The laboratory assessment of lipid disorders

Lipid guidelines developed by several professional organisations and bodies serve to simplify and standardise the clinical approach to and management of patients at risk for CHD.

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All updated lipid guidelines published so far focus on the concept of 'global' or 'total' risk assessment of coronary heart disease (CHD) risk.[1] Traditionally, risk-factor guidelines have been concerned with unifactorial assessment — in the management of hypertension or hyperlipidaemia — and this has resulted in undue emphasis being placed on individual risk factors. In practice, physicians deal with the whole patient rather than one aspect of his or her risk and as clusters of risk factors may have a multiplicative effect, an individual with a number of modest risk factors may be at considerably greater risk than a subject with one very high risk factor. Clinical laboratories play an indispensable role in this integrated assessment and in monitoring the efficacy of any intervention.

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Hyperlipidaemia is the most common biochemical abnormality that requires laboratory investigations and monitoring.[2] There are also emerging risk factors that are receiving increasing research attention such as hyperhomocysteinaemia, clotting factor abnormalities, infectious agents and inflammatory markers such as C-reactive protein (CRP) that will impact on laboratory investigations and the role of the laboratory in the management of these disorders and the complications associated with them.[3,4] These emerging risk factors fall outside the scope of this review and the focus and discussion will be on dyslipidaemia.

LABORATORY APPROACH TO SUSPECTED LIPID DISORDERS

The laboratory approach to any patient with an abnormality must be conducted in a logical progression. The following three clinicopathological questions guide the diagnostic work-up of the patient:

- Is there a true hyperlipidaemia? Is the degree of abnormality contributing significantly to the patient's total risk and will the patient benefit materially by lowering the cholesterol ?
- Is the cause primary or secondary? These secondary causes should be screened by measuring thyroid-stimulating hormone (TSH), glucose, liver enzymes, and kidney function. They should be treated if possible, prior to initiating drug therapy for lipid disorders.
- What is the nature of this abnormality? The lipid abnormality may manifest itself either as a predominantly high cholesterol abnormality or at the other end as a predominantly high triglyceride problem. There are also cases where the patient suffers from a mixed or combined dyslipidaemia where both triglycerides (TGs) and total cholesterol (TC) are elevated.

IS THERE A TRUE HYPERLIPIDAEMIA?

To answer this question a number of issues require a closer inspection, e.g. how do we define hyperlipidaemia and more specifically the issues of cut-off points or reference ranges, intra-individual or biological variations as well as analytical and methodological variations, the standardisation of assays and lastly, which patients should be targeted for lipid screening.

Hyperlipidaemia and dyslipidaemia

Hyperlipidaemia is the term used to embrace the conditions that result in raised serum levels of one or more of TC, low-density lipoprotein cholesterol (LDLC), or TGs, or both TC and TGs. It is, however, not an easy task to define what is meant by 'normal', or low or high, or what the various action limits should be for a number of reasons. The long exposure period to the lipid abnormality before the disease becomes clinically manifest and the high prevalence of lipid disorder in the general population defy the use of statistical methods to establish reference ranges.

The alternative approach is to study the relationship between CHD risk and the serum concentrations of TC and LDLC in a population. Most of these investigations produced a curvilinear graph and epidemiologists identified three segments on this graph based on the steepness of the gradient, i.e. desirable, borderline-high risk and high risk for CHD.[5] These curvilinear graphs only apply to serum TC and LDLC. A serum cholesterol of <5.2 mmol/l for TC, and 3.4 mmol/l for LDLC were deemed desirable; a value between 5.2 and 6.2 mmol/l as borderline-high for TC and >6.2 mmol/l for TC as high risk. The corresponding cut-off points for LDLC are 3.4 - 4.1 mmol/l (borderline - high) and >4.1 mmol/l (high risk).

