

## Posters to be displayed on Thursday

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### A new UK reference range for thyroid function tests on the Abbott Architect analyser

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During transfer of thyroid function tests from the Abbott AxSYM to Abbott Architect analytical platform, good agreement was seen for TSH and free T4 results on the two platforms. However, approximately 14% of patients with normal TSH, free T4 and free T3 results from the AxSYM instrument, gave abnormally high free T3 results on the Architect. This discrepancy was also seen during routine use. This suggested that the upper limit of the reference range may be set too low.

Discussion with Abbott representatives, reveal that the quoted reference ranges supplied to UK users are derived from a USA population, which may not be applicable in the UK. We decided to calculate our own reference ranges for free T3.

After exclusion of patients that may be expected to give abnormal TFT results (hypothyroidism, hyperthyroidism, anti-thyroid drugs, radioactive iodine etc), those with confounding clinical conditions (e.g. pregnancy, auto immune disease, type 1 DM, lithium therapy etc.), those where no clinical details were given with the request and those with an abnormal TSH or free T4 result, 564 sets of data were analysed. Using the statistical package 'Analyse-It' a new free T3 reference range was calculated: 3.25-6.21 pmol/L vs. 2.63-5.70 pmol/L quoted by Abbott. This calculated range confirmed our suspicions that the original range was too high and has been in routine use in this laboratory since December 2003. Reference ranges for TSH (non-parametric) 0.50-3.70 mIU/L vs. 0.35-4.94 mIU/L (quoted) and free T4 (parametric) 9.92-17.96 pmol/L vs. 9.01-19.05 pmol/L (quoted), also showed tighter reference intervals than those previously quoted. Ranges for TSH, free T4 and free T3 are available for males & females for each decade of life from 20 to 60 years.

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### Review of thyroid function testing strategy when investigating subclinical hypothyroidism at the Southern General Hospital, Glasgow

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Currently we use TSH (ref range 0.5-4.0 mU/L) as a first line test with free T4 (11-22 pmol/L) generated automatically when the TSH is either >8.00 or <0.30 mU/L.

Further testing in the ranges 0.30-0.50 or 4.00-8.00 is at the discretion of the reporting biochemist. This strategy introduced after a previous review, aims to reduce unnecessary free T4 testing unless a follow-up TSH remains borderline elevated, at which point an ATPO antibodies may also be requested. All assays are carried out on the Chiron ACS:180 SE.

The aims of this review were to assess the effectiveness of this strategy and the value of additional free T4 and/or ATPO antibody measurements in investigating borderline elevated TSH results.

100 successive GP patients with first-time TSH in the range 4.00-8.00 mU/L were studied. 87% did not have a free T4 requested at this stage. A follow-up TSH was received for 75 of the patients within 3 months of which 36 (48%) had a TSH that remained borderline elevated. Free T4 was added by the duty biochemist to 64% of these patients however only 4% of the free T4 results were below the reference range. ATPO antibodies were added to 44% of the patients of which 37% proved to be antibody positive.

This demonstrates that the majority of patients with a first-time borderline elevated TSH results do not have a free T4 added but receive the appropriate follow up. Free T4 is requested on approximately two thirds of follow-up borderline elevated TSH results but is normal in the vast majority of cases. In contrast, ATPO antibodies were positive in over one third of the patients on whom it was requested. This suggests that when investigating possible developing hypothyroidism, requesting ATPO antibodies may be more helpful than measuring free T4.

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### An audit of TRH tests: is the 60 minute TSH sample necessary?

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Most protocols for the thyrotrophin releasing hormone (TRH) test advocate collection of specimens for thyrotrophin (TSH) analysis at baseline, 20 and 60 minutes. In hypothalamic disease, TSH is reported to be greater at 60 minutes than at 20 minutes.

The aim of this study was to ascertain how often suspected hypothalamic disease is the indication for TRH tests, and how often TSH is greater at 60 minutes than at 20 minutes.

Data from 55 consecutive fully completed TRH tests were reviewed. TSH increments greater than 2 but less than 25 mU/L at 20 minutes were regarded as normal.

Suspected hyperthyroidism and hypothyroidism

accounted for 20 and 12 TRH tests respectively. A further 15 tests were performed to assess pituitary reserve. Other clinical indications included suspected thyroiditis and thyroid hormone resistance. No test was identified in which hypothalamic disease was suspected. In two cases TSH was greater at 60 minutes than at 20 minutes. The first was a case of suspected hypothyroidism in which the increment in TSH at 20 minutes was within normal limits; the second a case of suspected thyroiditis, in which the increment in TSH at 20 minutes was less than 2 mU/L. TSH was the same at 20 and 60 minutes in a further four cases in which the response to TRH confirmed suspected hyperthyroidism, and in one case where it established absent pituitary TSH reserve. In the remaining 48 cases, TSH was less at 60 minutes than at 20 minutes.

Hypothalamic disease is rarely suspected or identified by TRH tests, and measurement of TSH at 60 minutes therefore adds little to the interpretation of the test.

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##### **The use of sweat patch and salivary cortisol in the assessment of adrenal function**

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The adrenal hormone cortisol is often used in tests of adrenal axis function. However, the sampling techniques used can be problematic. Venepuncture is invasive, which may lead to increased stress and falsely high results. 24-hour urine sampling is not invasive, but patient compliance is poor, which compromises the accuracy of the test. This study examined the use of salivary and sweat patch sampling, using a dissociation-enhanced lanthanide fluoroimmunoassay (DELFLIA®) method, in the assessment of adrenal function in a group of normal control subjects and five patients with diagnosed hypercortisolism (Cushing's syndrome). Both methods were compared with the results obtained for 24-hour urinary free cortisol.

The intra- and inter-subject variations in sweat patch and salivary cortisol were comparable with those seen in plasma and urine. A significant correlation with 24-hour urinary free cortisol was found in both sweat patch cortisol and the area under the curve of the salivary cortisol day profile. Using the upper limit of the control data as a cut-off achieved a sensitivity and specificity of 100% for the diagnosis of Cushing's syndrome in sweat. Evening (23.00 h) salivary cortisol measurement afforded the best discrimination between patient and control groups, and gave a sensitivity and specificity of 100% for

the diagnosis of Cushing's syndrome when the upper limit of the control data was used as a cut-off. However, as the number of patients in the present study was small, further work is required with a larger patient group to confirm these findings.

In conclusion, sweat patch and salivary cortisol measurement represent a more convenient, less invasive method of cortisol measurement, compared with conventional techniques, and show promise as potential screening tests for Cushing's syndrome.

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##### **The overnight metyrapone test versus the short synacthen in detecting adrenal suppression in patients on prednisolone therapy**

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The standard synacthen test is commonly used to investigate suppression of the hypothalamo-pituitary-adrenal (HPA) axis resulting from long-term steroid treatment. This test only examines the adrenal response and does not test hypothalamic or pituitary function. The overnight metyrapone test examines the whole HPA axis and therefore may be a better test to investigate HPA insufficiency. We have examined the response to these two tests in 18 patients who had been treated with prednisolone for at least 3 months.

Nine controls aged 20-30 years, 9 controls aged >50 years and 18 patients who had been treated with >20 mg prednisolone for at least 3 months had a short synacthen test (SST) and an overnight metyrapone test (OMT). Baseline blood was taken for cortisol and ACTH measurement. After 250 µg synacthen blood was taken at 0, 30 and 60 min for cortisol measurement. Metyrapone (30 mg/kg) was given at 11 pm at night with blood taken at 9 am the next morning for cortisol, ACTH and 11-deoxycortisol measurement. There was at least one week between the tests.

Mature controls had a significantly lower ( $p=0.001$ ) baseline cortisol than young controls. Patients had a significantly lower mean baseline cortisol, 30 and 60 min cortisol and cortisol increment after a SST ( $p<0.01$ ) and a lower mean ACTH, cortisol, 11-deoxycortisol and total glucocorticoids after OMT ( $p<0.001$ ). There was a 22% discordance between 30 min cortisol and total glucocorticoid concentrations with the SST indicating fewer patients with HPA insufficiency. Dose of prednisolone correlated with the 11-deoxycortisol concentration.

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### Alcoholic presenting with hypercortisolaemia and hypokalaemia: a case presentation

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Alcohol abuse is associated with multi-organ dysfunction. We describe a patient with grossly elevated serum cortisol and a profound hypokalaemia and suggest a mechanism for these observations.

On presentation, a 60 year old divorced male complained of general malaise and lethargy and was apyrexial. On examination he was clinically dehydrated, incontinent of urine and faeces and appeared malnourished. He had faecal impaction and he was disoriented in time and place. Peripheral pulses in his left leg were absent and the limb was cold. Laboratory tests showed raised inflammatory markers (WCC and CRP) and abnormal LFTs. Diabetes was poorly controlled (BMs 15-20 mmol/L). Potassium levels were normal at admission but over one week and six days post thiamine fell to 1.8 mmol/L, with inappropriate urine potassium excretion (250 mmol/day). At this point cortisol was 2200 nmol/L with aldosterone 172 pmol/L (near lower limit) and an ACTH of 31 ng/L. On the advice of laboratory staff spironolactone was started and within 4 days the potassium was 3.3 mmol/L. Levels of urine tetrahydrocortisol were normal but those of tetrahydrocortisone were low suggesting 11 $\beta$ -hydroxysteroid dehydrogenase type 2 (11 $\beta$ -HSD2) deficiency causing an apparent mineralocorticoid excess (AME).

The 11 $\beta$ -HSD2 is a microsomal enzyme of renal origin oxidising cortisol to cortisone. Low activity is associated with severe hypokalaemia, low renin and aldosterone. With reduced activity of 11 $\beta$ -HSD2 the elevated cortisol binds to the renal type I receptor which is nonspecific and does not distinguish between aldosterone and cortisol. Cortisol acts as a mineralocorticoid causing a profound increase in potassium excretion.

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### Macroprolactin(s): composition and reactivity in immunoassays

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Little is known about the relationship between the composition of macroprolactin and its reactivity in immunoassays for prolactin. We studied these in an unusual serum sample which contained two peaks of macroprolactin on Sephacryl® S300 gel chromatography.

Peak 1 contained 62% of the prolactin loaded (Wallac

DELFLIA assay) and was similar to the most common form of macroprolactin, a glycosylated prolactin-IgG complex of molecular mass 150 kDa. Peak 2 eluted in the void volume (molecular mass 2000 kDa), contained 16% of the total loaded and was more highly glycosylated than Peak 1 (Concanavalin A chromatography). Protein A chromatography indicated a smaller immunoglobulin component than in Peak 1. A third peak corresponding to monomeric prolactin contained the remaining 22% of prolactin loaded.

When the same serum was issued through the UK NEQAS for Prolactin, method means were 1960 mU/L (Bayer ACS:180 and Centaur), 1591 mU/L (Roche E170), 770 mU/L (Wallac DELFLIA) and 730 mU/L (DPC Immulite 2000). This unusual pattern of immunoreactivity - most macroprolactins react less strongly in the ACS:180 and Centaur methods than in the Roche E170 - was also observed for Peak 1, but not for Peak 2. We found no differences in composition to explain this unusual pattern.

High molecular mass forms of prolactin are uncommon. In 75 patients with macroprolactinaemia we have studied, the median molecular mass was 144 kDa, and was >200 kDa in only two individuals. There were no examples of multiple high molecular mass forms.

This case highlights the variable behaviour of macroprolactin species and leads to two important practical conclusions: 1) the major macroprolactin component in this sample reacted in all prolactin assays tested, resulting in apparent moderate to marked hyperprolactinaemia; 2) the absence of a substantial difference in results obtained with two immunoassays does not necessarily exclude the presence of macroprolactin.

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### Prolactin, macroprolactin and rheumatoid arthritis

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Macroprolactin contributes to circulating prolactin in patients with rheumatoid arthritis (RA).

The aim of this study was to investigate for macroprolactin in RA.

It has been suggested that prolactin may have a pathogenetic role in RA, since *in vitro* studies have shown prolactin enhances inflammatory responses. Indeed, increased serum prolactin concentrations have been reported in patients with RA, but this is controversial. Prolactin circulates in several different molecular forms including predominantly monomeric prolactin and much lesser and variable amounts of macroprolactin. The binding of prolactin to an IgG antibody forms macroprolactin. Since low biological activity

macroprolactin is less effectively cleared than monomeric prolactin, the total concentration of prolactin increases. RA is associated with increased serum immunoglobulins. We, therefore, considered the possibility that macroprolactin could contribute to the increased serum prolactin reported in RA.

We compared serum prolactin in 60 women with RA (56.7 {12.9} yr, mean {SD}) and 31 female controls (53.1 {16.1}yr) before and after precipitation with PEG 6000 using the ARCHITECT prolactin assay [Abbott Laboratories, Diagnostics Division, Illinois, USA]. Prolactin recovery  $\leq 50\%$  is indicative of predominant macroprolactinaemia.

Before and after PEG precipitation serum prolactin concentrations were higher ( $p \leq 0.05$ ) in women with RA (225.6 {104.6} and 201.6 {95.4} mU/L respectively) compared to controls (175.0 {68.5} and 154.0 {60.9} mU/L respectively). Prolactin recovery was  $> 50\%$  in all women with RA and their controls.

These results confirm increased serum prolactin concentrations in patients with RA and indicate that these are not due to macroprolactin.

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### Prolactin, macroprolactin and infection with human immunodeficiency virus

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Macroprolactin contributes to circulating prolactin in patients with human immunodeficiency virus (HIV) infection.

The aim of this study was to investigate for macroprolactin in HIV +ve patients.

Increased serum prolactin concentrations have been reported in HIV +ve patients but this is controversial. Prolactin circulates in several different molecular forms including predominantly monomeric prolactin and much lesser and variable amounts of macroprolactin. The binding of prolactin to an IgG antibody forms macroprolactin. Since low biological activity macroprolactin is less effectively cleared than monomeric prolactin, the total concentration of prolactin increases. HIV infection is associated with an increased frequency of autoantibodies including anti-prolactin antibodies. We, therefore, considered the possibility that macroprolactin could contribute to the increased serum prolactin observed in HIV infection.

We compared serum prolactin in 32 HIV+ve subjects (14 female; 32.5 {31.3-37.9} yr, median {95% confidence intervals}) with 52 HIV-ve subjects (36 female; 35.5 {34.9-41.4} yr) before and after precipitation with PEG 6000 using the ARCHITECT prolactin assay [Abbott Laboratories, Diagnostics Division, Illinois, USA]. Prolactin recovery  $\leq 50\%$  is indicative of predominant

macroprolactinaemia.

Serum total prolactin concentrations were similar in HIV+ve and HIV-ve patients (167.0 {122.4-313.8} vs 206.5 {187.8-248.4} mU/L respectively). Following PEG precipitation, serum prolactin concentrations were lower in HIV+ve subjects than in HIV-ve subjects (112.0 {91.1-141.8} vs 171.0 {154.5-200.9} mU/L respectively;  $p \leq 0.0005$ ). Prolactin recovery was  $\leq 50\%$  in 6 (18.6%) HIV+ve subjects and 1 (1.9%) HIV-ve subject.

Macroprolactin significantly contributes to circulating prolactin concentrations in HIV+ve subjects. Our results also suggest that the contradictory serum prolactin results reported in the scientific literature could be due to use of different prolactin assays with varying detection of macroprolactin.

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### Antipsychotic-induced hyperprolactinaemia and macroprolactinaemia?

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A 42 year old woman with previously normal menstrual cycles first presented with one year of post pill oligomenorrhoea with no galactorrhoea, headache or visual field disturbance. Serum prolactin was 783 mU/L ( $\leq 480$ ); gonadotrophins and thyroid and renal function were normal. She was lost to follow up.

Two years later, she re-presented via a Psychiatry Outpatients Clinic with amenorrhoea and was taking risperidone, an anti-psychotic drug that blocks dopamine ( $D_2$ ) receptors. Total serum prolactin (Bayer Centaur) was 21257 mU/L, recovery after PEG precipitation 9% (Ref range  $> 60\%$ ) and calculated monomeric prolactin concentration 2584 mU/L. The raised prolactin (Wallac DELFIA, 26400 mU/L) was confirmed and a peak of macroprolactin (150-170 kD) contributing 80% of total prolactin immunoreactivity was demonstrated by gel filtration chromatography. Gonadotrophins were measured to investigate pituitary function and were consistent with perimenopause (LH 40 IU/L, FSH 74 IU/L).

Changing the antipsychotic agent to amisulpiride (another atypical antipsychotic) resulted in total prolactin 38835 mU/L (recovery 10.4%, monomeric prolactin 4829 mU/L). Five weeks after a further change to clozapine, a prolactin-sparing antipsychotic, total prolactin was 7376 mU/L (recovery 9.9%, monomeric prolactin 986 mU/L). Thyroid and renal function tests were normal and gonadotrophins were perimenopausal. Subsequent CT and MRI imaging were normal.

A single amisulpiride dose was administered to study macroprolactin kinetics once her baseline prolactin concentration was normal on clozapine (392 mU/L, recovery 20%). The total and monomeric prolactin

concentrations increased to a peak at 1 hour post-amisulpiride (5219 mU/L, 2573 mU/L respectively) but were still raised at 24 hours (1790 mU/L, 651 mU/L respectively). The recovery post-PEG precipitation initially increased (peak, 41% at 3 hours) due to acute increases in monomeric prolactin secretion before decreasing (26.9 % at 24 h) as the macroprolactin immune complexes formed.

This case of macroprolactinaemia and antipsychotic drug-induced hyperprolactinaemia has provided an interesting insight into the kinetics of macroprolactin formation.

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### Vitamin D: HPLC, manual RIA or automated chemiluminescent immunoassay?

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Automated chemiluminescent immunoassay methods have been developed to measure 25-hydroxyvitamin D (25OHD). These methods are more suitable for the increasing workloads for 25OHD analysis than the labour-intensive HPLC or manual RIA with extraction methods. However, they do not always recognise 25OHD<sub>2</sub> and 25OHD<sub>3</sub> equally. We compared one manual and two automated immunoassay methods for measurement of 25-hydroxyvitamin D with an HPLC method.

