metabolic blood and urine tests.

Laboratory Findings:

Routine haematology and biochemistry tests were all normal including blood gases and ammonia. Plasma amino acid analysis showed an increase in plasma citrulline, argininosuccinic acid (ASA) which is normally not detectable, ASA anhydrides and a relatively low arginine. Urine organic acid analysis showed the presence of orotate and uracil. These findings are consistent with a diagnosis of Argininosuccinic Lyase Deficiency (ASLD).

Patient B:

The three year old male sibling of patient A was noted as having isolated speech delay. He also had the 'brittle' hair growth which is characteristic of ASLD patients and also present in his little sister. His plasma and urine samples were therefore sent for metabolic investigations and showed the diagnostic amino acid profile and organic acids as in his sibling. He also had CSF amino acid analysis performed showing the relatively high concentration of ASA in the brain, relative to the blood, which is responsible for the neurological toxicity.

Discussion:

Urea cycle disorders are among the most common inborn errors of metabolism and can present at all ages and with variable biochemical and clinical phenotype. The severity depends on the mutation and amount of residual enzyme activity present. ASLD may not cause the same degree of elevation of ammonia and glutamine as other urea cycle enzyme defects but ASA is itself a neurotoxin and needs to be monitored. The treatment involves the restriction of protein intake, the elimination of ammonia and other nitrogenous compounds, and replenishment of the urea cycle with arginine.

Conclusion:

Investigations into the causes of delayed development in infants and children should include metabolic laboratory tests especially if there are accompanying clinical features such as poor feeding, vomiting, abnormal hair growth, lethargy or neurological symptoms.



Rhabdomyolysis in a young individual: When to think of inborn errors of metabolism

Borovickova I¹, Fitzimons PE¹, Knerr I², and Mayne PD¹

¹Department of Clinical Biochemistry, Children's University Hospital, Temple Street, Dublin

²Department of Metabolic Paediatric Medicine, Children's University Hospital, Temple Street, Dublin

Introduction:

Rhabdomyolysis results from skeletal muscle breakdown and is characterized by an acute increase in serum concentrations of CK, typically to more than five times the upper limit of normal when myocardial infarction has been excluded as a cause. It might be accompanied by the release of other intracellular components such as K⁺, PO₄, urate or myoglobin. The latter can precipitate in the renal tubules leading to the acute kidney injury. Hypocalcaemia may occur as a result of muscle necrosis.

Causes of rhabdomyolysis are often obvious such as ischaemia, trauma, burns, uncontrolled seizures, excessive exercise, some drugs/toxins (statins, fibrates, alcohol, ecstasy, heroin, carbon monoxide) and inflammatory myopathies. Patients with unexplained rhabdomyolysis after exclusion of acquired causes should be investigated for possible metabolic causes.

Case Report:

A 21-year old female presented to Tallaght Hospital with generalized muscle pains associated with muscle weakness precipitated by exercise. She denied drug use, trauma and was not taking any medication. Her serum CK level was raised to over 5000 U/L and she required a renal replacement therapy in ICU.

Clinical assessment and routine blood tests did not reveal any obvious cause of rhabdomyolysis which prompted a metabolic referral to CUH Temple Street to exclude inborn errors of metabolism.

Recommended first-line investigations in such cases include

- plasma lactate and amino acids
- dried blood spot acylcarnitine profile
- urinary organic acids
- CSF lactate (if respiratory chain disease suspected).

Additional testing (exercise testing, forearm exercise test, EMG, ECG, MRS) can be considered.

Her plasma lactate and amino acids were unremarkable. Urine organic acids showed non-specific pattern with no DCA or glycine conjugates present. Acylcarnitine/carnitine profile showed decreased free carnitine with deficient pattern and raised (C16+C18:1)/(C2) of 0.46. A diagnosis of carnitine palmitoyltransferase II (CPT2) deficiency, one of the fatty acid oxidation defects, was subsequently confirmed from skin biopsy by fatty acid oxidation flux assay and specific CPT2 enzyme assay. Glucose is the mainstay therapy and some authors recommend avoidance of C12-fatty acids.

