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Abstracts Joint Sessions and Biochemistry and Haematology Sessions

ABSTRACTS JOINT SESSIONS

What is 'A High Quality Pathology Service' when the Budget is Limited?

Prof. Peter N. Furness

Vice-Chair & Revalidation Lead, Academy of Medical Royal Colleges and President, The Royal College of Pathologists, UK

As a result of the impact of the international banking crisis, state-funded pathology services in the UK are currently facing a Government-imposed cut in funding equivalent to 20% of total cost. Even before this, we had seen calls for 'consolidation' of pathology services into fewer, larger laboratories, to improve efficiency. This process is now being forced forward, with the UK Government putting increasing emphasis on the involvement of private companies in what was previously a mainly state-run service. The simultaneous introduction of reorganisation and the introduction of the profit motive are generating great concern and controversy. In this situation, the role of the Royal College of Pathologists, as a charitable organisation, is to advocate the highest possible standards of pathology service for the benefit of patients. All concerned with these changes claim that the plans that they advocate will maintain or increase 'quality', even if resources are cut. They cannot all be right, but how should the quality of a pathology service be measured? Pathologists will usually emphasise getting the diagnosis right or producing accurate measurements. Clinicians may emphasise speed of delivery of results. Managers will emphasize cost and efficiency. Commercial organisations emphasise customer satisfaction; but who is the customer, and is the customer sufficiently well informed to decide what should generate such satisfaction? If we consider patient safety, data collected by the UK National Patient Safety Agency show that adverse patient safety incidents in relation to laboratory services almost all occur in getting the specimen to the laboratory and in getting the result to the clinician. How often are these 'interface' problems considered when laboratory quality is discussed? Ultimately, the only true measure of the quality of a laboratory service is the improvement of patient outcomes that it achieves. But patient outcome is influenced by so many factors that the contribution of the laboratory is impossible to assess; so surrogate measures have to be used. In which case, we need an open discussion of what such markers really assess and which of them are of value. It is clear that any simple method of measuring laboratory quality is likely to be incomplete or simply wrong. Identifying the best way to measure quality in pathology is a considerable challenge. Persuading all concerned that this really is the best way to measure quality is an even greater challenge.

Quality Assurance and Quality Management Systems in Laboratory Medicine

Dr. Robby Bacchus

World Association of Societies of Pathology and Laboratory Medicine, Education Secretariat (WASPaLM), UK

The pursuit and concern for quality in health care is an issue that transcends national boundaries. Of all the health professions, the laboratory based pathology disciplines have done most and continue to do most to monitor the quality of what they do, and to anticipate and correct sources of analytical error. In addition to recording their performance both within their own departments and as members of peer groups, they educate themselves, their peers and clinical colleagues in an effort to improve the effectiveness of the service they provide, set analytical goals and subject themselves to external proficiency testing. Quality assurance is the whole programme

of activities mounted by laboratories, regions, countries, professional groups, commercial and industrial companies in an attempt to improve clinical laboratory performance generally. They include: 1) encouragement of the constant use of internal quality control in every laboratory; 2) support for external quality assessment (EQA) schemes; 3) all measures taken to increase within laboratory reproducibility and between-laboratory comparability by means of training courses, conferences and other collaborative activities. These are based on hypotheses derived from internal quality control and from EQA results accumulated over time. There are now growing public demands for accountability, finite fiscal resources, provider resistance or apathy towards changes and an imbalance in the supply and demand equations for health care organisations in today's world. Active quality assurance programmes are critical to meet this challenge.

Research in Sickle Cell Disease, from Bench to Bedside

Dr. Salam Al Kindi

Associate Professor, and Head, Department of Haematology, Sultan Qaboos University Hospital, Muscat, Oman

In Oman, 5.7% of Omani people carry the gene for sickle cell disease (SCD) and about 0.2% manifest the disease. Although SCD is traditionally looked at primarily as a disorder of red cells, it is a disease demonstrating a model for red cell interaction with white cells and endothelial cells. Recent work from our laboratory on acute chest syndrome, which is one of the major causes of death in SCD and vaso-occlusive crises (VOC), the most frequent presentation of this disease, has demonstrated this. An alteration in the level of nitric oxide as well as lymphocytes and monocytes activation play a significant role in both conditions. Similarly the sickled red cells, causing perturbed platelet and haemostatic functions, play an important role in stroke development, complicating further the hereditary component of thrombophilia in this syndrome. These changes are promising focii for studies on the various therapeutic interventions that are available for this disease such as hydroxyurea, low molecular weight heparin, nicosan and other agents that are under study in clinical trials. Good progress made in reduced intensity conditioning (RIC) bone marrow transplant for patients with SCD is seen in the recent experience in our centre, enabling the sickled and normal cells to co-exist together and ameliorate symptoms of SCD. currently the use of stem cells is in progress to help patients with avascular necrosis of the hips (AVN), a crippling complication seen in some of our patients.

Benefits of Accreditation of Medical Laboratories – A global perspective

Paul Stennett

Chief Executive, Clinical Pathology Accreditation (UK) Ltd and Chief Executive of the United Kingdom Accreditation Service, UK

This paper covers the accreditation of medical laboratories starting with a detailed review of what the Clinical Pathology Accreditation (CPA) standard assesses during a laboratory visit and some typical outcomes of assessments. It also looks at how to prepare for accreditation, what is involved and an indication of the costs of accreditation. The role of Peer Assessors in the assessment is also examinded. Next, there is a review of the benefits that medical laboratories in the UK have reported in terms of reduced risk and improved quality. To conclude, there is a short preview of how the accreditation standards might be further improved to continue to meet the quality requirements of medical laboratories in the future.

The National External Qualtiy Assurance Programme in Oman

Dr. Suleiman Al Busaidi

Director of Laboratories, Central Public Health Laboratory, Oman

The presentation outlines the historical development of the National External Quality Assessment Scheme (NEQAS) in Oman. We will describe the goals and objectives of the program as well as currently available disciplines and future plans for the programme. In this presentation, we will focus on the microbiology scheme since NEQAS focuses on agents of diseases of public health and clinical importance. We will therefore, also give an overview of how panels are simulated, quality checked, packed and shipped to various participating laboratories as well as the strategic use of results in planning purposes. The evaluation guidelines and the marking scheme will be presented together with some examples of performance of laboratories in some panels. The microbiology scheme covers 26 laboratories including all Ministry of Health laboratories, other government sister institutions, some private laboratories and four international reference laboratories outside the country. The Quality Assurance Unit prepares the panels which consist of simulated samples as well as instructions for processing and response forms. The simulated specimens are spiked with relevant pathogens and inoculated in appropriate transport media. The specimens are quality checked for contamination and viability before and after dispatch. After passing the quality checking the specimens are packed and dispatched by courier to various participating laboratories. The participating laboratories process the specimens and report the results to the External Quality Assessment Unit at the Central Public Health Laboratory within two weeks. A confidential report on the expected results, together with overall performance (including marks obtained), is then issued to each participating laboratory.