Samples with a range of 25OHD concentrations were analysed by four methods: an in-house HPLC method with UV detection following extraction and separation (Manchester) that provided 25OHD<sub>2</sub>, 25OHD<sub>3</sub> and total 25OHD concentrations; a manual RIA following acetonitrile extraction (Diasorin); Nichols Advantage and Diasorin Liaison chemiluminescent immunoassays.

Ninety-two samples were analysed. Four samples were not analysed by the Diasorin RIA. One sample (25OHD 520 nmol/L by HPLC) was excluded from all analyses. The range of 25OHD concentrations in the remaining samples was 0-145 nmol/L by HPLC. Passing and Bablok method comparison showed RIA = 0.553(HPLC) + 2.56; Liaison = 1.24(HPLC) - 3.75 and Advantage = 1.28(HPLC) - 6.94. The RIA method was negatively biased but showed the closest association. The degree of scatter was greater for the Advantage automated method than the Liaison method.

For the RIA method, there was little difference in recovery between 25OHD<sub>2</sub> and 25OHD<sub>3</sub> although overall recovery was low. The Nichols Advantage recognised 25OHD<sub>3</sub> more than 25OHD<sub>2</sub> whereas the Liaison recognised 25OHD<sub>2</sub> more than 25OHD<sub>3</sub>.

The ability of each assay to detect vitamin D insufficiency (25OHD  $\leq$  37.5 nmol/L by HPLC) reflected the strength of the association of the assay with the HPLC method. Areas under the ROC curve were 0.982, 0.968 and 0.904 for the RIA, Liaison and Advantage methods respectively.

The automated immunoassay methods are capable of producing results quickly from a small sample volume but with a loss of diagnostic efficiency.

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### Vitamin D Status and redefining serum parathyroid reference range for intact PTH on DPC Immulite 2000

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Since DPC has changed the formulation of their Intact PTH assay (June 2001), there have been numerous debates concerning changes to the expected reference range. Reference range values have changed from 0.7-7.4 pmol/L to 1-5.3 pmol/L and more recently to 1.2-6.8 pmol/L. 25 hydroxy vitamin D (Vit D) status has not been taken into account when establishing the reference values for the current PTH assays. Vit D insufficiency is very common and remains undetected, in the general population. It induces mild secondary hyperparathyroidism. We have re-determined the reference range for the DPC Intact PTH assay using the Immulite 2000 analyser, excluding subjects with abnormal serum Vit D, corrected calcium, alkaline phosphatase and creatinine. Serum samples from 95 healthy subjects (47 men and 48 women aged 18-60 yrs) were analysed for Intact PTH on Immulite 2000. Vit D was analysed using the Diasorin kit and calcium, albumin, alkaline phosphatase and creatinine were measured on Olympus AU 640 analyser. Serum PTH was negatively correlated with Vit D ( $r = -0.27$ ;  $p < 0.007$ ). No correlation was found between PTH and age, corrected calcium or alkaline phosphatase. We found a significant difference in reference range between male and female subjects. The overall reference range for the (95% confidence interval) for PTH was found to be 0.8-5.3 pmol/L. For female subjects the range was 0.8-5.0 pmol/L and for male, 1.1-5.8 pmol/L. Vit D in these subjects ranged from 25-103 nmol/L. No subject was found to have Vit D insufficiency or toxicosis. Thus the PTH reference value obtained is a true reference interval.

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

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#### Oral Presentation

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.

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### **Is the relative change in IGF-1 and IGFBP-1 throughout pregnancy a better indicator of birthweight than a single measurement at 36 weeks gestation?**

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Insulin-like growth factors are important regulators of glucose and protein metabolism. In pregnancy low insulin-like growth factor 1 (IGF-1) concentrations have been linked to low birthweight while insulin-like growth factor binding protein 1 (IGFBP-1) negatively correlates with birthweight. We have compared maternal IGF-1 and IGFBP-1 at preconception, 12, 24 and 36 weeks to

see if the changes in these values correlates better to birthweight.

Serum samples were taken prior to conception and then at 12, 24 and 36 weeks of gestation in 41 healthy women with uncomplicated pregnancies. We measured IGF-1 using an automated chemiluminescent method. IGFBP-1 was analysed using a commercial enzyme-linked immunosorbent assay (ELISA).

IGF-1 at 36 weeks showed a significant positive correlation ( $p < 0.05$ ) with fetal birthweight, however when comparing the change from 12 to 36 weeks and 24 to 36 weeks this showed greater significance ( $p < 0.01$ ). IGFBP-1 at 36 weeks also showed a significant negative correlation ( $p < 0.05$ ). When the changes between preconception and 36 weeks and between 12 weeks and 36 weeks was measured, the significance was even greater ( $p < 0.01$ ).

The change in IGF-1 or IGFBP-1 at 36 weeks shows a greater correlation with fetal birthweight than the single measurement at this time.

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### **A review of clinical presentation and biochemical investigations in four paediatric cases of adrenal hypoplasia congenita**

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Adrenal hypoplasia congenita (AHC) is a rare inherited developmental disorder associated with hypofunction of the adult adrenal cortex. A retrospective study of four cases of AHC has been used to compare clinical presentation and biochemical investigations.

Congenital adrenal hyperplasia (CAH) has a well described clinical presentation and clear guidelines exist for its biochemical investigation. In contrast, our experience with AHC has shown a plethora of more complex clinical features in addition to the expected salt wasting. In addition there are no definitive guidelines on the sequence of investigations for those cases of salt wasting where a normal  $17\alpha\text{OHP}$  level has excluded CAH.

Comparison of the initial key biochemical investigations in our cases showed that all patients presented with hyponatraemia; hyperkalaemia was only documented in three cases. Low basal cortisol levels, or failure of the adrenal to secrete cortisol in response to synacthen was also documented in all cases.  $17\alpha\text{OHP}$ , renin and aldosterone levels were only investigated in three cases.  $17\alpha\text{OHP}$  levels were found to be low in two cases and normal in one case. Aldosterone levels were low in two cases and normal in one case, with renin levels being correspondingly high in two cases and normal in one case. Hypoglycaemia was documented in two cases.

The four cases serve to illustrate the spectrum of clinical features and biochemical changes which may exist in patients requiring investigation for AHC. A protocol for biochemical investigation and test prioritisation in cases of suspected AHC will be proposed.

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### The effects of metformin on adrenal steroids in polycystic ovary syndrome

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Metformin is effective in achieving ovulation in women with polycystic ovary syndrome (PCOS). The mechanism is unknown but changes in steroidogenesis are anticipated. Adrenal function has been evaluated in order to calculate androgen and cortisol outputs in 8 lean and 11 obese PCOS subjects basally, and after 3 and 6 months treatment with metformin. PCOS patients were characterised clinically, by ovarian ultrasound and endocrine tests. Eleven controls were age-matched. Tetrahydrocortisone (THE), tetrahydrocortisol (THF) allo-THE,  $\alpha$ -cortol and  $\alpha$ -cortolone excretion rates were determined by gas chromatography (GC).  $\beta$ -cortol and  $\beta$ -cortolone were determined separately for the first time by GC mass spectrometry with selected ion monitoring. Sum of all cortisol metabolites/sum of all cortisone metabolites was then used to assess 11 $\beta$ HSD (hydroxysteroid dehydrogenase) type 1 activity, and androstosterone/aetiocholanolone for 5 $\alpha$ -reductase activity.

All results are mean  $\mu$ g/24h and range. Combined androgen metabolites (androsterone + aetiocholanolone) were elevated in PCOS basally (controls 1560, 200-7300; lean PCOS 2830, 1060-5550; obese PCOS 2280, 360-7250). The total cortisol metabolites (TCM) were also elevated basally in lean (10660, 7880-14180) ( $p < 0.09$ ) and obese (13200, 4290-30170) ( $p < 0.04$ ) subjects compared with controls (7090, 1490-14840). The increase in cortisol production was accounted by enhanced activity of 5 $\alpha$ -, 20 $\alpha$ - and 20 $\beta$ -reductases and inhibition of 11 $\beta$ HSD1. In lean PCOS androgens increased with 6 months metformin administration (4650, 2430-9950). In obese PCOS the androgen metabolites were not elevated by metformin after 3 (2570, 1788-4640) or 6 months treatment (2390, 1100-4690). After 6 months treatment the TCM were further elevated in lean (15800, 9930-23880) and obese PCOS (16450, 5150-42320). Neither 11 $\beta$ HSD1 or 5 $\alpha$ -reductase activities were significantly altered with the administration of metformin over a period of 6 months.

In conclusion, hyperadrenalism was documented in a small subset of PCOS subjects due to derangement of cortisol catabolism. Several catabolic enzymes were

affected favouring a metabolic rather than or in addition to a genetic basis. Metformin was found to enhance the hyperadrenalism demonstrated basally and these findings warrant further investigations.

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### C-reactive protein relates to age and BMI in patients with polycystic ovary syndrome but not to insulin sensitivity

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Polycystic ovary syndrome (PCOS) is associated with increased biochemical risk markers for cardiovascular disease, particularly low HDL cholesterol and increased triglycerides. There is accelerated progression to type 2 diabetes and hypertension in middle age. These biochemical abnormalities associate with insulin resistance which is present to a greater extent than can be explained by obesity alone. Notwithstanding, hard endpoint data in terms of increased cardiovascular mortality remains elusive. Inflammatory processes are now recognised as being important in atherogenesis and the inflammatory marker C-reactive protein (CRP) has been shown to be a powerful predictor of cardiovascular morbidity and mortality, independent of conventional risk factors, in several studies. We defined PCOS as hyperandrogenaemia associated with menstrual disturbance, hirsutism and infertility in any combination. To further investigate cardiovascular risk in PCOS we studied serum CRP in patients and controls and its relationship to age, body mass index and insulin sensitivity.

CRP was measured by high sensitivity ELISA and insulin sensitivity (HOMA-S) was derived from fasting glucose and insulin pairs. Serum CRP in patients ( $n=37$ ) was 1.59 mg/L (interquartile range 0.53-3.38) and not significantly different to controls ( $n=21$ ) 1.27 mg/L (interquartile range 0.32-2.25). In the group as a whole CRP was significantly correlated with BMI ( $r_s=0.70$ ;  $p < 0.001$ ) and insulin sensitivity ( $r_s = -0.38$ ;  $p = 0.004$ ) but not with age ( $r_s=0.197$ ;  $p=0.077$ ). In a multiple regression CRP was correlated with BMI ( $p < 0.001$ ) but not with insulin sensitivity ( $p=0.448$ ) or age ( $p=0.075$ ). In controls, CRP was significantly correlated with BMI ( $r_s=0.52$ ;  $p=0.014$ ) but not with age or insulin sensitivity. In PCOS, CRP was significantly correlated with BMI ( $r_s=0.72$ ;  $p < 0.001$ ), insulin sensitivity ( $r_s=-0.38$ ;  $p=0.022$ ) and age ( $r_s=0.489$ ;  $p=0.005$ ). In a multiple regression, CRP was correlated with BMI ( $p < 0.001$ ) and age ( $p=0.005$ ) but not with insulin sensitivity ( $p=0.157$ ) in PCOS patients. These data suggest that the increased cardiovascular risk observed in PCOS may not be further exacerbated by accelerated inflammatory processes.

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### **Statistical investigation of interference in testosterone measurement in women**

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**Aim:** To investigate the degree of interference in testosterone measurement in women and ascertain if its distribution could provide information about the source of interference.

Direct testosterone assays can lead to spuriously high measurements in women. The presumed interference is reduced when testosterone is extracted into an organic phase before measurement is carried out. To avoid misinterpretation, we check all samples from women with a testosterone concentration >3.0 nmol/L on direct assay (Abbott Architect) by an extraction method.

Women who had a blood sample referred to Hope Hospital Clinical Biochemistry laboratory for measurement of serum testosterone concentration were included in the study. This included women who had been referred to a tertiary endocrine clinic with problems thought to be related to hyperandrogenaemia.

From a retrospective 8 month period, all samples which had required extraction were entered onto a database. Subtraction of the extraction result from the direct result provided the degree of interference. The frequency distribution of the degree of interference was plotted and analysed.

1271 results were obtained for analysis. This represented approximately 20% of women in whom testosterone was measured. The main finding was that the distribution of interference was unimodal. The median degree of interference was 1.5 nmol/L, (range 1.2 to 33.7).

Interference in testosterone measurement in women is a significant clinical problem. It can lead to misdiagnosis of androgen excess and inappropriate further investigations. The lack of a bimodal distribution suggests that it is unlikely that the absence or presence of a particular drug or clinical condition is the cause of the interference.

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### **Prospective clinical investigation of possible causes of interference in testosterone measurement in women**

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Our aim was to quantify the degree of interference in testosterone measurement in women and ascertain if any association with clinical diagnosis or medication history could be found, which might provide information about the source of interference.

Direct testosterone assays can lead to spuriously high measurements in women. The presumed interference is

reduced when testosterone is extracted into an organic phase before measurement is carried out. To avoid misinterpretation, we check all samples from women with a testosterone concentration >3.0 nmol/L on direct assay (Abbott Architect) by an extraction method.

Women who had a blood sample referred to Hope Hospital Clinical Biochemistry laboratory for measurement of serum testosterone concentration were included in the study. Consecutive testosterone results over a period of 6 weeks were used to recruit subjects into the study. A difference (direct minus extracted testosterone) of less than 1.0 nmol/L was used to define a control group, and a difference of 2.5 nmol/L or more was used to define a group with interference. Data on clinical diagnosis, symptoms, and medication were obtained by case-note review. Comparison between groups was made using chi-squared and Fisher's exact tests.

131 patients were recruited, in 83 of whom complete data were found: 29 in the interference group and 54 in the control group. Comparison of prevalence of diagnoses (including polycystic ovary disease, idiopathic hirsutism), symptoms (including hirsutism, acne, weight gain), or medication (including glucocorticoid treatment, oestrogens, metformin) revealed no statistically significant differences between the two groups.

No diagnostic, symptomatic, or medication variable was able to discriminate between the interference and the control group. This increases the likelihood that the interference is caused by an endogenous, inactive compound, such as a steroid conjugate.

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### **Evidence-based improvement in the use of androgen assays in men**

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The numbers of requests submitted for androgen status investigation in males has increased over recent years. There has also been considerable debate among health care professionals regarding appropriate investigation of male androgen status. The aim of this study was to investigate the current requesting of testosterone and related assays in men, and then undertake definition of both the optimum approach to investigation of androgen status in the hypogonadal male and the role of biochemical measurements in monitoring hypogonadal men undergoing testosterone replacement.

Three investigative strategies were pursued. Firstly, a retrospective review of over 500 sequential requests that included testosterone was performed. Androgen status investigation was approached very differently by urologists, oncologists, endocrinologists and general practitioners. Secondly, a prospective investigation of male

patients with requests for testosterone measurement was undertaken. Total testosterone (TT), SHBG, albumin, free androgen index (FAI) and free testosterone index (FTI) were determined in 493 patients. Relationships between TT and FAI and TT and FTI were shown to be similar: however, TT correlated better with FTI ( $r=0.87$ ) than FAI ( $r=0.48$ ). FTI was seen to be particularly useful in patients with a low TT but of significantly less value if TT was  $>15$  nmol/L. Then,  $5\alpha$ -dihydrotestosterone ( $5\alpha$ -DHT) concentrations were measured in 200 of these patients. Most patients with low TT had  $5\alpha$ -DHT within the reference interval.  $5\alpha$ -DHT and TT in patients with secondary hypogonadism and age-related hypoandrogenism differed significantly.

Currently, FAI is used routinely in Tayside, but the evidence generated showed that FTI may be a more reliable index and is being introduced to clinical practice. In addition,  $5\alpha$ -DHT may add significant information to investigation of androgen status although analytically challenging to perform routinely.

### 31

#### Extracted testosterone: has anything changed in the last 20 years?

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Testosterone in samples from female subjects should, ideally, be measured after extraction with diethyl ether in order to remove interfering substances. This used to be standard practice in many laboratories when they used manual radioimmunoassays. With the advent of automated analysers, most laboratories have reverted to using direct testosterone assays because the extraction step is time-consuming. At Hull Royal Infirmary we measure testosterone using the Abbott Architect analyser, with an upper limit of the reference interval of 4.1 nmol/L, which is considerably higher than most other direct methods.

The aims of this study were to validate an upper limit for the reference interval for extracted testosterone (based on a method used at Hope Hospital, Manchester {courtesy Mr J Kane}, with testosterone measured on the Architect), compare the results with the direct method and examine the clinical outcomes for each of the patients.

Two groups of patients were studied. Group 1: female patients with direct testosterone  $>4.1$  nmol/L ( $n=42$ ). Group 2: age-matched controls ( $n=22$ ) with direct testosterone  $<4.1$  nmol/L ( $n=22$ )

All samples were re-analysed following extraction and the patient notes were examined to determine the clinical outcome.

All control samples gave an extracted testosterone result  $<2.0$  nmol/L, the limited data supporting the use of this value as the upper limit of the reference interval. Of the 42 patients with a raised direct testosterone result, 26 were classified as having ovarian/adrenal disease (23 polycystic ovarian disease, 1 adrenal source, 1 case of ovarian hyperthecosis and 1 ovarian tumour). 21 of these 26 patients had a raised extracted testosterone result. The remaining 16 patients were classified as not having ovarian/adrenal disease and 12 of these patients had an extracted testosterone  $<2.0$  nmol/L.

We reiterate that extraction of female samples with a raised direct testosterone improves the clinical utility of the test.

### 32

#### Development of a method for the measurement of testosterone in plasma by LC/MS/MS

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Close liaison with the Clinical Applications Group at Waters has resulted in the development of a simple, rapid, low cost method for measuring testosterone in plasma. This technique has now been established in a routine clinical biochemistry laboratory using an Alliance HPLC system and a Micromass Quattro micro API Mass Spectrometer. The method involves the addition of zinc sulphate in methanol to plasma/serum samples or calibrators. Deuterated testosterone is also added as internal standard. After thorough mixing and centrifugation, an aliquot of the supernatant is injected onto a 2.5  $\mu$ m C18 column and eluted using a methanol-water gradient. Retention time for testosterone is 2.95 minutes and a single sample is completed within 5 minutes. Assay calibrators are prepared in charcoal-stripped plasma with a range of testosterone concentrations from 0.25 to 50 nmol/L although the line is linear to at least 100 nmol/L. The software supplied allows assessment of results as they are generated or as a batch at the end of the run. Applying this technique, intra- and inter-assay imprecision at 1 nmol/L and above is  $<10\%$  but at 0.4 nmol/L is about 24%. NEQAS recovery pools gave values ranging from 108 to 96% for female samples and 101-88% for male samples. Estimations of GCMS targetted pools at 0.7361, 1.225 and 2.720 nmo/L gave 94, 81 and 87% respectively of target values. Comparison of results for clinical samples with our in-house extraction RIA gave a linear regression of  $y=0.9954x - 0.5477$  with a correlation of 0.99.

