Discussion Document

Recommendations for Lipid Testing and Reporting by Australian Pathology Laboratories

Australian Pathology Lipid Interest Group

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Abstract

The importance of measuring blood lipids in determining the absolute risk of a cardiovascular event is now well established. In Australia, the National Heart Foundation of Australia and the Cardiac Society of Asutralia and New Zealand (NHFA/CSANZ) have done much to educate doctors. In recent years the recommendations of the NHFA/CSANZ have been based on values for Low-density lipoprotein (LDL-C) as well as High-density lipoprotein cholesterol (HDL-C) and Triglyceride (TG). This change has been reflected in requests to pathology laboratories. However the interpretation of these results may be difficult and the NHFA guidelines outline desirable values for patients at high risk only. There are no formal recommendations for reference intervals or interpretive comments. With the availability of expert systems, some pathology laboratories are now in a better position to provide specific comments to assist with the interpretation of test results.

An ad hoc committee of private and public chemical pathologists met to draft recommendations for lipid testing and reporting by Australian pathology providers, on the basis of current guidelines and their own expertise. Provisions in the current Medicare Benefits Schedule (MBS) for lipid testing were reviewed, and the indications for lipid testing, recommended tests, the logistics of managing specimens, methods of analysis and availability of specialised tests have been documented. Recommendations are made on the provision of desirable values for lipid tests. Suggestions are provided on interpretive comments which could accompany reports of lipid test results, including categorisation of the likely associated lipoprotein abnormalities, their causes, contribution to risk for cardiovascular disease (CVD) and targets for treatment. Current and future approaches to the assessment of risk for CVD are discussed.

Note: These recommendations are published to encourage lipid testing and reporting in Australia to be more closely aligned to the NHFA/CSANZ Position Statement on Lipid Management and the PBS Criteria for eligibility for lipid-lowering therapy. Comments are sought, particularly from pathologists and laboratory scientists . The recommendations will then be reviewed and endorsement sought from the relevant professional bodies. It is proposed that the final document will be published as Guidelines for lipid testing and reporting by Australian pathology laboratories and supported with a doctor education program.

Introduction

CVD remains the leading cause of death in Australia as well as a significant burden on the healthcare budget.[1] Abnormalities in lipid metabolism are now established as a major treatable risk factor to reduce CVD progression and ultimately reduce morbidity and mortality for people who are at high risk of events.

Successive Lipid Management Guidelines have been published by the National Heart Foundation of Australia and the Cardiac Society of Australia and New Zealand (NHFA/CSANZ), in 2001[2] and most recently in 2005.[3] The 2005 position statement is an update based on new epidemiological data and recent publications of major clinical trials. The main themes from the 2005 Update are:

1) a greater focus on vascular prognosis in terms of absolute risk (i.e. population numbers suffering an event within a specified period) as opposed to relative risk (i.e. percentage increase in risk in comparison to a healthy individual of the same age) and
2) a greater emphasis on LDL-C and HDL-C rather than total cholesterol (TC).

The 2001 Lipid Management Guidelines and the December 2005 Update deal comprehensively with lipid targets in patients considered to be at high risk of CVD. Unfortunately, these publications do not give recommendations concerning advice on lipid reporting by pathology laboratories.

An ad hoc committee of both private and public pathologists (Australian Pathology Lipid Interest Group) was convened to meet and discuss this topic more broadly. The group had representation from the three major private pathology providers in Australia which are responsible for most lipid testing in the community. The process was commenced in January 2006.

The availability of expert commenting systems has allowed pathology laboratories to provide the medical practitioner with comments on the report based on the patient's individual results. Employing the concept of best practice it was agreed that the pathology laboratories should aim to become better standardised in their reporting of lipid results following the precedent that was set with the criteria for the diagnosis of diabetes in Australia.

In the case of lipid results, formal recommendations for reference intervals and appropriate comments are lacking. Thus, as an action from the meeting it was agreed to write a set of recommendations with the view of addressing this gap.

Accuracy and consistency are considered to be of paramount importance for pathology testing laboratories. All laboratories are required to participate in a quality assurance program as part of their registration as approved pathology laboratories. Laboratories are also accredited through the medical testing program provided jointly by the National Association of Testing Authorities and the Royal College of Pathologists of Australasia, with face to face assessments on a regular basis. These processes all help to ensure that lipid and lipoprotein testing is performed by Australian pathology laboratories at an accepted standard.

Whilst the measurement of these values is likely to be highly accurate and precise, common questions for these pathology providers include:-

- what are the most appropriate reference limits for these tests; and
- what are appropriate and useful comments which could be provided to the clinicians to assist with the management of their patients?

The aims of these recommendations are as follows:-

- to suggest broad guidelines about the testing of lipids, logistics and handling of specimens and methods of analysis;
- to outline reference limits for both high risk and low risk patients; and
- to present a list of comments for both (a) common lipid profiles and (b) rare lipid profiles that require specific attention. These, or similar comments may then be considered for inclusion in lipid pathology reports, including incorporation into the various expert commenting systems that are already in place in major pathology providers.

Currently in Australia

MBS for Lipid Testing

Serum or plasma TC and TG form part of the basic biochemistry item number, 66500, a group including 26 common biochemical tests as well as several less frequently ordered analytes. Medicare provides a refund for a maximum of 6 tests from this item number in any one day.

A clinician may order these on a patient with a specific request using approved terms or abbreviations such as "CHOL" and "TRIG", "FATS" or "Lipid studies". On such a request, it is not legal for a laboratory to bill Medicare for any other lipid-related test. That is, if a doctor requires cholesterol fractions as well, he must specifically order HDL-C. Requests for "lipid fractions, lipid studies, lipid profile, lipid risk factors", etc are not considered legal requests for HDL-C. (See PATHOLOGY SERVICES CATEGORY 6, page 617

section PQ.4 of the MBS Book Effective 1 November 2006.)

