Rationalisation of the Biochemistry Laboratory Which way forwards?

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Introduction

The eternal problem faced in service provision is that of coping with an ever-increasing work-load within the relative constraints of keeping costs down to a minimum. The reasons for the increase in the work-load are multiple and include the need to expand the repertoire of tests available if so justified. The justification in medical practice is not only limited to evidence-based proof of the use of medicinals, but is also extendable to the usage of laboratory tests. Regrettably, laboratory usage still appears to be rooted in the mentality that if the test is available, then its request may just be justified in the case that it throws up an abnormal result. This article aims to look at some commonly requested and possibly over-requested tests as well as discusses the value of emerging tests that may well help the rationalisation of other over-stretched medical services.

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To oGTT or not oGTT

The oral glucose tolerance test has seen its usage change with the review in the diagnostic criteria for diabetes mellitus. It is a cumbersome and lengthy test, that is subject, for its accuracy in diagnosis, in the patient remaining on a carbohydrate-rich diet in the days prior to its undertaking.

The oGTT seeks to make a diagnosis using two different criteria; (a) the fasting blood glucose which in some individuals eg obese subjects may be sufficient to diagnose diabetes and (b) the 2 hour value which is more likely to be diagnostic in other individuals (eg the elderly).[1] Current guidelines recommend screening adults over the age of 45 years for diabetes with a fasting blood glucose (FBG) and the oGTT is only indicated in those with FBG between 6.1 and 7.0 mmol/l. The FBG cut-off of 7.0mmol/l was chosen because it corresponds roughly with a 2 hour value of 11.1mmol/l. However nearly one third of Europeans with diagnostic 2 hour values have "normal" (≤6mmol/l) FBGs simply because they are a less obese population than the ones from which the new diagnostic criteria were established.[2] To extend the oGTT in an open-ended fashion to this population group is frankly impossible in the current setting.

The role of the HbA1c as a diagnostic tool has been repeatedly looked at. A meta-analysis of 34 studies confirmed that that while an HbA1c result above the upper reference limit is relatively specific for glucose intolerance,[3] a "normal" value does not exclude it.[4] The HbA1c cannot be used as a screening test in its own right but may be combined with FBG to try and avoid unnecessary oGTTs[5]. In patients pre-selected on the strength of risk factors of obesity, dyslipidaemia, hypertension, previous history of impaired glucose tolerance and a positive family history of diabetes, the authors found that subjects with a FBG ≤6mmol/l and a HbA1c of 5.6% had a sensitivity of 72% and a specificity of 77% to predict a 2 hour plasma glucose level ≥7.8mmol/l.[5]

Liver enzymes – what's normal?

The liver function profile is frequently used to screen for liver disease in patients with non-specific symptoms and is also used as a baseline investigation prior to the commencement of potentially hepatotoxic drug therapy eg statins. The finding of persistent, unexplained mild to moderate increases in liver transaminases is a frequent occurrence raising questions as to what the normal upper reference limits should be.[6] A recent

large-scale survey in the USA showed that the prevalence of elevated ALT is 7.9% of which 69% is unexplained.[7] In both sexes, otherwise unexplained elevated ALT is significantly associated with with higher body mass index, waist circumference, serum triglycerides, fasting insulin and lower HDL-cholesterol, all features of the metabolic syndrome. Patients with non-alcoholic fatty liver disease (NAFLD) show hepatic and peripheral insulin resistance of a degree similar to that observed in diabetes mellitus type 2.[8] Apart from being a marker for NAFLD, serum ALT, but not AST, can also predict the future development of type 2 DM independently of other known risk factors.[9] Men with ALT levels >29U/l at baseline have a 3-fold risk of developing DM compared to those with levels <17U/l. Serum gamma-GT correlates with ALT and is similarly associated with metabolic syndrome and predicts the development of type 2 diabetes mellitus.[10]

PSA – a victim of its own success?

Prostate-specific antigen is the most widely requested among the various tumour markers available. In this age of evidence-based medicine, it becomes extremely relevant to re-evaluate our position as to whether we are using or abusing this diagnostic tool. The number of requests that come through to the biochemistry laboratory has shown phenomenal growth, indicating the increased awareness of prostate cancer as one of the primary cancers affecting men and the general desire to pick this up at as early a stage as possible.