## 33

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

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**Oral Presentation**

Serum testosterone measurements are usually performed by immunoassay. Commercial non-isotopic immuno-assays 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.

## 34

**Unusual urinary steroid profiles in morbidly obese males: preliminary observations**

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Experience shows that urinary steroid secretion is positively correlated with body mass. This may be due to

enhanced ECF volume and/or increased metabolism in adipose tissue. In a previous study we showed that ratios of cortisone over cortisol metabolites were higher in obese female subjects and this would predict an enhanced cortisol metabolic clearance rate.

The aim of this study was to examine the effect of increased adiposity on the urinary steroid profiles of 3 male patients of a similar age (41, 42 and 44 years) with documented morbid obesity (BMI 54.2 to greater than 60).

Twenty-four hour urinary steroid metabolite excretion was quantified using gas-chromatography.

The results for total cortisol metabolite concentrations were 250±40, 21768 and 5038 ug/24h. The normal male adult value (mean ±SD) is 7618±2761.

For androgen metabolites (androsterone and aetiocholanolone), results were 2170, 2460 and 694 µg/24 h with a normal value of 2834±962. The latter value was for a patient with known primary gonadal failure.

Ratios of steroid metabolites were normal. Therefore, whilst two patients showed very high levels of total cortisol metabolites as expected, the third patient showed no increase.

Also, levels of androgen metabolites (androsterone and aetiocholanolone) were found to be unexpectedly normal. This is in contrast to obese females who have significantly raised levels of these metabolites.

These preliminary findings suggest interesting trends in the urinary steroid profiles of the morbidly obese male and questions the influence of adipose tissue on steroid metabolism. Considerably more work needs to be done to establish these results.

## 35

**Evaluating a method for measuring bioavailable oestradiol**

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Oestradiol (E2) is the most potent oestrogenic hormone secreted by the mammalian ovary and is one of 20 oestrogens that have been identified. E2 circulates in the bloodstream in one of three forms: bound to SHBG; bound to albumin; and unbound/free. The albumin bound plus the free fraction are defined as bioavailable E2 (bioE2). Elevated or reduced concentrations of bioE2 have been found to be associated with several disease states when there is no such association with total hormone concentrations. Accurate methods for measuring bioE2 are therefore required, and these need validating.

BioE2 was measured by a salt precipitation technique with <sup>3</sup>H-E2 as a tracer. Replicate analysis was used to assess inter-assay precision, drift and response to interference. Interassay precision was found to be 4.1% and

3.1% at 70 and 270 pmol/L respectively. No change in concentration (drift) was detected throughout the assay. No significant change in concentrations of bioE2 was found in response to potential interfering hormones (oestriol, oestrone, ethinylestradiol, oestrone-3-sulphate and testosterone) within physiological ranges. Precision was affected when increasing amounts of triglycerides were added to the samples. Increasing the albumin concentration for a constant amount of E2 and SHBG caused an increase in bioE2. Increasing the E2 concentration for a constant amount of albumin and SHBG caused an increase in bio E2. Samples spiked with SHBG showed decreasing concentrations of bioE2 with increasing concentrations of SHBG. These findings indicate that the assay measures bioE2 as defined above.

### 36

#### Evaluation of a radioimmunoassay kit for determination of plasma metadrenaline and normetadrenaline

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Phaeochromocytoma is a rare but treatable cause of secondary hypertension. Current biochemical screening tests mainly rely on assaying various combinations of catecholamines and or their metabolites in 24-hour urine collections. These have variable diagnostic characteristics due the different methodologies and reference ranges used. Increasingly measurement of plasma metadrenalines has been proposed as the preferred test for screening patients due to its high sensitivity. Clearly this approach avoids the inherent problems of timed urine collections.

The aim of this study was to evaluate a plasma metadrenalines radioimmunoassay kit (Labor Diagnostika Nord, Quidel Diagnostics, Oxford) for use in our laboratory. Patients were seated for 15 minutes, and blood samples collected into lithium-heparin tubes, immediately centrifuged and plasma frozen until use. The assay procedure employed a protein precipitation step followed by acylation of the supernatant and then a competitive radioimmunoassay with <sup>125</sup>I-labelled antigen.

Intra-assay CV (n=18) for plasma metadrenalines (M) was 22.2% at 27.2 pg/mL and 10.1% at 736.9 pg/mL and for plasma normetadrenalines (NM) 12.8% at 33.2 pg/mL and 14.9% at 1424.4 pg/mL. Inter-assay CV (n=5) for M was 26.3% at 43.2 pg/mL, 27.4% at 539.3 pg/mL and for NM 19.8% at 41.4 pg/mL and 11.9% at 1200.1 pg/mL. The assay was linear for M in the range 61.4-1875.8 pg/mL (r=0.998) and for NM in the range 78.1-6557.3 pg/mL (r=0.999). Spiking normal samples gave mean recovery values of 105.0% for M and 101.6%

for NM. The detection limit for M was 9.9 pg/ml and for NM was 14.1 pg/mL. The range of values for 12 normotensive volunteers (aged 24-57 years) was 12.8-56.6 pg/mL (M) and 20.3-72.2 pg/mL (NM).

In conclusion this is a relatively simple method for assaying plasma metadrenalines. However it needs to be further evaluated in phaeochromocytoma patients in direct comparison with more established biochemical approaches.

### 37

#### Regulation of secreted plasminogen activator activity and expression in human thyroid cells

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Plasminogen activators (PAs) have a central role in a wide range of biological processes involving extracellular proteolysis, in particular in tumour invasion and metastasis. They are serine proteases of tryptic specificity that liberate growth factors sequestered within the extracellular matrix, activate proteases e.g. MMPs, and are involved in the synthesis of the endothelial cell growth inhibitor, angiostatin. We have investigated the expression and activity of urokinase-type PA (uPA) and tissue-type PA (tPA) in primary cultures of human thyroid cells.

Cells were maintained in serum-free media, and function was maintained by the addition of TSH (300 mU/L) and insulin (0.1 mg/L). Subsequently, function was measured as the uptake of <sup>125</sup>I and PA activity was measured using a colorimetric assay with S2251 as a substrate. PA secretion was maintained for up to 17 days in culture with differential effects observed in the presence of growth factors and PKC activators. In particular, exogenous insulin and TSH had inhibitory effects on PA secretion (647 U/mL ± 36.5 and 439.8 U/mL ± 22.6, respectively; n=3) and this was inversely related to thyroid function. These inhibitory effects were further evident upon comparison with PA secretion by control samples (720.9 U/mL ± 26; n=3). In contrast, epidermal growth factor stimulated PA activity (1196.8 U/mL ± 26.7; n=3), while inhibiting uptake of <sup>125</sup>I. Activation of protein kinase C by TPA was also a potent inhibitor of <sup>125</sup>I uptake and stimulator of secretion of PA activity (1135 U/ml ± 17; n=3). Western blots indicated that uPA activity correlated closely with its expression. tPA was expressed, however its regulation by TSH and growth factors was less marked than that of uPA.

In conclusion, both uPA and tPA are secreted by human thyroid cells and their expression/activity correlates inversely with thyroid function. Inhibition of PA activity with aprotinin and inhibition of expression by TSH is correlated with increased thyroid function indicating that PA substrates may regulate iodide uptake by thyroid cells. PAs may thus have autocrine effects.

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### Assessing an in-house radioimmunoassay method for peptide YY (PYY)

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PYY is a gut hormone that physiologically inhibits appetite. Currently only radioimmunoassay (RIA) techniques are available for its estimation. The aim of the study was to evaluate our in-house assay for precision, detection limit, interference of haemolysis, suitability of serum versus plasma, freeze-thaw cycle, storing samples at 4 Celsius and biological variation within one subject.

All samples were obtained from one subject on two occasions following an over night fast and the consumption of 1000 and 3000 kcal meals on the respective days. Plasma was aliquoted in multiple samples. Coefficient of variation (CV) was measured at three concentrations (13.4, 16.4, 31.7 pmol/L). The influence of haemolysis was evaluated by diluting severely haemolysed blood with non haemolysed blood obtained at the same venepuncture. All plasma samples were obtained in lithium heparin tubes with added trasylol. Serum samples were obtained in a tube with trasylol and a clot activator. Carry over and the detection limit of the assay was also evaluated.

Fasting samples obtained from the same individual on two occasions were comparable. The CV at 13.4, 16.4, 31.7pmol/L was 19.7%, 13.4% and 12.5% respectively. Severe haemolysis affected the measurement, but slightly haemolysed plasma gave results similar to non haemolysed samples. Freeze-thaw cycles and storing samples at 4 Celsius for 12 h did not significantly alter measurements. Plasma samples were superior to serum samples. There was no evidence of significant carry over and the detection limit of this assay was 5 pmol/L. Conclusions: Plasma is the preferred source for sample for this assay which has a detection limit of 5 pmol/L.

Only severe haemolysis should be avoided. Carry over, freeze-thaw cycles and storing at 4 Celsius do not seem to be a problem and the CV of the assay is acceptable within the physiological range of PYY.

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### How hands can play havoc with your hormones

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The Department of Biochemistry at Morriston carries out over 250,000 tests on a twin E-170 (Roche Modular Analyser). During January 2003 significant internal QC failures were observed in the oestradiol assay. These continued on a regular basis, new reagents were loaded, repeat calibrations were performed but the problem still

persisted. Internal QC performance was acceptable on days 9-13. A sample of the reconstituted QC material, together with a lyophilized bottle set, were forwarded to Roche UK for analysis. Elevated values were found in the reconstituted bottles, but the lyophilized material made up at Roche UK and analysed over a 7 day period performed within acceptable QC limits. No other assay during this period was affected. Roche concluded that the contamination was probably due to dirty pipettes/water. On day 14 the internal QC failure returned once again. On further investigation it was discovered that the MLSO working on the E-170 section during this period was using a HRT preparation, which involved applying a gel formulation to her hands. A series of small experiments were undertaken to try and prove whether this was the source of the QC contamination. These experiments subsequently confirmed that the oestradiol in the QC material was being erroneously elevated via a tip contaminated with oestradiol from the MLSO's hands. Further implications arose as the Department has a Roche Modular Pre-analytic System that is linked to the twin E-170 System. Routine daily maintenance involves the manual replacement of disposable tips. The MLSO concerned does not carry out this form of maintenance as contamination could occur at this point in the process.

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### Reproducibility of serum hCG measurements in early pregnancy using the Bayer Centaur total hCG method

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Interpretation of changes in serum hCG concentrations requires a knowledge of the analytical imprecision of the method used at the concentrations measured. As our internal quality control materials only covered the range 0-180 IU/L, we decided to study the reproducibility of our total hCG measurements over a four week period using clinical specimens with hCG concentrations up to 20000 IU/L.

Thirty-three serum specimens collected during early pregnancy were stored for up to 4 weeks at -20°C and +4°C. Total hCG was measured at weekly intervals on the Bayer Advia Centaur immunoassay analyser. The significance of the changes observed was assessed using the Wilcoxon Signed Ranks Test.

The reproducibility of hCG results below 1000 IU/L was poor (mean CV for refrigerated specimens 17.4%, for frozen aliquots 9.3%, n=18) but apparent total hCG concentrations increased by up to 68% (mean 52.5%) during storage at +4°C. Eight specimens also showed significant changes during storage at -20°C. However,

day-to-day precision with QC materials and within-day precision for clinical specimens were excellent (all CVs  $\leq 5\%$ ) and no significant changes were observed for specimens with initial hCG concentrations above 2000 IU/L.

In specimens with an initial hCG concentration below approximately 1000 IU/L, hCG results increase during storage when the current Bayer Centaur total hCG assay is used. Further experiments confirmed that significant changes can occur within the first two days. We think the problem may be due to a change in the conformation of a species of hCG (possibly hyperglycosylated hCG) which forms a greater proportion of total hCG in early pregnancy. If the Centaur Total hCG method is used for early pregnancy monitoring, specimens should ideally be analysed on the day of collection. These findings may also explain recent poor performance of the method in the UK National External Quality Assessment Scheme.

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### Comparison of positive macroprolactin samples in Bayer Immuno 1 and Centaur assays

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Samples which had tested positive (29) or equivocal (1) for macroprolactin following PEG precipitation using Immuno 1 were selected for study. A further aliquot was analysed on the Centaur analyser following PEG precipitation. The protocols adopted followed the guidelines issued by Bayer for both analysers.

All except 2 of the samples had prolactin concentrations of  $>700$  mU/L by Immuno 1 - the threshold used for assessing macroprolactin status in patients with confirmed hyperprolactinaemia.

Prolactin results using the Centaur assay were significantly lower in most cases. Indeed, the measured prolactin in 15 of the 30 samples was below the threshold (700 mU/L) which would have initiated a macroprolactin request. This included one sample with a measured prolactin of 5428 mU/L by Immuno 1 and 386 mU/L by Centaur. All except 1 of the samples (including the equivocal one by Immuno 1) had recoveries of below 60% post-PEG precipitation and therefore were in the range where macroprolactin could contribute significantly to the measured prolactin.

Monomeric prolactin results calculated by both methods were in good agreement.

(Linear regression: Centaur =  $42.8 + 0.999$  Immuno 1,  $n=30$ ).

#### 42

### Measurement of intact PTH as a marker for vitamin D deficiency

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Osteomalacia due to vitamin D deficiency (VDD) may be identified by measuring 25-hydroxycholecalciferol (25-OHD). As patients with this condition can develop secondary hyperparathyroidism, measurement of parathyroid hormone (PTH) is a suggested alternative. The 1,293 samples analysed for PTH and 25-OHD between January 2001 and March 2002 were studied retrospectively. Only the earliest sample was included where multiple samples were received from the same patient, and samples from paediatric, renal and gastrointestinal units were excluded, leaving 1,106 samples for study. Patients were classified according to Asian or European ethnicity and gender, and results outside the laboratory reference ranges for 25-OHD (10-45  $\mu\text{g/L}$ ) and PTH (10-54 ng/L) were used to determine VDD and hyperparathyroidism. No significant differences ( $p>0.05$ ) in mean 25-OHD or PTH levels between the genders were found in either ethnic group. There were significant differences between the ethnic groups irrespective of gender. The overall sensitivity and specificity of raised PTH as a marker for VDD was found to be 70% and 60% respectively. This study confirms previous findings that measurement of PTH alone is neither a sensitive nor specific marker for VDD.

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### The relative effects of renal function, age, PTH and vitamin D on bone turnover in elderly hospital attendees

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Almost 75% of community dwelling elderly people over 70 years will have a degree of renal impairment. In this study we examined the relative effects of renal function (RF), age, parathyroid hormone (PTH), 25-hydroxyvitamin D (25OHD), and creatinine clearance (CrCl) on bone turnover in an elderly group of hospital attendees.

153 patients referred from the Care of the Elderly Service for DEXA bone scanning were recruited. All patients had calculated CrCl (Cockcroft Gault), serum CTx (a marker of bone resorption), serum osteocalcin (OC) (a marker of bone formation), PTH, and 25OHD assessed. The relationship between bone markers, RF, PTH, and 25OHD was examined in the group as a whole and also in subgroups of patients with CrCl  $<60$  mL/min (moderate renal failure) and  $<30$  mL/min (severe renal failure).

36 patients were male and 117 female. Mean ( $\pm$ SE) overall age was 79.5 (0.7) years. Mean ( $\pm$ SE) CTx, OC, PTH, 25OHD concentrations were 0.502 (0.03) ng/mL, 28.6 (1.72)  $\mu$ g/L, 44.7 (2.4) pg/mL, 24.4 (1.3) ng/mL. Mean ( $\pm$ SE) CrCl was 46.4 (1.6) mL/min. Overall, CTx and OC were positively correlated with age ( $p < 0.005$  and  $p < 0.003$  respectively) and negatively correlated with 25OHD ( $p < 0.0001$  and  $p < 0.002$  respectively). In the whole group CrCl correlated negatively with CTx ( $p < 0.0002$ ) but this correlation was not evident when CrCl was  $> 60$  mL/min. Similarly, CrCl correlated negatively with OC overall ( $p < 0.0001$ ) but this correlation no longer held when CrCl was  $> 30$  mL/min.

This pilot study suggests that moderate to severe renal impairment may increase bone marker concentrations and, given the prevalence of renal impairment in the elderly, CrCl should be estimated to avoid misinterpretation of biochemical bone turnover.

#### 44 Within-subject variation of CTx in mailed urine samples

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In order to assess its suitability for monitoring the therapy of women with osteoporosis, we have estimated the within-subject variability of the urinary bone resorption marker CTx-I (cross-linked C-terminal telopeptide of type I collagen) by measuring it in successive samples of urine sent through the post by women (30 so far) attending our out-patients clinic for metabolic bone disease, before beginning any treatment. None of those included here were known to have recently undergone a change in therapy or medical history which might affect their bone turnover. Many had osteoporosis or other mild metabolic bone disease, but at this time their diagnosis is not complete. Evident cases of Pagets Disease of bone were excluded. Patients were asked to collect 2 second morning void samples, separated by at least 2 weeks, and post them to our laboratory the same day. Mean age of the women was 62.5yr (SD=11.6). CTx-I was measured using the urine  $\beta$ Crosslaps<sup>®</sup> ELISA kits from Nordic Bioscience, kindly donated by IDS Ltd, Boldon, Tyne and Wear, UK and expressed as a ratio of creatinine to adjust for variation in urine dilution. Analytical coefficients of variation for CTx-I were 8.6% within-run for duplicates and 9.7% between-run. Mean CTx-I was 318  $\mu$ g/mmol creatinine (SD=193) for the first sample and 326 (SD=216) for the second. Mean interval between samples was 22.9 days (SD=10.4). Mean within-subject coefficient of variation (total) for CTx-I was 38% (SD=29) which gives a target for the least significant

change (LSC)(one-tailed) of -89%. This compares with our earlier findings of a LSC of -34.5% for NTx-I (after log transformation) and -34.3% for the free deoxypyridinoline collagen cross-link (DPD).

