Quantification of Lipoprotein(a) in Dried Blood Spots and Screening for Above-Normal Lipoprotein(a) Concentrations in Newborns

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Lipoprotein(a) [Lp(a)] is considered an additional, independent, and largely genetically determined risk factor for the development of premature coronary heart disease. Analogous with increased Lp(a) concentrations that represent an additional risk factor in adults, above-normal concentrations of Lp(a) can be detected in five- to seven-day-old newborns. We describe a simple enzyme-linked immunosorbent assay for measuring Lp(a) in dried blood spots collected by heel-prick in five- to seven-day-old infants. Lp(a) could be quantitatively recovered from blood spots. We chose a cutoff value of 100 mg/L for identifying the newborns at risk, based on the Lp(a) distribution in 180 such infants.

Additional Keyphrases: enzyme-linked immunosorbent assay • cutoff value • heart disease • dyslipoproteinemia

Among the inherited metabolic diseases, familial dyslipoproteinemia has one of the highest frequencies and can result in premature coronary heart disease (1). Recently, lipoprotein(a) [Lp(a)] has been identified as an additional, independent, and largely genetically determined risk factor for developing premature coronary heart disease (2, 3). Analogous with increased Lp(a) concentrations in adults, above-normal concentrations of Lp(a) can also be detected in five- to seven-day-old newborns (4–7). To define these relatively increased Lp(a) concentrations, we set a cutoff limit of 100 mg/L for newborns seven days postpartum, by comparison with the mean value and distribution in a large group of infants (6).

Screening procedures for detecting dyslipoproteinemia-
mia, based on the measurement of either apolipoprotein B or the apolipoprotein A-I/B ratio in dried blood spots (8-12), could be combined with screening for Lp(a) (13). Above-normal Lp(a) values in newborns should lead to a systematic family investigation, including collecting a family history of cardiovascular problems through use of a questionnaire and determining lipoprotein profiles. An above-normal concentration of Lp(a) in an individual with abnormal lipoprotein patterns should be one more factor to consider in recommending dietary and drug treatment.

The purpose of this report is to demonstrate the feasibility of such a screening program for Lp(a) by an enzyme-linked immunosorbert assay (ELISA) performed with dried blood spots, which are routinely collected for other screening purposes.

Materials and Methods

Samples. Blood samples were collected by heel-prick in five- to seven-day-old infants and spotted onto filter paper (no. 2992; Schleicher and Schuell, Keene, NH). Twenty to 30 μL of blood was adsorbed onto the filter paper, dried in the air at room temperature, and kept at 4 °C for no more than one month in a plastic bag. The routine screening was carried out with a 6-mm-diameter disc punched out of the spots, containing ~5 μL of plasma.

Blood with Lp(a) concentrations varying from 50 to 700 mg/L was collected in the presence of disodium EDTA (final concentration 1 g/L). The subjects were 10 healthy newborns (seven days postpartum) and 10 healthy adult volunteers. Exactly 10 μL was spotted on the filter paper, and hematocrit values of the spotted samples were determined.

Assessment of Lp(a) recovery from the dried blood spots. The amount of Lp(a) eluted from the blood spots was determined by comparison with the values measured in the plasma of newborns. Various supports, designed for low adsorption of proteins, were compared to improve desorption. Besides filter paper, we tested low-adsorbing carboxymethylated cellulose (Whatman C/CM 50 and C/CM 30; Whatman Paper Ltd., Maidstone, U.K.) and a glass-fiber support (Whatman GF/QA 30). We spotted 10-μL blood spots on the different supports, cut out the entire spots, and eluted them for 2 h in 300 μL of sodium phosphate buffer (0.05 mmol/L, pH 7.4) containing 0.15 mol of NaCl and 0.5 mL of Tween 20 (Bio-Rad Labs., Richmond, CA 94804) per liter. The percentage of Lp(a) recovery was calculated for the supports, and the stability of the spots was evaluated after 0 to three weeks of storage at 4 °C.

Measurement of Lp(a) by ELISA. Lp(a) was measured in plasma and in blood spots by the ELISA previously described (12). The assay was adapted for the screening by using standard spots made of equal volumes of washed erythrocytes and of standardized plasma diluted with lipoprotein-free serum (8). For Lp(a) determination in the blood spot samples, we routinely diluted the eluates 80-fold.

Results

Lp(a) elution from dried blood spots. To optimize the assay, we tested various supports and lengths of storage. We eluted the spots for 1 h under gentle shaking with the sodium phosphate buffer containing NaCl and Tween 20. Analytical recovery, expressed as the percentage of the plasma concentration of the same sample, was higher for the carboxymethylated celluloses (Whatman CM 30 and CM 50) than for the filter paper or for the glass-fiber material (Whatman GF/QA 30).

Average analytical recovery, measured on 10 samples, was 97%, 84%, and 72% for the CM-cellulose, filter paper, and glass fiber. The CVs for the 10 elutions ranged between 6% and 8%. When measured in 20 samples having concentrations between 50 and 1000 mg/L, recovery from the carboxymethylated cellulose supports was between 90% and 115% (SD 15.7%). Because the aim of the screening is to detect newborns with Lp(a) concentrations >100 mg/L, compared with an average value of 17 mg/L, these recovery percentages are acceptable.

The effect of sample storage at 4 °C on reproducibility of the Lp(a) measurements was investigated by ELISA. Figure 1 shows that we could quantify Lp(a) in fresh blood spots stored at 4 °C without significant loss of recovery for at least three weeks. The intra- and interassay CVs measured for 10 samples assayed in duplicate in one day and in four separate assays for three weeks were 8% and 13%, respectively. These CVs are higher than for the plasma ELISA (14) because they include both the elution and assay errors. The stability of Lp(a) in the
dried blood spots is therefore sufficient for a screening program.

Results of the Lp(a) screening by ELISA. The calibration curves for the ELISA of Lp(a) from plasma and from blood-spot standards are depicted in Figure 2, which shows a slightly higher response of Lp(a) in plasma than in blood spots. When Lp(a) was assayed in 20 blood spots, with both plasma and blood spots used as standards, the values were close to the corresponding plasma concentrations and did not depend on the kind of standard used.

The mean Lp(a) value measured in 180 infants at five days postpartum was 17 mg/L. On the basis of previous measurements in newborn plasma (6), we selected a cutoff value of 100 mg/L; infants with higher concentrations were recalled for follow-up.

Discussion

The ELISA of Lp(a) in dried blood spots allows an easy and meaningful screening for above-normal Lp(a) values in five- to seven-day-old infants. About 12% of the infants screened had a concentration >100 mg/L. In comparison, 14% of the adults screened in our local population had a value greater than the adult cutoff value of 250 mg/L (14). The increase of Lp(a) concentrations with age (6) accounts for the differences in cutoff values selected to define infants at risk and adults at risk.

Selecting an appropriate support material for blood collection and using a detergent in the elution allow the quantitative recovery of Lp(a) from blood spots kept at 4 °C for three weeks. This technique, therefore, meets the practical requirements of a screening program for newborns.

Because Lp(a) is present and detectable by five days postpartum, its quantification and that of other lipoproteins known to be risk factors should allow the refinement of a risk profile for individuals and their families. The Lp(a) assay could usefully complement other lipid and apoprotein determinations in screening programs and in follow-up studies. Although no therapeutic or dietary treatment is currently in place to normalize increased Lp(a) concentrations (15), detection of this independent risk factor and of other lipoprotein abnormalities in infants and in their families could help clinicians decide whether to treat accompanying lipid disorders, thereby decreasing the patients' total risk of developing cardiovascular disease.

References