Markers of kidney disease

11th Bergmeyer Conference - IFCC Master Discussion "Improving the clinical value of laboratory data"*

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Interchangeability of results over time and location would contribute considerably to improvements in healthcare because results of clinical studies undertaken in different locations or at different times could be universally applied. Standardization of laboratory measurements, ensuring the interchangeability of results, is basic in enabling effective cooperation between laboratory workers and clinicians, often producing guidelines for diagnosis and therapeutic intervention.

With the main objective of achieving even better comparability and compatibility of laboratory results the IFCC organizes Bergmeyer Conferences. This series of IFCC Master Discussions of experts started 20 years ago and is still generously sponsored by Roche Diagnostics, thanks to the availability of a fund established by Prof. H. U. Bergmeyer after his retirement in 1985.

The 11th Bergmeyer Conference, held in March 2008, in Eibsee, near Garmisch-Partenkirchen/Germany, was entitled "Markers of kidney disease" and focused on epidemiological, clinical, analytical (including standardization) issues and future trends in kidney diseases. The incidence and prevalence of end-stage renal disease and kidney failure treated by dialysis and transplantation have increased significantly during the past 25 years. The development of best practice in relation to detection and management of kidney disease, in the hope of slowing its progression, is therefore an important contribution by which analytically reliable and clinically relevant laboratory tests for evaluation of renal function can take a central role. The Conference programme reflected the multidisciplinary approach in attempts to promote a closer working relationship between laboratory professionals, nephrologists and other clinicians practising in renal medicine. It is significant that so many distinguished scientists representing, among others, guideline setting organizations, governmental bodies, academic centres, hospitals and diagnostic manufacturers have accepted an invitation actively to conduct discussions and gain further insight into this important subject. The Conference comprised speakers from various countries worldwide and invited participants, meeting for three days for wide-ranging discussions. A summary of different presentations is reported.

SESSION I - EPIDEMIOLOGY AND CLINICAL ISSUES

Acute renal failure: epidemiology and assessment. *John Kellum, Pittsburgh, USA*

Precise clinical and biochemical definitions of acute renal failure were never proposed and until recently there has been no consensus on the diagnostic criteria. Depending on the definition used (at least 35 definitions in literature), acute renal failure has been reported to affect from 1% to 25% of intensive care unit (ICU) patients and has lead to mortality rates from 15-60%. Furthermore, the need to classify the severity of the syndrome rather than only consider the most severe form was emphasized. Following such advocacy and through the persistent work of the Acute Dialysis Quality Initiative (ADQI) group, such a system was developed through a broad consensus of experts. RIFLE (Risk, Injury, Failure, Loss, End Stage Kidney Disease) criteria were developed to achieve diagnostic standardization. The three increasing severity grades (Risk, Injury, Failure) are defined on the basis of the changes in serum creatinine or urine output, the two outcome criteria (Loss and End Stage Kidney Disease) are defined by the duration of loss of kidney function. Furthermore, the term 'Acute Kidney Injury' (AKI) has been proposed to encompass the entire spectrum of the syndrome from minor changes in renal function to requirement for renal replacement therapy. The RIFLE criteria provide a uniform definition of AKI and have now been validated in numerous studies. The population incidence of AKI is approximately 2000-3000 patients per million population per year. The incidence of AKI is increasing and ICU patients with AKI have a longer length of stay, and, therefore, generate greater costs. In addition, AKI is associated with increased mortality, even after correction for covariates. Patients with AKI who are treated with renal replacement therapy still have a mortality of 50 to 60%. Of surviving patients, 5-20% remain dialysis-dependent at hospital discharge.

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The National Kidney Disease Education Program (NKDEP) and other related efforts in United States. *Andrew Narva, Bethesda, USA*

The NKDEP, an initiative of the National Institutes of Health, works to reduce the morbidity and mortality caused by chronic kidney disease (CKD) and its complications through educational efforts targeted towards at risk communities, patients, and health care professionals. Barriers to achieving these goals include confusion and misunderstanding of the laboratory tests used to identify and monitor patients with CKD. Through the Laboratory Working Group (LWG), NKDEP has collaborated with the clinical chemistry community to standardize creatinine determinations, promote the routine reporting of estimated glomerular filtration rate (eGFR), and standardize the measurement and reporting of urine albumin. The LWG recommends that clinical laboratories report creatinine results to two decimal places when using mg/dL and to the nearest whole number when using μmol/L; calculate eGFR using the Modification of Diet in Renal Disease (MDRD) study equation; use the isotope dilution mass spectrometry (IDMS)-traceable MDRD study equation at the same time they commence to use a creatinine method that has its calibration traceable to IDMS; routinely report eGFR with all serum creatinine results for patients 18 and older, whenever appropriate and feasible; report a numeric result only when the eGFR is <60 mL/min 1.73 m^2. In order to facilitate the use of urine albumin NKDEP LWG and the IFCC initiated a process to address lack of standardization in urine albumin determination and reporting. It is hoped that these efforts will improve screening, clinical care, and research in CKD and facilitate the implementation of evidence-based care recommended by the National Kidney Foundation and others.

