

## Editorial

# The value of accurate analytics in the management of cardiovascular disease!

Accurate measurement of serum cholesterol levels, as well as other analytes that are causally related to the pathogenesis and progression of cardiovascular disease has become of utmost importance for the timely assessment of risk and the monitoring of treatment. If the physician is not cognisant of the possible magnitude of the intra- and inter-laboratory variations in the measurement of an analyte, as well as the potential biological within- and between-individual variations in blood concentrations of the analytes, the application of cut-off levels for diagnosis and treatment goals becomes ludicrous.

LDL cholesterol is regarded as the major risk factor for initial classification of coronary heart disease risk status, based on the Adult Treatment Panel (ATP) guidelines of the National Cholesterol Education Program (NCEP),<sup>1,2</sup> and the lowering of LDL cholesterol has been identified as the primary goal of therapy.<sup>1</sup> The Panel recommends a complete lipoprotein profile (total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides) as the preferred initial test, rather than screening for total cholesterol and HDL alone.

To re-emphasise: the ATP III panel classifies LDL cholesterol concentrations of below 2.6 mmol/l (100 mg/dl) as optimal, 2.6–3.33 mmol/l (100–129 mg/dl) as near optimal/above optimal, 3.36–4.11 mmol/l (130–159 mg/dl) as borderline high, 4.13–4.88 mmol/l (160–189 mg/dl) as high, and concentrations of over 4.91 mmol/l (190 mg/dl) as very high. Total cholesterol concentrations of below 5.17 mmol/l (200 mg/dl) are desirable, 5.17–6.18 mmol/l (200–239 mg/dl) are borderline high, and above 6.21 mmol/l (240 mg/dl) are high. HDL cholesterol concentrations of below 1.03 mmol/l (40 mg/dl) are classified as low and concentrations of above 1.55 mmol/l (60 mg/dl) as high.<sup>1</sup>

Major risk factors (exclusive of LDL cholesterol) that modify LDL goals are:

- cigarette smoking
- hypertension (BP over 140/90 mmHg or on antihypertensive medication)
- low HDL cholesterol (below 1.03 mmol/l); (HDL cholesterol above 1.55 mmol/l counts as a 'negative' risk factor; its presence removes one risk factor from the total count)
- family history of premature coronary heart disease (CHD) (CHD in male first-degree relative under 55 years; CHD in female first-degree relative over 65 years)
- age (men over 45 years; women over 55 years).

It is important to note that in ATP III, diabetes is regarded as a CHD risk equivalent.<sup>1</sup>

The target levels for LDL cholesterol to be applied in the three risk categories defined according to the ATP III algorithm are as follows: CHD and CHD risk equivalents, below 2.6 mmol/l; multiple (2+) risk factors, below 3.36 mmol/l; and zero to one risk factor, below 4.13 mmol/l. Patients with diabetes and concordant cardiovascular disease fall within the highest risk category and statin therapy is to be initiated regardless of baseline LDL-C levels, with a treatment goal of below 1.8 mmol/l (70 mg/dl) where indicated.<sup>3</sup> The American Diabetes Association recommends initiation of statin therapy in diabetics over the age of 40 with total cholesterol concentrations of over 3.5 mmol/l (135 mg/dl), to achieve an LDL reduction of approximately 30% regardless of baseline LDL levels.<sup>4</sup>

Accuracy of measurement (accuracy reflects the true concentration of the analyte) and precision (consistent findings are obtained with repetitive measurements) are of paramount importance for reliable classification of patients and for monitoring therapy. The analytical coefficient of variation (CV) describes the degree of fluctuation (i.e. imprecision) of the measurements, and may be ascribed, among other reasons, to slight differences in volume measurements, variations in instrument function, and between-batch variations in reagents used. An analytical CV of below 4% is probably readily attainable and acceptable.<sup>5</sup> The analytical CV should be less than one-half the average within-subject biological variation.<sup>5</sup> Cholesterol levels fluctuate during the day and the within-subject biological CV for LDL cholesterol is reported to lie within the range of 6 and 11% and averages 8.2%.<sup>6</sup>

Another laboratory error, called bias, is a method-specific constant over- or under-estimation of an analyte. Bachorik<sup>6</sup> defines laboratory bias as 'the average deviation of the measured value from the actual value'. This issue is of particular concern to the physician who must interpret LDL cholesterol measurements done in different laboratories. The NCEP recommends that the total error for LDL-C measurements should be under 12%, which includes an imprecision of up to and including 4% and a bias from the reference method of up to and including 4%.<sup>6</sup>