However, it seems that clinicians have understood the importance of reviewing all lipid fractions. According to some laboratories, full lipid profiles including HDL-C are requested by clinicians in over 88% of cases (personal communication). It is not possible to quantify these numbers as Medicare does not have the required data. It only retains the number of tests reimbursed rather than the number of tests requested.

Since the MBS revision of 1 November, 2001, there has been no limit to the number of HDL-C tests for which the laboratory may bill Medicare in any 12 month period, nor has there been a restriction based on other lipid test results, the patient's clinical history or drug therapy.

At present, there is no MBS Item for the reimbursement of LDL-C, whether calculated using the Friedewald formula (see later) or directly assayed.

Since the MBS revision of 2001, assay and reporting of apolipoprotein A-I and/or B (or related information such as the ratio) has not been listed. This would suggest that the apolipoproteins no longer attract a Medicare reimbursement. However review of the Pathology Services Category 6 index continues to list Apolipoprotein B/A-I ratio as refundable under item number 66536 (the HDL-C item number in which it was formerly included). Most laboratories appear to continue to bill for this request under this item number. If a doctor requests both HDL-C and apolipoprotein B/A-I ratio, Medicare will reimburse only one claim under Item 66536.

Lipoprotein electrophoresis continues to attract reimbursement from Medicare but currently this test is rarely requested.

Apolipoprotein E genotyping is widely available but is infrequently requested except by neurologists to address a question of Alzheimer's disease. This test attracts no Medicare reimbursement and therefore may attract a charge.

Specific genetic testing for mutations associated with familial hypercholesterolaemia (FH) is a resource-intensive test that is performed by specialised laboratories. It is designed to assist family cascade screening by identifying the mutation in index cases so that other affected members of the family may be identified with certainty. Clinical features highlighted by publications from the Netherlands and the United Kingdom help to define the diagnosis as required for the Pharmaceutical Benefits Schedule (PBS).[4,5] Mutation detection is not cost-effective for diagnosis of FH when there is clinical uncertainty. It is unlikely that this test will attract a Medicare reimbursement, so routine patients will be likely to have to carry an out-of-pocket expense if they undertake such testing.

How do clinicians use the lipid results?

There seems to have been a change in the attitude toward lipid management by Australian clinicians from disinterest or even disbelief in the late 1970's and early 1980's to acceptance of an "upper limit of normality" of serum cholesterol of 5.5 mmol/L up to the late 1990s. Few were keen to look at the lipid pattern critically, assess other CVD risk factors and treat the patient based on assessment of the overall risk. The advent of the Framingham data in the form of the New Zealand Risk Calculator [6] assisted in addressing this, but many clinicians persisted with the view that a serum TC exceeding 5.5 mmol/L required treatment whereas a value of below that threshold did not.

Laboratories have considered performing the NZ Risk calculation "in-house" and reporting this as part of the lipid report. This is perceived as a significant addition of value to the report. However the difficulty arises with accurate inclusion of the patient's blood pressure, diabetic and smoking status, particularly in larger laboratories whose numbers of daily lipid reports are in the thousands. Clearly, a patient can be harmed if an erroneous report (based on incorrect clinical information) forms the basis for commencement or withholding of lipid-lowering therapy.

Alternatively, this calculation could be performed automatically by the surgery's patient database computer.[7] The role of pathology laboratories in facilitating this process is being discussed with several practice software developers.

PBS Eligibility Criteria for Lipid-lowering Drugs

The PBS criteria for the eligibility of lipid lowering drugs was modified and came into effect on the 1 October 2006 (Table 1).[8] These new criteria were developed following a meeting between the Pharmaceutical Benefits Advisory Committee and major stakeholders in 2004. One aim of this meeting was to reduce the disparity between National Heart Foundation (NHFA) recommendations with PBS eligibility and thus address a significant gap and source of confusion within primary care.

The recent revision of PBS eligibility criteria for subsidy of lipid lowering drug therapy is a positive development. General practitioners felt liable to penalty if they prescribe PBS-funded therapy for patients who do not meet the criteria. The revision is important because it goes a long way toward reducing the disparity between the NHFA recommendations and PBS eligibility.

Table 1. Excerpt from the Schedule of Pharmaceutical Benefits December 2006.[8]

Patients identified as being in one of the following very high risk categories may commence drug therapy with statins or fibrates at any cholesterol level:

- coronary heart disease which has become symptomatic
- cerebrovascular disease which has become symptomatic
- peripheral vascular disease which has become symptomatic
- diabetes mellitus with microalbuminuria (defined as urinary albumin excretion rate of >20ug/min or urinary albumin to creatinine ratio of >2.5 for males, >3.5 for females)
- diabetes mellitus in Aboriginal or Torres Strait Islander patients
- diabetes mellitus in patients aged 60 years or more
- family history of coronary heart disease which has become symptomatic before the age of 55 years in two or more first degree relatives
- family history of coronary heart disease which has become symptomatic before the age of 45 years in one or more first degree relatives

Other patients are required to meet the lipid levels shown in the following table:

Patient Category	**Lipid Levels for PBS Subsidy**
Patients with diabetes mellitus not otherwise included	TC >5.5 mmol/L
Aboriginal or Torres Strait Islander patients Patients with hypertension	TC >6.5 mmol/L or TC >5.5 mmol/L and HDL-C <1 mmol/L
Patients with HDL cholesterol <1 mmol/L	TC >6.5 mmol/L
Patients with Familial Hypercholesterolaemia identified by: • DNA mutation; or • tendon xanthomas in the patient or their first or second degree relative Patients with: • family history of coronary heart disease which has become symptomatic before the age of 60 years in one or more first degree relatives; or • family history of coronary heart disease which has become symptomatic before the age of 50 years in one or more second degree relatives	If aged 18 years or less at treatment initiation: LDL-C >4 mmol/L If aged more than 18 years at treatment initiation: LDL-C >5 mmol/L or TC >6.5 mmol/L or TC >5.5 mmol/L and HDL-C <1 mmol/L
Patients not eligible under the above: • men aged 35 to 75 years • postmenopausal women aged up to 75 years	TC >7.5 mmol/L or TG >4 mmol/L
Patients not otherwise included	TC >9 mmol/L or TG >8 mmol/L

Lipid Testing

Indications for Lipid Testing

Serum lipids should be measured in all adults over 45 years of age as part of assessment of overall CVD risk.[2] The yield of testing will be low in those less than 45 years of age and this should be reserved for those who are at increased risk because of their personal or family history, or the presence of other disease such as diabetes mellitus or chronic kidney disease.[2] If a patient is started on lipid-lowering medication their serum lipids should be measured at 6 monthly intervals to check that they are reaching their desirable values.

Recommended Tests

Serum TC and serum TG in a fasting specimen are the simplest and cheapest lipid measurements. However, the measurement of HDL-C and LDL-C, as markers of the anti- and pro-atherogenic lipid particles, gives more information and should be requested routinely. The reason for this is that some people with mildly increased TC may actually be at lower risk because their HDL-C is high and their LDL-C is relatively low. People with low serum TC generally have low LDL-C, but they may also have low HDL-C concentrations.

Other tests such as lipid electrophoresis and apolipoprotein measurement should be reserved for those with unusual lipid disorders. Diabetes Australia recommends that people aged 45 and over who are obese (BMI $\geq$30), have hypertension or clinical evidence of CVD will need their fasting blood glucose measured or an oral glucose tolerance test performed.[9] Hypothyroidism and rarely the nephrotic syndrome will need to be excluded in cases of unexplained hypercholesterolaemia.

People taking lipid-lowering medication should be monitored with LDL-C, HDL-C and TG measurements. Liver function tests and creatine kinase (CK) should be measured at baseline, and repeated during therapy only if adverse reactions to medication are suspected. Although recent reviews are encouraging about the safety of longterm lipid-lowering therapy,[10] treatment is generally interrupted if ALT exceeds 2 to 3 times the upper limit of normal, or if unexplained CK rise exceeds 5 to 10 times the upper limit of normal. Lower thresholds are applied if the patient is symptomatic, particularly if symptoms relate to muscles or liver.

Clinical Information

With most pathology computing systems, it is now possible to record extensive notes on individual patient's profiles in addition to the basic demographic information. Unfortunately, this potentially useful information is rarely provided. Whilst at present there is no plan for pathology providers to calculate absolute risk on an individual patient, it is still highly desirable for the clinician to provide additional clinical information to the laboratory. This could include risk factors such as:-

- ethnicity – particularly Aboriginal and Torres Strait Islander patients
- the presence of high risk factor states such as ischaemic heart disease, CVD, diabetes mellitus, hypertension, Familial Hypercholesterolaemia, family history of CVD
- whether the patient is on lipid-lowering therapy

It should be emphasised to the clinician that the pathology request form should be similar as any referral letter to a medical specialist. Therefore these requests should contain all the medical information about the patient which is relevant to the tests that have been requested.

Logistics for Lipid Testing

Patient Preparation

To facilitate assessment and monitoring of lipid status, especially for comparison with desirable values, it is preferable to obtain a fasting specimen. It should be noted, however, that a fasting state is not a specific requirement described for lipid evaluation in current Lipid Management guidelines or PBS criteria or in the MBS.

The patient should fast for a minimum of nine hours, ideally about twelve hours and to a maximum of 15 hours.[11] However in some patients prolonged fasting may not be feasible or the patient may present having not fasted. In these situations, the test report should indicate clearly that the patient has not fasted. If the testing returns abnormal results, then it is recommended that the patient should be further assessed by collecting a fasting specimen.

During the fasting period only water and medications as directed by the clinician should be taken. In order to obtain a valid assessment of lipid status, it is important that optimal conditions such as posture (seated or supine) and tourniquet use (not too tight or too prolonged) apply to specimen collection. Clinical factors that may affect lipid results include but are not limited to the following:

- acute or recent illness, e.g. myocardial infarction or severe infection
- disorders in which treatment is yet to be optimised, e.g. hypothyroidism or diabetes mellitus
- medication, e.g. oral contraceptives or corticosteroids
- pregnancy or phase of menstrual cycle.

It is also important to consider the effect of intra-individual biological variation when assessing a patient's lipid status.[12] This variation can be up to 6% for TC, 7% for HDL-C, 8% for LDL-C and 21% for TG levels so it may be necessary to perform more than one specimen collection in order to obtain

valid results for the purpose of clinical management. Other factors that have the potential to affect lipid values include diurnal variation, alcohol, smoking, posture, exercise and prolonged tourniquet use. Therefore it is not only desirable that fasting conditions apply but also that specimen collection occurs in the morning from a resting patient using an optimal venipuncture technique.