UK guidelines for identification, management and referral of chronic kidney disease. *Edmund Lamb, Canterbury, UK*

The great majority of patients starting renal replacement therapy have progressed from earlier stages of CKD: most could therefore have been identified earlier, with possible improvements in outcome. Recent years have seen kidney disease move up the health-political agenda in the UK. This process began with the publication of the National Service Framework for Renal Services, which was underpinned by UK guidelines for the identification, management and referral of CKD in adults. Implementation of these strategies was encouraged by a national roll-out of eGFR reporting. National variation in eGFR reporting was addressed through a UK National EQAS 'harmonisation' process. UK CKD guidelines for assessment of excretory kidney function recommend that: kidney function in patients with CKD should be assessed by eGFR preferably using the MDRD equation; all clinical biochemistry laboratories should report eGFR alongside measurements of serum creatinine; when eGFR exceeds 90 mL/min 1.73 m^2, it should be reported as '>90 mL/min 1.73 m^2', specifying that when eGFR is between 60 and 89 mL/min 1.73 m^2 it does not indicate CKD unless there is other laboratory/clinical evidence of disease; laboratories should provide comparable creatinine results, ideally by the use of identical methodology, they should be audited by IQC procedures and satisfactory performance in a national EQAS. Proteinuria testing recommendations stated that there is no need to perform 24 h urine collection in primary care; a positive dipstick test should result in a urine sample (preferably early morning) being sent to the laboratory for confirmation by measurement of the total protein:creatinine ratio or albumin:creatinine ratio (simultaneously, a sample should be sent for culture to exclude urinary tract infection); urine protein:creatinine ratio ≥45 mg/mmol or albumin:creatinine ratio of ≥30 mg/mmol should be considered as positive tests for proteinuria; positive tests for proteinuria should be followed by tests to exclude postural proteinuria, by analysis of an early morning urine sample; patients with two or more positive tests for proteinuria, preferably spaced by one to two weeks, should be diagnosed as having persistent proteinuria. In autumn 2008 it is anticipated that the National Institute for Health and Clinical Excellence (NICE) will publish its clinical guideline on CKD.

Australasian Position Statement. *Graham Jones, Sydney, Australia*

The reporting of an eGFR with every requested serum creatinine has been successfully introduced as routine practice in Australia and New Zealand. This change in laboratory practice has been linked with a major educational initiative in the diagnosis and management of CKD as well as standardisation of a range of laboratory measurement and reporting issues. The process has been collaborative between renal physicians, chemical pathologists and laboratory scientists and their respective professional bodies and the relevant decisions have been made collectively on the best available evidence. The initial guidelines were released in August 2005 and these have been followed up in 2007 with further recommendations to address issues arising since that time. The first consensus meeting recommend to report serum creatinine as μmol/L and eGFR as mL/min; at least for eGFR <60 mL/min 1.73 m^2 the use of MDRD equation was acceptable estimate of GFR, probably at least equivalent to Cockcroft and Gault formula; the lack of studies in racial groups other than Caucasians and African-Americans was noted; the eGFR was to report routinely in all patients over 17 years of age, including hospital inpatients, with results above 60 mL/min 1.73 m^2 reported as ">60". It was therefore agreed that a significant educational program was required to maximise the benefit of the tool. Factors agreed to include in this program should be: changes in eGFR with age; decreased accuracy of eGFR above 60 mL/min 1.73 m^2; implications of body surface area for consideration of drug doses; decreased accuracy of eGFR in acute/unstable conditions; imprecision of eGFR results lack of applicability to dialysis dependent patients; an appropriate management pathway for those with an eGFR <60 mL/min 1.73m^2; particular patient groups and clinical setting where eGFR is not validated (different racial groups, severe malnutrition or obesity,

extremes of body size and age, disease of skeletal muscle, paraplegia, rapidly changing kidney function).