PSA has been around for more than 20 years and when originally described in the New England Journal of Medicine, a 40 to 50% relationship was found to exist between the blood PSA and the size of the cancer. A study spanning 20 years, using data from 1300 prostate tissue samples collected at Stanford University, divided the numbers sampled into four consecutive five-year periods and compared the volume and the grade of cancer with the clinical findings found on digital rectal examination and the blood PSA levels. Over time, the correlation weakened from a predictive ability of 43% in the first 5 years to 2% in the last 5 years.[11]

Given these values, PSA can no longer be considered to have any significant relationship to prostate cancer except for 2% of men. PSA is now related only to benign prostatic hypertrophy for levels of blood PSA between 2 and 10 µg/l and possibly in a lot of cases of PSA levels up to 20µg/l. It is difficult to explain exactly why this phenomenon has happened but it is probably due to over-screening and the removal of all of the big cancers. Also, it is important to realise the incidence of prostate cancer and its ubiquitous nature. 8% of men in their twenties already have cancer cells in their prostate and the numbers grow with each decade until 70% of men in their seventies have prostate cancer. Despite these terrifying figures, the death rate from prostate cancer is 226 per 100,000 men over the age of 65 years.

The low specificity of PSA for prostatic cancer results in a high proportion of unnecessary biopsies. In the absence of alternative and better tumour markers for screening purposes, the use of complexed and free isoforms of PSA may serve as a potential method of increasing the ability to discriminate between those patients with benign disease and those with prostate cancer. In unscreened populations, there are significant differences in the total and complexed PSA concentrations, in the total-to-total PSA ratio and in the free-to-total PSA ratio in patients with prostate cancer compared with those with benign disease. The corresponding specificities are similar. In such a population, the use of PSA isoform concentrations and their ratios does not provide additional discriminatory power. In the subset of patients with total PSA levels between 2 and 10 µg/l, the free-to-total PSA ratio at a cut-off point of 16.5% is superior to total PSA concentration (42 vs. 20%) in discriminating between benign and malignant disease. PSA remains a very good tool for the monitoring of treatment for patients already diagnosed with and treated for prostate cancer by either surgery or irradiation. It is so not on account of its cancer specificity but on account of its prostate specificity.

Remaining on the theme of prostatic carcinoma, the primary prevention of this condition is an attractive and appropriate target because of its incidence, prevalence and disease-related mortality as well as its long latency and molecular pathogenesis. Epidemiological data indicates that a number of environmental factors modify the risk. Selenium and Vitamin E have been shown in SELECT (Selenium and Vitamin E Cancer Prevention Trial) to prevent prostate cancer.[12] Vitamin D may also be preventative and reduced blood levels of active vitamin D result in a higher incidence and a higher mortality rate from prostate cancer. Preclinical work has also demonstrated that vitamin D exerts an antiproloferative effect on prostate cancer cells.[13] Daily use of soy products has also been associated with a 70% reduction in the risk of developing prostate cancer. Lycopene, derived in food-form but not as a pure pharmaceutical agent, is also associated with a lower risk of prostate cancer. Green tea, on account of its polyphenol content, also explains the low incidence of prostate cancer in those Asian men who show a high dietary intake.[14] Unfortunately not all of what is usually considered good is indeed good for prostate cancer. Men with the highest quartile levels for folate and vitamin B12 show odds ratios for prostate cancer risk of 1.6 and 2.3 respectively, compared to subjects in the lowest quartiles.[15]

Cardiovascular disease – drowning under its own labour-intensive work-load!

Biochemical markers for better coronary risk stratification

Despite the intense interest and massive funding into cardiovascular disease, a number of unanswered questions and worrying statistics remain with us even now. Approximately 30% of coronary heart disease (CHD)-related deaths occurs in individuals with no prior indications of the disease, partially because more than 65% of acute myocardial infarctions occur

in vessels with less than 50% stenosis. This accentuates the importance of the identification of biochemical markers for the early diagnosis and treatment of CHD. The standard risk factors e.g. total cholesterol, LDL-cholesterol, HDL-cholesterol, age, gender, etc explain only a small percentage of CHD deaths. Total and LDL-cholesterol are not prerequisites for coronary heart disease. The discovery of new risk factors and causal pathways continue to aid our understanding of atherogenesis. Some novel risk factors for atherosclerosis include homocysteine, fibrinogen, impaired fibrinolysis, platelet reactivity, hypercoagulability, infections, inflammation, antioxidants and oxidation of LDL and body iron.

