Technical method

Estimation of lipoproteins using capillary blood

A. HART, H. COHEN, and F. ALLISON *Salford Royal Hospital*

The standard methods of measuring plasma lipoproteins are electrophoresis and ultracentrifugation, but comparable results are obtained (Stone *et al*, 1971) with the nephelometric method which is simpler, requires inexpensive equipment, and can be used to examine many samples quickly. The principle of the nephelometric method is based on the fact that the amount of light scattered when a beam is passed through a dilute solution is dependent on the size and concentration of the particles suspended in the solution. In a dilute sample of plasma or serum the majority of the light scattering intensity (LSI) is accounted for by lipoprotein particles. Plasma or serum from an intravenous blood sample is diluted and the LSI is measured using a nephelometer (Stone and Thorp, 1966). Membrane filtration to remove large particles and then measurement of the LSI of the filtrate and chemical cholesterol analysis permits identification of the levels of three lipoprotein fractions (table). A modification using a smaller amount of capillary blood has been devised for use as a screening procedure.

Method

The usual nephelometric method has been modified so that a single measurement (LSI reading) is taken from diluted unfiltered plasma obtained from a small capillary sample to screen for lipoprotein abnormalities. Capillary and venous blood samples were obtained simultaneously from two groups of subjects:

- 1 Sixty healthy controls, chosen so that there were equal numbers of males and females with 10 subjects in each decade from the second to the seventh.
- 2 A diabetic group comprising 63 females and 37 males, 30 being treated with insulin, 51 with tablets, and 19 with diet alone.

To evaluate the capillary method as a simple screening technique, venous and capillary samples were obtained simultaneously from the normal subjects and the diabetic patients. From each individual, 2-3 hours after the midday meal a 5-10 ml sample of venous blood together with 0.6-1 ml of capillary blood was collected into EDTA. Samples were centrifuged at 3000 rev/min for 20 minutes. In the case of the capillary blood, 0.1 ml amounts of plasma were removed respectively for cholesterol and lipoprotein estimation. From the venous blood, 0.5 ml of plasma was used for lipoproteins and 0.1 ml for cholesterol estimation. Plasma cholesterol was measured by chemical analysis using the method of Watson (1960); plasma lipoproteins were measured by the nephelometric technique of Stone and Thorp (1966). The basis of this method is that the LSI of an appropriately diluted sample of serum or plasma is proportional to the concentration of lipoproteins. The first nephelometric reading on an unfiltered sample corresponds to the total value of the large (L), medium (M), and small (S) lipoprotein fractions (table). The S fraction due to the small particle size has little influence on LSI readings in dilute samples. If either the L or M fraction (or both) is raised, the nephelometric reading will be elevated. Membrane filtration may then be carried out to separate the L particle fraction, and a further nephelometric reading is taken to estimate the M and S particle levels. Subtraction from the first reading thereby gives the L particle level. The S particle fraction is calculated from the second reading after filtration and the cholesterol level. Elevation of the S fraction is accompanied by a high plasma cholesterol

Received for publication 27 November 1975

Lipoprotein	Density (g/ml)	Approximate Particle Size (μ m)	Terminology used for Each Analytical Method		
			Paper Electrophoresis	Analytical Ultracentrifugation	Membrane Filtration and Nephelometry
Low density	1.063-1.006	ca. 0.02	Beta	S 0-20	S Particles
Very low density	1.006-0.96	0.028-0.045	Pre-beta	S 20-400	M Particles
Very low density (chylomicrons)	< 0.96	0.11-0.4	Chylomicrons	S > 400	L Particles

Table Comparison of terminology used for fractions of total low density lipoproteins

level as the biggest proportion of plasma cholesterol is contained in the S lipoprotein particles.

Results

The upper limits of normal for S, M, and L plasma lipoprotein fractions have been taken from those given by Stone *et al* (1971) for subjects in the age group 19-29. These are S particles—500 mg per 100 ml, M particles—230 mg per 100 ml, L particles—28 mg per 100 ml. The normal level for plasma cholesterol in our laboratory is up to 250 mg per 100 ml.