Specimen

Serum specimens collected in either a plain or gel tube are commonly used for routine lipid analysis which may be carried out in conjunction with other investigations such as liver function tests and CK measurement for patients on lipid lowering therapy. Heparin or EDTA plasma are also usually acceptable for testing, however, this should be confirmed by referring to the method specifications. In circumstances where there may be a prolonged delay before analysis occurs, specimens should be centrifuged, separated and maintained at 4 ^{0}C.

Specimens other than serum or plasma may be required for other laboratory investigations of lipid disorders (see Table 2). It is important to ensure that the conditions which are specified by referral laboratories for any of these investigations are observed in terms of patient preparation and the collection, handling, transportation and storage of specimens.

Methods of Analysis

The common four lipid measurements of cholesterol, HDL-C, LDL-C and triglycerides require two quality characteristics. Firstly, the results need to be reproducible so that the laboratory measurement imprecision doesn't add significantly to the intra-individual variations observed in patients. There are different ways of judging the analytical goal for imprecision and while it is generally considered desirable that the imprecision is less than half of the intra-individual biological variation, another approach is to follow the recommendations of professional groups such as the Center for Disease Control (CDC) from the USA. Secondly, the results need to be accurate or a true indication of the lipid level that is free from method related biases. The international body named the Joint Commission for Traceability in Laboratory Medicine (JCTLM), serves the global pathology laboratory community by keeping records of best ways to estimate trueness. For most biochemical measurements, isotope dilution mass spectrometry serve as the best methods but other methods, used by the CDC for example have also been acknowledged as reference methods. This is important as many assay manufacturers, and laboratories, have been certified via CDC and this provides traceability for the clinical outcome data in clinical trials which use those methods.

Cholesterol

Cholesterol is known to react with sulfuric acid and acetic anhydride to produce an intense blue colour (the 'Lieberman' colorimetric method). Although the JCTLM, the international authority on pathology laboratory values, lists the CDC Lieberman-based "Abell-Kendall' method for cholesterol as a reference method for defining the true cholesterol value,

Table 2. Specialised tests for diagnosis of specific lipoprotein abnormalities or assessment of CVD risk.

Apolipoprotein Quantitation:-
- Apolipoprotein A-I
- Apolipoprotein B
- Apolipoprotein C-III
- Apolipoprotein E genotyping

Specific Lipoproteins:-
- Lipoprotein (a)
- Lipoprotein X

Lipoprotein Electrophoresis

Lipid Ultracentrifugation

Enzymes (functional assays):-
- Lipoprotein lipase
- Hepatic lipase
- LCAT (lecithin-cholesterol acyl transferase)

Receptors (functional assays):-
- LDL receptor

Molecular Assays
- LDL receptor gene (mutation screening)
- Apolipoprotein B-100 gene (specific mutations)
- Apolipoprotein E gene (polymorphisms)
- Lipoprotein lipase gene (polymorphisms/mutations)

Other Assays
- Lp(a) genotyping
- Beta quantitation
- Non-esterified fatty acids
- High sensitive CRP
- Homocysteine

definitive methods such as isotope dilution mass spectrometry are ideal for defining the calibration point for assays.

Methods using the enzyme cholesterol oxidase represent an adequate approach for the routine requests received by clinical laboratories. All pathology laboratories use the one-step enzymatic method that breaks down cholesterol esters with cholesterol esterase, and the subsequent reaction with cholesterol oxidase which generates hydrogen peroxide that can react with many different indicators to give a measurable colour.

The CDC states that it is desirable that assays for TC have an acceptable reproducibility with a between run Coefficient of Variance (CV) of less than 3%. Virtually all pathology laboratories can achieve this precision, but some point of care instruments may not. The CV for TC measurements using cholesterol oxidase methods varies from 1.5 to 3.5%. Laboratories usually report results in mmol/L to one decimal place as these assays are not capable of differentiating the second decimal place. However for TC measurement, as with all assays, laboratories should consider using an extra decimal place for quality control values to improve their ability to detect early shifts.

HDL Cholesterol

Ultracentrifugation is the definitive method to separate the various cholesterol carrying particles of the blood according to their density. However this technique is cumbersome, expensive and impracticable in a pathology laboratory performing large numbers of lipid assays daily.

Separation of the particles by electrical charge using electrophoresis is also possible but not precise enough for quantitative purposes. Another approach is to precipitate the larger low density particles using agents such as heparin-manganese chloride, dextran sulphate or polyethyleneglycol, and separate the high density lipoprotein particles remaining in the supernatant for cholesterol measurement. Separation implies a moderately cumbersome two-step procedure, so new non-separation techniques have been developed based on using a cyclodextrin or polyanion to chemically alter the lipoprotein particles so as to enhance the reactivity of the cholesterol in HDL particles to the enzymes used in the standard enzymatic cholesterol assay. Two thirds of laboratories in Australasia currently use these methods. These methods are constantly under review to improve their accuracy and precision in the presence of atypical lipoprotein particles, a situation where accurate HDL-C measurement is even more critical.

The CV for measuring HDL-C using cholesterol oxidase methods generally varies from 3.5 to 5.0% indicating that most laboratories easily fit below a desirable performance standard of around 4.0% both according to biological variability and the CDC. Such reproducibility indicates that laboratories are capable of defining the first decimal place but would have little confidence in a second decimal place for measurements in mmol/L. The manufacturers of the direct HDL-C methods include cautions to the effect that "*there is no model available that can mimic interference by TG as triglyceride levels in patient specimens behave unpredictably. Therefore it is not possible to exclude interference by triglycerides in patient specimens*".