These cut-off points became firmly entrenched in the minds of the public and medical fraternity during the 'era of unifactorial assessments' and even today it is not unusual to find many patients who have baseline or native TC levels of slightly over 6.0 mmol/l and with no other risk factor for CHD, but who are on statin therapy in an attempt to drive their serum TC below 5 mmol/l! This approach is inherently flawed or even dangerous since it can lead to over-treatment or inappropriate treatment of individuals who are not at risk, or otherwise under-treatment of patients who are truly at high risk and who may require a far more aggressive approach to bring their TC concentration to levels well below 5 mmol/l. The fallacy of treating the TC cut-off points of 5.2 and 6.2 mmol/l as absolute risk cut points regardless of the clinical risk profile is further illustrated by several studies which found that as many as 20% of men with confirmed CHD have serum TC <5.2 mmol/l.[6] Most of these subjects with almost desirable TC levels, however, had low HDLC levels (<0.9 mmol/l). It is for this reason that the term 'dyslipidaemia' is regarded as more appropriate than 'hyperlipidaemia', although many use it interchangeably. The aforementioned realisation of the limitations and inappropriate use of the proposed cholesterol cut points prompted researchers to redefine the concept of hyperlipidaemia or dyslipidaemia.

A 'contextualised' definition of abnormal serum cholesterol or hyperlipidaemia

The integrated risk approach which is currently emphasised by all guidelines[1] has provided the background and platform to define abnormal or undesirable cholesterol levels. This operational definition is required when assessing the need for lipid lowering therapy and states that dyslipidaemia is present when serum TC is at least 5 mmol/l, or LDLC at least 3 mmol/l or HDLC <0.9 mmol/l *and* the 10-year risk of developing CHD is >30%.[7] The essence of this definition is that the lipid values should be judged and acted upon against the clinical background and the total risk estimate for CHD.

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Global risk assessment tools

The assessment of the 10-year CHD risk is done by using one of several risk assessment tools (See Website addresses, Table I). The different risk assessment tools require slightly different sets of data, but most include the following: gender, age (years), smoking status, presence or absence of diabetes, blood pressure (mmHg), TC (mmol/l), HDLC (mmol/l), presence or absence of ECG evidence of left ventricular hypertrophy.

The role of other lipid and lipoprotein fractions in the integrated assessment of the biochemical risk profile

Serum HDLC

The risk associated with high TC is primarily due to high levels of LDLC but there is a strong, independent and inverse association between HDLC and CHD risk.[8] Low HDLC levels increase the risk for CHD, even when TC is below 5.2 mmol/l, a pattern present in up to 20% of men with confirmed CHD.[6] In many studies, measures of HDLC or the ratio of TC/HDLC are better predictors of CHD risk than serum TC alone.[9]

Table I. Website addresses

CHD risk assessment tool	Source
Joint British recommendations coronary risk prediction chart	View http:/www.hyp.ac.uk/bhs/resources prediction chart.htm Download http://www.bhaonline.org.uk/download/crpc.doc
Joint British recommendations coronary risk prediction calculator	Download http://www.bnf.org/CalculatorHome,htm or http://www.hyp.ac.uk/bhs/resources guidelines.htm
Sheffield table for primary prevention of CHD (3rd edition, corrected)	View http://bmj.com/content/vol320/issue 7236/images/large/wale.3599.fl.jpeg
Framingham function for risk estimation	Online calculator http://www.cardiocrisk,org.uk/ (hint, look at the bottom of the screen and click on the numbers 1,2,3,4,5)
New Zealand tables for absolute 5-year risk of a cardiovascular event	View http://cebm.jr2.ox.ac.uk/docs/prognosis,html
A suite of algorithms and risk prediction tools	Download http://www.medal.org
UKPDS risk engine: risk in people with type 2 diabetes	Download http://www.dtu.ox.ac.uk/
Indana risk calculator: risk in people with hypertension	Download http://www.riskscore.org.uk

Serum triglycerides

The importance of TGs has remained uncertain for a long time. In a meta-analysis, but not always in individual studies, increased TG values have been shown to be an independent CHD risk factor, especially in women.[10] Increased TG values are correlated with decreased HDLC values. The combination of high TG values and low HDLC values often occurs in association with other CHD risk factors such as hypertension, diabetes mellitus or milder forms of insulin resistance.[11] This forms part of what has become known as syndrome X or plurimetabolic syndrome. The National Cholesterol Education Program (NCEP) Adult Treatment Panel II suggests that a serum TG level of between 2.3 - 4.5 mmol/l is compatible with a borderline to high risk for CHD.[12] More recent epidemiological data suggest that a fasting TG level >1.7 mmol/l already denotes a borderline to high-risk value.[13]

Serum Lp(a)