In the normal group, a LSI reading of 14 or over indicated abnormality of the L and/or M lipoprotein fractions. However, values below this were sometimes associated with elevation of the S particle fraction alone. Ten subjects had capillary blood LSI values of 14 or more and in these, measurements on venous samples with filtration showed M lipoprotein elevation alone. A further six subjects had LSI values over 14 shown by analysis of venous samples to be due to elevations of the L fraction only. Four subjects had LSI values below 14 with cholesterol levels over 250 mg per 100 ml and thus had S lipoprotein fraction elevation with normal levels of L and M. All the abnormal levels were revealed by both the capillary and venous tests. In 10 subjects, simultaneous capillary and venous sampling was repeated five times within a two-hour period. Results from these showed that the range of differences between duplicates given as a percentage of the mean was $\pm 5\%$ for the larger and $\pm 10\%$ for the smaller samples. The correlations obtained between venous and capillary samples in 100 diabetic patients are shown in fig 1; 48 had LSI values over 14. Figure 2 a,b shows the M and S lipoprotein fraction levels obtained from capillary and venous samples after filtration and further analysis in the 30 diabetics who were taking insulin.

Discussion

Hart *et al* (1971) have shown that samples collected early in the afternoon after a normal lunch do not differ greatly in L lipoprotein fraction level from those collected after an overnight fast. Our usual results show that satisfactory lipid estimations can be obtained using capillary blood plasma in greater dilution. In some instances there was an elevation of S lipoprotein fraction with normal L and M levels. The LSI was 14 units or less but the serum cholesterol was then over 250 mg per 100 ml. Thus neither

Fig 1 Comparison of LSI values for unfiltered capillary and venous plasma samples collected simultaneously.

(a)

(b)

Fig 2 Comparison of (a) plasma M particle and (b) plasma S particle concentrations in capillary and venous blood collected simultaneously from 30 diabetics.

the measurement of LSI alone nor plasma total cholesterol alone is satisfactory as a screening test for all lipoprotein abnormalities. Some subjects had an LSI of over 14 units due solely to an elevation of the L lipoprotein fraction. In the clinical situation these subjects would need to be re-tested in the post-fasting state for clarification.

The differences observed in LSI readings between capillary and venous samples were not systematic and thus are most likely due to the reduced precision inherent in the capillary method. A small variation in LSI reading from the samples has a large effect on the calculation of L, M, and S levels.

Conclusion

To screen patients using the method described it is necessary to measure the plasma cholesterol and LSI of a diluted unfiltered capillary sample. If the LSI is 14 or less and the plasma cholesterol 250 mg per 100 ml or less, the sample is normal. If the LSI is over 14 or the cholesterol over 250 mg per 100 ml, the sample is abnormal and then filtration of the diluted serum sample with full lipoprotein analysis is necessary. Using this method, we have estimated lipoprotein fractions from capillary samples in over

30 patients at one clinical session with only one technician, the results being available before the end of the clinic. The use of lipoprotein estimations, hitherto limited by the available techniques, can thus be extended to the screening of large numbers of patients at clinics and in surveys.

This work was supported by a research grant from the British Diabetic Association to A. Hart and F. Allison.

Our thanks are expressed to the Imperial Chemical Industries Limited, who provided the nephelometer, and to Mr J. M. Thorp, of the ICI Pharmaceutical Research Laboratories, for advice and technical assistance and for permission to reproduce the table.

References

- Hart, A., Cohen, H., and Thorp, J. M. (1971). Lipoprotein and fibrinogen studies in diabetes. *Postgrad. med. J.* (June suppl.), **47**, 435-439.
- Stone, M. C. and Thorp, J. M. (1966). A new technique for the investigation of the low-density lipoproteins in health and disease. *Clin. chim. Acta*, **14**, 812-830.
- Stone, M. C., Thorp, J. M., Mills, G. L., and Dick, T. B. S. (1971). Diagnosis and classification of abnormal lipoprotein patterns. *Clin. chim. Acta*, **31**, 333-354.
- Watson, D. (1960). A simple method for the determination of serum cholesterol. *Clin. chim. Acta*, **5**, 637-643.