Laboratory Services in Oman – Past, present and future

Dr. Nayyar Ali

Head of Diagnostic Laboratory Services, Ministry of Health, Oman

The Diagnostic Laboratory Services of the Ministry of Health have seen rapid development and a dramatic increase in laboratories and staffing. By end of 2009, there were 198 laboratories with a staff of 1,147 and the number of pathologists has increased to 80. There has also been a marked increase in sophistication and modernisation of the service with the introduction of automation to most extended health centres and hospitals. As a result of the increase in the numbers of laboratories, the increase in automation and the general population trends, there has been a significant increase in the numbers of tests done. In 1992 there were just under 6 million tests done. This had increased to 13.8 million by end of 2009. In conclusion, the laboratory service has come a long way in a relatively short time. Our aim is to provide the highest quality laboratory services at the lowest possible cost offering a comprehensive diagnostic service relevant to the needs of the people and available close to all communities in the country. Therefore, we do recommend the introduction of external quality control, an increase in staff to meet the increased work load and the introduction of new tests at regional hospitals.

Are we Ready to Use Portfolios as an Assessment Tool of Professional Development?

Dr. Arundathi Kurukulasuriya

Senior Specialist, Department of Haematology, Royal Hospital, Oman

Assessments currently in practice use comparative achievements to place knowledge, skills and attitudes of individual students in a rank, by using grades, test scores and grade percentiles, which are often obtained at summative end of course assessments. They are designed by trainers often with no constructive feedback to the learner. Usually, the lower cognitive learning styles, such as memorising and reproducing, are used by the student when preparing for these assessments. Portfolios are self portraits of students demonstrating personal development over a period of time, with guidance by the trainers and peers, where the learning is student centered. They foster skills in self evaluation, problem solving, lifelong learning, communication, writing and reflective practice. They promote higher cognitive learning strategies such as application, interpretation and reflection.

The portfolio can be also be integrated easily with instruction; however, the guidelines for developing them must be aligned well with the learning outcomes of the course. Trainers must also provide guidance and monitor the development of the portfolio in order to avoid the student spending too much time developing it, and neither learning from it nor producing a worthwhile collection of his/her work for assessment. Hence it is vital to train the trainers to guide students in portfolio development. Oman is in a phase of nation building and need professionals who have been educated by modern educational tools, such as assessment by portfolio, rather than by the archaic summative assessment.

ABSTRACTS BIOCHEMISTRY SESSIONS

Second and Third Generation PTH Assays in Secondary Hyperparathyroidism worth the Upgrade

Dr. Daniel Holmes

Consultant, Department of Pathology and Laboratory Medicine, St. Paul's Hospital, Vancouver, Canada

It is well-known that second generation "intact" PTH (iPTH) assays have cross-reactivity with C-terminal PTH metabolites, often collectively referred to as "7-84PTH". This becomes particularly relevant in patients with chronic kidney disease (CKD) because these fragments accumulate in the plasma. This has led to several companies developing third generation assays variably referred to as "whole PTH", "biointact PTH" or "1-84PTH". Is the move to a third generation PTH assay worth the effort? We have recently performed intact PTH, 1-84PTH (Diasorin Liaison), 25(OH)VitD, Bone ALP, Ca, and PO4 in a large cohort (~2500) of patients from the CanPREDDICT study. Estimated GFR in this cohort was between 14 and 45 ml/min (stage 3 and 4 CKD); none were receiving dialysis at the time of recruitment. Relationships between these bone markers and both iPTH and 1-84PTH will be reviewed. Based on this data, we will look at the performance and value of the 1-84PTH assay in the setting of non-dialysis-dependent CKD.

Vitamin D: Growing Role in Medicine

Dr. Meenu Kaur

Specialist Biochemist, Central Public Health Laboratories, Oman

Vitamin D deficiency has serious health consequences beyond rickets and osteomalacia. Evidence from clinical, epidemiological studies and randomised controlled trials shows that vitamin D has a role in prevention of many chronic diseases and its deficiency is associated with an increased risk of several diseases like osteoporosis, autoimmune disorders, diabetes, hypertension, autism, infections and various cancers. More than 200 genes are known to be regulated by 1,25-dihydroxyvitamin D[1,25(OH)2D], the active metabolite of

vitamin D. The serum concentration of 25-hydroxyvitamin D[25(OH)D] is the indicator of vitamin D nutrition status. Although the cut-off value to define vitamin D deficiency remains controversial, the current consensus points to a goal of ensuring the 25(OH)D level of 30-100ng/ml. Studies addressing vitamin D deficiency, supplementation and toxicity issues indicate the need to amend the existing advice on vitamin D requirements in all age groups. Vitamin D supplementation is also advised for the maternal and child health care. Lack of sun exposure is widely accepted as the primary cause of worldwide epidemic of vitamin D deficiency. Other causes include dark pigmentation, use of sunscreens, skin covering, obesity, old age, insufficient dietary intake, malabsorption and certain medications. Sunlight exposure, food fortification and supplementation with vitamin D are effective in preventing and treating vitamin D deficiency. Monitoring 25(OH)D levels in patients receiving high doses helps to guard against toxicity.

