

Oral Presentations

Thames Regional audit on creatinine clearance

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Advice from the National Kidney Foundation, and in the draft Renal NSF, is that serum creatinine alone is not an adequate marker of renal function and estimated GFR should be reported. The draft NSF also states measurement of creatinine clearance, using urine and serum, is no longer the gold standard for detecting and monitoring renal disease.

Laboratory practice in the Thames regions was audited, covering reference ranges for serum and urine creatinine, creatinine clearance and whether creatinine clearances were still measured or whether GFR was estimated from formulae, such as Cockcroft and Gault.

Forty-nine laboratories responded to the questionnaire; 40 measured creatinine clearance, using serum and urine, and only 5 estimated GFR using a formula.

The audit showed 54% of laboratories did not use a sex-related reference range for serum creatinine and 32% did not use age-related ranges. The upper and lower limits of adult reference ranges varied widely (males: lower limit range 20-80, upper limit 107-144 $\mu\text{mol/L}$; females lower limit range 20-62, upper limit 93-130 $\mu\text{mol/L}$). Urine ranges varied from 4-12 as the lower limit to 12-25 mmol/L as the upper limit.

There was confusion about measured and calculated creatinine clearance, with laboratories often unaware of the formulae for calculating GFR from a serum creatinine.

Samples distributed as part of the audit showed wide variation: urine mean concentration = 9.57 mmol/L ; range 7.7 to 12.1, CV 10.4%; serum mean concentration = 217.3 $\mu\text{mol/L}$; range 195-270, CV 6.6%; mean measured clearance = 30.7 ml/min ; range 23-40, CV 13.3%; mean estimated GFR = 24.2 ml/min ; range 19.6-26.9, CV 6.1%.

Standards proposed were 1) that creatinine clearance should be calculated using a formula, not measured, 2) creatinine assays should be calibrated against an IRP, 3) ideally, laboratories should report an estimate of GFR as well as a serum creatinine.

Serum free light chain assays can identify minimal residual disease in multiple myeloma patients who are immunofixation negative

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Measurement of serum free light chain (FLC) concentrations has been shown to be more sensitive than

electrophoresis for detection and monitoring of nonsecretory multiple myeloma and light chain only multiple myeloma (LCMM). After treatment, however, complete response (CR) is currently defined as an absence of paraprotein in serum and urine by immunofixation electrophoresis (IFE). The aim of this study was to determine whether the serum free kappa/lambda ratio is a more sensitive measure of CR in LCMM and intact immunoglobulin multiple myeloma (IIMM). FLC concentrations were measured in the sera of 85 patients from the MRC VI and VII Myeloma trials who were determined to have achieved CR by IFE. Of the 85, 31 had LCMM and 54 had IIMM. 11/31 LCMM and 37/54 IIMM patients, had normal serum FLC ratios, in agreement with the IFE results. The remaining 20/31 LCMM patients and 17/54 IIMM patients had abnormal FLC ratios indicating the presence of residual disease. Where available ($n=44$), overall survival (OS) data, indicated that patients who had abnormal FLC ratios, had shorter OS than those who were IFE negative with normal FLC ratios (mean OS of 990 versus 1188 days for LCMM patients and 891 days versus 1430 days for IIMM). A t-test analysis of the data revealed that the differences reached significance for the IIMM patients ($p<0.03$) but not the LCMM patients. It has previously been shown that serum FLC assays are more sensitive than urine FLC tests for the determination of CR in LCMM and this increased sensitivity is due, in part, to normal renal function preventing light chains entering the urine. The current study indicates that serum FLC assays also identify residual disease in some IFE negative IIMM patients and that abnormal FLC ratios may be predictive of a reduced OS.

Can the insulin-like growth factor axis be used to discriminate prostate cancer from benign prostatic hypertrophy?

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Prostate cancer (CaP) is one of the most common malignancies occurring in males in the Western World. Screening for the disease with prostate specific antigen (PSA) is limited due to the inability to clearly differentiate men with CaP from those with benign prostate hyperplasia (BPH) which is a common phenomenon associated with ageing. Increasing evidence implicates the insulin-like growth factor (IGF) axis in the pathogenesis of CaP. The IGF axis consists of a number of components: two peptide growth factors insulin-like

growth factor-I (IGF-I) and insulin-like growth factor-II (IGF-II), two types of IGF receptors, at least six binding proteins (IGFBPs) and several IGFBP proteases. Although there is evidence of an association of higher circulating levels of IGF-I with an increased risk of CaP, the association of other IGF axis components to the pathogenesis of CaP remains unclear. Potentially measurement of IGF axis components could be useful as adjuncts to current diagnostic tools to distinguish between benign and malignant prostate disease.