An increased Lp(a) value is likely to be a significant independent CHD risk factor.[14] This concept is supported by many case control studies and 9 of 13 prospective studies.[15] Problems associated with Lp(a) measurement include lack of assay standardisation and considerable heterogeneity within the protein.[16] When calibrated to total mass of the particle, an Lp(a) value of >40 mg/dl is clearly above the 90th percentile, but many authorities feel that a value >30 mg/dl is already a high-risk value.[17] Familial Lp(a) excess (>90th percentile) is a highly heritable disorder found in 15 - 20% of kindreds with premature CHD. Niacin administration has been shown to lower Lp(a) levels as well as to reduce CHD morbidity and mortality. For this reason certain authors recommend that Lp(a) levels be measured in patients who are candidates for drug therapy, especially those with CHD and a strong family history of CHD.[18]

Serum apolipoproteins

In some studies low Apo A1 values and increased ApoB concentrations as well as smaller LDL particles have been reported to be superior to HDLC and LDLC as markers for CHD.[19] Others, again, have found that particle size is not an independent risk factor after controlling for the effect of β-blockers and LDLC and HDLC concentrations.[20] Because of cost considerations these determinations cannot be recommended for CHD risk assessment at this stage. More data from large-scale prospective studies

are required before this parameter can be included in the biochemical risk profile assessment.

Who should be investigated for hyperlipidaemia?

It is not worth while to measure cholesterol concentrations in people whose risk is less than 15% over 10 years. This statement is based on the assumption that these individuals have average cholesterol values. Testing for dyslipidaemia should be targeted at those groups at the greatest risk so that candidates for treatment can be efficiently identified.

What variables can influence the validity of the results?

Accurate determination of serum lipids and lipoproteins is dependent on control of both analytical and pre-analytical factors. Pre-analytical variation in subjects results from differences in lifestyle, altered metabolism due to disease, the source of the specimen and the conditions of sample collection. Variation can arise from biological, behavioural and clinical factors as well as variability in specimen collection and handling. (See Table II for recommendations for minimising pre-analytical variation).

Serum TC is the most stable lipid analyte. Between days biological variation averages 6.1%, although some individuals may vary up to 11%. There is no diurnal variation and the seasonal variation is no more than 2.5%.[21] In women TC may fluctuate during the menstrual cycle, averaging 10 - 20% lower in the luteal and menstrual phases. For the average woman, TC levels increase by about 0.31 mmol/l around ovulation and fall to a nadir during menstruation.[21]

TG levels show marked intra-individual variation. Even excluding the marked postprandial fluctuation, fasting TGs differ by an average of 23% over one or more months, and in some persons may fluctuate as much as 40% around the mean value.[22] The average diurnal variation is ± 30% with the nadir at approximately 03:00 and the peak values in mid-afternoon. Because of this marked random variation, it is difficult to assess seasonal, menstrual and other fluctuations of TGs.[23]

HDLC levels show an average intra-individual variation of 7% over one month to a year, but seldom differ by more than 12%. Seasonal variation in HDLC is comparable with that of TC.[24]

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Whether LDLC is measured directly or derived from measurements of TC, HDLC and TG, biological variability is similar at an average of 9.5%.

Lp(a) shows an average biological variation of 8.6%.[18]

The impact of lifestyle elements on lipid, lipoprotein and apolipoprotein levels

The major behavioural factors that exert an effect on these parameters are: diet, obesity, cigarette smoking, alcohol and caffeine intake, exercise and stress. Since these lifestyle elements are controllable, it is imperative that subjects maintain their behaviour for several days before blood specimens for lipid testing are obtained. The effect of food ingestion on lipid measurements has long been established. A controlled dietary study showed that ingestion of a typical fat-containing meal caused a significant increase in TGs that persisted for at least 9 hours.[25] VLDLC also increases while LDLC falls significantly. Both changes persist for at least 9 hours.

The duration of fasting has profound effects on TC and TG levels. TC and TGs increase an average of 25% after 1 week of fasting, falling to baseline after 3 weeks. Refeeding causes a 13% fall in TC but an 86% increase in TGs.[26]

Anticoagulants and preservatives

Current recommendations for cut-off points are based on ordinary serum samples (red top vacuum tubes). Because of its osmotic effect, EDTA causes a method-dependent artefactual fall in most lipid and lipoprotein concentrations, but a paradoxical rise in HDLC.[27] The average difference between serum and plasma using tubes with EDTA is now close to 5% because of increases in the content of EDTA in the blood collection tubes by the manufacturers.[28] Use of other anticoagulants such as oxalate and citrate is associated with even greater osmotic fluid shifts. Heparin does not produce fluid shifts and appears to be an acceptable alternative for TC measurement.[29] However, it activates lipoprotein lipase both *in vitro* and *in vivo*. Most serum separator tubes do not appear to affect the results of lipid measurement and are gaining favour as they are more convenient for routine chemistry laboratories.