Standardisation of Endocrine Assays

Dr. Catherine M. Sturgeon

Clinical Biochemist, Royal Infirmary of Edinburgh, UK

High quality health care provision encourages the development of evidence-based guidelines, many of which include recommendations about laboratory testing. For some tests, analyte concentrations that define the need for clinical intervention are specified (e.g. parathyroid hormone in chronic kidney disease), This commendable drive to improve comparability in clinical practice will only succeed if the analytical results on which these recommendations depend are properly standardised. Improving between-method comparability is particularly challenging in immunoassays. Difficulties encountered generally reflect the characteristics of particular analytes, contributory factors include errors of calibration, antibody selection, assay design and properties of the assay matrix. The lack of established international standards for some analytes is also problematic. It is interesting to consider how these factors influence between-method comparability for several representative analytes, together with some of the international initiates addressing these issues. Achieving comparability of results for analytes with well-defined structures should, in theory, be possible. However, despite the availability of highly purified analyte preparations, isotope-dilution gas chromatography-mass spectrometry (ID-GCMS), reference methods and reference laboratories, poor comparability of results for these analytes is still regularly demonstrated in external quality assessment (EQA) schemes. While short incubation times and absence of automated extraction steps probably also contribute, isotope dilution gas chromatography-mass spectrometry (ID-GC-MS) targeting exercises confirm that poor assay calibration is responsible for much of the poor agreement observed. ID-GCMS methods may ultimately replace immunoassay of steroids in the routine hospital laboratory. In the meantime, encouraging manufacturers to use the available reference measurement systems to assess and improve their assays is a major priority that is being actively addressed. Establishing internationally recognized reference panels of patient sera and assigning these ID-GC-MS target values, as has been done for cortisol under the auspices of the International Federation of Clinical Chemistry (IFCC), should also help to encourage between-method comparability. While the same factors that contribute to poor between-method agreement for the steroid hormones are relevant to molecularly heterogeneous analytes such as the peptide hormones, the relative importance of these factors differs. EQA data demonstrate coefficients of variation of >10% for luteinizing hormone (LH), follicle stimulating hormone (FSH), human chorionic gonadotrophin (hCG) and prolactin, but incorrect assay calibration does not appear to be the major cause of poor between-method agreement for these analytes. This suggests that while correct calibration is essential for improved method comparability, antibody specificity and assay design are also of considerable importance. Addressing these requires knowledge both of what present assays are measuring and of what is clinically desirable to measure. In a prototype IFCC project, highly purified international reference reagents for six important hCG-related molecules have been prepared and calibrated in molar units. Their primary purpose initially is to assist manufacturers and users alike in characterising what current hCG assays are measuring. By combining the results of such studies with those of antibody-mapping studies carried out by the International Society of Oncology and Biomarkers (ISOBM), broad recommendations have been made regarding hCG antibody combinations likely to be most appropriate for particular clinical applications. Assessing results obtained for panels of patient sera should now permit validation of the predictions made. This two pronged approach with hCG, aimed both at standardising antibody specificity and improving the accuracy of calibration, may serve as a model for a broad strategic approach to improve between-method comparability of other molecularly heterogeneous analytes, and hence their clinical utility.

Tumour Marker Guidelines

Dr. Catherine M. Sturgeon

Clinical Biochemist, Royal Infirmary of Edinburgh, UK

Increasing pressure to provide health care based on “best practice” has stimulated the development of guidelines in cancer medicine. The recommendations of the American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN) have recently been complemented by more detailed guidelines from the National Academy of Clinical Biochemistry (NACB) in the United States and the European Group for Tumor Markers (EGTM) which provides a more laboratory-oriented perspective. In the pre-analytical phase, as well as ensuring the integrity of specimen collection and identity, and maintaining awareness of the effect of treatment or other conditions that may influence interpretation, the laboratory can encourage appropriate test requesting by promoting the recommendations made by the NACB for fifteen major cancer types. The NACB and EGTM guidelines also include detailed recommendations for best practice in the analytical phase, where well validated methods, rigorous internal quality control (IQC) procedures and participation in well-designed external quality assessment (EQA) schemes are all essential. Excellent precision and reproducibility (intra-assay variability <5%; inter-assay variability <10%) are important especially at concentrations close to critical clinical decision points, as is long-term assay stability. Awareness of clinically relevant interferences is highly desirable, with active dialogue between laboratory and clinical staff facilitating early identification of erroneous results. In the post-analytical phase, laboratory

reports should include fully cumulated results, an appropriate reference interval and the name of the assay method used, together with an indication of whether any change in marker level is significant and whether any change of method is likely to have affected interpretation of the trend in marker level. Laboratories should also undertake on-going audit of the clinical utility of the tumor marker results, linking this with clinical outcome.

Freelight Chain Utility in Clinical Practice

Dr. David Nkansa-Dwamena

Clinical Biochemist, SQUH, Oman

The serum immunoglobulin-free light chain assay measures levels of free kappa and lambda immunoglobulin light chains. The International Myeloma Working Group guidelines recommend the use of free light chain assays in the evaluation and management of multiple myeloma and related plasma cell disorders for screening, prognosis, monitoring response in patients with oligosecretory plasma cell disorders and assessment of stringent response in all patients who achieve complete response.

The urinary immunoglobulin free light chain test is not recommended for monitoring patients. The free light chain assay has technical limitations which makes its use as a serial measurement potentially problematic.

Wilson's Disease: A Treatable Inherited Disorder Pathogenesis & Diagnosis

Dr. Arunodaya R. Gujjar

Associate Professor of Neurology, Department of Medicine, Sultan Qaboos University, Oman

Wilson's disease (WD) is an autosomal recessive disorder of copper metabolism with protean manifestations, with a prevalence of 12–29 cases per million. It occurs due to mutations in the ATP7B gene, which codes for a membrane bound copper transporting ATPase protein. The mutant gene impairs biliary copper excretion, leading to excessive copper accumulation in the body, particularly in liver, brain, and musculoskeletal tissues. The clinical manifestations of WD are varied and its diagnosis challenging. In a study of 282 cases of WD (male:female ratio, 196:86) followed up over about three decades, clinical presentations were: hepatic, 42 (14.9%); hepato-neurologic, 10 (3.5%); neurologic, 195 (69.1%); pure psychiatric, 7 (2.4%); osseomuscular, 6 (2.1%); and "presymptomatic," 15 (5.3%). WD is known to cause hepatic disease at an earlier age (first decade) and neuropsychiatric or osseomuscular manifestations later (early second decade). Most patients would have symptoms for several months before diagnosis. Predominant neurologic features are: parkinsonism, dystonia, cerebellar ataxia, pyramidal signs, choreoathetosis, myoclonus and behavioral abnormalities. Hepatic dysfunction may range from fulminant hepatitis to indolent cirrhosis. Kayser-Fleischer (KF) rings which are diagnostic of WD, are evident in almost all patients with neuropsychiatric manifestations and more than 80% of those with others. Positive family history is present in half the patients. Serum ceruloplasmin was decreased in >90% and 24-hour urinary copper excretion is increased in >65% of patients. These are used as common diagnostic tests in the appropriate clinical context, while the gold standard is liver biopsy with quantitative copper assay. Most patients with neuropsychiatric manifestations have changes in CT or MRI scans of the brain, typically involving the basal ganglia. D-penicillamine or trientene, which are chelating agents, and zinc sulphate, which blocks intestinal copper absorption, are the common therapeutic agents. Diligent treatment can result in meaningful improvement in more than 70% of patients. Poor follow-up often leads to progression or relapse. Advances in treatment such as liver transplantation or gene therapy may improve the outcomes in near future. WD is now a treatable inherited disorder, mandating early diagnosis and diligent management.