A second meeting was held to review progress and consider issues raised locally and overseas subsequent to the initial implementation. Major issues included the introduction of assays aligned to IDMS and the revised MDRD formula for use with these assays; the effect of age on interpretation of the eGFR; the level up to which eGFR results should be reported numerically; and issues to do with race and drug dosing. It was agreed to follow the advice from the LWG of the NKDEP to use the revised formula with IDMS-aligned assays. Reporting numerical values for the eGFR up to 90 mL/min 1.73 m^2 was accepted. Subjects over 75 years of age, with eGFR between 45 and 60 mL/min 1.73 m^2, would be treated in the same manner as same age patients with an eGFR between 60 and 90 mL/min 1.73 m^2, with recognition that this fall in GFR would remain important for drug dosing decisions. It was recommended that the eGFR is suitable for most drug dosing decisions unless the drug was known to be dose critical or the patient was an inpatient. The process of consideration and implementation of the routine reporting of eGFR and associated factors in Australia and New Zealand has been a fruitful exercise. Through the link with laboratories, the public health issues of CKD detection and management has been able to be implemented in a uniform manner in most locations in the two countries. This collaborative approach can provide a template for future activities where consensus approaches driven by informed experts are preferable to patchwork local implementations at the laboratory or laboratory network level. This consistent approach optimises the use of laboratory services to disseminate laboratory-related health interventions as widely as possible.

SESSION II - THE ROLE OF LABORATORY MEDICINE IN THE EVALUATION OF KIDNEY FUNCTION

GFR estimate: equations, algorithms and their implementation in clinical practice

Reliability of GFR formulas based on serum creatinine, with special reference to MDRD study equation. *Josef Coresh, Baltimore, USA*

The performance of the MDRD study equation has now been studied extensively (>20 papers including over 10,000 individuals). From these data, several conclusions about eGFR can be made. First, in CKD populations or patients evaluated for low GFR, the MDRD study equation often outperformed the Cockcroft and Gault equation and both equations are clearly superior to serum creatinine alone. Second, studies that calibrated the serum creatinine to that of the MDRD study laboratory showed improved performance. The effect of uncalibrated estimates is largest at the higher GFR range. Third, at higher GFRs several studies have reported that the MDRD study and the Cockcroft and Gault equations are less accurate at predicting GFR. The MDRD study equation often underestimated GFR, while the Cockcroft and Gault equation errors were related to age and body weight. Direct measurement of GFR will be useful in certain clinical situations where estimating equations are known to be inaccurate or clinical decision making requires a greater accuracy than expected from eGFR. Standardization of creatinine measurements by clinical laboratories should be in place soon and will improve accuracy of GFR estimates. Standardized values should be incorporated into GFR estimating equations as was recently done for the MDRD study equation. The NKDEP is actively working on a national program for such standardization and proficiency testing. The National Institute of Diabetes and Digestive and Kidney Diseases is currently funding a research group, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), to develop improved estimating equations for GFR. The scientific community has supported this effort and the CKD-EPI collaboration has published data on 5,504 individuals evaluating the MDRD study equation. New equations will be developed in this dataset and validated in independent populations. The effect of different sources of error (creatinine assay, GFR measurement, population characteristics) in equation performance within and across different study populations will be quantified, but these remain important limitations. Additional markers are needed for improved prediction with a focus on decreasing the proportion of individuals with a large error in estimating GFR (>30%). Finally, more work is needed on evaluation on the longitudinal performance of estimating change in GFR.

How does MDRD equation perform compared to serum creatinine in routine healthcare? *Anders Kallner, Stockholm, Sweden*

The use of MDRD-eGFR to diagnose CKD is based on the assumption that the algorithm will minimize the influence of age, gender and ethnicity that is observed in serum creatinine concentrations and thus allow a single cut-off at which further diagnostic and therapeutic actions should be considered. This hypothesis was tested in a retrospective analysis of outpatients (n=93,404) and hospitalised (n=35,572) patients in UK and Sweden, respectively. An algorithm based on the same model as the MDRD-eGFR algorithm was derived from simultaneously measured serum creatinine concentrations and iohexol GFR in a subset of 565 patients. The combined uncertainty of using this algorithm was estimated to about 15%, which is about three times that of the creatinine concentration results. The diagnostic performance of serum creatinine concentration was evaluated using the iohexol clearance as the reference procedure. It was shown that the diagnostic performance of MDRD-eGFR, as it stands, has no or little added value compared to serum creatinine alone. The gender and age differences of the creatinine concentrations

in the dataset persist after applying the MDRD-eGFR algorithm. Thus, a general use of the MDRD-eGFR does not seem justified. Furthermore, the claim that the eGFR is adjusted for body area is misleading, as the algorithm does not include any body size marker. MDRD-eGFR is thus a dangerous marker to use to guide drug administration.