Conclusion:

Unexplained or recurrent exercise intolerance or rhabdomyolysis should be investigated for possible metabolic myopathy. Glycogenoses (eg GSD V-McArdle's disease, GSD II), fatty acid oxidation defects, purine cycle defect and respiratory chain disorders should be excluded with first-line biochemical investigations as highlighted above, followed by additional tests tailored from the clinical assessment and preliminary investigations.

Ferroportin Disease – A Report On The Clinical Utility Of An In-House Mutation Scanning Assay of SLC40A1

¹B MacNamara, ¹JJ Phelan, ¹A Balfe, ²N Breslin, ¹VEF Crowley

¹Biochemistry Department, St James's Hospital, Dublin 8

²Department of Gastroenterology, AMNCH, Tallaght, Dublin 24

The identification of mutations in genes implicated in iron transport and storage has led to a genetic reclassification of iron-overload syndromes. While mutations in HFE have been clearly associated with recessive hereditary hemochromatosis (HH), in the last decade there is an increasing awareness in clinical practice that mutations in SLC40A1 can cause an autosomal dominant iron-overload syndrome, referred to as Ferroportin disease (FD) or Type 4 HH. Consequently a method for SLC40A1 mutation scanning has been developed in Biochemistry Department, St James's Hospital, to facilitate the diagnosis of FD in the Republic of Ireland. Herein we describe our experience with this method to date including the identification of a pathogenic mutation.

To date four subjects with suspected FD have undergone SLC40A1 mutation scanning using a method of PCR amplification and direct nucleotide sequencing followed by Megalign analysis. Subjects were selected based on various criteria including the presence of an elevated serum ferritin but normal or only mildly elevated transferrin saturation, biopsy evidence of iron-overload, a negative HFE genotype status, and in one case a family history of hyperferritinaemia.

In three subjects non pathogenic genetic variants were identified including the CGG insertion RS3833570 in the 5'UTR and the SNP RS:2304704 in exon 6. One patient, with marked hyperferritinaemia (S Ferritin >3000 µg/L) and a positive family history, was heterozygous for a pathogenic mutation c.230 C>A (Ala77Asp) located in Exon 3. This mutation has been previously described as being associated with FD in different populations.

The results to date have confirmed the clinical utility of this mutation scanning assay, including the identification of a known pathogenic mutation. The sensitivity of the assay will improve with more stringent application of selection criteria for analysis and over time this will provide an insight into the prevalence and genetic nature of FD in Ireland.

Development and Clinical Utility of Mutation Scanning Assays for SDHB, SDHD and VHL in the Genetic Diagnosis of Pheochromocytoma-Paraganglioma Syndrome

¹L Carleton, ¹B MacNamara, ²ML Healy, ¹VEF Crowley

¹Biochemistry Department, St James's Hospital, Dublin 8

²Department Endocrinology, St James's Hospital, Dublin 8

It is now widely acknowledged that over 30% of apparently sporadic Pheochromocytoma/Paraganglioma tumours are caused by an underlying germline mutation. Consequently, genetic classification is now regarded as an important component in the multidisciplinary management of these tumours, facilitating better prognosis for patients and their relatives. Our objective was to develop and optimise molecular diagnostic mutation scanning assays for three of the most common genes (VHL, SDHB AND SDHD) implicated in the pathogenesis of inherited pheochromocytoma/paraganglioma.

The assay design was based upon the gold standard technique of polymerase chain reaction (PCR) amplification of a target region of DNA followed by direct nucleotide sequencing of the target amplicon. Initially, 16 de novo PCR primer sets covering all 15 coding and exon-intron flanking regions within the genes of interest, were designed. These were then successfully optimised using control DNA samples. To clinically validate the assays DNA extracted from whole blood of four patients, with a personal or family history of Pheochromocytoma in whom genetic testing was clinically indicated, were amplified and sequenced. Although no pathogenic mutations were uncovered during the clinical validation process, a common polymorphism (SNP) in at least one of the genes was identified in each patient, and these findings correlated with the results obtained from an external genetic reference laboratory using automated clonal sequence analysis and direct sequencing. This established the clinical utility of our assays. In addition a mutation detection method using reverse transcriptase PCR (RT-PCR) was also developed for SDHD and this may offer some advantages in terms of cost-effectiveness.