Proteinuria: Current Trends in Detection and Monitoring

Dr. Waad-Allah S. Mula-Abed

Consultant, Clinical Biochemistry, Royal Hospital, Oman

Measurement of proteinuria is a traditional and established test for kidney injury. It facilitates risk stratification and appropriate management of patients with chronic kidney disease identified based on estimated glomerular filtration rate (eGFR) that will prompt early referral and initiation of renal protective therapy. Testing for proteinuria or albuminuria in at-risk groups has been recommended by many international guidelines. Measurement based on reagent strips is accompanied with many pitfalls and should be abandoned in favour of laboratory measurements reflected as urine protein or albumin concentration as a ratio to urinary creatinine. Methods for total protein are more sensitive to albumin, having poor precision at low concentrations, being insensitive, non-specific and subject to a range of false-positive and false-negative problems. Urinary albumin measurement provides a quantitative, relatively standardised measurement for the loss of an important single protein in most nephropathies. It is widely accepted as the test of choice for the detection of diabetic nephropathy that is commonly requested by physicians, although total protein measurement continues to be used by specialists investigating kidney disease particularly in non-diabetics. The use of urine albumin measurement as a front-line test for proteinuria appears to offer the best chance of improving the sensitivity, quality and consistency to the early detection and management of kidney disease.

External Qualtiy Assessment

Dr. Catherine M. Sturgeon

UK National External Quality Assessment Service (NEQAS) [Edinburgh], Department of Clinical Biochemistry, Royal Infirmary, Edinburgh

The primary function of an external quality assessment (EQA) service must always be to provide each participating laboratory with an objective assessment of its own performance, enabling comparison with that of other laboratories. Data from EQA schemes influence laboratory decisions when considering changes of method, particularly for complex analytes such as tumour markers and the glycoprotein hormones. Increasingly, data generated by EQA schemes inform the strategic decisions of professional organisations, including those involved in setting standards for laboratory accreditation, those working to improve analytical standardisation and comparability of clinical results for specific analytes (e.g. the International Federation of Clinical Chemistry) and those specifying quality requirements for health programmes (e.g. the National Health Service Prostate Cancer Risk Management Programme in the United Kingdom and the Kidney Disease: Improving Global Outcomes (KDOGI) initiative in the United States). Such reliance on EQA data places a considerable responsibility on EQA providers to ensure that specimens distributed are appropriate, i.e. as similar as possible to patient specimens and of clinically relevant concentrations. The validity of target values (usually trimmed consensus means) should also be regularly confirmed, by assessing recovery of international standards, by assessing linearity on dilution, and by determining their reproducibility on repeat distribution of the same pool. Assessment of long-term assay stability is particularly important for tumour markers. Experience suggests that major factors contributing to between-method differences in results include errors in calibration, differences in antibody specificity, and method design. Between-method agreement has improved for some analytes, including prostate specific antigen (PSA), for which between-method coefficients of variation in the UK National External Quality Assessment Scheme for PSA have fallen from 21.9% to 9.5% following the widespread adoption of International Standard 96/670 for PSA and highly commendable efforts by diagnostic companies to calibrate their PSA methods accurately in terms of this standard. However there are still significant method-related differences in results for many other analytes. Attention to method design is also required, e.g. to achieve equimolarity of recognition of different isoforms where relevant (as is desirable for complexed and free PSA and for HCG and its free beta-subunit) and to minimise the risk of clinically relevant interferences (e.g. heterophilic antibodies and high dose hooking). Through occasional issue of 'special' EQA samples designed to probe these potential pitfalls, aspects that need to be addressed, e.g. better blocking in vulnerable methods or protocols to identify hooking in some laboratories can be highlighted to manufacturers and users. This essentially educational remit of EQA can be further extended beyond the monitoring of analytical performance to incorporate assessment of other aspects of laboratory provision. In the pre-analytical phase, EQA surveys of practice provide a means of assessing the quality of advice given by participants to clinical users about test requesting. Interpretative exercises in which participants are asked to interpret their EQA results in the context of a given clinical history enable assessment of post-analytical advice including reference intervals, recommendations for further testing and interpretative comments.

Disorders of Aldosterone

Dr. Daniel Holmes

Consultant, Department of Pathology and Laboratory Medicine, St. Paul's Hospital, Vancouver, Canada

The laboratory serves as the main diagnostic tool for screening, diagnosis and tumour localisation in patients with primary aldosteronism. Since aldosterone and plasma renin activity (PRA) methods demonstrate such wide inter-method bias, appropriate aldosterone, PRA and aldosterone:PRA ratio thresholds need to be established at each laboratory. These cannot be taken from literature without careful comparison of methods. Appropriate use of screening tests will be reviewed along with the use and interpretation of provocative diagnostic tests. Strategies for successful analysis and useful reporting of samples taken from selective venous sampling of the adrenal veins will be reviewed.

Paediatric Growth Hormone Disorders

Dr. Aisha Al-Sinani

Paediatric Endocrinologist, Royal Hospital, Oman

Growth assessment is essential in child care and short stature can be recognised only with prompt history and accurate measurements of growth data. The hallmark of endocrine disease is linear growth failure that occurs to a greater degree than weight loss. There are three key diagnostic features of growth hormone disorders (GHD): abnormal growth demonstrated by auxological evidence of height < -2 SD, subnormal growth velocity and low GH in response to two dynamic tests. Primary insulin-like growth factor (IGF) deficiency can be missed as constitutional short stature if the dynamic stimulation is not interpreted cautiously. Measurement of IGF-1 and IGFBP-3 can be performed in our laboratory at the Royal Hospital, which is helping us to establish a diagnosis in IGF deficiency. Unfortunately, genetic analysis for growth hormone receptor genes is not available; it is recommended for those patients with access to laboratories expert in these techniques.