LDL Cholesterol

After a fast of 9 to 12 hours, when chylomicrons should be absent, the particles that are normally in the blood include HDL, LDL and Very Low Density Lipoproteins (VLDL). The routine measurements above can provide the TC and the HDL–C concentrations. Also, as all the TG are carried by VLDL in the fasting state and the molar ratio of triglyceride to cholesterol in these particles is 2.2:1, dividing the TG value by 2.2 will give the VLDL cholesterol concentration. Using the equation developed by Friedewald[13] the concentration of cholesterol in the unmeasured fraction (LDL-C) can be determined:

LDL-C = TC – HDL-C – TRIG/2.2 (all values in mmol/L).

The equation can only be used if unusual triglyceride-carrying particles are not present (e.g. remnant particles), and this is likely to be the case as long as the triglyceride concentration is below 4.5 mmol/L.

Most laboratories use this calculation to report LDL-C as reference methods listed by JCTLM and CDC generally require ultracentrifugation techniques which are beyond the capabilities of routine laboratories. However, increasingly laboratories are measuring LDL-C directly by using antibodies and other chemicals to modify the non-LDL particles so that they will not react in the cholesterol oxidase reaction.

Both calculated and measured LDL-C methods have CVs of about 4.5%, which generally match the CDC and desirable 'biological' performance standard of 4.0%, however this may be an area for improvement in some laboratories. Once again this performance would justify one decimal place but not two.

Triglycerides

TGs are predominantly carried by chylomicrons after a meal and by VLDL particles at other times. The TG levels in the blood will vary after a meal, so the convention is to measure

them after a 9 to 12 hour fast, by which time the chylomicrons will have been cleared and only VLDL remain.

Separation of the fatty acids from triglycerides can be achieved with caustic soda or potassium hydroxide, but is usually simply achieved by using the enzyme lipase. This reaction leaves free a variety of fatty acids but also the common glycerol backbone of triglyceride. Glycerol can then be measured either by reaction with periodate to form formaldehyde or more usually by enzymatic approaches such as glycerol kinase which generates glycerol phosphate and ADP which can in turn be measured.

The CV for enzymatic TG methods varies from 2.5 to 5.0% indicating that most laboratories easily fit below the CDC target even the most ideal or optimal biological performance standard for TG of around 5.0%. This performance justifies reporting results in mmol/L to one decimal place but is not good enough for two decimal places.

Specialised Lipid Tests

Other tests (Table 2) may be performed to assist with diagnosis or CVD risk assessment. These include lipoprotein tests such as lipoprotein (a) (or apolipoprotein (a)), LDL pattern (so-called "small dense" or "pattern B" LDL), apolipoproteins A-I and B, apolipoprotein E genotyping and others. LDL pattern reflects reduced diameter, reduced cholesterol content and increased density of LDL particles which often occurs in the presence of elevated TG. This is a situation in which LDL-C may underestimate CVD risk. CVD risk is more accurately reflected by the number of LDL particles, which is reflected by apo B levels.[14] Apo B measurement may be helpful in the presence of hypertriglyceridaemia, but care must be taken to avoid interference from lipaemic sera. Non-lipid tests may reflect other risk factors or different aspects of the atherothrombotic process. These tests include homocysteine and an increasing array of inflammatory markers such as high sensitivity C-reactive protein. It is questionable whether or not tests such as these provide any improvement in CVD risk prediction.[15] Many pathology laboratories perform these tests. Details of availability can be obtained from the ROSA File maintained by the Australasian Association of Clinical Biochemists.[16]

Lipid Reporting

Reference Intervals, Desirable Values and Lipid Test Categories

Most laboratories do not provide conventional Reference Intervals (e.g. population means ± 2.0 standard deviations) for lipid tests, particularly for LDL-C and HDL-C, as the risk for coronary heart disease has a continuous relationship with these measurements. Instead, many laboratories give desirable values which can be used in individuals to indicate the likelihood of increased risk of CVD. These values are based on extensive epidemiological data that have been collected over many years. With the exception indicated below, it is recommended that this approach should continue.

Although desirable values for TC have been promoted in the past, some individuals with a serum TC concentration above this limit may be incorrectly classified as 'increased risk' when the increase in TC has resulted solely from an increase in HDL-C, which is protective. Provision of a desirable value for TC may be misleading when assessing individual risk for coronary heart disease. In recent years, epidemiological studies and drug trials have involved the measurement of HDL-C and LDL-C, such that reliable data are now available to categorise risk associated with these parameters. Accordingly, and in line with the NHFA 2005 lipid update, the provision of a desirable value for TC is not given.[3]

For the assessment of lipid abnormalities, particularly those that affect risk for CVD, it is recommended that laboratories perform measurements of TC, fasting TG and HDL-C and provide an assayed or calculated LDL-C value using the Friedewald equation.[13] Results of these measurements and calculations should be expressed as mmol/L, reported to the first decimal place only and printed in the following order with the indicated desirable values:--

TC	
HDL-C	>1.0 mmol/L
LDL-C	<2.0mmol/L (for patients at high risk)
	<2.5mmol/L (for patients at lower risk)
TG	<1.5 mmol/L

Age and, more particularly, gender may affect lipid and lipoprotein results. Some risk factor guidelines take these factors into account, but those used in Australia do not do so at the present time. Paediatric lipid levels, which tend to be lower, have been reported,[17] and female HDL-C levels, which tend to be higher, have been reported.[18] It should be noted that lipid values may vary when tested repeatedly in the same individual. These variations include biological and analytical factors, and must be taken into consideration when assessing changes induced by dietary modification or treatment with lipid-lowering drugs.

