toward possible B-cell malignancy. In comparing serial measurement results from the same patient, PA-induced modifications of quantitative data should also be considered.

References


7. Marien G, Bossuyt X. Response to the comment of K. Day and J. Zakowski on “Clinical capillary electrophoresis by isoelectric focusing (IEF) (1) is the preferred procedure to screen for congenital disorders of glycosylation (CDG).” HPLC (2) and capillary electrophoresis (CE) (3) are recently introduced techniques that offer mainly the advantage of automation. The analyses are usually performed in specialized laboratories, and serum samples are sent primarily via courier. Stibler and Cederberg (4) showed that blood samples spotted on Guthrie cards could be used instead of serum for investigating carbohydrate-deficient Tf. We had the same experience with Guthrie cards sent for Tf isoform profiling. In fact, a substantial proportion of the samples we have investigated (886 of 9654 samples) were transported this way (our unpublished data).

Tf obtained by extraction from Guthrie cards may show on IEF an isoform profile that corresponds to a known CDG pattern (Fig. 1A, lane 2). The Tf profile on CE is unusable because of the high degree of hemolysis. However, when the Tf concentration in serum is low (Fig. 1A, lane 3) or when only a small amount of blood has been spotted on the Guthrie card, the Tf bands are mostly too faint to exclude a CDG. This is particularly true for type II CDG (CDG-II) profiles because of the smaller increases in asialo- and monosialo-Tf. Increasing the amount of sample on the agarose gel is not useful because it increases the background (Fig. 1A, lane 4).

We obtained 393 abnormal IEF profiles suggestive of CDG among 8768 serum samples (4.5%) and 9 abnormal profiles among 886 blood samples on Guthrie cards (1%; our unpublished data), and investigated whether the application of serum instead of whole blood could combine the usefulness of Guthrie cards with the accuracy of serum analysis.

The protein concentration in each sample was determined by the BIORAD assay. IEF and CE analyses of sialo-Tf were performed as described previously (3).

Sera spotted on the Guthrie cards originated from serum pools that were obtained from control samples (permanently stored at −20 °C) and from patients with CDG-I or CDG-II. After 250 μL of serum was spotted on a Guthrie card, the card was enclosed in a plastic bag and sealed after air removal. After 2 weeks at room temperature or −20 °C, spots were cut and snipped and dispersed in a volume (in microliters) of physiologic saline–2 mL/L Triton X-100 equal to 10 times the weight of the paper (in milligrams); this surfactant solution is used for homogenization of intestinal mucosal biopsies (5). Protein was extracted overnight at room temperature under constant stirring. After centrifugation for 10 min at 13 000g, the supernatant was lyophilized and the residue was dissolved in 50 μL of physiologic saline. Tf isoform profiling was performed by IEF and by CE.

The mean (SD) protein recovery with this extraction procedure was 10 (0.3)% (n = 10). Thus, ~300 μL of serum should be applied on the Guthrie cards for IEF and CE analysis to provide the equivalent to 30 μL of serum in the extract. As this would correspond to at least 0.5 mL of blood, it is clear that in many cases the amount applied on Guthrie cards is too low for Tf analysis.

The intensities of the Tf isoforms in the samples stored at −20 °C were slightly higher than the intensities in samples stored at room temperature (Fig. 1B). The CE profiles (Fig. 1C) corresponded to the expected Tf patterns for the same control (trace 1), CDG-I (trace 2), and CDG-II (trace 3) sera on the Guthrie cards after 2 weeks of storage at −20 °C.

We have observed neither analyti-
In conclusion, analysis of a sufficient amount of serum spotted on a Guthrie card, stored at –20 °C and transported by air, gives information about the Tf profile similar to that obtained by analysis of serum. Although serum samples are preferable, we recommend the present procedure if Guthrie cards are used.

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References

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To the Editor:
Guanidinoacetate methyltransferase (GAMT; EC 2.1.1.2) deficiency (OMIM 601240) is an autosomal recessive disorder of creatine biosynthesis, characterized clinically by mental retardation, language delay, extrapyramidal movements, epilepsy, and autistic behavior (1). Biochemically, GAMT deficiency is characterized by depletion of creatine and accumulation of guanidinoacetate (GAA) in the brain and body fluids (2). Treatment by creatine supplementation (combined with arginine restriction and ornithine supplementation) partially restores (~70%) cerebral creatine, reduces seizures, and improves behavior, but it does not reverse the mental retardation (3). We have described a method to measure GAA and creatine in plasma and urine by liquid chromatography–tandem mass spectrometry (LC-MS/MS) (4). In the present study, we validated this method for measurement of GAA and creatine in amniotic fluid, and we report the first GAMT prenatal diagnosis based on a combination of molecular and biochemical investigations.

We adapted a previously reported method to measure GAA and creatine in plasma (4) for amniotic fluid. Briefly, 50 μL of supernatant of amniotic fluid or aqueous calibrators were mixed with internal standards [d4-creatine (CDN isotopes) and [13C2]GAA (Dr. Ten Brink, VU University Medical Center, Amsterdam, The Netherlands)]. Samples were ex-

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Prenatal Diagnosis of Guanidinoacetate Methyltransferase Deficiency: Increased Guanidinoacetate Concentrations in Amniotic Fluid