Capillary versus venous blood

Conflicting results have been obtained from lower TC levels in capillary blood, to similar results to higher values.[30] The use of a standardised protocol for collecting capillary samples can produce results that agree closely with venous plasma.

Haemoconcentration

A patient who stands for 5 minutes will experience an apparent increase in lipid concentration of 9% and a further increase to 16%

Table II. Recommendations for minimising pre-analytical variation

- Lipid and lipoprotein profile should only be measured when the individual is in a steady metabolic state.
- Subjects should maintain their usual diet and weight for at least 2 weeks prior to the determination of their lipids or lipoproteins.
- Multiple measurements should be performed within 2 months, at least 1 week apart, before making a medical decision about further action.
- Subjects should not perform vigorous physical activity during the 24 h prior to testing.
- Fasting or non-fasting specimens can be used for TC testing. However, a 12-h fasting specimen is required for TG and recommended for lipoproteins.
- The subject should be seated for at least 5 min before specimen collection.
- The tourniquet should not be kept on more than 1 min during venepuncture.
- TC, TG, and HDL-C concentrations can be determined in either serum or plasma. When EDTA is used as the anticoagulant, plasma should be immediately cooled to 2 - 4°C to prevent changes in composition and values should be multiplied by 1.03.
- For TC testing, serum can be transported either at 4°C or frozen. Storage of specimens at –20°C is adequate for TC measurement. However, specimens must be stored frozen at –70°C or lower for TG and lipoprotein/apolipoprotein testing.
- All blood specimens should be considered potentially infectious and handled accordingly.

after 15 minutes.[31] This can be minimised by seating a patient for at least 15 minutes before venipuncture. A tourniquet can also cause significant haemoconcentration. After 1 minute there is no significant change in TC or protein concentrations. However after 2 minutes the apparent TC concentration increases up to 5% and after 5 minutes apparent increases of 10 - 15% can occur. If a tourniquet remains for 15 minutes during phlebotomy, lipid measurements increase by 20 - 40%.[32]

Specimen storage

Specimens for most lipid testing are stable at 0 - 4°C. Serum does not fully freeze until about -40°C.[33] ApoB can decrease slightly when frozen, but other lipid components appear stable when frozen for up to 6 months.

Analytical considerations

TC measurements

In 1988 the laboratory standardisation panel of the NCEP released its report on the status of the measurement of serum TC in the USA.[34] For the first time guidelines were established for the accuracy and precision of serum TC determinations. As of 1992 laboratories were expected to be able to attain TC values that are within 3% of the 'true value' as defined by a definitive method, and to have a total imprecision (CV) of < 3%.[33] These comparatively tight standards were established in order to minimise the possibility of inappropriately reporting a TC value. For example, if the total imprecision were 10% a specimen in the range of 5.2 could be reported as 6.2 and, conversely, a high TC level could be reported as acceptable. Both possibilities have their own set of undesirable consequences.

A patient who stands for 5 minutes will experience an apparent increase in lipid concentration of 9% and a further increase to 16% after 15 minutes.

Measurements of TGs

At this stage, no true definitive or reference method has been established for the measurement of TGs.[34] Due to the broad biological variability of TGs, the accuracy of measurement has not been of overwhelming importance in the clinical laboratory, except for the calculation of the LDLC by the use of the Friedewald equation. With the emergence of direct measurements of LDLC the value of the measurement of TGs may be lessened further. It should be borne in mind that the Friedewald formula cannot be used for values obtained on serum from non-fasting individuals or those whose TGs values exceed 4.5 mmol/l. Moreover, there is considerable variability in calculated LDLC concentrations when TGs concentrations are 2.3 - 4.5 mmol/l as compared with values obtained by ultracentrifugation.[35]

HDLC measurements

The importance of accurate HDLC measurements was emphasised by the following statement: 'One has to be able to measure accurately in the range below 1.30 mmol/l as a difference of less than 0.13 mmol/l is used to define an individual's risk.'[36] The NCEP has set the following analytical goals for HDLC measurements: precision <4%; accuracy <5% and total allowable error <13%.