Evidence of use of cystatin C and derived equations to estimate GFR. *Anders Grubb, Lund, Sweden*

Cystatin C- and creatinine-based prediction equations for GFR are more useful for estimating GFR than simple concentrations of the analytes. However, during recent years hundreds of GFR-prediction equations have been proposed, which has caused confusion about the application of such prediction equations and delay in their introduction in the clinical laboratory routine. The reasons for the present multitude of GFR-prediction equations are related to: differences in "gold standard" methods for GFR determination; use of different creatinine calibrations; use of different methods with differing dose-response curves and accuracies for determination of creatinine or cystatin C; use of different statistical models to generate the prediction equations; and use of different patient cohorts.

How to estimate GFR in specific populations (e.g., children). *Joris Delanghe, Gent, Belgium*

The availability of a worldwide reference system for creatinine is an important milestone for the improvement of GFR estimations for adults. However, an unacceptable interlaboratory variation is still observed which is mainly due to differences in calibration, especially in children and infants, where reference concentrations for serum creatinine are low. Compensating calibration in Jaffe assays to IDMS standards results in an underestimation of serum creatinine due to the lower concentrations of plasma proteins in children. Low molecular mass marker proteins like cystatin C and β-trace protein (BTP) can be regarded as an attractive practical alternative for assessing GFR. Studies comparing serum cystatin C to serum creatinine as a marker of GFR in pediatric populations generally showed diagnostic superiority or equivalence of serum cystatin C, especially in the so-called 'blind range' of creatinine concentrations. Formulas have been developed allowing reliable estimation of GFR based on cystatin C. Serum cystatin C reflects renal function in children independent of age, gender, height, and body composition and because of its low individuality, cystatin C has fewer inherent limitations as a screening test for detecting deteriorating GFR than serum creatinine. However, clinicians should be cognizant of extrarenal conditions (thyroid dysfunction, upregulation in certain tumours) and pharmacological factors (glucocorticoid treatment) that can influence serum cystatin C. Furthermore, serum creatinine is probably still the better assay for following sequential changes in an individual with confirmed renal disease. Serum creatinine concentrations are lower in malnourished children and lead to overestimation of GFR, while cystatin C concentrations are unaffected. Agreement between the Schwartz formula and gold standard GFR showed considerable bias and a trend towards overestimation of the GFR with lower GFRs. Analysis of the eGFR derived from cystatin C showed a negligible difference and no trend towards overestimation. From an analytical point of view, cystatin C can easily be measured using immunochemical methods in a highly reproducible manner. International standardisation for cystatin C is still lacking. However, validation of a candidate primary recombinant reference material by an IFCC Working Group is ongoing. Recently, BTP has been introduced for the measurement of kidney function in the creatinine 'blind range'. BTP, like cystatin C, is independent of age and gender.

Additional CKD markers

Evidence for use of urinary albumin as marker of kidney involvement in unselected population. *Matthew McQueen, Hamilton, Canada*

Results from PREVEND (Prevention of REnal and Vascular ENd stage Disease) study suggest that a population screening for small amounts of urinary albumin leakage may identify early those who would benefit from blockade of the renin-angiotensin-aldosterone system. Such screening is costly, but does it have the potential to be cost-effective compared with the financial and human resources costs of treated, poorly treated or untreated patients at end stage renal disease and cardiovascular disease. The data require confirmation from multicentre trials applied on other populations.

What place for the β-trace protein? *Rainer Woitas, Bonn, Germany*

Endogenous filtration markers may offer advantages with respect to estimation of GFR in clinical settings. An optimal endogenous marker for GFR should have a constant production rate and should be freely filtered in the glomeruli without reabsorption or tubular secretion or extrarenal elimination. Although, serum creatinine is most widely used to predict GFR, it does not fit these criteria. Since even sophisticated GFR equations based on creatinine failed to counteract the disadvantages, there is an ongoing search for new endogenous GFR markers. It has been proposed that virtually all BTP is excreted in urine and serum concentrations depend on GFR. BTP has been reported to be a better indicator of reduced GFR than serum creatinine in the creatinine 'blind range', but not better than cystatin C. Putative advantages of BTP may be its usage as filtration marker in patients after renal ischemia where tubular back leak of creatinine leads to dissociation of creatinine and GFR. However, it is still under debate which endogenous biomarker is less affected by glucocorticoid treatment. Very recently, two studies generated BTP-based calculation formulae for GFR in renal transplanted patients. Finally, there is evidence that BTP is hardly removed by hemodialysis

or peritoneal dialysis. This makes BTP a promising candidate for evaluation of residual renal function, which is an important factor influencing mortality, nutrition status, and quality of life in patients undergoing renal replacement therapy.

