Neural Tube Defects Cannot Be Diagnosed Prenatally by Electrophoresis of Amniotic Fluid Transferrin Isoforms

To the Editor:

Prenatal diagnosis of neural tube defects (NTDs) is based on ultrasonography; but uncertainty exists in a few cases, and biochemical analysis of amniotic fluid (AF) is required. Electrophoresis of AF acetylcholinesterase is a specific and sensitive method for diagnosis of myelomeningocele (1, 2). Although false-positive results are rare, another biochemical technique would be of great value.

Adult and infant cerebrospinal fluid (CSF) is characterized by a specific marker, asialotransferrin or $\beta_2$-transferrin, because it migrates more slowly in electrophoresis than $\beta_1$-tetrasialotransferrin, the main isoform in all biological fluids (3). The specific behavior of $\beta_2$-transferrin is used for the detection of CSF leakage from the subarachnoid space into the nasal or aural cavity (4, 5). Assuming that CSF would leak from a myelomeningocele affected fetus into the AF, we screened AF for asialotransferrin.

We undertook a retrospective study of 12 AF samples from NTD-affected fetuses (16–35 weeks of gestation) and 36 AF gestational age-matched controls. Controls were from newborns or fetuses (collected by in utero puncture at 22 and 32 weeks for fetal karyotyping), and CSF controls were from newborns or infants (normal biochemical and bacteriologic findings). Electrophoresis was performed on agarose with the Hydragel 6 CSF® assay (Sebia), followed by immunostaining with a polyclonal anti-transferrin antiserum conjugated to peroxidase (Sebia). Dilutions were used to obtain a transferrin concentration of ~10 mg/L.

Control sera and AF (regardless of gestational age) produced the classic $\beta_1$-transferrin band corresponding to the tetrasialotransferrin isofrom, whereas control CSF produced 2 bands, a major band of $\beta_1$ mobility and a lighter band of $\beta_2$ mobility, corresponding to the asialo isoform (Fig. 1). AF from fetuses with NTDs gave the same pattern as controls without detectable $\beta_2$-transferrin.

To find an explanation for this unexpected result, we compared fetal and newborn CSF electrophoretic patterns. Fetal CSF (n = 8; 20 to 35 weeks of gestation) was collected by cranial puncture after medical termination of pregnancy (terminal renal failure, skeletal abnormalities, or trisomy 21). We observed that the fetal CSF pattern depended on gestational age. Before 27 weeks, there was a single $\beta_1$ band, whereas from 32 weeks, a smear appeared between $\beta_1$ and $\beta_2$. However, CSF from a newborn delivered at 37 weeks of gestation displayed the classic CSF pattern, with 2 distinct transferrin bands, rather than this peculiar pattern.

This study indicates that electrophoresis of AF transferrin isoforms is inadequate for prenatal diagnosis of NTD. There are 2 possible explanations for the absence of the $\beta_2$ band: (a) inadequate sensitivity of the technique because of unknown in vivo dilution of CSF leaks in AF; and (b) an absence of asialotransferrin in fetal CSF. The CSF fetal patterns agree with this hypothesis, showing the progressive appearance of the $\beta_2$ brain isoform from the 32nd week of gestation. The processing of brain glycosylation appears to be mature at birth as the same pattern can be observed in newborn and adult CSF. Similar findings have been reported for the abnormal glycosylation of transferrin in carbohydrate-deficient glycoprotein syndrome (6). Blood samples from affected fetuses display the classic $\beta_1$ pattern of unaffected fetuses, suggesting a false negative. The carbohydrate-deficient pattern appears only after birth.

Although negative, the present findings may be of interest to pediatric physicians, particularly when they are using protein glycosylation abnormalities for prenatal diagnosis.
plasma protein-A (PAPP-A) immunoassays developed for use during pregnancy to study acute coronary syndromes (ACS). That report and the authors’ related article in Clinical Chemistry (2) have important implications, because PAPP-A has been suggested to be a prognostic marker of cardiac risk (3). We have reported on the direct relationship between serum PAPP-A concentrations and both the extent and complex morphology of angiographic coronary artery stenoses in patients with chronic stable angina pectoris (4, 5). These studies were performed with the same assay that was used in the first clinical report on PAPP-A and ACS by Bayes-Genis et al., who described a relationship between PAPP-A and unstable atherosclerotic plaques (3). This assay was based on a polyclonal capture antibody and a combination of monoclonal detection antibodies. It was calibrated with WHO reference standard 78/610, which was derived from serum collected from pregnant women. The findings of Qin et al. (1, 2), raise questions concerning the appropriateness of assays used in studies predicting cardiac risk. These authors have clearly demonstrated that the circulating molecular form of PAPP-A, with respect to its associated complexed protein (PAPP-A/ proMBP), differs in ACS from the form that typically circulates at low concentrations in nonacute patients and at higher concentrations in pregnant women. The selection of a suitable combination of antibodies, directed against appropriate epitopes, is, therefore, paramount for developing assays for use in this setting. Recently, 2 highly sensitive assays have become available for PAPP-A, and have produced discordant findings in relation to ACS. It has been shown that the assay manufactured by DRG Co. (Germany) failed to detect any difference in serum PAPP-A concentrations between healthy controls and patients with ST-segment elevation myocardial infarction (6). By contrast, with the assay from Diagnostic System Laboratories, Inc. (DSL), PAPP-A concentrations predicted cardiovascular events after renal transplant (7) and cardiovascular events in patients who had presented to the emergency department with acute chest pain (8).

We compared the assay used by Bayes-Genis with the DSL assay in 61 patients with chronic stable angina, by use of serum collected immediately before diagnostic coronary angiography. Fasting blood samples were collected into Vacutainer® SST tubes. Serum was separated after clot development, <1 h after blood collection. Each sample was then divided into 3 fractions (to avoid freeze-thaw cycles) and frozen immediately at −80 °C for storage. Specimens were frozen and thawed only once before analysis. We did not investigate the stability of PAPP-A in the SST tubes, and this was not remarked upon in either of the reports detailing the assay and analyte characteristics (4, 9) or within the manual provided by DSL. We found the limit of detection of the DSL assay to be in line with the manufacturer’s stated detection limit of 0.06 mIU/L and the within- and between-assay CVs were <3% and <8%, respectively, at concentrations of 2.5 and 10.0 mIU/L. The noncommercial assay had a detection limit of 0.03 mIU/L, and the within and between assay CVs were 10% and 15%, respectively. Details of the assay performance for these 2 assays have been described previously (4, 9). The correlation between the 2 assays (Spearman R) was 0.645 (Fig. 1A), and the analysis of residuals showed a mean difference of 0.63 (SD 2.34) mIU/L. A Bland-Altman plot (Fig. 1B) shows that these differences are positively skewed, with larger positive differences with higher mean PAPP-A values. Twenty-seven data points were below the limit of detection for the DSL assay and were not included in the statistical analysis. Both assays detected substantially higher PAPP-A concentrations in patients with coronary stenosis than in patients without stenosis. These major differences in assay performance may help explain the contrast in clinical findings observed when these 2 assays were used to investigate risk in patients with ACS.