#### 45 Serum CTx-I in men, variability, healthy reference range and a comparison with untreated vertebral osteoporosis

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Clinical interpretation of biochemical markers of bone turnover is often difficult and both biological and analytical variation add to these uncertainties. Our previous findings, using urinary collagen crosslinks, suggested that bone breakdown may not be increased in male osteoporosis. Here we report: (i) the within-subject variation ( $CV_T$ ) in serum CTx-I, a product of cathepsin K-mediated cross-linked type I collagen breakdown, in healthy men; (ii) a healthy male reference range for sCTx-I, and (iii) a comparison of sCTx-I in healthy men with patients with untreated idiopathic vertebral osteoporosis defined by the presence of vertebral fractures.

CTx-I was measured using the  $\beta$ Crosslaps<sup>®</sup> ELISA (Nordic Bioscience, kindly donated by IDS Ltd, Tyne and Wear, UK). Bloods were drawn at undefined times throughout the working day with no control over food-intake etc. Inter- and intra-assay imprecision was  $< 15\%$  and  $< 4\%$  respectively.

For within-subject variability, 2 serum samples were collected from each of 13 healthy men over time intervals of between 0.88-1.55 yrs, (mean=1.07 $\pm$ 0.15 yrs). From these,  $CV_T$  was 33%. In 78 healthy men (20-79 yrs) mean sCTx-I was 0.37 $\pm$ 0.33  $\mu$ g/L. Stratification into 20yr-cohorts showed little change with increasing age: 20-39 yrs, 0.47 $\pm$ 0.49  $\mu$ g/L (n=12), 40-59 yrs, 0.38 $\pm$ 0.3  $\mu$ g/L (39), 60-79 yrs, 0.31 $\pm$ 0.3  $\mu$ g/L (27) respectively. Mean sCTx in 18 men (age=60 $\pm$ 9yrs, range 45-79 yrs) with untreated vertebral fractures was not significantly different to 57 age-matched (61 $\pm$ 10yrs) healthy men (0.34 $\pm$ 0.3  $\mu$ g/L vs 0.34 $\pm$ 0.3  $\mu$ g/L respectively).

In conclusion, within-subject variation of sCTx-I measured in healthy men in a typical clinical setting was 33%. sCTx-I values were not found to be significantly changed by age in men, and in men with vertebral fractures were no different to controls suggesting that idiopathic male osteoporosis is not associated with excessive bone loss. sCTx-I provides a convenient measure of bone collagen degradation that, in men, is little changed by age or vertebral fracture.

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### The effect of treatment on Pearson correlations between bone markers in the plasma of patients with Paget's disease of bone

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We have measured nine bone markers in plasma from 103 patients with Paget's disease of bone [PDB], [M 53, F 50; mean age 72.3 y, range 51-89 y]. Patients were divided into 2 groups depending on treatment and total alkaline phosphatase [TAP], if TAP was >150 U/L [upper reference limit for this age group] they were assigned to the biochemically active PDB, group 1 and those with TAP <150 U/L post treatment were assigned to the treated PDB, group 2. The formation markers were: osteocalcin [OC], bone specific alkaline phosphatase [BAP], amino and carboxy terminal extension peptides of type 1 procollagen [P1CP, P1NP]; and the resorption markers were: the osteoclast derived tartrate-resistant acid phosphatase [TRACP], the beta form of the carboxy terminal telopeptide of collagen 1 [CTX] and the carboxy terminal telopeptide region of type 1 collagen [CTXmmp]. The soluble form of the receptor activator of nuclear factor kappa B ligand [sRANKL] and osteoprotegerin [OPG] were measured as the regulator of, and decoy receptor for osteoclast activation respectively.

In both groups BAP correlates with TAP [ $p < 0.01$ ], sRANKL shows no significant correlation with any marker. In group 1 OPG correlates significantly with OC, CTX, CTXmmp and P1CP [ $p < 0.01$ ] whilst the remaining markers excluding sRANKL show significant correlation [ $p < 0.05$ ] with the 7 other markers. In group 2 no significant correlations exist for OPG, BAP, and OC and only between P1NP, beta-CTX and TRACP is the significance [ $p < 0.01$ ] maintained.

The results suggest that in active PDB even though bone turnover is increased a coupling remains between formation and resorption. In treated PDB the significant correlations between the markers particularly those estimating osteoblast and osteoclast activity disappear whereas those for collagen formation and resorption remain. When following PDB patients during treatment P1NP, beta-CTX, TRACP, or all three can be used because the correlation significance [ $p < 0.01$ ] is maintained.

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### Peripheral bone density and bone turnover in postmenopausal women with type 2 diabetes

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Menopause is associated with increased bone turnover, which decreases bone mineral density (BMD) and

predisposes these women to fractures. Several studies have suggested that diabetes affects BMD. It is therefore important to know if diabetes modulates the rate of bone loss in postmenopausal women and influence their risk for sustaining fracture.

We compared BMD in the distal non-dominant radius and markers of bone turnover in 60 postmenopausal females with type 2 diabetes and 48 non-diabetic controls, matched for age, activity levels, alcohol intake and HRT usage.

Bone mineral density (BMD) was significantly higher in patients with type 2 diabetes than controls ( $0.51 \pm 0.08$  vs.  $0.47 \pm 0.08$  gm/cm<sup>2</sup>,  $p < 0.01$ ). The diabetic group has lower values than the control group for: serum osteocalcin ( $5.6 \pm 2.4$  vs.  $9 \pm 3.8$  ng/mL,  $p < 0.0001$ ), serum  $\beta$ -cross laps ( $0.26 \pm 0.17$  vs.  $0.41 \pm 0.31$  ng/mL,  $p < 0.01$ ), urinary NTx: (median 34.7 [range: 3.6-166] vs. 42 [range: 11.2 - 370] nM BCE/mM Cr,  $p < 0.05$ ) and urinary helical peptide: median: 33.1 [range: 1.9-145.6] vs. 48 [range: 12.6-478]  $\mu$ g/mmol Cr,  $p < 0.005$ .

The diabetic group has higher BMI values than the control group ( $33.7 \pm 5.9$  vs.  $26.8 \pm 4.4$ ,  $p < 0.0001$ ). After adjusting for BMI by multiple regression analysis, the difference in BMD levels between the diabetic and the non-diabetic group became non-significant.

Postmenopausal women with type 2 diabetes apparently have higher BMD when compared to non-diabetic controls. This is likely due to a slower bone turn over rate, which leads to relative preservation of bone. However, the difference in BMD between the two groups became non-significant after adjusting for the effect of BMI. This study therefore does not provide evidence that type 2 diabetes per se affects bone mineral density in postmenopausal women.

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### A case of acute intermittent porphyria resulting in severe neurological damage

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A 22 year old lady was referred, as an emergency, to this hospital for neurological assessment. She had been admitted to a neighbouring hospital where she presented with severe abdominal pain and vomiting. She became increasingly irritable and paranoid, and fitted.

She was rapidly transferred to ITU as ventilation was required. She developed limb jerks, nystagmus and total motor neuropathy.

Initial investigations showed that she was hyponatraemic (Na 115 mmol/L) and hypokalaemic (K 2.8 mmol/L), with high creatine kinase 4539 U/L and marginally elevated transaminases. Other routine pathology

investigations showed no abnormality. She had an abnormal MRI.

Urine darkened on standing. Ehrlich's test showed large excess of porphobilinogen, and spectrophotometric scanning of acidified urine showed a large porphyrin absorption peak. 5-aminolaevulinic acid was 207  $\mu\text{mol}/\text{mmol}$  creatinine (normal  $<3.8$ ) and porphobilinogen 215  $\mu\text{mol}/\text{mmol}$  creatinine (normal  $<1.5$ ). Erythrocyte hydroxymethylbilane synthase activity was 11 nmol uroporphyrin/mL (normal 20-42).

Following haem arginate treatment, the 5-ALA was lowered, but still remained 10-20 times the upper limit of normal.

History revealed that she had recently returned from an "alcoholic" week in Majorca with other girls including her sister. Abdominal pain while on holiday was ascribed to food poisoning.

She required 8 weeks on ITU with ventilation, and 6 months following the crisis, she is still receiving neurological inpatient therapy.

This AIP crisis was probably triggered by alcohol, but compounded by anti-epileptic treatment given for fitting. The case illustrates the severe neurological damage that can be caused by an AIP crisis. MRI abnormalities are rare in AIP. An acute porphyria should be considered when investigating abdominal pain and planning anti-epileptic therapy.

#### 49

### Rhabdomyolysis in a patient with acute intermittent porphyria

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There are several conditions associated with rhabdomyolysis such as muscle injury, viral infections, metabolic disorders and toxic effects from ingestion of drugs, for example, alcohol, opiates, cocaine or heroin. We report on a patient who was admitted to hospital after a visit to her general practitioner and local A&E on two previous occasions with symptoms of lower back pain and vomiting.

She was admitted to hospital with a raised serum creatinine 161  $\mu\text{mol}/\text{L}$  (reference range: 45-120  $\mu\text{mol}/\text{L}$ ) and serum aspartate aminotransferase (AST) 530 IU/L (10-50 IU/L). In view of the raised serum AST a serum creatine kinase was requested and was found to be elevated: creatine kinase 57,400 IU/L (0-150 IU/L). Additional tests were requested to find the cause of the rhabdomyolysis and unresolved lower back pain. These included routine porphyrin analysis, drugs of abuse screen, paracetamol, thyroid and cortisol measurements. There was no history of alcohol abuse and constant denial of the use of illicit drugs.

The drugs of abuse screen showed no drugs other than those prescribed by her physicians and serum paracetamol, thyroid stimulating hormone and cortisol levels were normal. Porphyrin analysis showed a marked increase in urinary 5-aminolevulinic acid (ALA) 27.9  $\mu\text{mol}/\text{mmol}$  creatinine ( $<3.8$   $\mu\text{mol}/\text{mmol}$  creatinine) and urinary porphobilinogen 73.5  $\mu\text{mol}/\text{mmol}$  creatinine ( $<1.5$   $\mu\text{mol}/\text{mmol}$  creatinine) excretion.

The patient was found to have previously undiagnosed acute porphyria that was investigated with additional specimens, blood and faeces, and shown to be acute intermittent porphyria (AIP). Her symptoms resolved following appropriate treatment and she was referred to the Porphyria Clinic at King's College Hospital for advice on the management of her disease.

Biochemical data will be illustrated and the possible causal link between rhabdomyolysis and porphyria will be discussed.

#### 50

### A comparison of CSF D-dimer measurements and spectrophotometry in suspected subarachnoid haemorrhage

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Semi-quantitative tests for the D-dimer fragment of fibrin in cerebrospinal fluid (CSF) have been used to distinguish subarachnoid haemorrhage and traumatic tap. The release of sensitive automated immunoassays therefore raises the possibility of an objective and specific alternative to CSF spectrophotometry for on-call use. The aim of this study was to compare D-dimer measurement with spectrophotometry in patients with suspected subarachnoid haemorrhage but negative computed tomography (CT) scans.

Ninety-seven CSF specimens were collected from patients presenting with sudden severe headache but normal CT scans. D-dimers were measured using the Dimertest Gold microtitre plate enzyme immunoassay (American Diagnostica). A provisional reference range of 0-80  $\mu\text{g}/\text{L}$  was defined. Inspection of the CSF and spectrophotometry were initially performed prior to the publication of the nationally recommended method, so the net bilirubin absorbance at 476 nm ( $\text{NBA}_{476}$ ) was measured on the retained hard copies of the scans. The results of further investigations and final diagnoses were collected at the end of the study.

All tests were positive in the one patient subsequently shown to have an intracranial aneurysm by 4-vessel cerebral angiography. Only one other specimen had both increased D-dimers and a  $\text{NBA}_{476} >0.007$  AU, but in this case angiography was normal. Two specimens were incorrectly described as clear and colourless on visual

inspection. Five specimens contained increased D-dimer concentrations but had a  $NBA_{476} < 0.007$  AU and two specimens had an increased  $NBA_{476}$  but normal D-dimer concentrations.

The nationally recommended method for spectrophotometry appeared to be the most specific test for the detection of an intracranial aneurysm visible at angiography, but only one of four patients with a  $NBA_{476} > 0.007$  AU had a demonstrable aneurysm. Increased CSF D-dimer concentrations may provide additional evidence for subarachnoid haemorrhage but the significance of positive results in patients with normal CSF by spectrophotometry is uncertain.

## 51

### **Bedside microalbuminuria in 431 patients admitted to ICU compared with mortality, inotrope requirement, SOFA and APACHE II scores**

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Accurate risk assessment of patients on admission to the ICU is essential to make optimum use of expensive resources and to allow early intervention in patients likely to develop complications. Microalbuminuria is associated with systemic microvascular dysfunction, organ failures and death in acute inflammatory conditions such as surgery, trauma and sepsis.

This study aimed to compare microalbuminuria on ICU admission with mortality, inotrope requirements and two established illness-severity scoring systems which require multiple laboratory and physiological measurements: Sequential Organ Dysfunction Assessment (SOFA) score and Applied Physiological and Chronic Health Evaluation score (APACHE II) using admission data.

Urine albumin creatinine ratio (ACR: normal  $< 2.3$  mg/mmol) was measured by nursing staff (DCA 2000, Bayer Diagnostics Ltd) within 15 minutes of admission (ACR 1) and again 4-6 hours later (ACR 2) in 431 patients (194 medical and 237 surgical).

Median (95%CI) admission and 4-6 h ACRs were 11.2 (8.7-13.2) and 5.4 (4.7-6.8) mg/mmol respectively ( $p < 0.0001$ ). ACR 2 for 49 patients who later required inotropes was 16.6 (7.8-22.4) mg/mmol compared with 5.1 (4.6-5.8) mg/mmol for the remainder. ( $p < 0.0001$  Mann-Whitney). Using a cut off for ACR 2 of 10 mg/mmol the positive and negative predictive values for later inotrope requirement were 21% and 93% respectively. Median ACR 2 for 90 non-survivors was 12.4 (8.2-18.9) mg/mmol and for 341 survivors 4.8 (3.9-5.4) mg/mmol respectively ( $p < 0.0001$ ). Using a cut-off for ACR 2 of 2.9 mg/mmol the positive and negative predictive values for death on the ICU were 27% and

94% respectively. Measurement of ACR on ICU admission was a better predictor of outcome than SOFA or APACHE II scores.

This is the largest evaluation of microalbuminuria in ICU patients to date, and indicates that bedside measurement by nurses provides a powerful tool to rule out patients at risk of microvascular dysfunction, organ failure and death. ACR measurement may help optimal use of scarce ICU beds.

## 52

### **The use of plasma DNA concentrations in the prognosis of intensive care patients**

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The assessment of prognosis in intensive care patients allows the treatment of those that are most likely to benefit, whilst preventing the use of therapies that will only prolong suffering. The development of analytical systems such as the Roche Lightcycler has allowed the evaluation of molecular species such as plasma DNA for use as a prognostic marker. Plasma DNA has been found to increase in trauma patients and in those with cancer. It may be released into the circulation from apoptotic or necrotic cells following cell lysis. Paired plasma samples were taken from 53 patients admitted to the intensive care unit. Following DNA extraction, samples were assayed for plasma DNA using real time quantitative polymerase chain reaction. After development and optimisation of the plasma DNA assay, results were compared with patient data on outcome, length of stay and other biochemical markers of prognosis. The median plasma DNA concentration of patients who died and those who survived was 263.5 ng/mL and 68 ng/mL respectively. Using a cut-off of 190 ng/mL, the sensitivity of plasma DNA analysis for the prediction of death was 71%, with a specificity of 86%. Plasma DNA analysis was found to be a superior marker of outcome than the current scoring system used in the intensive care.

In conclusion, plasma DNA is increased in patients admitted to the intensive care and can potentially be used as a prognostic marker in these patients.

## 53

### **Cardiac troponin T levels and myocardial involvement in children with severe respiratory syncytial virus lung disease**

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Lower respiratory tract infection by respiratory syncytial virus (RSV) is one of the most important causes of death

and morbidity in infants worldwide. A previous retrospective study on the prevalence of hepatitis associated with RSV disease revealed that 13% of children admitted to the paediatric intensive care unit (PICU) with RSV had significantly increased aspartate aminotransferase levels (AST), potentially from myocardial origin (Eisenhut M and Thorburn K. *Scan J of Infect Diseases* 2002; **34**: 235). The aim of this study was to determine the prevalence of myocardial damage in severe RSV disease as evident from increased cardiac specific Troponin T (cTnT) levels. This was a prospective observational cohort study of infants with RSV infection admitted to the PICU at Royal Liverpool Childrens Hospital during the winter season of 2002-2003. The cTnT measurements were performed using a third generation monoclonal sandwich immunoassay (Roche Diagnostics).

34 children were included in this study, 12 (35%) had increased cTnT levels. The levels measured after admission had a median (IQR) of 50 pg/ml (37.5-67.5). There were no significant differences between patients with and without increased cTnT levels with regards to gender, gestational age at birth, previous history, presence of congenital heart disease, chronic lung disease, ionotrope requirements, duration of ventilation, death, fractional shortening on echocardiogram or arrhythmias. Children with increased cTnT levels were significantly younger [median (IQR) 1.4 months (0.8-2.0)] than children without [4.0 months (1.7-6.6)] ( $p=0.04$ ). The systolic blood pressure on admission was lower in infants with increased cTnT compared to those with undetectable cTnT ( $p=0.01$ ).

In conclusion, myocardial involvement is common in infants with severe RSV lung disease without congenital heart disease. Increase in cTnT level was associated with hypotension.

#### 54 Interleukin-9 production in the lungs of infants with severe respiratory syncytial virus bronchiolitis

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Respiratory Syncytial Virus bronchiolitis is the most prevalent acute wheezing disorder in infants and is associated with recurrent wheeze and asthma in childhood. Recently, IL-9, a type 2 cytokine has been proposed as a key cytokine determining asthma susceptibility. There is also increasing evidence that IL-9 may stimulate mucus production in the lung.