Standardization issues

Standardization of creatinine measurement: theory and practice. *Gary Myers, Atlanta, USA*

Central to the improvement of serum creatinine measurement for the reliable estimation of GFR is achieving standardization of measurement results worldwide. To achieve this, standardization state requires that serum creatinine measurements must be traceable to an order of references comprised of high-order reference measurement procedures and reference materials. The International Organization for Standardization (ISO) has developed the 17511 standard that details a pathway for establishing traceability of clinical laboratory measurement results. The components of this reference system are linked together in a systematic fashion that is referred to as the "traceability chain". In this context, traceability means metrological traceability and is a prerequisite for achieving the needed comparability and reliability of laboratory results for patient care.

Both gas chromatography-isotope dilution mass spectrometry (GC-IDMS) and liquid chromatography (LC)-IDMS methods are approved and listed by Joint Committee on Traceability in Laboratory Medicine (JCTLM) as reference measurement procedures for serum creatinine. The NIST SRM 914a is crystalline creatinine intended for use only as a primary calibrator for these high-order reference methods and is not intended for use in calibrating routine measurement procedures. Among other creatinine materials, only NIST SRM 967 (creatinine in frozen human serum) has been documented to be commutable with native clinical specimens in routine methods. The SRM 967, prepared according to Clinical and Laboratory Standards Institute (CLSI) C-37A document, was developed in collaboration with the NKDEP and the College of American Pathologists to meet the need for improved calibration of clinical instruments and procedures for measuring serum creatinine. Commutability was evaluated using criteria described in CLSI EP14-A2 document. The complete list of reagent instrument systems included in the commutability study can be found on the NKDEP website.

To improve the performance of serum creatinine measurement, a number of recommendations have been suggested by the NKDEP LWG. IVD manufacturers should recalibrate routine serum creatinine methods to be traceable to the reference measurement system. After recalibration to IDMS, a realistic total error goal for creatinine measurement should be such that only a maximum 10% increase in the relative error in eGFR results. Routine methods could achieve this recommended goal if analytical bias compared to an IDMS reference procedure is <5% and analytical imprecision is <8% (including between-laboratory calibration variability) at all serum creatinine concentrations ≥88.4 μmol/L (1.0 mg/dL). The 4-variable MDRD study equation was reexpressed for use with standardized serum creatinine assays. In addition to the efforts by the NKDEP LWG, the IFCC has created a Working Group on Standardization of GFR Assessment (WG-GFRA) with the main goal to disseminate information, recommendations and programs internationally so that an accurate estimate of GFR is routinely reported in all pertinent clinical settings.

How to implement traceability of creatinine results: a manufacturer's experience. *Hans Joachim Kytzia, Penzberg, Germany*

In 1997, Roche Diagnostics has introduced an enzymatic creatinine assay based on the enzymatic conversion of creatinine. This method is a priori free of the well known unspecific reactions of the classical creatinine "Jaffé" method. As a consequence, it can be standardised to give a very good correlation with negligible intercept to the IDMS reference method. The discrepancy between the Jaffé and the IDMS creatinine results is caused by the reaction of the alkaline picrate reagent with a variety of endogeneous sample components in addition to that with creatinine. That results in an intercept in the method comparison that cannot be corrected simply by appropriate calibration. However, the difference is fairly constant at approximately 0.3 mg/dL (26 μmol/L) for serum or plasma samples over the entire measuring range. This finding led Roche to introduce the so-called creatinine Jaffé "compensated" method that automatically corrects each result by -0.3 mg/dL. Using this correction, a good correlation with the reference method results is also obtained with the Jaffé method.

While with the implementation of the compensated Jaffé method, the creatinine routine methods showed a satisfactory agreement with and the desired traceability to the reference method, a new problem arose with the determination of the GFR. According to the recommendations of NKDEP, GFR is calculated by the MDRD study equation. However, upon switch from the classical Jaffé to an IDMS traceable creatinine method, the eGFR increased considerably. As a consequence, the so-called reexpressed 4-variable MDRD study equation was developed and is meanwhile recommended by the NKDEP for use with IDMS traceable creatinine methods.

Enzymatic assays for creatinine: time for action. *Mauro Panteghini, Milano, Italy*

Standardization of calibration, i.e. implementation of calibration traceability to high-order reference measurement procedures and reference materials, does not correct for analytical interferences of field methods (non-specificity bias). To account for the sensitivity of alkaline picrate-based methods to non-creatinine chromogens, some manufacturers have adjusted the calibration to minimize the pseudo-creatinine contribution of plasma proteins, producing

results more closely aligned to the reference method, but this strategy makes an assumption that the non-creatinine chromogen interference is constant among samples, which is an oversimplification. Thus, analytical non-specificity for substances found in individual patient samples affect the accuracy of eGFR computed from serum creatinine values for any alkaline picrate method including the so-called "compensated" Jaffé methods. The use of assays more specific for serum creatinine determination, such as those based on enzymatic reactions, may provide more reliable eGFR values.

