Clinical Topics

Current clinical laboratory practice: investigation of plasma lipids—which tests and when?

W J MARSHALL, FIONA C BALLANTYNE

Abstract

Clinical interest in the lipoproteins stems mainly from the association between serum cholesterol concentrations and coronary heart disease. Investigations of lipoproteins should be performed in patients with premature coronary heart disease, with a strong family history of coronary heart disease, or with certain cutaneous stigmata of hyperlipoproteinaemia and when fasting serum samples are seen to be lipaemic. Family studies should be performed in appropriate cases to identify relatives at increased risk of developing coronary heart disease. Patients with conditions known to cause secondary hyperlipoproteinaemia should be investigated if they fall into one of these categories but only after treatment of the underlying condition. Non-specialist laboratories should be able to measure total cholesterol and triglyceride concentrations and high density lipoprotein cholesterol concentrations. Lipoprotein electrophoresis has a limited role in such laboratories and is not necessary as a routine procedure. Specialist laboratories should in addition be able to measure individual lipoproteins and identify apolipoprotein E phenotypes.

Introduction

The plasma lipoproteins contain cholesterol, triglyceride, and phospholipids complexed with specific apoproteins. Chylomicrons, which are not normally present in fasting serum, and very low density lipoproteins (VLDL) carry most of the triglyceride; the triglyceride in chylomicrons is exogenous, being derived from dietary fat, while that in VLDL is endogenous, having been synthesised in the liver. The bulk of the cholesterol in plasma (roughly 80%) is transported in low density lipoprotein (LDL), with 15-20% in high density lipoprotein (HDL) and small amounts in VLDL and chylomicrons.

Triglyceride is an important energy substrate, while cholesterol is an essential component of the membranes of cells and organelles as well as being the precursor of steroid hormones. The major clinical interest in the lipoproteins, however, lies in the association, shown by epidemiological surveys, between increased plasma concentrations of both total and LDL cholesterol and the risk of development of coronary heart disease. Also, and independently, there is an association between coronary heart disease and a low concentration

of HDL.^{1,2} The precise basis of these associations remains imperfectly understood, and other important risk factors for coronary heart disease exist—for example, smoking and hypertension. Hypertriglyceridaemia per se is probably not a risk factor for coronary heart disease,^{3,4} but it is often associated with reduced concentrations of HDL.

Hyperlipoproteinaemia may be primary—that is, genetically determined—or secondary to other conditions such as hypothyroidism, diabetes mellitus, obesity, and excessive alcohol consumption. These other conditions may also exacerbate the lipid abnormalities in primary hyperlipoproteinaemia.

In whom should lipids be measured?

It is generally agreed that plasma lipid concentrations should be measured in (i) patients with evidence of premature coronary heart disease (that is, in those aged under 40); (ii) subjects with a strong family history of coronary heart disease; (iii) patients with xanthomas (but not xanthelasmas); and (iv) patients whose fasting serum is lipaemic. Measurement of cholesterol concentration may also be included in voluntary screening programmes available to men in the age group prone to coronary disease (25-65 years) to identify those at risk, who may then be offered appropriate treatment. Economic considerations preclude biochemical screening for hyperlipoproteinaemia except in these at risk groups. Although mild hypercholesterolaemia (>7.5 mmol/l; >290 mg/100 ml) is common, it is often related to both genetic and environmental factors, and whether its control would reduce the risk of coronary heart disease remains to be established. Mild hypertriglyceridaemia (>1.9 , <4.0 mmol/l) (>190 , <350 mg/100 ml) is also common and also often related to both genetic and environmental factors; it is of little clinical importance in the absence of clinical manifestations (for example, xanthomas, lipaemia retinalis, recurrent abdominal pain), so screening for it cannot be justified.

Routine determination of lipoprotein concentrations in patients with conditions known to cause secondary hyperlipoproteinaemia is unnecessary unless the patients additionally fall into one of the above categories. Even then, investigation should be delayed until the underlying condition has been treated, as this should result in normalisation of any secondary changes. The finding of abnormal lipid concentrations plays no part in the diagnosis of these conditions.

When secondary causes have been eliminated family studies merely to support the diagnosis of a primary abnormality are unnecessary in patients with only mild hyperlipoproteinaemia as they will not have any effect on management. Family studies are indicated only to identify other subjects at risk, who may then be offered treatment. How vigorously such studies should be pursued is problematical, but if a person is found to have a serum cholesterol concentration in excess of 8 mmol/l (310 mg/100 ml) and secondary causes have been eliminated it is advisable to screen his siblings and

Department of Chemical Pathology, King's College School of Medicine and Dentistry, London SE5 8RX

W J MARSHALL, MRCP, MRCPATH, senior lecturer

Department of Biochemistry, Royal Infirmary, Glasgow G4 0SF

FIONA C BALLANTYNE, PHD, MRCPATH, principal biochemist

Correspondence to: Dr Marshall.

offspring. If there is a strong family history of coronary artery disease the threshold may be lower.