Some laboratories report TC/HDL-C ratios. As TC is a composite which includes HDL-C, this ratio is not universally accepted as a reliable indicator of risk although it is a factor in the New Zealand Risk Calculator. Although the LDL-C/HDL-C ratio may appear to be more appropriate in this respect, the LDL-C is subject to increased variability, whether directly

measured using currently available methods or calculated on the basis of the Friedewald equation using three separate measurements (each of which is subject to analytical error). An alternative approach is to measure the Apolipoprotein B/Apolipoprotein A-I ratio, as measurements of these proteins are subject to less analytical imprecision and can be calibrated against international standards. In either ratio the two components represent independent risk factors for CVD and therefore each component should still be considered separately. Although the group do not recommend the use or reporting of ratios, the current recommendation is that this be left to the discretion of the individual laboratories.

Use of Comments on Lipid Reports

Pathology laboratories are encouraged to provide interpretive comments on all laboratory reports on serum lipid measurements. Laboratories may elect not to provide comments on lipids reports where a specialist or any other clinician clearly experienced in lipid disorders has requested the tests. Interpretive comments should include (1) a statement of the likely lipoprotein abnormality, in terms of which lipoproteins are likely to be increased and/or decreased, and (2) a list of the possible causes of the abnormality. This latter component should be based on clinical information available to the laboratory, such as clinical notes included on the request form, previous comments provided by the requesting doctor and recorded in the laboratory's data base or diagnostic tests performed currently or previously by the laboratory (e.g. glucose tolerance tests, thyroid function tests, glycated haemoglobin measurements).

Additional information on CVD risk, approaches to management and treatment goals can also be included as part of the report on serum lipids. Many laboratories now use computer-based 'expert' systems to provide interpretive comments on test results. Use of these systems to produce individualised comments with pertinent information to assist the requesting clinician in achieving the desired clinical outcome of reduced CVD risk is encouraged.

Comments for Lipid Test Categories

The following tables are provided to assist pathology laboratories in developing interpretive comments for reports on serum lipid measurements. The comments are provided as suggestions only. They are comprehensive and designed to cover most possibilities. Laboratories can modify these as a basis for their own comments which may be tailored to the individual patient's situation and the needs of the clinician who submits requests for serum lipid measurements. It is also recognised that comments on reports may not be required for patients who are already diagnosed or who are undergoing treatment.

Table 3 outlines the general comments for when TC and/or TG have been requested as the only lipids measured or when moderate to severe hypertriglyceridaemia is detected.

Table 4 lists the likely lipoprotein abnormalities associated with various abnormalities in the measured serum lipids, particularly HDL-C, LDL-C and TG. Hyper- or hypo-lipidaemia can then be translated into hyper- or hypo-

Table 3. General comments for inclusion on lipid reports when only TC and/or TG have been requested.

Lipid Abnormality	Comments
TC >4.0 mmol/L when cholesterol is the sole lipid measurement.	TC alone is insufficient to assess lipid-associated risk for CVD. TG, LDL-C and HDL-C are required in addition, with all lipid measurements performed after 12 hours fasting.
TC (any value) and TG (any value) when these are the only lipid tests requested.	TC and TG alone are insufficient to assess lipid-associated risk for CVD. LDL-C and HDL-C are also required, with all lipid measurements performed after 12 hours fasting.
TG >4.4 mmol/L in any situation.	When TG >4.4 mmol/L, calculation of LDL-C by the Friedewald equation is invalid. Additional lipoprotein assessment may be necessary to identify lipoprotein abnormalities.
TG >10.0 mmol/L in any situation	Triglyceride >10.0 mmol/L is associated with an increased risk of acute pancreatitis.[19]

Table 4. Common lipid abnormalities with associated lipoprotein changes and possible causes.

Lipid Abnormality	Lipoprotein Abnormality And Associated Conditions	Category
Category A - Abnormalities involving HDL-C		
Increased HDL-C >2.5 mmol/L	HDL increased familial, pregnancy, oestrogen, anticonvulsant use, pesticide exposure.	A1
Normal HDL-C and TG 1.6 – 4.4 mmol/L	Chylomicrons and/or VLDL increased non-fasting specimen, pregnancy, exposure to alcohol, oestrogens, bile acid sequesterants	A2
Decreased HDL-C and TG 1.6-4.4 mmol/L	HDL reduced and VLDL and/or chylomicrons increased. non-fasting specimen, obesity, metabolic syndrome, type 2 diabetes, renal impairment, hepatic impairment, drugs, intercurrent illness, familial forms of hypertriglyceridaemia	A3
Decreased HDL-C and TG <1.6	HDL reduced familial hypo alpha-lipoproteinaemia including ABCA1 transporter defects, intercurrent illness	A4
Category B - Abnormalities of LDL-C		
LDL-C >2.0 mmol/L to 4.0 mmol/L (HDL-C and TG normal)	LDL exceeds target for high risk patients and may be excessive in some individuals	B1 (a)
LDL-C >4.0 mmol/L (HDL-C and TG normal)	LDL is increased polygenic hypercholesterolaemia Familial Hypercholesterolaemia Familial Defective Apo B-100 secondary causes (nephrotic syndrome, hypothyroidism, cholestatic liver disease etc.)	B1 (b)
Decreased LDL-C <1.0 mmol/L	LDL decreased lipid-lowering therapy abnormalities of lipoprotein-B or microsomal transfer protein deficiency intercurrent illness	B2
LDL-C >4.0 mmol/L and markedly abnormal LFTs	Lipoprotein-X present cholestatic liver disease LCAT deficiency	B3
Category C - Abnormalities in TG		
TG 1.6 - 4.4 mmol/L and TC usually <5.0 mmol/L	Chylomicrons and/or VLDL increased non-fasting specimen, causes listed for A2 and A3	C1
TG >4.4 mmol/L and TC <5.0 mmol/L	Chylomicrons increased lipoprotein lipase deficiency	C2
TG >4.4 mmol/L and TC >4.9 mmol/L	IDL (remnant lipoproteins) increased familial dysbetalipoproteinaemia (see Table 5)	C3
	VLDL and LDL increased – (see Table 5) causes listed for A2, A3	C4
	VLDL (LDL changes vary) and Chylomicrons increased – (see Table 5) causes listed for A2, A3, C2 and C3	C5