A further difficulty associated with the standardization efforts is the need to develop scientifically sound and globally useful reference intervals for serum creatinine concentrations. For the production of common reference intervals the method specificity is crucial. Thus, only serum creatinine reference values obtained with standardized, specific assays, such as those based on enzymatic principles, should be considered for the establishment of reference intervals, as these methods have the unique analytical specificity to guarantee traceability of each reference individual result to the reference measurement system for creatinine measurement, especially at the low serum creatinine concentrations found in children.

In summary, reporting an accurate eGFR in all pertinent clinical situations requires the use of more specific assays. The frequently raised issue of reagent costs is a false problem. In the near future, as more and more companies begin providing commercial enzymatic assays for creatinine, there will be a more competitive situation in the marketplace and, ultimately, prices may be driven lower. More importantly, the cost aspects in clinical laboratories must be considered in the wider overall context of health economics and not within the more blinkered area of pure laboratory economics. Otherwise, a cent saved in the laboratory can paradoxically cost euros in the clinic.

Standardization of cystatin C: achievements and further steps. *Søren Blirup-Jensen, Lund, Sweden*

Serum cystatin C has been shown to be an excellent marker for GFR. However, in the clinical routine using different methods varying results are obtained due to lack of standardisation. The goal of the IFCC Working Group on Standardisation of Cystatin C is to produce and characterise a primary and a secondary reference material for cystatin C. A primary reference material has been produced using pure, recombinant cystatin C. Dry mass determination of the preparation resulted in a cystatin C concentration of 5.20 g/L. Agarose- and SDS-electrophoresis as well as N-terminal sequencing verified the purity, homogeneity and identity of cystatin C in the reference material. For the secondary reference material a serum pool was collected and stabilised. A pilot batch was made to verify the selected procedure and the spiking with the pure, recombinant cystatin C. The final secondary material is now produced and ready for value assignment, further characterisation and stability studies. Its cystatin C concentration will be determined using the primary reference material with the use of a carrier serum in order to ensure the same matrix in both preparations. The value assignment will be carried out using single radial immunodiffusion, turbidimetry and nephelometry. The secondary preparation is expected to be released in the last part of 2008, whereafter the commercial calibrators can be adjusted accordingly.

Urinary albumin: measurement standardization and recommendations for sample collection and result reporting. *W. Greg Miller, Richmond, USA*

A joint work group of the NKDEP and the IFCC has the objective to improve the standardization of sample collection, measuring and reporting for urine albumin. The group held a conference in March 2007 to frame the issues and identify solutions for standardization. A manuscript is in development to report the proceedings and recommendations from that conference. Current routine measurement procedures use immunoassay with nephelometric, turbidimetric or enzyme linked measurements. EQA programs in several countries have shown a lack of agreement in results and in units used to report results among different methods and laboratories. Surveys of clinicians and clinical laboratories have identified a lack of uniformity in sample collection, result reporting and interpretation practices. A standardization program is needed for urine albumin because diabetes and kidney disease professional societies have developed clinical practice guidelines for interpreting the results. There is no JCTLM listed reference measurement procedure for urine albumin. A LC-tandem-MS procedure has been developed at the Mayo Clinic, that quantitates the intact N-terminal fragment of urine albumin. This procedure has attributes of a candidate reference measurement procedure. A candidate urine albumin reference material has been developed under the auspices of the Japan Society of Clinical Chemistry and the Japanese Committee for Clinical Laboratory Standards and is in the process of validation and credentialing. The candidate reference material is a lyophilized preparation of purified monomeric human albumin in an aqueous buffer. The material was value assigned by traceability to dilutions of serum protein reference material CRM 470 (Institute for Reference Materials and Measurements of the European Commission) using a routine immunoassay that had uniform antibody binding to albumin in the reference material and in CRM 470. The NKDEP/IFCC joint working group intends to develop a standardization program and an educational initiative for urine albumin and urine creatinine measurement to improve use of urine albumin and the albumin/creatinine ratio in clinical practice.

New markers for kidney disease

Fatty acid-binding protein (FABP). *Maurice Pelsers, Maastricht, The Netherlands*

The assessment of biomarkers to detect renal injury has been studied for many years, but sensitivity and specificity of urinary and serum profiles of these markers still need to be improved. One of the promising new markers for detection of renal injury is the family of 15 kDa cytoplasmic fatty acid-binding proteins (FABPs). Remarkably, however, the application of FABP as marker for renal injury due to ischemia, toxic heavy metals or in end-stage renal failure has only recently been investigated. Two types of FABP in the kidney, heart-type (H-FABP), located in the distal tubular cells, and liver-type (L-FABP), located in the proximal tubular cells, may be considered for sensitive detection of renal injury. H-FABP has been shown to be a proper marker for detection of ischemic injury in tissue perfusates of non-heart beating donor kidneys. Urinary L-FABP has been evaluated more explicitly showing significant elevations in progressive end-stage renal failure as well as after ischemic injury due to renal transplantation or cardiac bypass surgery. H- and L-FABP have been shown to be useful markers for rapid detection and monitoring of renal injury. Performance of diagnostic and prognostic studies of these FABP types will require commercialization of automated and rapid assays for proper clinical application. As renal disease also appears to be an independent risk factor for CVD, earlier detection by these biomarkers can stratify treatment and reduce death by CVD.