To investigate the role of the IGF axis in prostate disease, serial pre- and post-operative serum samples were obtained from 22 patients undergoing transurethral resection of the prostate. Initial experiments demonstrated there was no significant differences in the serum levels of IGF-I, IGF-II, IGFBP-1 and IGFBP-3 levels between CaP and BPH patients. However, pre-operative total PSA and IGFBP-2 levels differed significantly between CaP and BPH patients.

Currently no commercial methods exist for the measurement of serum levels of IGFBP-5. A Western immunoblotting procedure was established to measure serum IGFBP-5. Serum IGFBP-5 concentrations were significantly higher in CaP than in BPH and were correlated to pre-operative total PSA but not to IGF-I or IGF-II. Following surgery, IGFBP-5 levels were significantly reduced.

In conclusion, IGFBP-2 and IGFBP-5 may assist discrimination between BPH and CaP but larger studies are required.

A study of the immunoreactivity of caeruloplasmin using a resonant mirror technology

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The aim of this study was to investigate the immunoreactivity of caeruloplasmin from reference material compared with caeruloplasmin from fresh patient serum.

Caeruloplasmin assays can show considerable method dependant variation. Recent work highlights an example of this phenomenon. Fresh UKNEQAS samples gave readings of 0.479 g/L by nephelometry and 0.368 g/L by turbidimetry. When retested 4 months later the same samples gave readings of 0.449 g/L by nephelometry and 0.413 g/L by turbidimetry. This implies that the caeruloplasmin changes its immunoreactivity over time, perhaps related to a change in structure.

To test this theory, resonant mirror technology was used to measure the kinetic parameters of the antibody binding reaction to caeruloplasmin. This was performed

on an IASys (ThermoLabsystems). Four biosensors were produced by immobilizing antibodies from four sources on the reactive surface of carboxymethyl dextran cuvettes. When caeruloplasmin is added to the biosensor, the binding reaction to the antibody is monitored through the interaction of the Ab-Ag complex with an evanescent wave on the reactive surface of the cuvette. The kinetic parameters measured were k_a , k_d and affinity. Two different reference sera representing aged caeruloplasmin and two different patient sera representing fresh caeruloplasmin were analysed by the four different biosensors.

The four different biosensors showed different kinetic characteristics due to the different antibodies used. In all cases, the patient sera showed at least a 10-fold reduction in affinity compared with aged caeruloplasmin for a given biosensor. For all biosensors, the average affinity for reference sera was 2.66×10^{-14} and 1.23×10^{-12} for patient sera ($p < 0.05$).

There was a clear difference in the immunoreactivity of caeruloplasmin from aged and fresh samples to a given antibody. This indicates a change in the epitope expression and hence a change in the protein structure. This would explain why the results for UK NEQAS samples appear different on retest after 4 months.

Availability and reporting of lipid analyses: a national audit

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National guidelines to improve mortality from coronary heart disease have been developed in all parts of the United Kingdom. We assess the current provision of services by Clinical Chemistry laboratories to support their implementation.

Current guidelines were used to develop audit standards. A questionnaire was circulated by ACB regional audit leads to Clinical Chemistry laboratories throughout the UK. Replies were received from 108 laboratories and were assessed against current guidelines. Routine lipid profiles included triglycerides, HDL, LDL and total:HDL ratio in 98%, 85%, 72% and 44% respectively. Only 33% and 27% analysed triglycerides and HDL respectively when asked simply for a cholesterol measurement. 76% stated on the report whether the patient had fasted prior to specimen collection.

An HDL lower reference limit was quoted by 55% of laboratories, and a triglyceride upper limit by 69%. 46% quoted a reference range for total cholesterol, and 42% for LDL. Ranges were age-related in 20%.

45% of laboratory reports referred to the need (for primary prevention) to consider other risk factors; 20% referred explicitly to national guidelines. 9% provided a service to calculate coronary heart disease risk. Secondary prevention treatment thresholds for total cholesterol were quoted by only 18 laboratories (LDL by 17); 5 quoted a threshold for the total:HDL ratio.

50% of laboratories occasionally added extra tests, and 32% added comments. However, 5% appeared to provide no input from senior medical/scientific staff into report validation.

These results indicate scope for improvement in analysis of lipids, especially in the provision of assays and of information to support interpretation and clinical management.

Should anti tissue transglutaminase alone be used for the serological detection of coeliac disease?

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Increasingly anti tissue transglutaminase antibodies are being used to detect coeliac disease. Here we address the question as together they should be used alone or in combination with other serological tests

1364 consecutive samples received in one year from the gastroenterologists for coeliac serology were analysed for IgA and IgG antibodies to gliadin, IgA antibodies to endomysial and IgA antibodies to tissue transglutaminase (TTG). The pathology computer was searched for histology results of small intestinal biopsy.