Vitamin B12 – Insight

Dr. Manal Al-Kindi

Chemical Pathologist, Royal Hospital, Oman

Serum B12 remains the most common vitamin investigation in clinical practice, it included in investigations of patients who present with symptoms of anaemia, glossitis or neurological dysfunction. Vitamin B12 acts as a cofactor for enzyme that catalyses methyl group transfer.This includes DNA methylation and the conversion of homocysteine to methionine. B12 is transported in blood predominantly bound to heptocorrin and smaller amount transported by transcobalamin. Only B12 carried by transcobalamin is available for cellular uptake and hence it is consider physiologically relevant. Understanding the pathophysiology of B12 and the different causes of B12 deficiency helps in solving the challenges in medical diagnosis and management. Biochemical tests for diagnosis of B12 deficiency, including total serum B12, holotranscobalamin, intrinsic factor antibodies and homocysteine, will be discussed in the presentation. Lack of standardisation in B12 assay results in a wide variation in B12 results between different laboratories. In addition different laboratories use different methods, report in different units and use different reference intervals making the use of the serum total B12 test alone to diagnose B12 deficiency of limited value.

ABSTRACTS HAEMATOLOGY SESSIONS

Bone Marrow Transplantation in Thalassaemia – How can we improve outcome?

Prof. Alok Srivastava

Professor of Medicine, Head, Department of Haematology & Centre for Stem Cell Research, Christian Medical College, Vellore, India

Allogeneic bone marrow transplantation (BMT) is the only currently established way to cure beta thalassaemia major and other severe haemoglobinopathies. The rate of success of BMT in these patients has been traditionally correlated with their risk stratification based on the extent of liver damage prior to BMT as assessed by the adequacy of chelation (whether started within 2 years of first transfusion), degree of hepatomegaly (^{3} 3 cm) and presence or absence of fibrosis on liver biopsy. The long term event free survival for class I patients (least risk) is over 90%; for class II patients (intermediate risk) ~85% and for class III patients (high risk) ~60%. The causes of failure of BMT, particularly in class III patients, are mostly related to two major categories of complications – regimen related toxicities and immunological problems such as graft versus host disease (GVHD) or graft rejection. Our work over last few years has been directed at understanding their biological basis and finding ways to mitigate them. We have extensively evaluated the pharmacokinetics of busulfan and cyclophosphamide, (Balasubramaniam et al., 1999) assessed their genetic basis and correlated them with clinical outcomes (Srivastava et al., 2004; Chandy et al., 2005). We have recognized that our class III patients can be further classified into those who are at extremely high risk of failure of treatment. (Mathews et al., 2007). We are now able to dose adjust busulfan doses based on the first dose kinetics of the individual. We are also attempting to use newer conditioning regimens in these patients. We have also evaluated the immunological aspects of GVHD and graft rejection and shown the role of dendritic cells and natural killer cells in these events. (Rajasekar et al., 2009, 2010).

Laboratory Aspects of Bone Marrow Transplantation

Dr. David Dennison

Senior Consultant and Director BMT, Sultan Qaboos University Hospital, Oman

Haematopoietic stem cell transplantation has advanced considerably over the last four decades to provide curative therapy for patients with a wide range of disorders. The success of any transplant program is dependent upon high quality laboratory support with both basic and specialised services. A transplant program linked to a busy multispecialty hospital has the advantage that the four basic laboratory disciplines of haematology, biochemistry, microbiology and histopathology should be already well-established and can provide the routine services required for the transplant patient. The technology and expertise within these laboratories need to be enhanced over time to cope with the development of the transplant programme. Rapid viral diagnostics and expertise in the histopathology of graft versus host disease are some examples. The haematology laboratory is unique for the spectrum of support it provides for a transplant programme. In centres worldwide, the haematology laboratory services could range from routine blood counts and peripheral smear examinations to immunomagnetic t-cell depletion or CD34 selection in haploidentical transplants, DNA fingerprinting for chimerism and sophisticated techniques for the detection of minimal residual disease following haematopoietic stem cell transplantation for malignant disorders. The laboratory aspects of haematopoietic stem cell transplantation are diverse. A successful transplant program needs a well-coordinated basic and specialized laboratory support. Finally, the interaction of the transplant clinician and the biomedical scientist is important for growth and development of any transplant service.

The Incidence of Alloimmunisation in the Omani Sickle Cell Disease Patients Undergoing Red Cell Exchange Transfusion

Dr. Sulayma Al Lamki

Senior Consultant, Haematology, Royal Hospital, Oman

Patients with sickle cell disease (SCD) require several transfusions in their life time and hence are prone to develop multiple antibodies. The incidence of alloimmunization among such patients varies in different countries. We analysed patients with SCD in our centre who had exchange transfusions between December 2001 and May 2007. The incidence of alloimmunisation was 14.5%, and 75% of these patients had developed only one antibody. The most frequently observed alloantibody was anti K followed by anti C and anti E. These findings are similar to the data observed internationally.

Clinical Transplantation of Stem Cell Research – The way forward

Prof. Alok Srivastava

Professor of Medicine, Head, Department of Haematology & Centre for Stem Cell Research, Christian Medical College, Vellore, India

The last decade has seen major advances in our understanding of the biology of embryonic (ESC) and adult stem cells (ASC). What has evolved more recently is our ability to differentiate them ex-vivo into cells, tissues and potentially organs of our interest. However, considerable controversy exists with regard to the source of ESC. The science of differentiating them into therapeutically relevant cells also needs to develop further. Finally, ways will need to be found to overcome the immunological barriers after transplantation. Therefore, much effort has also being directed towards identifying, characterising and expanding adult stem cells from different organs. Many organ specific stem cells are also being evaluated for the treatment of several degenerative disorders. This has led to the development of a whole new science that has been termed regenerative medicine. The recently recognised immunomodulatory properties of mesenchymal stem cells have also opened new possibilities of their use for tolerance-induction in organ transplantation and treatment of other disorders associated with immune dysfunction. In the last few years, there has been tremendous excitement around the potential of reprogramming adult stem cells by introducing certain critical transcription factors that confer to them the property of pluripotent differentiation similar to embryonal stem cells. These have been called induced pluripotent stem cells. The science of these cells is rapidly evolving and holds tremendous promise for regenerative medicine. However, as we tread this path to developing stem cell therapies, it is critical that we understand that none of these potential applications, apart from HSC transplants for haematological diseases, has reached the status of being widely offered as standard therapy at present. Their safety and efficacy need to be assessed in preclinical models and clinical trials. Offering such therapies at this time, therefore, through advertised services that give the impression of efficacy beyond what is established is wrong. The scientific and medical community and indeed the regulatory authorities in every country need to guard against such exploitation of vulnerable patients. Apart from potential harm to the individual, this approach will bring disrepute to science in the long run. The International Society for Stem Cell Research (ISSCR) has developed guidelines (http://www.isscr.org/clinical_trans/pdfs/ISSCRGLClinicalTrans.pdf) for clinical translation of stem cell research as well as a website to provide appropriate information to patients (http://www.isscr.org/about/Stem_Cell_ Treatments.htm). While the path may seem long and difficult, we need to remain positive and persevere to develop these therapies in an ethical and scientific manner.