Laboratories will also be required to assess the response of patients to treatment. Dietary regimens or lipid lowering drugs, or both, may be used, and the positive findings of the Lipid Research Clinics coronary primary prevention trial have given renewed impetus to those who support aggressive intervention to lower plasma cholesterol concentrations.^{6,7} It must be remembered, however, that this study, which compared the combined effects of the lipid lowering drug cholestyramine and a cholesterol reducing diet with those obtained using the same diet together with a placebo, was performed on a highly selected group of men with serum cholesterol concentrations in the top 5% of the range. It may therefore be unwise to extrapolate recommendations for the general population from these results.

Collecting samples and interpreting data

For all the tests mentioned below each laboratory should determine its own reference range, although this may not be the same as the desirable range. The upper limit of the reference range for plasma cholesterol concentration in young men in the United Kingdom is generally taken as about 6.5 mmol/l (250 mg/100 ml) but this value is associated with a small but important risk of coronary heart disease and an optimum concentration of as low as 4.5 mmol/l (175 mg/100 ml) has been suggested,⁸ although the optimum in different populations is hard to predict.⁹ The accuracy and precision of cholesterol assays at present may not be adequate for clinical purposes. The overall coefficient of variation of such assays among laboratories participating in the United Kingdom external quality assessment scheme in 1982 was only 6.3%.¹⁰

Blood samples for assessment of lipoprotein concentrations should be collected after an overnight fast if triglyceride is to be measured, although this is unnecessary if only total or HDL cholesterol is to be measured. The patient should not have changed his diet during the previous two weeks. He should be questioned about alcohol consumption as excessive alcohol consumption causes hypertriglyceridaemia. Posture should be standardised as it affects the concentration of all proteins in plasma, and at least two samples should be analysed before treatment is started. Cholesterol can usefully be measured in the first 24 hours after a myocardial infarction,¹¹⁻¹³ but thereafter total and LDL cholesterol concentrations decrease considerably and results may be misleading; stable values are reached after a variable period, but certainly after three months.¹¹⁻¹³

Serum or plasma is suitable for the simpler tests, but for some more specialised tests plasma is necessary, with edetic acid (at a final concentration of 2.5-4.0 mmol/l) as the anticoagulant. Lipoproteins are labile, and analysis should be carried out soon after collection or within a few days if samples are stored at 4°C.

What tests should be used?

Details of test methodology are outwith the scope of this paper but have recently been comprehensively reviewed.¹⁴

NON-SPECIALIST LABORATORIES

Total cholesterol and triglyceride—Measurement of total cholesterol concentration, which reflects mainly LDL cholesterol, is essential for reasons outlined above. Actual measurement of total triglyceride concentration is probably necessary only in plasma samples seen to be turbid on inspection. The presence of turbidity suggests a considerably raised triglyceride concentration (>4 mmol/l; >350 mg/100 ml). If plasma is left undisturbed overnight at 4°C chylomicrons float to the surface while VLDL remains in suspension. This makes it possible to distinguish between hypertriglyceridaemia due to fasting chylomicronaemia and that due to an

increase in VLDL without the necessity to perform electrophoresis.

Electrophoresis—Routine electrophoresis of all samples received for analysis of lipoproteins is unnecessary. Nor is electrophoresis required if the only abnormality is an increase in triglyceride concentration. If the serum total cholesterol concentration is above 10 mmol/l (>390 mg/100 ml) the excess is almost always in the β fraction (the equivalent of LDL). An exception to this is familial dysbetalipoproteinaemia, which may be diagnosed on electrophoresis (preferably on agarose) by the presence of a broad β band. Patients with this condition, however, differ clinically from those with other types of hypercholesterolaemia, and it could be argued that electrophoresis is required when the cholesterol is above 10 mmol/l only if this condition is particularly suspected (for example, because of palmar or tuberous xanthomas). Electrophoresis may be performed in patients with smaller increases in total cholesterol concentration to determine whether the excess is in the β fraction (common) or the α fraction (uncommon). An increase in α lipoprotein cholesterol is seen in the rare familial hyper- α -lipoproteinaemia and also as a secondary phenomenon—for example, in some patients treated with oestrogens for carcinoma of the prostate. Identification of such patients is important as they do not require treatment for hypercholesterolaemia. An increase in α lipoprotein should be confirmed by specific measurement of HDL cholesterol.