lipoproteinaemia and the likely lipoprotein abnormality stated in the first part of the interpretive comment. That is, a statement is made on the likely lipoprotein abnormalities that account for the observed abnormalities in the serum lipids. Conditions that produce these lipoprotein abnormalities are also listed, which in conjunction with clinical information available to the laboratory can be used to generate further comments on the lipid report.

Comments are provided in Table 5 for the common lipid abnormalities listed in Table 4. These comments are more comprehensive and designed to include most possibilities. The likely lipoprotein changes that account for the serum lipid results are stated and possible causes are then described. In some instances a further comment is made on CVD risk, followed by suggested actions.

Risk Assessment

In Australia, two independent sets of guidelines have been crucial in shaping the clinical management of patients with serum lipid and lipoprotein abnormalities. The PBS has had the task of providing quantitative thresholds beyond which CVD risk justifies the use of subsidised lipid-lowering therapy. On the other hand, the NHFA Guidelines for Lipid Management have aimed to provide ideal targets for avoidance of future CVD events. Historically, in addition to differing functional roles, the review processes for the individual guidelines were not formalised or synchronised, so disparities emerged. A Consensus Conference in 2004 set the stage for resolving such disparities. Initially, the need to address evidence of the benefit of aggressive LDL-C reduction meant that the 2005 update of the NHFA guidelines broadened the gap. The 2006 changes in the PBS have diminished many of these disparities.

Both guidelines highlight the critical need to identify and treat patients with symptomatic CVD. These patients have already demonstrated their susceptibility to their ambient risk factor profile. The PBS places no threshold on the qualifying lipid levels for these patients whilst the NHFA guidelines recommend that they reduce their LDL-C to less than 2.0 mmol/L. Both guidelines also recognise the importance of lipid control in diabetic patients. The NHFA places them in the highest risk category and also recommends treatment for patients with the metabolic syndrome if their calculated absolute risk of CVD exceeds 10% over the next 5 years. The PBS prioritises diabetic patients with microalbuminuria, Aboriginal or Torres Strait background or age over 60, for whom there are no threshold lipid levels. There are minor differences between the guidelines in terms of heritable risk. Both identify Familial Hypercholesterolaemia as indicative of the highest level of risk, but the PBS includes diagnostic criteria. Both guidelines also emphasise the need to identify and treat the close relatives of those with premature CVD, but there are minor differences such as the specific age limits. The main area where the guidelines continue to differ is in their approach to multiple risk factors. The NHFA recommends the calculation of absolute risk whereas the PBS uses the combination of risk factors and lipid thresholds, probably because the facilities to calculate absolute risk are not universally available at this stage.

The process of up-dating guidelines is demanding, requires extensive review and cannot be undertaken too frequently. Even if multiple updates were possible, frequent alteration might cause confusion and undermine credibility. This means that guidelines may lag behind new evidence. This is not currently the case in Australia, but new developments are anticipated. Lipid-lowering intervention is one of the fastest-moving areas of clinical research. In future, guidelines are likely to feature an increased capacity to consider all modifiable risk factors simultaneously by means of absolute risk assessment.

Conclusion

Pathology services play a key role in the management of patients with dyslipidaemia although there have been no comprehensive guidelines for laboratories on the provision of lipid reports in the past. In addition, technological advances have allowed pathology services to tailor specific comments to lipid results to assist the clinicians in the management of their patients. The recommendations shown above are based on a review of current medical literature and take into account recent changes in PBS criteria in Australia. It is suggested they may be used as a template for lipid reporting in Australian pathology laboratories although it is acknowledged that local requirements may vary and that revision will be needed in future to accommodate changes in knowledge, treatment and regulations.

Acknowledgement

This initiative was in part supported by an unrestricted grant from Pfizer Australia.

Competing Interests: David Sullivan is a Member of several advisory panels within the pharmaceutical industry including Pfizer Australia, AstraZenica, Merck Sharp and Dohme, Schering Plough, Sanofi Aventis etc. David Tognarini is occasionally employed as a consultant to Pfizer Australia.

Table 5. Suggested comments for reports on various lipid abnormalities.