The aim of this study was to investigate whether IL-9 was produced in the lungs of infants with severe RSV disease, and if found, from which cells it originated. 150

non-bronchoscopic bronchoalveolar lavages (BAL) over the course of ventilation on 24 term and 21 preterm infants ventilated for RSV bronchiolitis and on 10 control infants ventilated for non respiratory causes were studied. IL-9 mRNA was measured using an RNase protection assay and IL-9 protein measured using a newly developed in house ELISA. Immunostaining was used to identify the cells that produce IL-9. IL-9 mRNA expression, which persisted over the course of ventilation was demonstrated in all infants with bronchiolitis, and additionally in 3 of the control group. IL-9 concentration on day 1 [1.9 (0.1-36.2) ng/ml; median(range)] was significantly greater in term infants with bronchiolitis than either preterm infants [0.4 (0.1-2.9) ng/ml;  $p<0.05$ ] or the control group [0.7 (0.4-2.5) ng/ml;  $p<0.05$ ]. There was a trend for IL-9 concentration to decrease over time in term but not preterm infants. Immunostained cell smears revealed that the majority of IL-9 expression in BAL was in neutrophils.

In conclusion, in term infants with RSV bronchiolitis large amounts of IL-9 mRNA and protein were present. Neutrophils appear to be the main source of this type 2 cytokine. This IL-9 production may contribute to the pathogenesis of RSV disease. These findings may be relevant to other disease processes in the lung where neutrophils are the predominant inflammatory cell type.

#### 55 Ward-based pregnancy testing revisited

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A number of wards at Arrowse Park Hospital have used Guest Medical One-Step Sticks to test urine samples for several years. External quality assurance schemes have highlighted problems with the current situation. This study was undertaken to formally evaluate the performance of fifteen other pregnancy test kits at hCG concentrations around the manufacturer's cut-off concentrations. Seven of the kits were also tested for high dose hook effects.

Five urine samples with hCG concentrations of 0, 10, 25, 50 and 100 mIU/mL were tested using all of the kits. Two urine samples known to produce a high dose hook effect with the Guest Medical One-Step Sticks (hCG >50,000 mIU/mL) were also tested, both neat and diluted tenfold, using seven of the kits. The manufacturer's instructions were followed exactly and results noted as correct, incorrect or potentially misleading.

Five of the kits produced no potentially misleading results. Potentially misleading results included those where there was a very faint test line present which could be missed, and those where the manufacturer's instructions were unclear as to when the result should

be read from the kit. One kit produced incorrect results. Of the seven kits tested for high dose hook effects, only one appeared free from the effect.

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### Evaluation of Rx Monza point of care analyser

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Much point of care (POCT) equipment is now robust, simple to use and if properly implemented, may improve clinical and economic outcomes. The aim of this study was to evaluate the Rx Monza POCT analyser in which a pen-like cartridge contains reagents for the measurement of troponin I, myoglobin, CKMB, CRP and HCG. The principle of the measurement is an immunofluorometric assay in which all the specific reagents are provided in a dry stable form within an assay cup. Serum samples were assayed for CRP (Abbott Aeroset using Randox reagent), HCG (Abbott AXSYM), CKMB (Abbott Aeroset), and Troponin T (Roche Elecsys 1010) and results compared with whole blood samples taken simultaneously and measured on the Rx Monza analyser. Of the 17 samples assayed for Troponin, 15 were classified as normal (below the acute coronary syndrome cut-off) with both methods. One sample was classified as borderline (result 0.029 µg/L, cut off 0.03 µg/L) with the Roche Troponin T method and above the ACS cutoff (result 0.042 µg/L, cut-off 0.025 µg/L) with the Rx Monza. The other sample was classified as above the ACS cut off (result 0.042 µg/L) with the Roche Troponin T method and above the ACS cut-off (result of 0.043 µg/L) with the Randox Rx Monza. Both samples were from patients with normal serial cardiac enzymes. Of 11 samples compared for CRP, 9 were classified as normal with both methods and 2 as abnormal with both methods whilst all 21 samples compared for CKMB were classified as normal with both methods. 15 samples were compared for HCG. One was classified as normal with both methods and 14 were classified as abnormal with both methods. This preliminary study indicates that the Rx Monza agrees well with existing laboratory methods in terms of patient classification. The system was easy to use and maintain. First result is available in 18 minutes and every 3 minutes thereafter.

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### An audit of the performance of point of care blood glucose monitoring using a data management system

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The Abbott MediSense PCx system has been operational throughout our hospital for one year. There are 42

meters on 30 sites and all are networked to the MediSense QC Manager Point-of-Care Data Management Software.

Aim: To assess (a) the performance of the meters and operators, (b) the frequency and the causes of errors and (c) the compliance of operators with guidelines.

Methods: Glucose monitoring was audited over a period of 4 months in 4 wards, differing in patient profile, case mix and workload.

Results: The performance of the meters with regard to QC measurement was acceptable, with a C.V. ranging from 6.5% to 9.9% for the low QC and from 4.7% to 7.1% for the high QC. The performance of the meters in the EQA Scheme was satisfactory; 92.3% of results being within the target range. QC was performed by a total of 150 operators; 83% had excellent performance (no value outside target range), while the remainder had adequate performance (>80% within target range). The frequency of outliers was 1.5% (34 from a total of 2261 QCs performed). The compliance with the training guidelines varied with the clinical setting. Compliance with entry of patient's hospital number was 90% in 3 wards but 54% in the one acute ward. The confirmation of patient outlier results (values  $\leq 3$  or  $> 20$  mmol/L) was very infrequent. In 2 of the wards examined only 5.4% and 8.7% of outlier results were confirmed. The performance of daily QC was high in the acute and critical wards but low in the medical and surgical wards. The overall monthly return rate of EQA results was 91 % throughout the hospital.

Conclusion: The overall performance of meters and operators was acceptable. However, the audit identified certain areas which need attention, e.g. non-compliance of specific guidelines.

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### Improving the quality of blood glucose POCT throughout the healthcare community in Cornwall

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A survey of POCT in GP surgeries in Cornwall found 9 different blood glucose meters in use, obtained from various sources. Only 33% of surgeries performed any QC and relevant training was often lacking. It was concluded that the quality of the POCT blood glucose service could be improved by: standardising the equipment used; centralising purchasing; initiating regular QC/QA programmes; implementing training programmes. There was considerable interest in these improvements from GP surgeries and PCTs, but there was concern regarding the funding.

The key to all the improvements was the standardisation of equipment and therefore a proposal regarding

this issue was formulated and disseminated to the entire healthcare community. Presentations were made to the clinical governance groups, Clinical Modernisation Group, Medical Forum and the Local Medical Committee, which culminated with all groups being in favour of this proposal, but funding laboratory support and QC/QA programmes remained a problem.

An innovative approach was required involving an inclusive quality service package. A joint hospital/community tender for blood glucose testing was initiated, which included the provision of blood glucose meters and multi-user finger pricking devices to all sites together with laboratory support and a structured QC/QA programme. This was to be directly funded through the purchase of blood glucose strips and lancets, with the diagnostic company contributing to the funding of the laboratory support. This approach has been widely accepted across the healthcare community with benefits to all involved with the testing.

In conclusion, improving the quality of blood glucose at POCT sites throughout the healthcare community is a major, but rewarding challenge that benefits from a partnership between the laboratory, healthcare community and the commercial sector and can act as a model for future developments in POCT. This partnership has improved the image of Pathology and enhanced the links with its users.

## 59 Point of care testing for CRP in a rheumatology clinic

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C-reactive protein (CRP) is a marker of the acute phase response and can be extremely useful in monitoring inflammatory diseases. Simple and rapid point of care testing (POCT) methods for CRP have been developed and have been shown to perform satisfactorily in primary care. We conducted a pilot study to investigate whether measuring CRP in a rheumatology clinic by POCT leads to an increase in change in patient management compared to conventional and fast-track laboratory analyses.

Sixty-four patients with rheumatoid arthritis were studied in 3 arms, conventional laboratory CRP analysis (n=21), fast-track laboratory analysis (n=24) and CRP by POCT (n=19) in randomised clinics. Conventional and fast-track (urgent) laboratory CRP were measured by immunoturbidimetric method (Olympus, Southall, UK). CRP by POCT was measured using the Orion Diagnostica QuikRead CRP system (Bio-Stat, Stockport, UK).

POCT CRP results showed good agreement with laboratory method (r=0.95, n=34) with a mean turnaround

time of 5 mins (range: 4-10 mins) compared to 74 mins for fast-track laboratory analysis (range: 35-125 mins).

4/64 patients overall had a change in management attributable to their CRP result (6.3%, confidence interval: 2.5-15%). 6/19 patients having POCT CRP measurements had changes in patient management, 2/6 initiated on the basis of their CRP result. Overall, of the 27 patients who had a change in management, 4 were as a result of their CRP (14.8%, confidence interval: 5.9-32.5%).

POCT for CRP is fast, accurate and easy to perform. Fast-track laboratory analysis of CRP did not achieve an adequate turnaround time for an outpatient clinic. Few patients had a change in management as a direct result of the CRP. However, POCT testing for CRP may be beneficial in new patients or for patients experiencing an acute flare of their disease. Further studies are required to examine the health economics in these settings.

## 60 Evaluation of four point of care testing devices for microalbumin estimation

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Four devices for measuring microalbumin concentration were compared to an immunoturbidimetric method (Roche Tinaquant) using 20 patient samples. Two devices were semi-quantitative dipstick tests; Roche and Bayer (creatinine also estimated). Two were immunoturbidimetric methods (Orion and HemoCue meters).

Urine samples were analysed within 24 h of receipt by one analyst. All samples tested negative for haemoglobin. For the meter methods, a dilution series was also tested in duplicate to assess linearity, imprecision and bias.

The Bayer dipsticks overestimated microalbumin concentration by 1 or more colour blocks in 30% of samples. Creatinine was also overestimated by 1 block in 35%. Interpretation using the chart provided generated 2 false positives and no false negative results. The Roche dipsticks overestimated as many samples as were underestimated (20%) by no more than 1 block. There were no false negatives for either test.

The Orion meter results correlated well with laboratory results ( $r^2=0.982$ ,  $Sy.x=4.49$  patient samples;  $r^2=0.999$ ,  $Sy.x=4.5$  linearity samples) and had low imprecision (5.7% at 16 mg/L and 7.3% at 48 mg/L. Range = 2.4 to 5.9% linearity samples). The mean bias for patient samples was -2.65 mg/L (range -16 to 5 mg/L) and for the linearity samples 7.5 mg/L (range = 3 to 11 mg/L).

The HemoCue meter also correlated well with laboratory results ( $r^2=0.944$ ,  $Sy.x=8.54$  patient samples;  $r^2=0.986$ ,  $Sy.x=5.98$  linearity samples) but not as well as the Orion meter. Imprecision was also worse (9.0% at 73 mg/L and 23.8% at 113 mg/L. Range 1.0 to 16% linearity samples). Bias was increased compared to the Orion meter for both the patient and linearity samples (means = -1.45 and 7.9 mg/L, ranges = 14 to 24 mg/L and 2 to 24 mg/L respectively).

Careful timing was required to use the dipsticks and the wide concentration intervals between blocks made interpretation difficult. The dipsticks are not sufficiently sensitive to be useful in monitoring microalbuminuria but could be a useful screening tool. Both the performance and the cost of the meter tests were comparable with the laboratory assay but creatinine values would not be available. Compared to the Orion meter, the HemoCue was easier to use but the results were less precise.

## 61

### Point of care test to monitor adherence to anti-tuberculous treatment

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In the fight against the global tuberculosis epidemic, it is essential to ensure that patients adhere to the treatment prescribed. As the treatment is given for a minimum of 6 months it is common for patients not to take their drugs regularly. Strategies are therefore needed to assess adherence to treatment. One established method is to examine the patient's urine for the presence of drug metabolites. A rapid point-of-care test would overcome some of the drawbacks with currently available methods.

A rapid, safe, point-of-care test for isoniazid metabolites has been developed and used to assess adherence to treatment in a busy clinic for tuberculosis patients in South London.

Urine samples (n=191) were examined from patients receiving isoniazid, usually in combination with rifampicin and other antituberculous drugs. The isoniazid test was positive in 93.2% of patients, suggesting that 6.8% might be poorly adhering to treatment. By contrast, examining the same urine samples for evidence of rifampicin ingestion gave positive results in only 43.5%, due to the fact that this test is only positive for a few hours after drug ingestion.

The isoniazid test has been shown to provide a rapid, safe and effective point-of-care test for assessment of drug adherence in patients with tuberculosis.

## 62

### Qualitative and quantitative assessment of smoking in relation to risk factors for cardiovascular disease

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Smoking is a major risk factor for cardiovascular disease. Patients who smoke frequently deny smoking or under-report their cigarette consumption. To overcome this bias biochemical verification of smoking by measurement of nicotine metabolites, specifically cotinine, is increasing. To facilitate easier measurement we developed a 6-minute point-of-care urine test called SmokeScreen, which can provide qualitative, semi-quantitative and quantitative measurements of nicotine intake. The aim of this study was to compare qualitative and quantitative assessment of smoking with biochemical and haematological risk factors for heart disease.

New patients attending an inner-city cardiology out-patient clinic were recruited (n=85, 33 current smokers and 52 never-smokers). Urine samples were obtained and analysed for nicotine metabolites, while blood samples were analysed for cholesterol, HDL and triglycerides; urea and electrolytes and liver function, together with a routine haematological screen.

None of the parameters measured in the biochemical analysis were associated with smoking habit or assessment of nicotine intake. However, white blood cell count (wbc) was significantly higher in smokers ( $p=0.002$ ), in particular, neutrophils ( $p=0.01$ ) and eosinophils ( $p=0.02$ ). Lymphocytes, monocytes and basophiles were higher but failed to reach significance. Quantitative assessment further revealed a positive correlation with wbc ( $p=0.0001$ ), neutrophils ( $p=0.001$ ), eosinophils ( $p=0.004$ ) and lymphocytes ( $p=0.02$ ), with monocytes approaching significance ( $p=0.7$ ).

We conclude that smoking or the amount of tobacco consumed does not appear to influence the biochemical risk factors for coronary artery disease. However, smoking does seem to increase many of the immune cells associated with both the formation and destabilisation of the atheromatous plaque. This is a possible mechanism whereby a reduction in nicotine intake reduces the risk of myocardial infarction.

## 63

### A urine dipstick quality assessment audit

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A second quality assessment audit for urinalysis in the wards of Bradford Royal Infirmary and St Lukes Hospital Bradford was performed, with a comparison between the

results acquired for visual reading and Clintek strip readers. Due to a belief that misalignment was resulting in incorrect results after the first audit, a 'urine' sample was prepared to maximise detection of these misalignment errors and sent to each ward.

Returned results were scored +1 per colour block away from the target. The components of the 'urine' sample gave the following percentage errors (1 colour block away from the target), and additional gross errors (2+ colour blocks away from the target: glucose - 35.5%, 16%; bilirubin - 3%, 0%; ketones - 32%, 3%; specific gravity - 39%, 10%; blood - 45%, 16%; pH - 19%, 3% and protein - 65%, 10%. This gives an error reading of 35%, and a gross error reading of 9%, an increase in both error types compared to the previous audit. In comparison no gross errors were detected with the electronic Clintek strip readers, and the total percentage of minor errors was 9%.

The increase in errors from the visual readings supports the hypothesis from the previous audit that most of the errors arise from misalignment of the stick with the colour chart. Other factors such as timing, misunderstanding of the training and incorrect recording may also play a role. Development of bench-top colour block charts that may enable easier interpretation of the results and retraining will be performed before the next audit. These measures will hopefully produce a decrease in the error rates, especially those from misalignment of the strip with the results chart.

## 64

### To rationalise or not to rationalise: an audit of urine dipstick analysis in Barking Havering and Redbridge Hospitals NHS Trust

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Urine analysis can be used to monitor carbohydrate metabolism, kidney and liver function, urinary tract function and acid base balance. This often involves semi-quantitative analysis of urine parameters using reagent strips. An audit questionnaire was circulated to 128 clinical areas chosen using stock sheets supplied by the pharmacy department. The purpose was to examine 1) the dipsticks used in the trust and assess the case for rationalising the range available; 2) the influence of this form of testing on clinical action; and 3) the need for additional training. The most widely and frequently used sticks were Multistix SG and 8SG. Pharmacy also supplies Diastix, Ketodiastix, Labstix and Multistix 10SG. These are not widely used and those that use them do so infrequently. The analytes they measure overlap with those measured by Multistix SG and 8SG. A positive result for glucose, protein, ketones, nitrites or leucocytes

for the majority of respondents triggers clinical action. Few act on positive results for pH or SG and no respondents act on positive bilirubin or urobilinogen results. The primary response triggered is further clinical/laboratory testing though a significant proportion of respondents claimed clinical intervention might also occur. Nursing staff carry out the majority of training. However, there are areas where no training occurs. This is particularly evident in locations remote to the main hospital sites. Staff do identify urinalysis as a training need with 70% of respondents claiming staff would benefit from training in this area. This audit has shown that urinalysis is a useful trigger for further clinical action and there is clearly a case for rationalising the dipstick methods available. In light of the low usage of some sticks and overlap in the analytes measured the clinical and financial justification for using so many types of stick must be questioned. Training in this area of analysis is largely underdeveloped but has been clearly identified as a training need. Efforts must now be made to meet the obvious demand.

## 65

### Urine collection and preservation

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This survey was conducted to determine current practice for urine collection and preservation. It was confined to the dozen or so most commonly requested analytes for 24 hour urine collections. A single A4 page questionnaire incorporating tick-box responses was distributed to 72 laboratories in the North & South Thames areas. The format was chosen to encourage a good return rate (63%).

There was disagreement on whether a preservative should be used for specific analytes and whether this should be issued with the container or added on receipt. The most commonly employed preservative was hydrochloric acid (HCl) although a great variety of volumes and concentrations were in use. The same acid preservative tended to be used by individual laboratories for all analytes requiring such preservation. Warnings for hazardous preservatives were uncommon.

The majority did provide patient information sheets which were almost exclusively instructions for collecting a 24 hour urine. Where dietary restrictions were stated (5-HIAA, HMMA, catecholamines) these were often combined and historical from days when spectrophotometric methods were more common.