Kidney injury molecule-1 (KIM-1). *Joseph V. Bonventre, Boston, USA*

There is an urgent need for the detection and monitoring of kidney injury both in the acute and chronic disease setting. Urinary kidney injury molecule-1 (KIM-1), a type 1 transmembrane protein, is not phisiologically present but is expressed on the proximal tubule apical membrane with injury. KIM-1 has proven to be an outstanding indicator of kidney injury in the rat outperforming urea and creatinine concentrations in serum as predictors of histopathological changes in the proximal tubule in response to many pathophysiological states or toxicants. Studies in man indicate that tissue expression and urinary excretion of the ectodomain of KIM-1 are sensitive and specific markers of injury as well as predictors of outcome.

Neutrophil gelatinase-associated lipocalin (NGAL). *Prasad Devarajan, Cincinnati, USA*

The incidence of both AKI and CKD is reaching epidemic proportions. In both situations, early intervention can significantly improve the prognosis. However, the paucity of early, predictive, non-invasive biomarkers has impaired our ability to institute potentially effective therapies for these common clinical conditions in a timely manner. One of the most promising novel biomarkers is represented by neutrophil gelatinase-associated lipocalin (NGAL). NGAL is emerging as a center-stage player in a variety of clinical situations leading to AKI (cardiac surgery, kidney transplantation, contrast nephropathy, hemolytic uremic syndrome, and in the intensive care setting). Large multicenter studies to further define the predictive role of plasma and urine NGAL as a member of the putative "AKI Biomarker Panel" have been initiated, using robust assays that have been developed for widespread clinical use. A growing literature suggests that NGAL is also a marker of kidney disease and severity in CKD (lupus nephritis, glomerulonephritides, obstruction, dysplasia, polycystic kidney disease, IgA nephropathy). The availability of validated clinical tools for NGAL measurements could revolutionize renal diagnostics. In this regard, a standardized point-of-care kit has been devised for the measurement of plasma NGAL (Triage NGAL Device, Biosite Inc.). In addition, a urine NGAL immunoassay has been developed for a standardized clinical platform (Architect, Abbott Diagnostics). It will be important in future studies to validate the sensitivity and specificity of NGAL measurements in clinical samples from large cohorts and from multiple clinical situations. Such studies will be facilitated by the widespread availability of automatic commercial tools in the near future.

SESSION III - KIDNEY AND NONRENAL DISEASES

Early diagnosis and treatment of diabetic nephropathy. *Eberhard Ritz, Heidelberg, Germany*

Type 2 diabetes mellitus (DM) is a major public health issue. It is associated with excessive cardiovascular mortality and has become in most countries the most frequent condition requiring renal replacement therapy. In end-stage diabetic nephropathy, the effect of interventions is not satisfactory: they delay, but fail to halt, progressive loss of renal function, emphasizing the need for early diagnosis and treatment. Albuminuria and metabolic syndrome predict the onset of type 2 DM. The best predictor of renal damage is albuminuria. The classical definition of "microalbuminuria" is not satisfactory, since any degree of albuminuria increases the cardiovascular and renal risk. It has therefore been argued that the term "microalbuminuria" should be completely abandoned and that urinary albumin should be treated as a continuous variable as, for instance, serum cholesterol. Direct precise measurements of GFR are laborious and/or invasive. There has been a great need for estimates of true GFR and two formulas, first Cockcroft-Gault and later the modified MDRD equation, have been introduced. Both are unsatisfactory in the near physiological range and are unreliable for GFR values above 60 ml/min 1.73 m^2, and this is particularly true in diabetic patients. An accurate estimate of true GFR in individual patients would be highly desirable because even minor reduction of GFR causes a dramatic increase in the cardiovascular risk at least in the general populations and presumably also in diabetic patients. Cystatin C is superior to estimates of GFR in predicting cardiovascular risk, but so far has not found routine

use. One has to distinguish between interventions in early and in advanced diabetic nephropathy: in the early stages, control of glycemia (particularly, of postprandial glycemia) is of paramount importance in type 1 DM. The evidence is less stringent, but plausible, in type 2 DM. Finally, observational studies suggest benefit from cessation of smoking.