For diagnosis, treatment and follow-up purposes, reliable baseline levels that truly represent the individual's LDL cholesterol concentrations need to be established. As quoted from the guidelines of the NCEP<sup>6</sup> and depending on whether

two or three serial samples are collected for measurement to establish efficacy of treatment, the following guidelines apply:

- *Three serial samples:* with three serial samples, each referred to the same laboratory and assayed once, and assuming a biological CV ( $CV_b$ ) of 8.2% and an analytical CV ( $CV_a$ ) of 4%, the observed CV for the mean LDL cholesterol value is 5.3%, and the difference between the means of sequential series of three samples should not exceed 14.6%, 95% of the time. The difference between sequential individual values in each series should not exceed 25%. If they are further apart, analytical error or a change in the physiological steady state of the patient should be suspected and another sample may be warranted, depending on the patient's LDL cholesterol concentration and its proximity to the concentrations used for decision-making.
- *Two serial samples:* for reasons of convenience and considering economic factors, the ATP II report recommended the use of at least two serial samples. With two serial samples, each referred to the same laboratory and assayed once, and assuming a  $CV_b$  of 8.2% and a  $CV_a$  of 4%, the observed CV for the mean LDL cholesterol value is 6.5%. The difference between the means of each sequential series should not exceed 17.9%. The difference between individual values in each series should not exceed 25%, 95% of the time. If they are farther apart, analytical error or a change in the physiological status of the patient should be suspected. Another sample may be warranted depending on the patient's LDL cholesterol concentration and its proximity to the concentrations used for decision-making.<sup>6</sup>

The NCEP proposes that '... with two serial measurements and considering a cut-off point of 3.36 mmol/l (130 mg/dl), a patient's LDL cholesterol can be confidently assumed to be above or below the cut-off point when the mean value is below 3.75 mmol/l or above 2.97 mmol/l (> 145 mg/dl or < 115 mg/dl), respectively'. The same applies when a cut-off point of 4.13 mmol/l (160 mg/dl) is used and the mean value is above 4.60 mmol/l or below 3.67 mmol/l (> 178 mg/dl or < 142 mg/dl).<sup>6</sup>

Variation in pre-analytical procedures, for example, seasonal and postural changes, use of tourniquet, and serum-versus-plasma collection, may also affect cholesterol and other lipid values significantly. This is evident from a study that assessed the effect of variation in the pre-analytic procedures, on the estimation of population distribution of total cholesterol and the prevalence of hypercholesterolaemia.<sup>7</sup> Taking the population distribution of total cholesterol into account, differences in pre-analytic procedures was reported to explain a difference of up to 1.12 mmol/l in the mean total cholesterol between populations, and 41% of the difference in the prevalence of hypercholesterolaemia ( $\geq 6.5$  mmol/l). Standardisation and training are advocated to reduce these differences.<sup>7</sup>

At the annual Roche Diagnostics forum in Johannesburg, October 2005, Robert M Kisabeth, MD of Mayo Medical Laboratories, Rochester, Minnesota, reported on case studies performed by their laboratory as well as other laboratories, starting with what they regarded 'critical analytes'. Among

these were total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol.

It was astounding to observe the magnitude of inter-assay variation and the inter-laboratory variation for control samples with given values as well as for selected patient samples. Dr George Klee of the Mayo Clinic reportedly performed an experiment in which he evaluated the effect of varying amounts of bias on the number of patients with cholesterol values exceeding the cut-off point. A change in bias of 1% resulted in a 5% change in the number of patients exceeding the 200-mg/dl cut-off. A change in bias of 3% resulted in a corresponding change of 15%.

Comparisons of measurements obtained with repeated determinations of specific patient samples for total cholesterol using different methods and apparatuses served to illustrate the fact that methods are by no means interchangeable. According to Dr Kisabeth, greater emphasis is generally placed on precision in most laboratories and not on accuracy. Clinicians appear to be oblivious of these facts. Their primary concern is to get actionable answers and they are, furthermore, not concerned about 'decimal points'. His plea is that accuracy be emphasised more strongly, and that methods be standardised to promote inter-changeability among methods.