The following initial standards are proposed. Clean, disposable container issued in a re-sealable plastic bag. General 24 hour urine collection instructions and warning (preservative) on adhesive labels. Urine refrigerated

during collection. 30 mL 6M HCl used for analytes requiring acid preservation and issued in the container. pH confirmed on receipt and adjusted to <3 if not already so. Urine stored at 4°C if analysed within 24 hours or 20°C if not. Urine at ambient temperature and well mixed before an aliquot is taken.

Any dietary restrictions should be appropriate for analyte and method used for measurement.

## 66

### **An analysis of non-conformities found from vertical audits**

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The new CPA standards require laboratories to have Quality Systems and perform internal audit. Data from the first four vertical audits performed in the Department of Chemical Pathology, Southampton General Hospital, has been analysed in order to determine with which sections of the standards we had most problems.

Analysis of the data showed that most of our non-conformities occurred in Section E (pre-examination process) and Section B (personnel).

Although the same two non-conformities recurred throughout all four vertical audits, the first four vertical audits did reveal 31 different non-conformities, showing that vertical audit is a powerful tool for highlighting non-conformities.

Analysis also revealed that some sections of the new CPA standards (sections C and F) are less well covered by the CPA vertical audit report form than other sections. However, as CPA also require us to perform horizontal audits to cover all sections of the standards, these can be refined to ensure that they provide satisfactory coverage of standards not covered so extensively by vertical audits.

## 67

### **A systematic approach to auditing clerical errors in the clinical laboratory and the effects of feedback on error rate**

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Errors in the sample receipt and booking in section can compromise patient results. Assessing the error rate on a regular basis is important in this area of the laboratory. This study has designed and implemented a tool to assess the integrity of the input of data onto the laboratory computer and the presence of an electronic image of the original paper request.

On one day each month one hundred forms (approximately 10% of the workload) are randomly selected from those received in the previous 12 hours. For each form 15 data fields are checked for accuracy of data input to the computer and where the information has been transcribed incorrectly an error is noted. An error is also counted if there is no electronic image present on the Pathology server. The number of errors for a field is multiplied by the error factor to give a relative error, and these are then summed to give a final score, which we have termed the CJ score. We have studied work undertaken during routine periods (Monday to Friday) and also weekend and bank holiday work.

Over the first 9 months results have shown a considerable improvement in accuracy on routine days, with the CJ score of 165 (4.5% error) in month 1 reducing to a CJ score of 60 (1.4% error) in month 5, an improvement of 36%. Performing the audit on non-routine days demonstrated markedly higher error rates with a weekend CJ Score of 176 (3.9% error) and a bank holiday CJ score of 360 (9.1% error).

The audit is continuing, and its value has been proven by the reduction in errors following feedback. It has also highlighted a problem when booking in is performed by non-dedicated staff out of normal working hours.

## 68

### **An audit investigating the number of urgent request forms entering the laboratory**

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A relatively large number of urgent request forms are received in our biochemistry department. Senior members of staff felt that too many urgent sample requests were being made within hours i.e. 09:00-17:00 h. This could possibly explain the build up of non-urgent samples during the day, thus leading to a reduced turn around time of these samples, as the urgent samples are given precedence. The objectives of this audit were to determine which wards were sending urgent requests to the laboratory and whether it was considered reasonable for them to do so; also to assess the ability of clinicians to fill out request forms in the appropriate manner. Each individual urgent request form sent to the laboratory between 03/08/03 - 09/08/03 was scrutinised and the following information extracted: the laboratory number, the accident and emergency (A&E) number or unit reference number, the time the request was made, the ward from where the request came, the consultant code, the clinical details if given, requests made by the clinician for the sample and finally requests for troponin-T were checked to see if hours post chest pain were given on the request form.

The results showed that the number of urgent request forms that were sent to the laboratory between 04/08/03 - 08/08/03 was 832, 47% of these were requested within hours. The number of urgent tests requested was 1203, 45% within hours. Out of all the in-patient request forms, 50% were requested urgently; this is considered excessive. The wards, which were urgently requesting tests the most were as would be expected for example, A&E and intensive care. Certain wards were requesting urgent tests irresponsibly. 20% of urgent request forms did not provide the laboratory with clinical details. 21% of request forms for troponin-T did not give hours post chest pain. Recommendations for requesting laboratory tests urgently have been drawn up and are being shown to clinical directorates within the hospital.

## 69

### A national survey of the quality of information provided on GP request forms to biochemistry laboratories

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Interpretation of results is an integral part of a clinical biochemist's role, a function valued and desired by GPs and their nursing staff. While NEQAS provide a scheme to support this activity it has never been established how well GPs provide the basic patient information and clinical details for safe interpretations to be made. To investigate this, a questionnaire was sent to all UK biochemistry laboratories requiring 100 GP request forms to be examined for the presence of patient details (forename, surname, DoB, sex, address, NHS No), GP details (name, return address), sample details (date, time, clinical details) and legibility.

120 laboratories completed the questionnaire with the following results. 99% of GP requests contained sufficient patient details for unique identification on the LIS although legibility was problematic in some laboratories. However, NHS No, patient address and gender were less consistently supplied. 96% of all requests contained the name and return address of the requesting GP although for some laboratories this information was available on <50% of request forms. Only 2 laboratories did not require the date or time of a sample to be supplied yet this was information that was poorly provided. Overall 86% of requests were dated and 49% were timed, the range was large and some laboratories had <20% of GP requests dated or timed. The provision of clinical details was also variable, overall 83% of requests contained some clinical information but this ranged from 7% to 100% depending on the laboratory. Again legibility was a problem with only 87% of requests containing clinical details that were decipherable.

Laboratories try to ensure the quality of reported results however incomplete and illegible patient and

clinical details may detract from the overall value of a report due to failure to accumulate results or a misleading interpretation.

## 70

### Benchmarking GP requesting practices

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In 1996 an audit was undertaken into the requesting practices of GPs for TFT, PSA, lipids and FSH. The request rate was presented as numbers of requests per 1000 patients per 6 months for each GP practice. This ensured that practice size did not bias the results and enabled comparison between surgeries. This formed the basis of dialogue on the absolute and relative requesting practices. In 2003 a re-audit was undertaken to see whether there were any changes in requesting. The 2003 audit was expanded to include 10 tests most frequently requested by primary care. These represented 78% of all primary care requests for laboratory medicine. Data from both audits were presented to Primary Care in an anonymised graphical format where only individual GP practices knew their results. Shift analysis was performed on the data included in both the 1996 and 2003 audit.

Large differences in requesting patterns were evident between GP practices. The total number of requests ranged from 331 to 2896 tests per 1000 patients, an 8.7-fold range in requesting patterns. Marked differences were also seen for other test groups: urea and electrolytes (request range 39-302 per 1000 patients), FBC (request range 46-307), LFT (request range 18-302), TFT (request range 26-300), lipids (request range 32-282), glucose (request range 24-296), bone (request range 0.5-297), PSA (request range 0-42), FSH (request range 3-24), and HbA1c (request range 7-68). Shift analysis revealed that median request rates for TFT, lipids and PSA increased by between 100 and 360%.

The audit provided useful benchmarking allowing GPs to compare their own requesting practices with that of others over time. A combined primary and secondary care diagnostics group has been set up. Benchmarking and shift analysis are being used for strategic planning of pathology testing in primary care.

## 71

### Effect of variations in laboratory automation on routine sample turnaround times

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The turnaround time for the analysis of sodium from "booking in" to "clinical validation" for routine samples received from in-patients in the department of Clinical

Biochemistry, Grampian University Hospitals was audited. The time period covered was the month of November from 1998 to 2002; this period encompassed major changes in both laboratory equipment and degree of automation.

The department had embarked on a programme of instrument replacement and consolidation to replace 3 instruments from 3 manufacturers (Beckman CX7, Dade Behring Dimension and Bayer Dax 72) to 3 instruments from 2 manufacturers (Dade Behring Dimension and 2 Bayer Advia 1650s). The final aim was to consolidate both chemistry and immunoassay in an "island of automation" composed of 2 Bayer Advia 1650s and 2 Bayer Centaur analysers linked by the Bayer Workcell.

The introduction of the Workcell has resulted in an improvement in the efficiency over the Advia 1650s when operated both with and without rackhandlers. There has also been an overall improvement in the percentage of samples turned around within an hour but deterioration in the percentage of samples turned around within 3 hours. This deterioration in sodium turnaround times may in part be due to the delaying effect of immunoassay tests on the release of results from the Workcell.

## 72

### Newborn screening audit: how well do we perform?

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The Yorkshire Neonatal Screening Laboratory was computerised (Specimen Gate, PerkinElmer) in 2000, funded by a pathology modernisation project grant. This meant that, for the first time, it became possible to establish an audit trail for each sample from its receipt to printing of the report. Samples were received from ten districts. Data was obtained for results reported between 1st July 2002 and 30th September 2002 from the computer database (Lifecycle). After export to spreadsheets (Excel), macros were used to assess compliance with the Streetly (1998) and draft UK Newborn Screening Programme Centre standards ([www.newborn-screening.bloodspot.org.uk](http://www.newborn-screening.bloodspot.org.uk)).

In eight districts samples were taken at the recommended time (between five and eight days of age) but in only three districts did the samples reach the laboratory by the time the baby was sixteen days of age. However, results could be reported to all but one district by twenty days of age. Between 33 and 90% of samples per district reached the laboratory within four days. This could be improved by sending samples to the laboratory by first-class post. It is currently impossible to identify, within the recommended time frame, untested babies or those whose samples have not reached the laboratory. Three of the Child Health Records departments regularly send lists of babies whose results are missing to the laboratory but

this process does not occur until the baby is 4-6 weeks of age. Considerable investment in IT is needed to create electronic data exchange links with the Child Health Record and Maternity Units to ensure that the data exchange in both directions can be enhanced and that results are available for babies' six-week health check.

## 73

### Audit of T3 measurement in patients with low but detectable TSH concentrations

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The lower limit of our reference range for TSH is 0.2 mU/L. Where fT4 is  $\geq 30$  pmol/L and TSH is  $\leq 0.04$  mU/L no further tests are performed. Currently T3 is measured in patients with TSH of  $\leq 0.2$  mU/L where fT4 is  $\leq 30$  pmol/L. This audit investigated the range of T3 concentrations seen in samples with TSH of  $\leq 179 > 0.04$  and  $\leq 0.2$  mU/L, sent to the Biochemistry Department at Stobhill Hospital, to assess if measurement of T3 contributed further to the establishment of their thyroid status. 188 samples (177 patients) were analysed for T3 in patients with TSH of  $\geq 0.04$  and  $< 0.2$  mU/L, over a seven-month period. 173 out of 188 samples (92%) had T3  $\leq 3.0$  nmol/L (upper limit of our reference interval). 124 repeat samples were sent and TSH was  $< 0.1$  in only 9 of these (fT4 and T3 were within the reference intervals). 15 out of 188 (8%) had T3  $> 3.0$  nmol/L. 13 repeat samples were sent, up to seven months later. Of these, in eight patients TSH had become  $> 0.2$  mU/L (fT4 was within the reference interval and T3 was not measured), four had results which were essentially unchanged and one patient had become thyrotoxic (original TSH concentration 0.06 mU/L). These results suggest that patients with TSH between 0.04 and 0.2 mU/L are unlikely to progress rapidly, if at all to having overt thyroid disease. Even in patients with a raised T3 concentration the majority appear to show TSH within the reference interval on repeat testing. Most of the samples were sent from GPs so this is unlikely to be an effect of severe acute illness. Measurement of T3 in patients with TSH  $\geq 0.04$  and  $\leq 0.2$  mU/L and fT4  $< 30$  pmol/L does not appear to clarify thyroid status and may lead to unnecessary follow-up and testing.

## 74

### An audit of FT3 use in a district general hospital

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The major clinical roles for FT3 are in the diagnosis of T3 thyrotoxicosis and in the monitoring of hyperthyroid

patients receiving antithyroid therapy. The aim of the audit was to establish whether FT3 determination, in addition to TSH and FT4, are useful in classifying patients. At Barnet District General Hospital first line TFTs are TSH and FT4. Current practice is to add FT3 to all requests in which TSH  $\leq$ 0.05 or 0.1 mU/L unless the patient is hypothyroid and on thyroxine. TSH, FT4 and FT3 are analysed on the DPC Immulite 2000. The reference ranges are: TSH 0.4-4.0 mU/L, FT4 10.3-24.5 pmol/L and FT3 2.8-6.5 pmol/L. 200 thyroid results, in which FT3 had been added during routine practice, were collected and relevant clinical detail from the request form was recorded. 105 requests (52.5%) had a normal FT4 and FT3. 15 requests (7.5%) had a normal FT4 and low FT3 most probably due to sick euthyroid syndrome. 12 requests (6%) had a normal FT4 but elevated FT3. FT4 in this group were all at the upper end of the range ( $>16$  pmol/L). 2 requests (1%) had a low FT4 and elevated FT3, these were from patients on T3 replacement. 35 requests (17.5%) had an elevated FT4 and a normal or low FT3. 30 requests (15%) had an elevated FT4 and elevated FT3. All requests that had an elevated FT4  $>40$  pmol/L had an elevated FT3. In conclusion the results from the audit show that the addition of FT3 was of value in 24.5% of requests. However the addition of FT3 when FT4  $\leq$ 14 pmol/L and  $>40$  pmol/L appears to be unnecessary and proposed cut-offs at these values have been implemented and cost savings will be made.

## 75 Audit of thyroid function testing as a result of thyroid antibodies detected during autoantibody screening, adding quality from joint working

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The incidence of thyroid disease is more common in people who are positive for antibodies to thyroid peroxidase (TPO). A thyroid function test (TFT) (free T4 and TSH) was added to patients with positive TPO antibodies, unless TFTs had already been requested.

A retrospective audit over 30 weeks (September 2002 to April 2003) was undertaken to evaluate the value and outcome of the process.

Fifty-seven TFTs were assayed during the evaluation period, 49 female and 8 male, age range 23 to 81 years, 17 greater than 60 years.

Thirty-six patients had no previous record of TFT testing during the last twelve months, 2 patients had known thyroid disease.

Free T4 was below reference range in 7 patients and above reference range in 1 patient. TSH was found to be between 5.5 U/L and 10.0 U/L in 4 patients and greater

than 10.0 U/L in 6 patients. Of the 6 patients with TSH greater than 10 U/L, 4 were subsequently prescribed thyroxine. Two patients (TSH 53.0 and 13.9 U/L) had no recorded follow up.

The practice of adding TFTs was considered to be effective and gave added quality when Immunology and Biochemistry were able to review each other's reports and work together.

To allow review of previous abnormal results a test check facility was introduced

## 76 Change in microalbumin workload in Biochemistry Departments in Scotland following the publication of SIGN guidelines for the management of diabetes

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Following the publication of SIGN Guidelines on the management of diabetes mellitus in late 2001, anecdotal evidence suggested that the number of microalbumin requests was increasing in Scottish laboratories. A questionnaire was distributed to assess the impact of the guidelines on the numbers of microalbumin requests. Twenty-three out of 25 laboratories responded. There was a marked difference in the numbers of microalbumin requests received in different trusts (range in 2002-2003: 189-31,859). The total number of microalbumin requests increased by 22% between 2001-2002 and 2002-2003. In individual laboratories the range of the change in workload seen was between a 15% fall and a 135% increase in the period examined. Although most laboratories saw an increase in both hospital and GP workloads the largest increases were in GP workload (range 14-351%).

A study was also performed to assess the number of individuals with Type 2 diabetes with previously undetected microalbuminuria, amongst patients attending the Diabetic Clinic at Stobhill Hospital between July 2002 and July 2003. Patients were excluded if they were already on an ACE inhibitor or A-II-receptor blocker. Three further EMU were analysed where the albumin/creatinine ratio was  $>3.5$  mg/mmol in the first sample. 236 of 416 patients tested were positive on the first sample and 112 of those had at least two out of three positive specimens.

The survey results suggest that the publication of the SIGN guidelines has led to a large increase in the numbers of microalbumin requests to laboratories. The results from the Diabetic Clinic suggest that there are likely to be further marked increases in microalbumin requests as patients who have not previously been assessed for microalbuminuria attend for follow-up. The

increases have major implications for laboratory budgets, as the publication of the SIGN guidelines was not accompanied by an associated increase in funding.

77

### Lithium requesting and the General Medical Services contract

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GPs accepted the General Medical Services contract in June 2003, which is to be fully implemented from April 2004. This sets standards for patient services, including ones for mental health care and lithium treatment. An audit was carried out to assess the extent of present compliance with these standards and to assess the need for a lithium register. Data was collected on all lithium requests during August 2003 and on the same cohort over the previous eighteen months. The data included times of lithium measurement, date of previous assay and lithium level; time since last thyroid and renal function checks. It was found that to a great extent the standards were already fulfilled, although the therapeutic range for lithium stated in the GMS contract differs from that in the BNF and the one recommended by our local consultant psychiatrists. 91% subjects met the GMS standard for checking renal function and 84% for assessing thyroid function. 94% met the standard for frequency of measuring lithium. As most GMS standards were already met it was not felt that a lithium register was needed at this time.

78

### An audit of female testosterone requests

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Measurements of female testosterone by commercially available non-extraction immunoassays have been heavily criticised in recent publications. The main factors contributing to this problem are reportedly: low levels of testosterone in females, interference caused by matrix effects (lipids, proteins, SHBG) and cross-reactivity with structurally related steroids. These problems commonly lead to overestimated testosterone concentrations.

We undertook a retrospective audit of 364 requests for female testosterone from a two month period, investigating their source, appropriateness and testosterone levels, which were determined on a Bayer Centaur. In another study we compared results obtained on our Bayer Centaur with results obtained independently by an extraction RIA method at West Park Hospital. The audit results showed that the majority of requests came from GPs (75%) and the remainder from fertility/antenatal

clinic (19%), endocrinology consultants (5%) and paediatric consultants (1%). The majority of requests were investigating infertility, menstrual irregularities and hirsutism. Less than 20 requests were considered inappropriate stating non-specific symptoms (overweight, TATT and patients on HRT). 83% of results fell within the quoted reference range (0.5-2.6 nmol/L). 9% of results were equal to or above 3.0 nmol/L.