Clinical role of estimation of urinary low-molecular weight proteins. *Walter Guder, Munich, Germany*

In traditional urinalysis, casts in the urinary sediment were the only specific signs of renal tubular injury. After tubulointerstitial fibrosis became the most predictive sign of renal outcome, tubular enzymes derived from proximal tubular brush border or lysosomes were used as early markers of nephrotoxicity and other tubular dysfunctions. More recently, the increase in low-molecular weight proteins in urine assumed to be freely filtered were reported to reflect tubular dysfunction. This can have prerenal, renal and postrenal causes. Among the prerenal causes, Bence Jones protein (immunoglobulin light chains), myoglobin and hemoglobin are signs of extrarenal diseases. On the other hand, β_2-microglobulin, α_1-microglobulin, retinol-binding protein and lysozyme were recommended as tubular markers. The urine outflow of these low-molecular weight proteins can be changed by prerenal, renal and postrenal causes. Because of its lower prerenal variability and higher stability in urine during storage in the bladder and urinary vessel, α_1-microglobulin proved to be most valuable in early detection, renal outcome prediction and easy inclusion into routine analytical programs. In addition, other markers of intrarenal inflammatory process may in addition help to mirror histological changes appearing in the kidney. A future guideline should therefore include low-molecular proteins as tubular markers.

Autoimmunity in kidney disease. *Ralph Kettritz, Berlin, Germany*

Renal involvement in autoimmunity has many facets. Glomerular, tubular, and vascular structures are targeted and damaged as a consequence of autoimmune processes. Most dramatic and life-threatening causes are observed with diseases that result in rapidly progressive glomerulonephritis (GN), frequently accompanied by involvement of additional non-renal organs. Typical diseases with these characteristics are anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitidis, anti-glomerular basement membrane (GBM) GN, and proliferative lupus nephritis. The leading cause of rapidly progressive GN is ANCA-associated GN. Two major ANCA antigens have been described, namely proteinase 3 (PR3), with a cytoplasmic immunfluorescence staining pattern, and myeloperoxidase (MPO), with a perinuclear pattern. ANCA-associated diseases include Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome, and a renal limited disease form that presents solely with necrotizing crescentic GN. Because of the strong association of ANCA with the aforementioned diseases, ANCA has become a valuable diagnostic tool for clinicians. Moreover, a variety of in vitro and in vivo findings have established the causal role of ANCA in the disease development. Recently, the membrane-PR3 expression pattern on neutrophils was shown to have clinical significance, suggesting membrane-PR3 as a novel biomarker. Furthermore, the data demonstrate that membrane-PR3 expression is restricted to a stable subset of neutrophils. This subset is determined by the existence of the glycosylphosphatidylinositol-anchored NB1 receptor (CD177). Better understanding of the PR3-NB1 interaction may have therapeutic implications with the development of more selective drugs.

Cardiac markers in CKD. *Paul Collinson, London, UK*

Cardiac biomarkers have a complex interrelationship with disease pathophysiology in patients with renal dysfunction. The underlying clinical condition results in a direct effect on the physiological release and clearance of cardiac troponins and natriuretic peptides. Although initial reports suggested that this might prove a major limitation in the routine clinical use of these markers, a combination of improved assay performance and a better understanding of the underlying biochemistry of these markers in health and disease have clarified the situation. Renal dysfunction does not provide a significant practical limitation to the use of cardiac biomarkers for diagnosis in acute presentation of cardiovascular disease. The direct relationship between cardiac biomarkers and renal dysfunction reflects the high incidence of CVD and cardiac death in patients with renal dysfunction and end-stage renal disease. Elevations of the cardiac troponins are prognostic in patients with renal dysfunction and represent global diffuse myocardial injury. Elevations of natriuretic peptides also occur due to abnormalities of ventricular function. In addition, background concentrations will be affected by fluid and electrolyte abnormalities due to renal dysfunction. This will directly affect vascular volume and fluid distribution altering atrial and ventricular wall tension and hence rates of natriuretic peptide release and production. Cardiac biomarkers have a diagnostic and prognostic role in patients with all stages of renal dysfunction. Although the pathophysiology of troponin release remains to be fully elucidated and some significant questions remain for natriuretic peptides, these do not preclude their use as diagnostic tests in patients with renal dysfunction. Their prognostic role remains to be fully utilised. Measurements of cardiac troponins and natriuretic peptides are measurements of CVD severity and risk of death in patients with renal dysfunction. The challenge is for the renal physician to translate the potential for CVD monitoring conferred by these biomarkers into improved patient management.