Evaluation of Calcium Measurement for Patients on Haemodialysis

P McGing, B Gillman, D Egan, Y O'Meara, D Sadlier, and M Fitzgibbon

*Department of Clinical Chemistry and Diagnostic Endocrinology, Nutrition and Dietetics, and Renal Medicine.
Mater Misericordiae University Hospital, Eccles Street, Dublin 7*

Introduction:

Bone Mineral disorders are an important clinical consequence of Chronic Kidney Disease (CKD). Determination of blood calcium is a key component of care for such patients. Ionised calcium is considered the best clinical measure but is not practical for routine use. It is usual to adjust measured total serum calcium in hypoalbuminaemic patients but there is much debate as to the most appropriate formula to use.

Methods:

For this study we measured calcium and albumin in 53 CKD patients undergoing haemodialysis (HD) as follows – Ionised Calcium (iCa) in whole blood by ISE (IL Gem4000), total Calcium (tCa) and BCP albumin (Abbott Architect c16000).

Adjusted calcium (adCa) was calculated using our in-house-derived routine equation (M-R) and HD-specific equation (M-HD) as well as a published HD-specific equation (P-HD).

The M-HD equation was derived from 76 HD patients (undertaken prior to the main study):

$$\text{adCa} = \text{tCa} + ((34 - [\text{Alb}]) \times 0.0191).$$

Results:

Of the 53 patients 25 had iCa below the manufacturer's reference interval (RI), 25 within the RI, and 3 were classified as hypercalcaemic. In contrast, the routine adCa (M-R) indicated only 6 patients as hypocalcaemic and 4 as hypercalcaemic. The in-house HD equation (M-HD) showed 14 patients below the RI while the published HD equation which correlated very well with iCa for hypocalcaemic diagnosis (23 pts below RI).

In total 5 patients were classified as hypercalcaemic by one or more measure (3 iCa, 4 M-R, 1 M-HD, 0 P-HD). All 5 patients were considered to be hypercalcaemic and were treated accordingly, with good clinical response

Conclusion:

In this study our standard formula for calculating adjusted calcium agreed best with ionised calcium for identification of hypercalcaemia in haemodialysis patients but underestimated hypocalcaemia. In the latter case a specific HD-derived equation appeared to correlate better with ionised calcium.

Development and Clinical Utility of a Mutation Screening Method for Gilbert's Syndrome

B MacNamra, A Naughton, VEF Crowley

Biochemistry Department, St James's Hospital, Dublin 8

The finding of an unexpected isolated hyperbilirubinaemia is a relatively frequent occurrence in the realm of adult clinical biochemistry practice. Further investigation of this abnormality commonly involves determining which fraction of the Serum Total Bilirubin (TBili) is elevated. The presence of an elevated serum unconjugated bilirubin should prompt haematological tests to rule out underlying haemolysis. By a process of elimination a diagnosis of Gilbert's syndrome (GS) may be attributed. This condition is an inherited, chronic and mild hyperbilirubinaemia caused by impaired hepatic bilirubin clearance and may be present in up to 7% of the population. GS is primarily caused by a dinucleotide insertion in the TATA promoter of UGT1A1. In clinical terms GS is important to recognise because on occasions patients can be inappropriately investigated for hepatic or haematological disorders and also it may have some pharmaco-therapeutic implications. Moreover, as the finding of abnormal LFTs is increasingly seen in routine laboratory testing, due to conditions such as NASH, it is unsurprising that in a significant proportion of such patients GS may be a comorbid condition.

A mutation screening assay based on PCR amplification and direct nucleotide sequencing of the TATA promoter of UGT1A1 was developed and optimised. The clinical utility was demonstrated in two patients with isolated hyperbilirubinaemia. Case 1 was referred from a hepatology clinic with a TBili of 70 $\mu\text{mol/L}$ and a Conjugated Bilirubin (CBili) 8 $\mu\text{mol/L}$. Case 2 was referred from a haematology clinic with TBili and CBili of 51 and 10 $\mu\text{mol/L}$ respectively. In both cases the patients were shown to be homozygous for (TA)₇ TAA genotype confirming a diagnosis of GS.

Gilbert's syndrome is very common and on occasion can cause diagnostic difficulties. We report the successful development of a method for direct detection of the most common variant causing GS in the Irish population to facilitate definitive diagnosis.