Female testosterone measurements on the Bayer Centaur showed a positive bias compared to the RIA extraction method. Centaur testosterone measurements greater than 3.0 nmol/L showed a mean bias of 0.45 (n=38) and measurements less than 3.0 nmol/L showed a mean bias of 0.13 (n=42).

This audit demonstrates that the majority of requests for female testosterone appear to be clinically relevant. The majority of our Centaur results fell within the reference range. We confirm that the Centaur overestimates raised female testosterone levels compared to an extraction RIA for testosterone. This highlights the need to confirm all female testosterone results greater than 3nmol/L by an extraction RIA method.

79

### Is SHBG a necessary measurement in men?

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Aims: measurement of male testosterone identifying whether/when SHBG is a necessary measurement; and to assess from the audit data and from the literature which free testosterone index should be used in men, if SHBG is found to be necessary.

All male testosterone results from 2002 were selected, giving 542 requests with complete results. The effects of age, time of year, time of day, gonadotrophin patterns and normalisation of testosterone results, using both the free testosterone index ( $FTI = 6.11 - 2.38 \log_{10} (SHBG) * 10 * \text{total testosterone result}$ ) and free androgen index ( $FAI = \text{total testosterone} / SHBG * 100$ ), on testosterone, were assessed.

Requesting patterns were influenced by age (most commonly requested in the 40-70 age group), but not time of year. No correlation was seen between total testosterone and age; however, there was a slight increase in SHBG with age. Excluding those under 20 and over 80 years old, all other age groups have at least 75% normal total testosterone. FTI has a tighter correlation with total testosterone than FAI. Low total testosterone, above 6 nmol/L, were normalised using free indices. Gonadotrophins were only useful in individual assessment. The circadian rhythm effect was seen, as eleven of thirteen patients had increased levels at 9 am ( $p \leq 0.05$ ) compared to later in the day. Using total

testosterone alone, the chance of a false negative was less than 0.5%.

A protocol for testosterone measurement has been drawn up based on the data achieved from the audit. Using this protocol, a service could have been provided for at least 83% of the samples in 2002 using total testosterone alone. The literature and audit data support a move to FTI for males, rather than FAI, when an index is appropriate.

## 80

### Do androstenedione and DHEAS contribute to the diagnosis of PCOS in GP patients?

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Our aim was to discover if the addition of DHEAS (dehydroepiandrosterone sulphate) and androstenedione aids diagnosis in GP patients with PCOS (polycystic ovary syndrome) as part of their differential diagnosis.

Over two months, our laboratory received samples from seventy-nine female General Practice patients whose symptoms/signs led to a differential diagnosis including PCOS. Of these, nine had a raised total testosterone and twenty-seven had a raised free androgen index (FAI) (18 with a normal total testosterone). Forty-four patients who had a normal total testosterone and FAI (if calculated) were selected from the seventy-nine and had androstenedione and DHEAS measured. Normally, female samples with a testosterone greater than 1.5 nmol/L (reference range 0.5-2.6nmol/L) are cascaded for SHBG (sex hormone binding globulin) measurement and subsequent FAI calculation ( $FAI = \text{total testosterone}/SHBG \times 100$ ). SHBG was also measured, if this test had not been cascaded, and the FAI calculated. Testosterone was measured using the Ciba-Corning ACS:180 Plus; DHEAS and SHBG were measured using the DPC:Immulite 2000 and androstenedione was measured using the DPC: Coat-a-count method.

Eight patients had an androstenedione level above the reference range; seven of which had androstenedione as the only raised androgen. One patient was found to have a raised FAI due to low SHBG, despite having a testosterone <1.5nmol/L, and one patient had a raised DHEAS.

Androstenedione was raised in 18% of the forty-four cases, much higher than predicted, and was almost always raised alone. From the original cohort of seventy-nine, thirty-six (46%) had at least one abnormal androgen result. This suggests that when PCOS is suspected and total testosterone and/or FTI is normal, androstenedione should be measured. This is particularly important when GPs adhere to a protocol where a patient is only referred for further investigation (ultrasound) if they have an abnormal androgen result.

## 81

### The short synacthen test: do we really need the +60 minute sample?

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The short synacthen test (SST), is a standard investigation for the evaluation of adrenal insufficiency. Our unit carries out morning SSTs, measuring plasma cortisol at 0, +30 and +60 minutes. Peak cortisol response >550 nmol/L at either +30 or +60 minutes, or an increment above basal of >170 nmol/L, is considered a pass, indicative of adequate adrenal reserve. Literature evidence suggests that the +30 minute plasma cortisol provides a better index of the hypothalamic-pituitary-adrenal axis than the +60 minute value, when compared with the gold standard insulin stress test (Hurel *et al*, 1996).

Our aim was to see whether the SST +60 minute cortisol value had an influence on the management of patients and if not to abandon this measurement.

We carried out a retrospective analysis of SSTs carried out between 1999-2003 for investigation of adrenal insufficiency. Patients with pituitary disease and on long term steroids were excluded.

There were 106 SSTs during the study period (31 males, 75 females, mean age 44 years (range 18-78 years)). 97 patients had a total cortisol of >550 nmol/L at both +30 and +60 minutes (pass). 12 patients had a cortisol <550 nmol/L at both +30 and +60 minutes (fail). The remaining 9 patients (8%) had a cortisol <550 nmol/L at +30 but >550 nmol/L at +60 minutes. Follow-up notes were not available on 3 patients at time of writing. The remaining 6 patients were deemed to have passed their SST by the treating clinician. Only two patients had cortisol increments above basal of <170 nmol/L (168 & 105 nmol/L respectively) at +30 minutes. Both were deemed to have passed their SST by the referring clinician.

The SST +60 minute cortisol value has little influence on the management of patients and we intend to abandon this in favour of 0 and +30 minute sampling only.

## 82

### Evidence of gender bias in an audit of adherence to the new diagnostic criteria for myocardial infarction

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By placing a greater emphasis on cardiac markers, publication of the new diagnostic criteria for myocardial infarction (MI) in 2000 was predicted to lead to an increase in the recorded incidence. After the diagnosis of MI itself, there is considerable evidence that females are

less aggressively investigated than males. This study has aimed to establish to what extent the new criteria are being applied at Hull Royal Infirmary and whether any gender or age bias exists in the diagnosis.

All 6,383 troponin T (TnT) samples collected during 5,620 admissions on 5,065 patients during 2002 were compared with the discharge diagnosis recorded by the NHS. These figures excluded patients with pulmonary embolism or creatinine >200  $\mu\text{mol/L}$ . When multiple TnT requests were made, the highest result was used.

Of 590 recorded MIs, 552 (94%) had raised (>0.05  $\mu\text{g/L}$ ) TnT values. However, only 552/1410 (39%) of patients with raised TnT were discharged with a MI diagnosis. This comprised 350/797 (44%) of males and just 202/613 (33%) of females ( $\chi^2=17.02$ ,  $p\leq 0.0001$ ). Multiple logistic regression showed gender to still be a separate predictor of MI diagnosis (F vs. M odds ratio 0.64,  $p=0.0001$ ) but patient age not so ( $p=0.16$ ), independently of a raised TnT ( $p\leq 0.0001$ ).

Thus, despite the new criteria defining all raised TnTs as indicative of an MI, this study has shown that the majority of these patients are still not recorded as having had an infarct. While patient age may not influence the likelihood of this diagnosis, we believe this is the first study to show that females are not only investigated less post-MI, they seem less likely to be diagnosed with an MI in the first place.

### 83

#### Audit of troponin I requesting in North Bristol NHS Trust

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Within North Bristol NHS Trust, a joint protocol has been developed between the Emergency, Cardiology and Clinical Biochemistry Departments for the investigation and management of patients with acute chest pain. The trust covers two major sites each with its own Emergency Department and Clinical Biochemistry Laboratory. The chest pain protocol involves stratification of patients by ECG findings and measurement of TnI on appropriately timed specimens. Only one of the three possible treatment pathways involves two TnI measurements. It was our impression that TnI was being requested more frequently in a large number of patients. This audit was designed to assess whether TnI requesting practice followed the protocol.

All TnI requests from the Cardiology and Emergency Departments over the course of one week ( $n=230$ ) and requests from all other locations over a period of one month ( $n=508$ ) were identified. Patient laboratory records were examined for clinical information and to identify patients with multiple TnI requests.

Requests were considered inappropriate if TnI had already been measured twice within a single episode, or if a previous TnI within 7 days was positive. A positive TnI was defined as >0.1  $\mu\text{g/L}$  by Bayer Centaur or >0.3  $\mu\text{g/L}$  by Abbott AxSYM. Overall, 16% of all requests were deemed inappropriate.

The acute chest pain protocol was designed for use in the emergency setting. However, a high proportion of TnI requests were from other locations (42% at Southmead Hospital and 27% at Frenchay Hospital). It was clear from this audit that a large proportion of TnI requests were from patients not presenting with typical chest pain. In these patients investigation according to the chest pain protocol may not be appropriate. This has highlighted that separate guidance for the use of TnI is needed in addition to the existing protocol, which is now under development.

### 84

#### Cardiac troponin I: clinical and laboratory audit

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We commenced cardiac troponin I (cTI) (Abbott AxSYM®) measurement in March 2003. Samples are run in twice daily batches (10:00 & 16:00 hours), with an expected laboratory turnaround time (TAT) of <24 hours. Hospital intranet guidelines ask for information on the request form as to the timing of the sample in relation to the suspected cardiac event. Recommendations are that blood samples should be taken at least 12 hours after an event or 12 hours after hospital admission.

Our aim was to assess adherence to the requesting guidelines, determine laboratory TAT, and examine cTI requesting and clinical outcome.

Information was collected from request forms for cTI received over two 5 day periods (April and June). Further clinical information and outcome data was collected from patients casenotes.

Complete data was available for 93/104 requests for cTI (71 patients). Mean number of requests/day was 9 (range 6-13). Mean laboratory TAT was 10 hr 5 min ( $\pm 9$  hr 45 min). Four samples (4%) had a TAT >24 hrs. 80/93 request forms gave no indication as to the timing of the sample. This information was subsequently found in only 46/93 patient casenotes. 36/93 provided no clinical details on the form. Repeat tests (x2) were requested on 11 and (x3) on 4 patients. Of the 11 patients (15%) found to have a positive cTI result (cTI >0.5  $\mu\text{g/L}$ ) 9 had an ECG with ischaemic changes, none had an exercise stress test, 1 had a cardiac perfusion scan, 6 had echocardiography and 3 had coronary angiography.

Clinical staff are not currently providing sufficient detail to assess the appropriateness of cTI requests. Patients with a positive result may not always be managed in line with accepted best practice. Staff have been re-educated (staff rounds) regarding the use of cTI and re-audit results are awaited.

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### **Audit of biochemical monitoring of hypertensive disorders of pregnancy**

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Hypertensive disorders of pregnancy range from mild, non-proteinuric hypertension through to pre-eclampsia and eclampsia. Severe forms of the disorders are one of the major causes of maternal death and are also associated with increased risk of stillbirth and neonatal death. The aim of this audit was to assess the usefulness of serum urate and biochemical monitoring in hypertensive disorders of pregnancy and also to assess the usefulness in predicting outcomes.

One hundred consecutive case notes of women in the third trimester of pregnancy who were admitted to the obstetric assessment unit with suspected hypertension of pregnancy were retrospectively reviewed. There were 420 requests (mean 4.2 requests/patient, range 2-20) generated. Of the 100 cases reviewed, only 35 cases (35%) met the criteria for hypertensive diseases of pregnancy (Royal College of Obstetrics and Gynaecology Guidelines). There was no correlation between serum urate concentration and diastolic blood pressure ( $r=0.1467$ ), although serum urate concentrations were significantly higher in the hypertensive group compared with the normotensive group ( $368\pm97$  vs.  $269\pm71$ ,  $p<0.01$ ). Moreover serum urate levels did not differentiate between the various hypertensive diseases of pregnancy, nor did serum urate help in predicting outcomes (development of pre-eclampsia, emergency delivery or commencement of oral anti-hypertension medication).

Our audit shows that the majority of biochemical screens are requested on patients who do not satisfy the criteria of hypertensive disorders of pregnancy. Uric acid, although higher in the hypertensive group, was not useful in differentiating between hypertensive states, nor was it useful in predicting outcomes.

86

### **Availability and reporting of lipid analyses: a national audit**

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#### **Oral Presentation**

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 an 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.

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### **Audit of HbA1c requesting in Bristol**

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A joint audit was undertaken across North Bristol and United Bristol NHS trusts to investigate the appropriateness of the requesting of HbA1c. It had been noted that a large percentage of low HbA1c values (<6%) were originating from patients with no history of diabetes, suggesting that HbA1c may be being inappropriately requested for investigating suspected diabetes. HbA1c is a mean index of glycaemic control and the WHO guidelines state that it should be routinely measured in all patients with diabetes mellitus. At present HbA1c measurement is not recommended for the diagnosis of diabetes.

All HbA1c requests over a 2 and 3 month period were investigated. Patients' records were examined for evidence of pre-existing diabetes. Previous biochemistry

results and clinical details were studied and the origin of the request was noted.

Approximately 13% of HbA1c requests from hospital sites across Bristol were from patients with no previous history of diabetes. Within the hospital setting the majority of these inappropriate requests originated from renal medicine and hepatology. Requesting of HbA1c at Bristol Children's Hospital in non-diabetic children originated mainly from CF respiratory physicians and paediatric endocrinologists. In general practice 9% of the total HbA1c requests were from patients with no previous history of diabetes. Over a quarter of these inappropriate requests originated from single general practices in each Bristol trust.

In conclusion, this audit has demonstrated that a significant proportion of HbA1c requests in both primary and secondary care is inappropriate. As a result clinicians requesting HbA1c inappropriately are being contacted to discuss the implications of HbA1c measurement in non-diabetic patients. The requesting of HbA1c will be re-audited following this contact.

## 88

### Inappropriate HbA1c testing

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Since the introduction of the NSF for Diabetes we have observed an increase in HbA1c and ACR requesting. Our standard operating procedure for HbA1c states 'Since the test is not used in normal subjects, a normal range is not quoted on reports. Good control would be indicated by a result of less than 7%'. However, a significant number of the HbA1c results were below this level.

We examined 4267 requests for appropriateness over a 3 month period from August 2003. 23.1% of these were generated within the hospital and 76.9% from general practice. Furthermore, 1878 (44%) had HbA1c values below 7%, 13% hospital-generated requests and 87% from GPs.

In order of frequency, the hospital requests came from diabetes, nephrology, paediatrics, surgery, care of the elderly, ANC/Obstetrics, general medicine and A/E. By hospital speciality, 12-60% of normal HbA1c requests were on patients with no record of diabetes-related treatment or attendance at diabetic out-patient department. The same figure from general practice was 50% (25% to 86% by practice).

The clinical details given with these requests included: weight loss, TATT, increased random glucose, FH diabetes, glycosuria and thirsty, suggesting that HbA1c is being used as a screening test. HbA1c measurement is recognised as a valuable tool for monitoring glycaemic control but is currently not recommended for the diagnosis of diabetes by WHO or NSF.

After discussion with our endocrinologists, local recommendations regarding the use of HbA1c testing have been established and have prompted an article in our GP Newsletter.

## 89

### A survey of age-related alkaline phosphatase reference ranges in use in the Wessex Region

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A questionnaire was circulated in March 2003 to examine the alkaline phosphatase reference ranges used by laboratories in the Wessex Region. Eight of the 11 laboratories replied.

All laboratories used *p*-nitrophenyl phosphate as a substrate, with five laboratories using AMP as a buffer (Vitros 250/700; Beckmann LX20; Roche Modular (2); Advia 1650), two using DEA (Olympus AU600; Bayer Advia 1650) and one laboratory carbonate buffer pH 10.5 (Vitros 250/950). There was close agreement between reference ranges using the same buffer system, but poor correlation between mean UK NEQAS bias index score (BIS) for specimens distributed in 2003 and URL. Two laboratories did not use specific age related reference ranges, but in 1 laboratory all paediatric results on GP patients receive a laboratory comment. Six laboratories employed age related reference ranges each derived from a different source with varying URL. Separate reference ranges for males and females during childhood were quoted in 4/6 labs. As expressed as a multiple of the upper reference limit of the corresponding laboratory adult reference range, enormous variability in the upper limit of these ranges was observed; at day 1 from 0.86 to 2.16, at 1 month 2.12 to 3.75, at 12 months 1.83 to 3.3, at 7 years 1.59 to 3.3, at 11 years 1.78 to 5.15, at 13 years 1.96 to 4.83 and at 18 years 1 to 2.4. In one laboratory further stratification of the reference range was observed in the 49 to 99 age group.

Alkaline phosphatase reference ranges are either derived in-house or taken from the literature however some are based on assay formulations no longer in use. Although laboratories attempt to align results when new methods are introduced, the results of this survey suggest that this may not have been appropriate in all cases.

## 90

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

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#### Oral Presentation

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.

Reference: *British Thoracic Society Thorax* 2003; 58 (suppl II): ii8-17

## 91

### Pleural and ascitic fluid: how logical is test requesting?

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Ascitic and pleural fluids are regularly sent for biochemical analysis. Tests requested by the clinicians frequently seem inappropriate. This may indicate a poor understanding of how to interpret the laboratory data.

Our aim was to establish test requesting patterns for pleural and ascitic fluids, and to compare these with standard test panels produced for both pleural and ascitic fluids following literature surveys.

A retrospective computer-based audit was performed of pleural and ascitic fluid analyses over a six-month period.

151 ascitic and 205 pleural fluid samples were received during the period covered by the audit. The following analyses were requested on ascitic fluid samples

(% of total number of ascitic fluid samples shown in brackets): protein (90%), glucose (79%), lactate dehydrogenase (42%), albumin (5%), and electrolytes (sodium, potassium, urea, creatinine) (13%). A simultaneous serum sample (collected within 2 hours) was received in 2 out of the 8 ascitic albumin requests. The following analyses were requested on pleural fluid samples (% of total number of pleural fluid samples shown in brackets): protein (96%), lactate dehydrogenase (74%), glucose (70%), electrolytes (7%) and albumin (3%). Only one simultaneous serum sample was collected (for protein). The standard panel of tests produced for ascites included albumin, total protein, lactate dehydrogenase and glucose as appropriate tests to determine the presence of portal hypertension, and to distinguish between spontaneous bacterial peritonitis and secondary bacterial infection. The panel of tests produced for pleural fluid included total protein and lactate dehydrogenase, allowing Light's criteria to be applied for identification of exudates, and fluid pH, for its role in determining which parapneumonic effusions are developing into empyemas.