Considering just LDL factors, univariate analysis indicates that LDL particle size and number are significantly associated with cardiovascular event risk in both men and women. However on multivariate analysis, particle size becomes non-significant and only LDL particle number remains a significant predictor. LDL particle testing should be restricted to high-risk patients in order to optimize their cholesterol profile.[16]

The association of oxidized LDL with atherosclerosis is significant. Patients with symptomatic ischaemic heart disease differ significantly from healthy persons with hypercholesterolaemia by having lower levels of LDL-oxidative potential and LDL-a tocopherol concentrations. No such differences exist in plasma anti-oxidant concentrations as measured by plasma a-tocopherol, plasma retinol and ascorbic acid or in parameters of body iron content e.g. ferritin, transferin, serum iron.[17]

It is important to realize that atherosclerosis is an inflammatory condition and managing inflammation may be just as important as lowering levels of LDL-cholesterol. Various inflammatory markers are now available and include hs-CRP.

CRP is directly involved in atheromatous plaque vulnerability and systemic inflammation is associated with multifocal plaque disruption and the development of acute coronary events. CRP plays a part in plaque build-up and use of the statin drugs in lowering cholesterol also lowers CRP levels, thereby slowing and even reversing atherosclerosis. Patients may achieve target cholesterol levels but if their CRP levels remain elevated, then the amount of benefit derived from statin therapy is limited. In heart disease patients, the goal should be to lower CRP levels to below 2mg/l.[18] Elevated CRP levels are known to be associated with raised concentrations of biochemical markers of endothelial and macrophage activation e.g. cellular adhesion molecules (CAMs) and matrix metalloproteinases (MMPs). These molecules play a pathogenic role in atheromatous plaque vulnerability and rapid coronary stenosis progression.[19]

Biochemistry markers in the diagnosis of ischaemic heart disease

The role of the biochemistry laboratory in cardiovascular disease now extends beyond coronary risk stratification and the retrospective detection of cardiac tissue necrosis by CK-MB and cardiac troponins. It can become an invaluable aid in the diagnosis of ischaemic heart disease and in the differentiation of chest pain of suspected ischaemic origin. Ischaemia-modified albumin (IMA) is one such marker that has the ability to modify the management of chest pain.

Differentiating transient myocardial ischaemia or angina from non-cardiac causes of chest pain is a major diagnostic challenge. Acute ST elevation myocardial infarction (MI) is in most instances picked up through examination of a standard 12 lead electrocardiogram. It is also possible to identify a non-STE MI, and acute coronary syndromes through detection of raised myocardial enzymes. But once either of these two conditions are excluded, chest pain patients cannot be informed whether they are at risk for any acute myocardial event. The inability to identify occult cardiac disease is compounded by the potential for sudden cardiac death in patients with unrecognised significant coronary artery lesions.

The classic approaches of clinical assessment, provocative testing, or advanced imaging studies do not truly meet the practice needs of emergency practitioners due to problems of false negative and positive outcomes [20], limited logistics in arranging such tests or limited access to the medical specialists needed to interpret them.

The IMA assay presents a quantitative accurate laboratory determination of the occurrence of an ischaemic myocardial event; angina pectoris. Unlike previous serum studies that identify myocardial damage after the fact, this test allows emergency physicians to determine which patients have potential coronary artery lesions before occlusion occurs. The IMA assay when combined with the ECG and troponin T demonstrates a 95% sensitivity, a value which matches that of any other diagnostic modality short of coronary artery catheterisation. Considering the potential consequences for missing the presence of clinically significant coronary lesions the introduction of an objective decisions tool for these patients is a welcome aid to even the most confident clinician.[21]

Biochemical markers in the diagnosis and management of congestive heart failure

In congestive heart failure, plasma levels of brain natriuretic peptide (BNP) are useful in differentiating dyspnoea secondary to cardiac dysfunction (BNP levels generally 1000 to 4000 mg/l) from dyspnoea due to pulmonary conditions (BNP levels < 100 mg/l).[22] Patients with congestive heart failure are clinically misdiagnosed 50-75% of the time. BNP is synthesized in the ventricles of the heart and secreted under conditions of myocardial stretch and abnormal wall tension. Use of BNP can help in the triage of patients and can serve as a valuable tool in the early diagnosis and treatment of patients with heart failure. It also optimizes the choice of patients who require echocardiography. Plasma BNP levels fall rapidly with successful anti-heart failure treatments,[23] this being most likely due the reversal of the pathological remodeling process that occurs following neurohormonal blockade.

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