Polychromatic Flow Cytometry in the Clinical Laboratory

Prof. Brent Wood

Pirector, Haematopathology Laboratory, Department of Laboratory Medicine, University of Washington, Seattle, USA

Multicolour flow cytometry has become a routine method for the multiparametric analysis of cellular populations in both the research and clinical laboratories. The advent of clinical instruments that allow for the routine analysis of up to 10 simultaneous fluorochromes is rapidly transforming clinical flow cytometry and offers a number of advantages including: improved assignment of cell lineage and maturational stage, more definitive assessment of immunophenotypic abnormality, increased laboratory efficiency and the ability to acquire large numbers of events, and ultimately standardisation of diagnostic approach. The increased degree of technical complexity of instrumentation and reagents, as well as decreased sensitivity for the simultaneous detection of multiple antigens on the same cell population for certain fluorochrome pairs, are the principle disadvantages. While it is now possible to generate reliably high-level multiparametric data, software tools to allow for the efficient and optimal use of this increased level of information have lagged behind and are now a primary focus of technology development. This talk will discuss technical details required for the successful performance of high-level multicolor flow cytometry, approaches under development for the analysis of multiparametric data in a clinical context, and recent technological advances likely to impact the field in the near future.

The Molecular Diagnosis of Haematological Disorders

Mr. Shoaib Al Zadjali

Senior Scientist, Department of Haematology, Sultan Qaboos University Hospital

Over the lst decades nucleic acid-based molecular diagnostic procedures have evolved astoundingly from the time-consuming labor-intensive Southern/Northern blot technology to cost-effective high throughput methodologies. This was made possible with the advent

of polymerase chain reaction (PCR) technology which allowed unlimited supply of target nucleic acid molecules. PCR-based diagnostic analysis (PCR-RFLP, Allele-specific PCR, Gene dosage, GeneScan, DNA Sequencing, Quantitative PCR and MLPA) are endowed with very high sensitivity, specificity and versatility. After an initial period of research and development, followed by quality assurance, the Department of Haematology of Sultan Qaboos University Hospital spearheaded the application of these technologies to a variety of inherited or acquired haematological disorders which include notably: chronic myeloid leukaemia (CML), acute lymphoblastic leukaemia (ALL), acute promyelocytic leukaemia (APL), myeloproliferative disorders, chimerism studies following bone marrow transplantation and haemoglobinopathies. Given the high rate of consanguinity of our society, further complicated by the genetic complexity of inherited disorders, a constant research component in designing/updating our diagnostic strategy was mandatory in order to translate them from bench to bed side. Such achievements were possible thanks to the fruitful and close interactions among the staff of the department at all levels.

Leukaemia Stem Cells in Acute Myeloid Leukemia: Implications for therapy and potential targeting strategies

Dr. Adhra Al Mawali

Chief of Medical Laboratory Science, Department of Haematology and Blood Transfusion, Royal Hospital, Muscat, Oman

A better understanding of leukaemic stem cells and molecular biology will lead to more effective therapies for leukaemic diseases. Malignant stem cells have been identified in acute myelogenous leukaemia, chronic myeloid leukaemia and some types of acute lymphoblastic leukaemia. Like normal stem cells, these leukaemic stem cells (LSCs) are able to self-renew, differentiate, and proliferate extensively. Evidence suggests that LSCs are critical for the initiation and perpetuation of leukaemic disease. Leukemia contains a subpopulation of cells that display characteristics of stem cells. These cells maintain tumour growth. The properties of LSC indicate that current conventional chemotherapy, directed against the bulk of the tumour, will not be effective. LSC are quiescent and do not respond to cell cycle-specific cytotoxic agents used to treat leukaemia and thus contribute to treatment failure. New strategies are required that specifically target this malignant stem cell population. LSCs are likely responsible for disease relapse and therefore represent an ideal target for effective therapy. Further characterisation of LSCs, using molecular and immunological techniques, is required to develop such therapies and monitor disease before relapse. LSCs are reported to over express the alpha subunit of the IL-3 receptor (CD123) compared to normal CD34+/CD38- haematopoietic stem cells, however, it has not been demonstrated whether the CD123 positive (CD34+/CD38-) subpopulation is enriched for any clonal markers of AML or any LSC properties. In our study, using five-colour flow cytometry, we confirm significant expression of CD123 in 32 /34 cases in the total blast population (median expression = 86%). CD123 was also strongly expressed in the CD34+/CD38- cells (96 ± 2% positive) from 28/32 primary positive specimens for CD123 taken from consecutive cases of adult AML at our institution. CD123 was not expressed/low in normal bone marrow CD34+/CD38- cells (median expression = 0%, range (0-.004%). Samples were tested for the presence of FMS-like tyrosine kinase 3 (FLT3) internal tandem duplication (ITD) by PCR as a tracking marker for leukaemic clones (10 positive /25). FLT3/ITD Positive AML samples were sorted into two putative LSC populations according to the expression of CD123 and then analysed for the presence of FLT3/ITD. Interestingly, FLT3/ITD was only detected in the CD34+/CD38-/CD123+ (7/7) and not in the CD34+/CD38-/CD123- subpopulation (6/7). These results suggest CD123 immunoprofiling provides further delineation of the FLT3 positive leukaemic stem cell clone and maybe useful to combine with CD34 and CD38 markers in tracking residual and relapsed disease at the level of the LSC. Combinational therapy targeting both CD123 and FLT3 in this population may result in more effective anti-LSC eradication.