The test requesting patterns observed suggest that some fluid analyses are requested with an incomplete understanding of the appropriate clinical questions to be posed, and of how the laboratory may address these questions.

## 92

### Analysis of CSF for bilirubin in SAH: an audit

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Introduction of the national guidelines for analysis of CSF bilirubin in the diagnosis of sub-arachnoid haemorrhage (SAH) led to the undertaking of an audit of the processes and procedures for handling and analysis of these samples.

An initial audit over the 11-month period of January to November 2002, shortly after the initial proposed guidelines were published, showed that of 148 specimens analysed, 28% of these were of insufficient volume for analysis. Of the remainder 8 (5%) showed a raised NBA value. Only 60% of specimens were analysed and reported within 24 hrs of receipt and 78% within 48 hrs. This data indicated a need to address systems and processes in order to comply with the then new guideline standards.

This involved re-education of medical staff with the publication of a protocol on the Trust Intranet and training of staff groups who undertake analysis and reporting. A spreadsheet was developed to enable reporting and interpretation to comply with standards and an autocoment process was introduced.

A re-audit covering January to November 2003 showed an increase in the number of investigations from 148 to 189. This may have been due to increased awareness of the medical staff to the need for this test within their practice. Only 21 (11%) of the 189 CSF samples were now of insufficient volume. NBA values  $>0.007$  AU were observed in only 3 of the patient cases. Turn around time improved with 80% of cases reported within 24 hrs and 95% within 48hrs.

Introduction of measures to enable compliance with the recommendations involved education of clinicians and laboratory staff, development of a computer based decision aid, and improvement of awareness of the importance of the investigation in clinical practice. These measures have led to a significant improvement in the delivery of the service.

### 93

#### Treatment and monitoring audit of patients with raised plasma homocysteine

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This laboratory has been offering a routine service for plasma homocysteine testing for twelve years. As awareness grows that an increased plasma homocysteine concentration may be a treatable, independent, risk factor for vascular disease the number of requests we receive for homocysteine analysis increases. The treatment of an increased homocysteine is usually by folate supplementation, but folate should not be given if vitamin B12 is deficient due to the risk of inducing neuropathy.

To investigate how patients with increased plasma homocysteine ( $>15 \mu\text{mol/L}$ ) are further investigated and monitored we examined results held on our laboratory computer system for the year 2001. Other than for patients attending the lipid clinic the results were similar regardless of source. Approximately 45% of patients with increased plasma homocysteine were also tested for B12 and folate status, 38% had a repeat homocysteine measurement and 29% of patients had a repeat homocysteine as well as investigation of B12 and folate. For the subgroup of patients attending the lipid clinic 89% were tested for B12 and folate status, 80% had repeat homocysteine measurements and 74% had folate, B12 and repeat homocysteine measurements.

To further investigate the use of our homocysteine service a questionnaire was then sent out (in 2003), to 502 doctors in Brighton; 175 replies were received

(35%). Of the 39 respondents who requested plasma homocysteine analysis, the main reason for request was investigation of premature vascular disease (49%), 74% used folate for treating hyperhomocysteinaemia but 46% used folate without checking B12 status, only 21% requested a repeat homocysteine after treatment. Patients were asked to fast by 33% of requestors but only 8% ensured rapid sample transport to the laboratory.

Conclusions: although requests for plasma homocysteine analysis continue to increase, patient investigation and follow-up is incomplete and sampling precautions are being omitted.

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#### An audit to assess the efficacy of soluble transferrin receptor as a marker of iron deficiency

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The differential diagnosis of iron deficient anaemia (IDA) from anaemia of chronic disease (ACD) is often difficult. Analysis of soluble transferrin receptor (sTfR) concentration may resolve this issue. Studies suggest that sTfR measurement is useful in detecting iron depletion reliably irrespective of inflammatory status and is therefore an aid in the differential diagnosis of IDA and ACD. It is also claimed to be the first analyte to signal iron deficient erythropoiesis (IDE).

The aim of this audit was to develop an immunoturbidimetric assay for sTfR (IDeA® sTfR IT, Orion Diagnostica, PO BOX 83, 02101 Espoo, Finland) on the Beckman LX20pro analyser. The correlation of this assay with other markers of anaemia, haematocrit (Hct), haemoglobin (Hb), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), iron, total iron binding capacity (TIBC) and ferritin was assessed.

Within-batch precision was 3.8% (mean=1.94, n=20), between-batch precision 3.8% (mean=1.89, n=16). 110 patient samples were investigated. Correlations with other markers were: Hct ( $r=-0.28$ ), TIBC ( $r=0.36$ ), Hb ( $r=-0.45$ ),  $\log_{10}$  Iron ( $r=-0.50$ ),  $\log_{10}$  ferritin ( $r=-0.53$ ), MCV ( $r=-0.57$ ), MCH ( $r=-0.68$ ) and MCHC ( $r=-0.75$ ).

The clinical decision levels for sTfR are  $>2.10 \text{ mg/L}$  for IDA and  $1.80-2.10 \text{ mg/L}$  for IDE. Analyses from 9 healthy individuals all gave results for sTfR of  $<1.40 \text{ mg/L}$ . Clinical review was undertaken on 21 patients. Selection criteria were Hb  $\leq 10.0 \text{ g/dL}$  and/or MCV  $\leq 80 \text{ fL}$ . Of 6 patients with IDA and no chronic disease, 5 had sTfR levels  $>2.10 \text{ mg/L}$  and 1 (treated) patient a

level <1.80 mg/L. Of the 15 patients with chronic disease, 7 had sTfR levels >2.10 mg/L, 2 had sTfR levels between 1.80-2.10 mg/L, and 6 had sTfR levels below 1.80 mg/L. Thus, in chronic disease, sTfR levels <1.80 mg/L with other markers indicating anaemia suggest ACD whilst sTfR levels >2.10 mg/L may suggest IDA or a mixed IDA/ACD pattern.

These data show that sTfR may assist in discriminating IDA in chronic disease.

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### Comparison of total iron binding capacity and transferrin as part of the iron profile

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This audit aimed to assess the impact of a change from use of total iron binding capacity (TIBC) to transferrin (Tf) in the biochemical profile used to assess iron status at the Royal Surrey County Hospital. It followed the perception by users, that Tf showed less tendency to climb above its reference range in iron deficient patients. TIBC results from 5597 iron profiles before the change and Tf results from 2529 profiles after the change were divided into those below, within or above their reference ranges. Ranges were consistent with published data. A marked difference between TIBC and Tf was observed in the distribution of results relative to their reference ranges. Only 5% of Tf results were above the reference range as against 23% of TIBC results. TIBC and Tf levels were examined in a subset of patients considered to have iron depletion on the basis of ferritin and percentage iron saturation measurements, who would have been expected to have raised Tf and TIBC levels. Tf was elevated in only 19% of patients with low ferritin and TIBC elevated in 60%. In patients with low percentage iron saturation, Tf was raised in 10% and TIBC in 55%. In order to compare the two data sets, a published conversion factor was used to calculate TIBC from Tf. This confirmed that the observed differences were not true differences in the study populations. Interconversion of the reference ranges indicated that they were not comparable. In conclusion, Tf measurements do not appear to be equivalent to TIBC, and can be misleading in the assessment of iron deficiency. This audit raises important issues about the appropriate use of tests available for the assessment of iron status. Following the audit, Tf and percentage iron saturation were dropped as tests for the assessment of anaemia at RSCH.

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### An audit of the performance of the protocol for lactose tolerance test

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Lactose tolerance tests involving the monitoring of blood glucose levels after an oral lactose load are of some assistance in the diagnosis of lactose intolerance. There are a variety of published protocols for performing a lactose tolerance test most of which specify measurement of blood glucose at 30 minute intervals for at least 2 hours after the lactose load. A normal response is usually defined as a rise in blood glucose of more than 1.1 mmol/L at some time during the test.

An audit of lactose tolerance tests performed over the period 1st January 2002 to 31st December 2003 was undertaken in order to assess the minimum number of samples required to correctly categorise the patient response according to the above standard. There were 216 tests each comprising a fasting sample and further samples taken at 30, 60, 90 and 120 minutes post ingestion of 50g of lactose.

142 tests had a normal response. Peak response was at 30 minutes in 102 cases, at 60 minutes in 30 cases and at 90 minutes in 10 cases. When the peak response was at 90 minutes consideration of only the earlier responses would not have altered the categorisation. 2 of the cases where the peak response was at 60 minutes would have been categorised as subnormal if the only the 30 minute response was considered. 74 tests had a subnormal response. Peak response was at 30 minutes in 43 cases, 60 minutes in 16 cases and 90 minutes in 10 cases.

A short lactose tolerance test of 3 samples (fasting, 30 and 60 minutes post lactose) gives exactly the same diagnostic information as a longer test. This can save phlebotomy time and is more convenient for the patient.

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### Audit of vitamin D requesting: can bone marker profiles be used to predict vitamin D status?

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A number of patients present to the Rheumatology clinic with complaints of arthralgia or generalised pain, which could be bony or muscular. A number of reasons could explain the cause, vitamin D deficiency being one of them. However, it is often difficult to ascertain when there is specific vitamin D deficiency based on clinical details alone. There are also no clear guidelines regarding the appropriateness of vitamin D requesting in

patients presenting with the non-specific symptoms described above.

This audit was, therefore, carried out to look at biochemical bone marker profiles to see if they could predict vitamin D insufficiency/deficiency.

Vitamin D has a known seasonal variation and therefore two sets of data were studied: winter and summer. The following parameters were measured as part of the bone marker profile: calcium, adjusted calcium, alkaline phosphatase, albumin and phosphate.

The results showed that vitamin D insufficiency/deficiency was not confined to the elderly population, the youngest patient being 23 years old. There was a weak correlation demonstrated between vitamin D and adjusted-calcium, but not with serum calcium, ALP or phosphate.

Receiver Operator Characteristics (ROC) analysis showed a sensitivity of 77.4% and a specificity of 28%, using an adjusted-calcium concentration cut-off of 2.4 mmol/L, with vitamin D deficiency being described by a concentration of <30 nmol/L. The sensitivity was increased to 85.7% and specificity to 29.4%, at the same adjusted-calcium concentration cut-off when vitamin D deficiency was defined by a concentration of <15 nmol/L. Areas under the ROC curves for all parameters included the median of 0.5 and could not, thus, positively distinguish between the positive and negative groups.

In conclusion, bone marker profiles do not accurately predict vitamin D status in an individual. However, an adjusted-calcium of >2.4 mmol/L makes severe vitamin D deficiency (<15 nmol/L) less likely.

## 98

### A regional audit of requests for the tumour marker prostate specific antigen

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Prostate specific antigen (PSA) is an established marker for monitoring patients with established prostate cancer and should only be requested for suspected prostate cancer on the basis of clinical examination or bone X-ray showing evidence of skeletal metastases or if there is a strong family history of prostate cancer. It is not recommended as a screening test for prostate cancer at the present time.

An audit looking at both the way the marker was being used clinically and the laboratory aspects of its measurement was carried out by means of a questionnaire sent to laboratories in the North Thames Region. 36 laboratories responded. 6 of these measured both total and free PSA in house if requested by their

Consultant Urologist, usually to assist them in distinguishing prostate cancer from benign prostatic from benign prostatic hypertrophy.

The majority allowed open access of the assay to general practitioners, did not have a policy as to when to request a PSA or issued guidelines regarding factors affecting sample collection.

Four laboratories recommended using PSA as a screening test for symptomatic males over 50 years of age. 21 laboratories quoted age-related ranges for their total PSA. All laboratories used methods calibrated against the reference standard IRP 96/270 and all participated in a recognised external quality assessment scheme.

The majority separated serum from whole blood within 8 hours and stored samples prior to assay at room temperature or 4°C. About 50% issued computer-generated comments on their reports but few produced cumulative reports.

As a result of the audit guidelines have been drawn up for both the clinical applications of PSA and the laboratory aspects of its measurement including conditions for sample collection.

## 99

### Thames Regional audit on creatinine clearance

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#### Oral Presentation

Advice from the National Kidney Foundation, and in the draft Renal NSE, 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  $\mu$ 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.

## 100

### Biochemical associations of hypomagnesaemia

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Some biochemical associations of hypomagnesaemia, such as hypocalcaemia and hypokalaemia, are well recognised. However, the association with other electrolyte abnormalities is less well established.

We sought in the current study to characterise the association of hypomagnesaemia with abnormalities of commonly measured electrolytes.

A retrospective computer-based audit was performed of serum magnesium measurements over a 12-month period. Where they had been measured, the results of the following serum analyses were documented in addition: sodium, potassium, bicarbonate, phosphate, calcium. Serum concentrations of magnesium (mmol/L) below the reference interval (0.70-1.00) were divided into bands as follows: 0.6-0.69, 0.50-0.59, 0.40-0.49, 0.30-0.39, 0.20-0.29. For each band of serum magnesium, results of other serum measurements were expressed in terms of median, range and the number of observations. All concentrations were expressed in mmol/L.

In order to facilitate comparison, results for other analytes are provided for serum magnesium within the reference interval of 0.70-1.00.

Magnesium 0.70-1.00: sodium 138 (116-170, n=2467); potassium 4.2 (2.2-9.1, n=2441); bicarbonate 26 (6-50, n=2418); phosphate 1.15 (0.17-3.07, n=2110); calcium 2.12 (1.03-3.85, n=2163).

Magnesium 0.60-0.69: sodium 138 (105-150, n=557); potassium 4.0 (1.8-6.3, n=553); bicarbonate 25 (10-43, n=548); phosphate 1.14 (0.11-3.71, n=491); calcium 2.03 (1.35-2.91, n=506).

Magnesium 0.50-0.59: sodium 138 (108-158, n=261); potassium 3.9 (1.8-5.9, n=258); bicarbonate 24 (5-46, n=260); phosphate 0.66 (0.33-1.46, n=13);

calcium 1.95 (1.36-2.71, n=237).

Magnesium 0.40-0.49: sodium 137 (107-157, n=73); potassium 3.8 (2.2-5.3, n=71); bicarbonate 22 (11-33, n=68); phosphate 1.01 (0.06-2.02, n=63); calcium 1.77 (1.05-2.66, n=67).

Magnesium 0.30-0.39: sodium 131 (104-145, n=17); potassium 3.4 (1.8-4.8, n=17); bicarbonate 23 (11-33, n=17); phosphate 0.53 (0.52-0.63, n=9); calcium 1.40 (1.35-1.60, n=9).

Magnesium 0.20-0.29: sodium 104 (101-122, n=9); potassium 1.9 (1.8-4.8, n=14); bicarbonate 11 (8-13, n=9).

The likelihood of severe electrolyte abnormalities increases at low concentrations of serum magnesium. These data give no indication of causality.

## 101

### An audit of the clinical management of severe dyskalaemia

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Untreated hypokalaemia or hyperkalaemia is associated with high morbidity and mortality. Cases have been reported of inappropriate treatment resulting in fatal outcomes. This audit retrospectively assessed the clinical management of severe hyperkalaemia (>7.0 mmol/L) and hypokalaemia (<2.5 mmol/L) in 2 hospitals.

Twenty cases of hyperkalaemia and twenty cases of hypokalaemia were identified from hospital inpatient laboratory computer records. General guidelines from the literature recommend that hyperkalaemia should be treated with iv calcium gluconate if necessary and 10-20 U insulin in 50 ml of 50% glucose. Calcium gluconate was not given to 2 patients despite showing ECG changes consistent with hyperkalaemia. Calcium resonium was used as the first line treatment in 1 patient and there was an inadequate delay in the treatment of 3 patients, 1 who suffered a myocardial infarction. It was noted that there was a difference between the 2 hospitals in the amount of insulin used, 15-20 U compared to 6-10 U. Adequate monitoring of serum potassium was performed daily and patients underwent ECG monitoring when appropriate.

Hypokalaemia was treated with potassium replacement but was insufficient in the majority of cases, <100 mmol/day in 19 patients and <50 mmol/day in 2 patients. Furthermore, 20 mmol KCl in 5% dextrose, which may further reduce potassium levels, was used for replacement on 3 occasions. The main difference between the 2 hospitals was the use of magnesium reflex testing following a low potassium result, in its absence magnesium was only monitored in 4/10 patients. Potassium levels were not monitored as frequently as in

hyperkalaemia patients with 4 waiting >24 h for a subsequent test and ECG monitoring was not always performed.

This audit shows that severe dyskalaemia was not always well managed and there is scope for the duty biochemist to give advice on management and monitoring and improve clinical outcomes.

## 102

### **Haemolysis in blood samples sent through a vacuum tube delivery system**

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An upgrade to the hospital vacuum tube delivery system resulted in transport pods setting off more promptly and at faster speeds. Soon afterwards it was noted that an increasing number of samples delivered by this system were haemolysed. We investigated the possible causes and solutions to this problem. Various strategies were tried to reduce the incidence of haemolysis including slowing down transit-rates and requesting that the

sample be allowed to clot for at least 5 minutes before transportation. These had limited effects on the number of haemolysed samples. Haemolysis was particularly affecting samples sent by the Accident and Emergency Department (A&E) and it was noted that transport pods containing single samples were more likely to be haemolysed. Possibly single samples were more subject to violent movement whilst in the pod resulting in increased haemolysis. When a pod contained many samples there was less room for the samples to move around. Foam rubber packing was put into some of the pods used by A&E with a request to use these pods when sending single samples. Over a period of 2 months we recorded if samples received in these pods were haemolysed and compared the results with samples received from A&E in the previous 2 months. In the previous two-month period 18.8% of samples received from A&E were haemolysed. With foam packing of the pods haemolysed samples were reduced to 4.6%. We issued foam packing to all vacuum tube terminals with a request to pack all pods when only a few samples are being sent but a more permanent solution is being sought with the manufacturer of the tube system.