HDL cholesterol—The total plasma cholesterol concentration yields sufficient information to allow clinical decisions to be made in most patients with hypercholesterolaemia. When an increased α lipoprotein band has been detected on electrophoresis a single measurement of HDL cholesterol concentration should be made to confirm that it is raised. Determination of ratios of HDL to LDL has been advocated as a useful marker of the risk of coronary heart disease in patients with excess LDL cholesterol, but this is of little value in the management of individual patients.

SPECIALISED LABORATORIES

VLDL, LDL, and HDL cholesterol—VLDL and LDL cholesterol concentrations may be calculated with reasonable accuracy from the total cholesterol, triglyceride, and HDL cholesterol concentrations provided that there is no chylomicronaemia, that the triglyceride concentration is not more than 4.5 mmol/l (400 mg/100 ml), and that the patient does not have dysbetalipoproteinaemia.¹⁵⁻¹⁶ The actual measurement of these fractions requires expensive, time consuming procedures entailing ultracentrifugation.¹⁷⁻¹⁸

Apolipoproteins—Although methods for measuring apolipoproteins are now available, there are few indications for their use except in research. Cross sectional studies in patients with proved coronary heart disease and controls have shown, for example, that apo-AI may be a better discriminator than HDL cholesterol for the presence of heart disease, whereas the data for apo-B in comparison with total LDL cholesterol are conflicting.¹⁹⁻²⁰ The relative merits of measuring apolipoproteins and lipoproteins as markers of the risk of coronary heart disease have not been elucidated²¹ and could be established conclusively only by long term prospective studies, the cost of which would probably be prohibitive. Measurement of apo-CII is required to diagnose apo-CII deficiency, a cause of the rare syndrome of fasting chylomicronaemia, although the management of this condition does not depend on knowledge of its cause. Phenotyping of apo-E should be available for the definitive diagnosis of familial dysbetalipoproteinaemia, although, again, this is not necessary for management. In most patients with this condition the phenotype is E2/E2 and the total plasma apo-E concentration is raised.²²⁻²³

Enzymes—An assay should be available to measure lipoprotein lipase, deficiency of which is a cause of fasting chylomicronaemia. The rare condition of deficiency of lecithin cholesterol acyl transferase requires that an assay for this enzyme should also be available. It is not necessary, however, for all specialised laboratories to be able to measure these enzymes, or the apolipoproteins, provided that they have access to these assays through cooperation with other laboratories.

Other tests—Future developments in the characterisation of lipoproteins may well provide clinically useful information. For example, women, who are less prone to coronary heart disease than men,²⁴ have higher HDL₂ concentrations than men, and men who survive myocardial infarction have lower HDL₂ concentrations than apparently normal men.²⁵

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Conference Report

Animal experiments

JANE DAWSON

A recent cartoon in *Private Eye* shows a gang of hooded animal liberationists "rescuing" pairs of elephants and giraffes from the Ark as the deluge begins. A less lighthearted mark of the vehemence with which some representatives of the animal welfare lobby are prepared to pursue their aims was the unaccustomed policemen on duty on the steps of the Royal Society when a press seminar was held to correct what is seen by many researchers as the misinformation of the public by journalists. Since fewer than a third of those attending the meeting were press representatives (with only two from the national dailies) no immediate change is likely.

The press was said to be interested only in polarisation between researchers and those whose antagonism to animal experiments was expressed as bomb threats. The low key, reasoned discussion of issues at the seminar between researchers and representatives from the RSPCA and moderate antivivisectionist organisations would make "bad television" said a man from the BBC.

Yet concern for animal welfare is increasing. The world's first professor of animal welfare has been appointed at Cambridge University veterinary school; initially his interest will be with farm and zoo animals but consideration of laboratory animals is a

possibility. The Animals (Scientific Procedures) Act received Royal Assent on 20 May. All laboratories where animal research is performed (over 500 throughout the country) must appoint a veterinary surgeon to be responsible for the care and welfare of their experimental animals. An Animals Procedures Committee, including lay representation, has been appointed to advise the Home Secretary, who has the power and responsibility to judge the scientific merit of the work he authorises.

Suggestions at the seminar that there should be local review committees, analogous to hospital ethics committees, were dismissed because the existing system of Home Office inspectors is designed to maintain standards nationally. The introduction of another control could lead to conflicts of advice and "variance of judgment if local review committees included lay representation." Countries, such as Sweden, that have local committees at each institution do not have a licensing system.

Scientists were criticised at the meeting for assuming that the media will intuitively understand research aims and practices without special preparation and revision of the material presented. Misunderstanding and the violence faced by some research workers can breed a laager mentality, and this defensive attitude was seen by Professor Colin Blakemore as causing researchers to slip into the "silly pattern" of emphasising potential applications of research work at the expense of basic research.

Knowledge of the promotion of carcinogenesis and of viral induction of carcinomas was derived almost entirely from work in