Detection of Minimal Residual Disease in Paediatric Acute Lymphoblastic Leukaemia

Prof. Brent Wood

Director, Haematopathology Laboratory, Department of Laboratory Medicine, University of Washington, Seattle, USA

Minimal residual disease (MRD) detection is increasingly recognised as an important prognostic factor for patients with pediatric precursor B cell lymphoblastic leukaemia/lymphoma (acute lymphoblastic leukemia, ALL). This presentation will review the experience of ALL MRD detection by flow cytometry with a focus on the experience of the Children's Oncology Group (COG). Data from the most recently completed generation of COG trials confirms the prognostic significance of ALL MRD in a large cohort of patients with pediatric ALL. In particular, these trials demonstrate a clear association between increasing levels of MRD detection and reduced event free survival when assessed at day 8 after initiation of therapy in peripheral blood, end of induction (day 29) in bone marrow aspirates or end of consolidation in bone marrow aspirates. The combined use of MRD assessment at day 8 in peripheral blood and day 29 in bone marrow allows for the identification of a subset of patients with very low risk of relapse for whom therapeutic reduction might be a consideration. MRD is also shown to be prognostically significant within subsets of good cytogenetic risk patients or trisomy 4 and 10, and is associated with both short and long term relapse after therapy. In a multivariate analysis, the detection of minimal residual disease by flow cytometry is found to be the single most important prognostic indicator. The current generation of clinical trials in COG is now focused on determining whether risk adapted therapy based in part on MRD detection can favorably impact outcome for this disease.

Diagnosis of Red Cell Enzymopathies

Dr. Kanjaksha Ghosh

Director, National Institute of Immunohaematology, Mumbai, India

Red cell enzymopathies, when severe, can present with diverse clinical presentations, hence a single diagnostic strategy is unlikely to suit all clinical situations. G6PD deficiency which reaches polymorphic proportions in many populations as a result of balanced polymorphism against falciparum malaria infection needs to be detected by simple population screening methods. For many haemolytic anaemias, due to red cell enzymopathies, the red cell enzyme level needs to be measured spectrophotometrically or fluorimetrically. However, certain red cell enzymes are present in high concentration in reticulocytes, neutrophils and lymphocytes hence the enzyme level is measured when the acute state of haemolysis has passed and the blood is depleted of white cells by using suitable techniques. Certain red cell enzymes like Pyrimidine 5' nucleotide deficiency can be suspected when coarse basophilic stippling is noted in blood films. This enzyme may become deficient in lead poisoning. Spectrophotometric measurement of red cell enzymes are performed in haemolysates made from leukocyte depleted blood samples and is used as the source of the red cell enzyme. A substrate solution for the given enzyme is incubated with the haemolysate along with coenzymes like NAD or NADP. After a constant period of incubation either the (i) remaining substrate (ii) amount of product produced by enzyme action or (iii) quantitative changes in the coenzymes, i.e. NAD NADPH2 and vice versa, are measured by using suitable colorimetric or fluorimetric techniques. Glycolytic pathway enzyme defects causing haemolytic anaemia used to be screened in the past by auto haemolysis with correction of haemolysis by different compounds; however, this was cumbersome. Complete deficiency of NADP dependent methaemoglobin reductase and certain glycolytic enzyme deficiencies associated with cytochrome B 5 deficiency in the nervous system may lead to severe mental retardation. Red cell catalase deficiency can be associated with recurrent mouth ulcers without haemolytic anaemia. Porphyrin synthesis in the red cells is driven by innumerable enzymes and finally leads to synthesis of haem. Acute erythropoietic porphyria and erythropoietic proto porphyria are two important conditions. Both of them can be associated with moderately severe haemolytic anaemia. The diagnosis of these cases is done by screening the red cells for porphyrin metabolites fluorimetrically. Hence a combination of clinical features, red cell morphology and screening tests discussed above should guide the investigators in selecting the diagnostic techniques for a specific red cell enzyme deficiency.

The Role of the Pathologist in the Diagnosis of Lymphoma

Dr. Ibrahim Al Hadabi

Consultant, Department of Histopathology, Sultan Qaboos University Hospital, Oman

Immunohistochemistry plays an important role in the classification and diagnosis of malignancies. According to the World Health Organization, the classification of Hodgkin"s and non-Hodgkin's lymphomas depends on many factors including clinical features, morphology, cell lineage, stage of maturation and immunophenotype. The panel of markers is selected according to the differential diagnosis initially made by morphology. The approach to interpretation and diagnosis using immunohistochemistry and the pitfalls in diagnosis by this tool is discussed.

Community Screening for Haemoglobinopathies: Technical and logistic considerations

Dr. Kanjaksha Ghosh

Director, National Institute of Immunohaematology, Mumbai, India

Community screening to identify carriers and couples at risk is an important component of a haemoglobinopathies control programme. An effective implementation of screening programmes first requires adequate outreach for information, education and communication (IEC) both in urban and rural areas. The target groups should include: 1) Medical professionals and nurses; 2) Medical Social Workers and Community Leaders; 3) Students and Teachers in schools and colleges, and 4) General population. A combination of different approaches would have to be used keeping in mind the social, cultural and religious sentiments of multiethnic populations. Mass media should reach even remote areas. Other approaches would include talks during conferences and CME programmes for medical personnel. Inclusion of topics on haemoglobinopathies in school and college curricula, booklets, pamphlets and posters for distribution in different regional languages are other means of the dissemination of knowledge. Once this is in place, community screening should be undertaken after due consideration of logistical issues. The following need to be considered. If resources are inadequate, preliminary screening can be done using a combination of NESTROFT, solubility test and DCIP test where carriers with beta thalassemia, HbS and HbE respectively will be picked up and HPLC analysis can be done in the individuals who are positive in any of the 3 tests. For screening of beta thalassemia carriers, the most suitable approach would be to do a complete blood count, HPLC on all individuals with MCV< 80fl and/or MCH <27 pg. At least 5 to 6 regional centres should be identified where training can be given for manpower development in that region as well as for undertaking regular quality control programmes to avoid any misdiagnosis. It is impossible randomly to screen the large population in India and it would be most appropriate to initially screen newlywed couples and women in antenatal care who would be at immediate risk of having an affected child. The other groups which could be screened are school and college going students as they are easily accessible. Multicentre studies have been undertaken to screen all these 3 groups by the Indian Council for Medical Research and a few centres in six different states, mainly in medical colleges under the Jai Vigyan Programme. These need to be expanded to other states and ultimately facilities should be established at the district level in every state. Eventually more centres for prenatal diagnosis need to be established to successfully initiate a community control programme for haemoglobinopathies at the

national level.