1180 samples were negative for all antibodies.

Of the 21 samples with negative endomysial and with TTG of above 3, histology was performed on 16. Ten of these showed active CD, 5 showed treated CD and only one was normal. The specificities of the two tests are virtually identical as all samples with a TTG below 4 were negative for anti endomysial antibodies

Of the 36 samples with positive IgA gliadin and negative TTG, biopsies were performed on 23. Sixteen of these were normal and the rest were known to have coeliac disease and were on treatment. Of the 30 samples with positive TTG and negative IgA gliadin, biopsies were performed on 20. Of these 18 had active coeliac disease, 1 had treated coeliac disease and one was normal.

Of the 21 samples with raised IgG gliadin and normal TTG, biopsies were performed on only 7. Four of these were normal and three were known to have coeliac disease and were on treatment. 1) Anti TTG is more sensitive and more specific than either IgA anti gliadin. or anti endomysial. 2) A raised IgG anti gliadin alone did

not detect any additional cases of coeliac disease. 3) Measurement of anti TTG alone is as sensitive for the detection of active coeliac disease as any combination of serological tests and is more specific.

Measurement of serum testosterone by isotope dilution LC tandem mass spectrometry using a Photospray™ ionisation source

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Serum testosterone measurement are usually performed by immunoassay. Commercial non-isotopic immunoassays do not perform well when compared to GCMS target means in EQA exercises due to the non-specificity of the antibodies.

We have developed a LC tandem mass spectrometry method for the measurement of serum testosterone. The equipment was an Agilent 1100 series HPLC system and API 3000 tandem mass spectrometer using Analyst™ software. The Photospray™ ionisation source using toluene as a dopant was used to facilitate ionisation of polar molecules. The mass spectrometer was operated in positive-ion mode detecting the protonated molecular ions of Testosterone and d3-Testosterone in the first quadropole (mz 289.4 and 292.4) and the fragmentation ion (mz 97.1) of both species in the second mass spectrometer. To aliquots of sample, standards or controls (100 µL), 50 µL of internal standard (d3-testosterone) was added and extracted with 1 ml of diethyl ether, then frozen in dry ice and the supernatant decanted and dried under a stream of nitrogen. The residue was dissolved in 300 µL of 50:50 water:methanol. 50 µL aliquots were injected onto a C8 reversed phase HPLC column and eluted with 0.1% formic acid and methanol using a gradient programme. The HPLC conditions separated testosterone from its stereo-isomer epitestosterone and other androgens with the same molecular weight.

Interassay CV (n=10) was 6.5%, 5.8% and 5.2% at dose levels of 3.23, 19.36 and 41.0 nmol/L respectively. A comparison of results (n=75 male and female) obtained by LC TMS and DPC coat-a-count immunoassay shows a regression line of $y = 1.133x + 1.02$ (r=0.99) however when the female data are analysed (n=25) the regression line is $y = 0.66x + 0.128$ indicating that the immunoassay is overestimating testosterone in females.

In conclusion we have developed a simple LC tandem MS method for the quantitation of testosterone in human serum which has sufficient sensitivity to determine testosterone concentrations in the male and female ranges.

An audit of pleural fluid analysis in the Wessex Region: comparison with the British Thoracic Society and Irish ACB guidelines

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A questionnaire on the biochemical analysis of fluids was circulated to all laboratories in the Wessex region in March 2003. Replies were received from 8 of the 11 laboratories.

All laboratories offered total protein to distinguish exudates from transudates, with only one laboratory offering any additional test (albumin). Three of the 8 laboratories quoted the sensitivity and specificity of these tests. Four of the 8 laboratories offered pleural fluid pH measurement. In two of the laboratories this was performed on a gas machine (with 1 of the 2 laboratories specifically validating their method for this application), whilst the other two laboratories were using a pH electrode. In the 2/4 laboratories offering pH measurement the test

was available to answer a specific clinical question. In one this was to support a diagnosis of bacterial infection and in the other lab more specifically in paediatric samples, to ascertain whether a parapneumonic effusion needs to be drained. Pleural fluid glucose was available in 7/8 laboratories. In 2 laboratories this was performed on all pleural fluids, in 4 laboratories glucose was available on request and in 1 laboratory performed as part of the paediatric parapneumonic protocol. Five different regimens were available for distinguishing chyle from pseudo-chyle. These ranged from visual inspection to a battery of tests including centrifugation, cholesterol, triglyceride, osmolality and protein. Finally, 3/8 labs had specific protocols for handling specimens from patients in which tuberculosis was on the list of differential diagnoses.

The results of the questionnaire were compared with guidelines prepared by the British Thoracic Society, the Irish Audit Group and advice obtained from the HSE.

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