The development of protocol-driven diagnostic and management pathways and the use of a multidisciplinary approach in primary and secondary healthcare are becoming increasingly evident. The NCEP guidelines serve as an example, with specific reference to the guidelines concerning 'adherence to LDL-lowering therapy', as quoted:

'Adherence to the ATP III guidelines by both patients and providers is a key to approximating the magnitude of the benefits demonstrated in clinical trials of cholesterol lowering. Adherence issues have to be addressed in order to attain the highest possible levels of CHD risk reduction. Therefore ATP III recommends the use of state-of-the-art multidisciplinary methods targeting the patient, providers, and health delivery systems to achieve the full population effectiveness of the guidelines for primary and secondary prevention.'

In his presentation 'Diagnostics in primary healthcare' at the annual Roche Diagnostics forum in Johannesburg in October 2005, Dr PO Collinson of the Department of Chemical Pathology and Cardiology, St George's Hospital and Medical School, London, UK eluded to protocol-driven diagnostics, as well as the fact that in the near future non-traditional users will have access to diagnostics. This requires a team-based approach to education and training, with the physician adopting a leadership or managerial role. This probably places greater demand on laboratory proficiency.

According to Dr Collinson, the drivers of change in the provision of diagnostic services are identified as being: accessibility, quality assurance and affordability. Models of healthcare delivery will be changing from traditional hospital-focused care biased towards hospital-based acute care, to community-focused care biased towards chronic care. Nurse practitioners, primary-care physicians and outpatient specialists will be mainly responsible for administering care, the reasons being a shortage of clinicians, an ageing patient population that is fitter, lives longer and has more chronic

disease, and the greater expectations being expressed by patients.

Accessibility implies physical as well as financial access and the assumption is that the patient will expect one-stop diagnosis, as well as immediate diagnosis and/or management decision. Laboratory options for testing include central laboratories, satellite laboratories, and point-of-care testing (POCT). The obvious implications concern test ranges, unit test costs and convenience, although technological changes appear to be blurring these boundaries.

Timely diagnosis is of utmost importance, especially where disease progression is rapid, where the condition is life threatening, and where the cost of treatment is expected to escalate disproportionately without timely intervention. Laboratory turn-around time could severely affect the personal lives of individuals as well as the economical burden of the disease. Fast turn-around time (TAT) should convert to significant clinical benefit for the patient and if TAT is important, for example, for cardiac enzymes, POCT greatly reduces TAT.

The decision on the most appropriate model of delivery of diagnostic service depends on an overview of the total process of care:

- is access a problem?
- is speed important?
- what level of diagnostic service is required?

Quality assurance is vital and in many instances 'an inaccurate test result is worse than no result'. Both false-positive and false-negative test results have serious implications, especially in terms of cost. Most laboratories have well-developed quality assurance protocols and schemes, and have been accredited. Dr Collinson suggests that a minimum level of acceptable analytical accuracy can be defined on the basis of biological variation. 'Tests must be good enough for purpose, not perfect.'

The final driver of change in the provision of diagnostic services is affordability. Although test costs are an insignificant part of the total healthcare costs for a patient, judicious use of testing does save money. Dr Collinson maintains that the focus should be on disease management, and in this respect, CHD should be regarded as a chronic disease that has acute manifestations, but is still a chronic condition that takes decades to develop and has to be managed appropriately.

Quality specifications for the reliability of performance characteristics of laboratory testing, particularly precision and bias, are necessary prerequisites for the creation and control of analytical quality. Many strategies have been promulgated for setting these specifications. Recently, the available approaches have been fixed into a hierarchical framework that has now been accepted by experts in the field to be the best current approach to a global strategy for setting quality specifications in laboratory medicine. They should be incorporated into quality planning strategies everywhere, irrespective of the settings in which laboratory medicine is practised, including the point-of-care testing. Models higher

in the hierarchy are preferred to lower approaches but lower approaches are better than none and should be used as the minimum standard.

## Conclusion

To summarise, the accuracy of laboratory measurement in cardiovascular disease management needs to be optimised within the constraints of cost and circumstance to ensure effective management of the disease within the individual as well as within the South African population as a whole. The stringent treatment goals for LDL cholesterol can only be applied if the analytical goals are maintained, as an increase in analytical CV will inadvertently impact on the individual's biological CV and this may compromise effective treatment. Accreditation of diagnostic medical laboratories helps ensure adherence to standards/guidelines for acceptable laboratory practice and external and internal proficiency testing can be used as a measure of an individual laboratory's performance. It may be advisable to include information on the analytical CV within the specific concentration range in which the patient's cholesterol levels were reported in the laboratory report.

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