Clinical Aspects of Bleeding Disorders

Prof. Alison Street

Vice President, Medical World Federation Haemophilia, Alfred Health, Melbourne, Australia

When reviewing a patient for symptoms of a bleeding disorder it is important accurately and precisely to define any abnormality both for the selection of appropriate treatment and for family counselling. Population screening by laboratory testing is neither specific nor cost-effective for the detection of clinically significant bleeding disorders. The most important clues come from the person's history of bleeding with past surgery, but where there has not been experience of such a homostatic challenge it can be very difficult to predict whether a person will bleed or not with any particular procedure. Working parties from various societies, such as the International Society of Thrombosis and Haemostasis have developed clinical questionnaires in attempts to standardise "bleeding scores", but there is a large overlap between the normal population and people with bleeding disorders. The history of past experience of bleeding remains the most important factor in deciding whether to proceed to targeted testing for the presence of a bleeding disorder. A family history of bleeding and the mode of inheritance may also help, for example transmission of haemophilia through the maternal line or an autosomal dominant pattern in families with von Willebrand disease (VWD). The type of bleeding, for example mucosal bleeds from nose, mouth and other parts of the gastro-intestinal tract may suggest a problem with platelet adhesion/aggregation such as VWD, whereas joint and deep muscle bleeds are more characteristic of a plasma factor deficiency. Algorithms for testing can be developed and the more specialised tests of von Willebrand factor function, genetic mutation analysis and identification of plasma factor inhibitors are often centralised to reference laboratories. Treatment is most safely and efficiently co-ordinated through centres where clinicians from multiple disciplines are co-located and trained to deliver comprehensive care. Treatment products are the most costly budget item for which protocols need to be locally designed, implemented and audited to maximise effectiveness of the investment.

Challenging Cases in Thrombosis and Haemostasis: SQUH experience

Dr. Khalil Al Farsi

Consultant, Department of Haematology, Sultan Qaboos University Hospital, Oman

Disorders of thrombosis and haemostasis are leading causes of morbidity and mortality world-wide. Thanks to the explosion in both clinical and translational research, our understanding of thrombosis and haemostasis has expanded from thinking of them as simple vessel leakages or occlusions to knowing the details of the molecular mechanisms behind them. The new insights gained from these, and the availability of specialists in the field with years of experience dealing with such disorders, have helped us establish some guidelines. These have been of great help in the diagnosis and management of such disorders. However, despite all this, we are still faced with cases that pose challenges both in the laboratory and the clinic i.e. in diagnosis and management. I will discuss a few challenging cases and try to provide a step-by-step approach that might be of help in managing such cases.

Developing Comprehensive Care Programs for the Diagnosis and Treatment of Haemophilia and other Bleeding Disorders

Prof. Alison Street

Vice President, Medical World Federation Haemophilia, Alfred Health, Melbourne, Australia

Comprehensive care is defined as the continuing supervision of all medical and psychosocial factors affecting the person with a bleeding disorder. Patients with bleeding disorders and their families are a "minority population" with special health needs for diagnosis, (including carrier status) and treatment. They develop trust and confidence in clinicians who show interest and develop competence in diagnosis and management of these disorders. The principles and practice of comprehensive care are those of "chronic disease management" with particular emphasis on training and multidisciplinary teamwork. The services provided within a comprehensive haemophilia care center will depend on locally available human and financial resources. They usually involve leadership from a haematologist or rehabilitation specialist, with input from laboratory technologists, nurses, physiotherapists, dentists, surgeons, etc. Services may also be integrated with those required for managing other inherited disorders such as thalassemia and haemoglobinopathies and thrombosis. A patient registry with systematic data entry supports effective and efficient clinical management both of patients and treatment products. Managing patients with haemophilia through a comprehensive care centre has proven benefit for both patient and financial outcomes. Adoption and adaptation of the services to local needs and resources requires trust and commitment between funders, clinicians and patients.

Screening Strategies for the Detection of Mutations in Haemophilia

Dr. Shrimati Shetty

National Institute of Immunohaematology (ICMR), Mumbai, India

Haemophilias represent the most common and severe inherited haemorrhagic disorders caused by mutations in the F8 and F9 genes,

which lead to deficiency or dysfunctional factor VIII and IX protein respectively. The F8 gene is 186 kb long; it has 26 exons and encodes a 9-kb mRNA transcript while the F9 gene is 33 kb in size with 8 exons and 7 introns with a 2.3 kb mRNA. Complicating the molecular characterisation of this disease is the complexity of the genes, the mutational heterogeneity, and technical limitations of the current mutation detection techniques. The mutations causing both haemophilia A and B are spread throughout the genes and are mostly represented by point alterations. However, the inversion of intron 22 was found in 40–50% of patients with severe HA and the inversion of intron 1 was reported with a prevalence of about 2-5% in different populations studied. Except the intron 1/22 inversions in severe HA cases, mutation detection in the F8/F9 genes is challenging so that it is only partially met by conventional screening methods such as single stranded conformational polymorphism (SSCP); conformational sensitive gel electrophoresis (CSGE) and chemical mismatch cleavage (CMC); high resolution melting analysis (HRM), and denaturing high performance liquid chromatography (DHPLC). Each of these have a varying applicability and efficiency; however, they all suffer from incomplete detection rates in the range of 70–90% . Moreover, each method places variable demands on the technical skills and time investment of the investigator. Direct sequencing of the gene is now the most accepted method of mutation detection in haemophilias. A F8 / F9 DNA micro array platform is an alternative gene mutation analysis approach that has a high sensitivity, and reproducibility. The methodology is, however, expensive and time consuming and, with the reduction in sequencing costs, direct sequencing is now the most cost and time efficient strategy for haemophilia A/B mutation analysis. Even extensive scanning of the exonic areas, promoter and splicing areas fail to detect mutations in 2-3% of the cases suggesting the role of other genes in reducing factor levels. Reports of double mutations in both haemophilia A and B add further to the complexity of genetic diagnosis in these disorders. These factors undetected, the phenotypic diagnosis has not been totally abandoned in many of the laboratories who routinely perform genetic diagnosis of haemophilia families.