Because such important treatment decisions are made on individual

lipid levels it becomes extremely important to have rigorous control over as many of the aforementioned variables as possible. All laboratories or phlebotomy points should have standard operating procedures in place to minimise the variability. Accuracy and repeatability of measurements are equally important. Clinical laboratories should ideally state their accuracy and precision with each lipid report produced on such patients so that dose response observations and adjustments can be made on credible and reliable laboratory data.

What laboratory tests should be performed on patients suspected of having hyperlipidaemia?

Initial tests. Non-fasting serum total cholesterol and HDL cholesterol (which are practically unaffected by meals).

Tests prior to treatment. Fasting lipid profile, including TG level. Serum lipid measurements are subject to biological variation and ideally require at least 3 measurements to assess their true mean levels. Levels of TG are raised by fat in recent meals and this invalidates the calculation of LDL levels.

- **Baseline tests.** Creatine kinase (CK) and aspartate aminotransferase (AST) or alanine aminotransferase (ALT) tests should be done before starting treatment with a statin or a fibrate.
- **Every 8 - 12 weeks while titrating therapy (statin or fibrate).** Fasting lipid profile, AST or ALT, CK.
- **Every year while on stable therapy (statin or fibrate).** Fasting lipid profile, AST or ALT, CK.

The frequency of metabolic monitoring should be increased in renal impairment and when a statin and a fibrate are co-prescribed.

IS THE DISORDER PRIMARY OR SECONDARY ?

Once a patient has been confirmed as having an abnormal lipid profile, secondary underlying causes of dyslipidaemia need to be excluded. The confirmation of hyperlipidaemia requires at least 3 measurements to assess their true mean values.

The following routine laboratory tests are indicated:

- fasting plasma glucose
- liver function tests
- renal function tests
- thyroid stimulating hormone
- muscle enzyme: creatinine kinase.

See also the article on secondary dyslipidaemias, p. 365 of this issue.

AETIOLOGICAL DIAGNOSIS OF DYSLIPIDAEMIA

The classification most suited to clinical practice is discussed in the article on the clinical approach to dyslipidaemia (p. 370 of this issue). It is based on the measurement of the 2 common lipids, defining the derangements as hypercholesterolaemia, hypertrigliceridaemia or mixed hypertrigliceridaemia.The measurement of HDLC and LDLC further defines the atherogenic risk. An exact aetiological diagnosis is not possible for the bulk of moderate hyperlipidaemias but specific diagnoses are of great importance in the presence of extremes of dyslipidaemia, very premature cardiovascular disease or tendinomas or cutaneous xanthomata. In many of these settings genetic counselling is required and special investigations may be performed at tertiary clinics.

References available on request.

IN A NUTSHELL

The older 'traditional' lipid guidelines have placed undue emphasis on individually high-risk factors such as cholesterol. The aim of the TC cut-off points of 5.2 mmol/l and 6.2 mmol/l was to identify as many subjects as possible who have borderline to high risk and high risk for CHD respectively. This approach created the impression that all subjects with serum cholesterol greater than 5.2 or 6.2 mmol/l are at a higher or high risk for CHD and it ignored the fact that a cluster of coexisting, mildly abnormal risk factors may confer a much higher risk on an individual than only one very high risk factor.

The latest guidelines suggest that these cut-off points should no longer be interpreted in isolation but rather against the background of the global CHD risk of a patient. This will avoid unnecessary treatment of patients who are not really at risk or alternatively identify patients who require aggressive lipid-lowering therapy even though their serum TC or LDLC may be only moderately elevated or may indeed be within the so-called desirable range.

The repertoire of laboratory investigations to fully assess the patient's total risk for CHD needs to be done in a logical progression in order to answer the following 5 crucial clinical questions:

- Which patients should be targeted for lipid screening?
- What variables can influence the validity of the results that need to be controlled?
- What laboratory tests should be done on them?
- What further laboratory tests are required in patients whose screening results justify possible intervention?
- What laboratory tests are indicated in monitoring patients who receive lipid-lowering drugs?

The subsequent management of the dyslipidaemic patient will depend on the nature and severity of the disorder, i.e. predominant hypercholesterolaemia, predominant hypertriglyceridaemia or a combined disorder.

More complex disorders such as type III (broad B band disease) and atypical presentations or clinical syndromes should be referred to specialist lipid clinics for further investigations.

Lipid guidelines and risk assessment algorithms should be used to supplement, not replace clinical judgement.