Prenalytical Stability of Urine Porphobilinogen and Porphyrins - A Pilot Study

N Brazil, S Savage, N Mulready VEF Crowley

Porphyrin Laboratory, Biochemistry Department, St James's Hospital, Dublin 8

The measurement of urine porphyrins and the porphyrin precursor porphobilinogen (PBG) are critical in the diagnosis of porphyrias and the acute porphyria attack, respectively. However, as these investigations are only available in specialist laboratories there may be delays in transporting samples to such reference centres. Moreover, it is recommended that all samples for porphyrin analysis must be protected from light to avoid degradation. This pilot study examined the stability of urine PBG and porphyrins with respect to time-to-analysis, storage temperature and light exposure.

A sample was obtained from a patient with known Acute Intermittent Porphyria and raised UrinePBG who is currently attending the Porphyria Clinic, St James's Hospital. After routine analysis the sample was aliquoted into a number of plastic tubes which were stored under varying conditions i.e. room temperature (RT), 4oC, -20oC, and light protected(LP) or non-light protected(NLP). PBG analysis by column chromatography was performed on Day2, 7 and 13 following collection. In addition, porphyrin analysis was performed on Day7 and 13 using both the RT-LP and RT-NLP samples.

Urine PBG levels showed a marked decrease in the samples which were not protected from light and stored at RT and 4oC. The final order of stability was: 4oC-LP = -20oC-LP > -20oC-NLP > RT-LP > 4oC-NLP > RT-NLP. In relation to porphyrin analysis, both samples showed increases in URO1, UROIII, and Copro1 which were more pronounced in the RT-LP sample. However, these samples showed wildly opposing responses for CoproIII with an increase noted for RT-LP and a decrease noted for RT-NLP.

These results clearly demonstrate that light protection, storage temperature and time to analysis can significantly alter both the levels of urine PBG and the pattern of urine porphyrins. The ideal conditions for preanalytical storage of urine samples for porphyrin/PBG analysis is either 4oC or -20oC with light protection.

Poster 39

CSF Xanthochromia: Visual Inspection vs. Spectrophotometry & an Audit of 12 Months Service in Beaumont Hospital

McBrierty D, Collier G, Tormey W.

Department of Chemical Pathology, Beaumont Hospital

Introduction:

Subarachnoid haemorrhages (SAH) are dramatic and devastating events that have a potentially fatal outcome. Most SAHs occur due to a ruptured aneurysm. The correct diagnosis of a SAH is vital for the outcome of the patient. Untreated ruptured cerebral aneurysms have an 81% mortality rate within 3 months of the SAH. CT scanning can detect SAH within the first 12 hours very accurately. After that time, cerebral spinal fluid (CSF) can aid in the diagnosis of SAH, especially in Computed Tomography (CT)-negative suspected SAH cases. Inspection of CSF for xanthochromia, and especially bilirubin, can aid in the diagnosis of SAH.

Methods:

10 water samples were spiked with varying concentrations of bilirubin, which were used to represent CSF samples with or without xanthochromia present. 27 members of laboratory staff were asked to visually inspect the samples for the presence of xanthochromia. Samples were identified as positive or negative for xanthochromia, or designated as 'unsure' where the individual was uncertain. The samples were also analysed by spectrophotometry and resulted as per UKNEQAS guidelines. An audit was also carried out to determine the number of requests for CSF bilirubin in the laboratory since the service was introduced.

Results:

The results demonstrated that spectrophotometry was superior for the detection of xanthochromia, and that uncertainty increased using visual inspection in samples with low levels of bilirubin present.

In Beaumont Hospital, 81 samples were received for xanthochromia analysis within a 12 month period. 12 of these samples were unsuitable for analysis (15% of samples). Of the 69 samples analysed there were 63 that showed no evidence of SAH, 2 that were inconclusive (due to large oxyhaemoglobin peaks), and 4 that were consistent with SAH.

Conclusion:

This study indicates that CSF xanthochromia should be analysed by spectrophotometry rather than visually. Also, there are ongoing issues with sample collecting and processing, leading to exclusion of samples from analysis, which need to be addressed. Importantly, sufficient samples are being processed to allow for expertise to be maintained in the interpretation of the analysis.