Category	Lipid Abnormality	Comment
	Abnormalities involving HDL-C	
A1	Isolated increase in HDL-C >2.5 mmol/L	Increased HDL is considered protective for CVD, but must be considered in context of all other risk factors. Increased HDL can be seen in pregnancy, oestrogen treatment, high alcohol intake, following prolonged intensive physical exercise, during anticonvulsant therapy, as a consequence of exposure to microsomal enzyme inducers such as pesticides, and as an inherited condition. Increased HDL-C does not exclude risk from increased LDL-C.
A2	Normal HDL-C (1.0 – 2.5 mmol/L) and TG 1.6 – 4.4 mmol/L	This pattern may be seen in non-fasting specimens, pregnancy, with oestrogen (HRT) or bile-acid sequesterant usage and in association with high alcohol intake.
A3	Decreased HDL-C and TG 1.6-4.4 mmol/L	Decreased HDL with increased VLDL. This pattern may be seen in the metabolic syndrome, type 2 diabetes, obesity, renal impairment, hepatic impairment, intercurrent illness, drugs, and familial forms of hypertriglyceridaemia. Decreased HDL is a risk factor for CVD. Suggest assessment of overall risk for CVD with the proviso that small dense LDL may be present, in which case LDL-C may underestimate CVD risk.
A4	Decreased HDL-C <1.0 mmol/L	Decreased HDL may accompany intercurrent illness. It is also seen in hypoalpha-lipoproteinaemia (various forms including Tangier disease) and in association with defects in the ABCA1 transporter protein. In most of these circumstances it is a risk factor for CVD.
	Abnormalities of LDL-C	
B1 (a)	LDL-C >2.0 mmol/L to 4.0 mmol/L (HDL-C and TG normal)	LDL exceeds target for high risk patients and may be excessive in some individuals
B1 (b)	LDL-C >4.0 mmol/L (HDL-C and TG normal)	Increased LDL can be seen in hypothyroidism and nephrotic syndrome. Primary causes include polygenic hypercholesterolaemia, Familial Hypercholesterolaemia, Familial Defective ApoB-100 and Familial Combined Hyperlipoproteinaemia. Increased LDL is a risk factor for CVD. Assess overall risk and consider treatment. LDL-C <2.0 mmol/L is the treatment target for very high risk patients (NHFA 2005).
B2	LDL-C <1.0 mmol/L	Decreased LDL can be seen with lipid-lowering therapy, severe illness or in rare inherited conditions (e.g. abnormalities in Apolipoprotein B or lipoprotein assembly).
B3	LDL-C >4.0 mmol/L and markedly abnormal LFTs	Lipoprotein X may be seen in patients with cholestatic liver disease and lecithin-cholesterol acyl transferase deficiency. In some cases an abnormal band, which is consistent with Lipoprotein X, is present on lipoprotein electrophoresis. Additional tests may confirm the presence of disk-like Lipoprotein X.
	Abnormalities in TG	
C1	TG 1.6 - 4.4 mmol/L and TC usually< 5.0 mmol/L	Mild hypertriglyceridaemia due to increases in chylomicrons and/or VLDL. This pattern can be seen in non-fasting or post-prandial states. Ensure patient has fasted 9 to 12 hours prior to performing lipid studies. Also consider pregnancy, exposure to alcohol, estrogens, bile acid sequesterants, intercurrent illness, other drugs, obesity, metabolic syndrome, type 2 diabetes, renal impairment, hepatic impairment, and familial forms of hypertriglyceridaemia. Assess HDL-C, which may be reduced.

C2	TG >4.4 mmol/L and TC <5.0 mmol/L	Chylomicrons increased. If patient has fasted 9 to 12 hours prior to performing lipid studies, increased chylomicrons may be due to a defect in lipoprotein lipase activity. This can be due to mutations in the gene for lipoprotein lipase or secondary to some autoimmune conditions. This condition poses a risk of pancreatitis. Plasma TG <10 mmol/L is recommended to minimise this risk. LDL-C and HDL-C are usually substantially reduced, but if chylomicronaemia is severe, TC may exceed 5 mmol/L.
C3 *	TG >4.4 mmol/L and TC >4.9 mmol/L (To determine which comment is appropriate in these categories additional tests may be necessary to determine the lipoprotein abnormality)	IDL (intermediate density lipoproteins) are also known as "remnant" lipoproteins because they are formed as the residual from the action of lipoprotein lipase on triglyceride-rich lipoproteins (VLDL and chylomicrons). Alternative names for this condition are remnant dyslipidaemia, broad – beta disease and Type 3 hyperlipidaemia. Increased IDL can be seen in individuals with obesity, diabetes mellitus, some autoimmune conditions and treatment with steroids, on a background of polymorphisms in the gene for Apolipoprotein E.
C4		Increased VLDL ± increased LDL can be seen in diabetes mellitus, obesity, oestrogen treatment, excess alcohol intake, hypothyroidism, renal impairment, other drugs, metabolic syndrome, hepatic impairment, Familial Combined Hyperlipoproteinaemia and familial forms of hypertriglyceridaemia. This pattern is usually associated with decreased HDL. Decreased HDL and increased LDL are risk factors for CVD. Suggest assessment of overall risk for CVD and consider treatment where appropriate. Targets for high risk patients are TG <1.5 mmol/L and LDL-C< 2.0 mmol/L (NHFA 2005).
C5#		Increased VLDL (LDL-C may vary) and increased chylomicrons can be seen in individuals with high dietary fat intake, obesity, estrogens, bile acid sequesterants, intercurrent illness, other drugs, metabolic syndrome, type 2 diabetes, renal impairment, hepatic impairment, high alcohol intake, or familial forms of hypertriglyceridaemia, There is a background of reduced lipoprotein lipase activity which may be hereditary or due to the saturation of enzyme activity by the excess VLDL and chylomicrons. This pattern is usually associated with decreased HDL. Decreased HDL-C is a risk factor for CVD. Suggest assessment of overall risk for CVD and consider treatment where appropriate. Targets for high risk patients are TG <1.5 mmol/L (which may be difficult to achieve under these circumstances) and HDL-C >1.0 mmol/L (NHFA 2005). This condition is associated with a risk of pancreatitis. Plasma TG <10 mmol/l is recommended to minimise this risk.

* Moderate to severe hypertriglyceridaemia and hypercholesterolaemia may be seen in this category. Often the TC and TG concentrations may be similar.

Gross hypertriglyceridaemia and severe hypercholesterolaemia may be seen in this category resulting from reduced clearance of chylomicrons and VLDL. Typically, TG may be 3 – 4 x TC concentration.

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