Estimation of Highly Increased Concentrations of Fetal Hemoglobin in Fanconi’s Anemia

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We report a case of Fanconi’s anemia with an extremely high proportion of fetal hemoglobin (Hb F). A three-year-old girl with multiple birth defects, mental retardation, and aplastic anemia consistent with Fanconi’s anemia showed Hb AF by electrophoresis; the Kleihauer smear showed Hb F in 70% of her erythrocytes. Total Hb concentration was 34 g/L, mean corpuscular volume 119 fl. The proportion of Hb F was 45% by densitometry, 36% by radial immunodiffusion, and 30% by cation-exchange microchromatography. The Hb A2 was 0.5%; glycated Hb was 7.8% by affinity chromatography. Sample volume was insufficient for alkali denaturation. As exemplified with this patient, we recommend microchromatographic cation-exchange assay when Hb F exceeds 30% by densitometry. Here the effect of contamination by Hb A, was lessened by the high proportion of Hb F. Cation-exchange microchromatography provides clinically relevant Hb F values more quickly than radial immunodiffusion and more conveniently than alkali denaturation.

Additional Keyphrases: aplastic anemia - hemoglobin variants - cation-exchange microchromatography - radial immunodiffusion and alkali denaturation compared

We report here a case of Fanconi’s anemia in which the proportion of fetal hemoglobin (Hb F) was extremely high.4 We applied cation-exchange microchromatography to determine the proportion of Hb F and propose that this method can be applied to determine Hb F in similar cases as well as in other hemoglobinopathies such as sickle-cell anemia, hereditary persistence of Hb F, and Hb C-associated conditions when the Hb F is greatly increased.

Case Report

A mentally retarded three-year-old black girl with multiple birth defects developed severe anemia. The Hb was 34 g/L, the mean corpuscular volume 119 fl, the leukocyte count 3.5 × 109/L, platelet count 22 × 109/L, and reticulocyte count 1.8%. Hemoglobin electrophoresis had an Hb AF pattern (see Figure 1). The blood smear demonstrated macrocytosis, the bone marrow aspirate and biopsy were hypocellular, and the Kleihauer smear showed that 70% of the cells contained Hb F (Figure 1). The quantity of blood in the microsample was insufficient to test by alkali-denaturation; however, the proportion of Hb F was 45% by densitometry and 36% by immunoassay. The Hb A2 proportion was 0.5%. Given the high value for Hb F, we redetermined it by cation-exchange microchromatography, finding it to be 30%. The proportion of glycated hemoglobin was 7.8% (normal range: 4.0–8.0%). The patient was treated by transfusion and with oxymetholone and prednisone. Eleven weeks later her Hb was 116 g/L, the mean corpuscular volume 107 fl, the leukocyte count 3.2 × 109/L, and reticulocyte count 2.0%. The proportion of Hb F was 30% by alkali denaturation, 34% by densitometry, 30% by immunoassay, and 35% by cation-exchange microchromatography.

Materials and Methods

The mean corpuscular volume, leukocyte count, and Hb content were determined with a Coulter Counter. Hemolysates were electrophoresed on both cellulose acetate and citrate agar (Helena Laboratories, Beaumont, TX 77704). The proportion of fetal cells was determined by the acid elution method of Kleihauer et al. (Biodynamics/bmc, Indianapolis, IN 46250) (1). The proportion of Hb F was estimated densitometrically (2) and by radial immunodiffusion after fourfold dilution (Helena Laboratories) (3). To determine Hb F by cation-exchange microchromatography, we used the method of Abraham et al. (4), which involves Bio-Rex 70 cation-exchange resin in a microcolumn (Isolab Inc., Akron, OH 44321). In that method, “fast hemoglobin” is eluted

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3 Nonstandard abbreviations: Hb, hemoglobin; Hb F, fetal hemoglobin; RID, radial immunodiffusion.

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early by low-ionic-strength buffer, the remainder of the hemoglobin by high-ionic-strength buffer. We also use these columns to determine Hb A1c. Alkali denaturation by the method of Betke et al. (5) was also used to determine the proportion of Hb F. We determined glycated hemoglobin by phenyl boronic acid affinity microchromatography (Isolab Inc.) (6), in which sorbitol competitively elutes the glycated hemoglobin. The proportion of Hb A2 was determined by anion-exchange microchromatography.

Discussion

Fanconi’s anemia is a congenital aplastic anemia associated with multiple birth defects and an increased familial incidence of malignancy; it represents a bone-marrow stem-cell defect (7) associated with extreme increases in Hb F (8, 9), reflecting reversion to fetal erythropoiesis consistent with macrocytosis, high Hb F, and low Hb A2. Hb F greater than 25% is rare after one year of age except in hereditary persistence of fetal hemoglobin, juvenile chronic myelogeneous leukemia, aplastic anemia, and erythroleukemia (9), disorders for which the determination of Hb F can be diagnostically important.

In this case, the quantity of the first sample was insufficient for alkali denaturation (5), but we were able to use densitometry, RID, and cation-exchange microchromatography to obtain values for the proportion of Hb F of 45, 96, and 30%, respectively. We believe the densitometric result is an overestimate, consistent with inadequacies of the method; e.g., the CV in a case similar to this would be 26% (10). Probably the RID estimate is the most nearly accurate of the three, the CV for this method being only 5% (3). The cation-exchange microchromatographic method may have slightly underestimated the Hb F, although the CV for this method in the absence of Hb A is <4% (4). Upon the patient’s return 11 weeks later, alkali denaturation, as well as densitometry, RID, and cation-exchange microchromatography were performed to determine Hb F. The results by these methods correlated well, and the lack of significant change from earlier results suggests that synthesis of both Hb A and Hb F was stimulated by therapy.

To our knowledge, this is the first reported application of cation-exchange microchromatography to Hb F determination in a bone-marrow stem-cell defect. The total “fast” Hb eluted from the column represents a combination of Hb F and Hb A1c. The determination by affinity chromatography of glycated Hb is important for two reasons: (a) the normal value obtained (7.8%) suggests a normal lifespan for the erythrocytes, as in Fanconi’s anemia, confirming that the anemia here is hypoproliferative, and (b) the glycated value is used to determine the theoretical proportion of Hb A1c; Hb A1c = Hb A x (glycated Hb + 2.19/140) (6), where Hb A = 100% - Hb F by RID.

In this patient the Hb A1c is 4.6%. The theoretical proportion of Hb F can be calculated as Hb F = “fast” Hb - Hb A1c = 29.8 - 4.6 = 25.2%. Hence, the correct Hb F proportion is only 25%. Whether the proportion of Hb F should be corrected is debatable; our data suggest that it need not be.

We believe the result for Hb F of 30% is clinically accurate. However, we recommend using “high-performance” liquid chromatography (11) to confirm the applicability of cation-exchange microchromatography for determining Hb F.

We conclude that cation-exchange microchromatography can be applied to the determination of Hb F in patients with substantial amounts of Hb A. Although this has been demonstrated for Hb C and hereditary persistence of fetal hemoglobin (12, 13), this represents its first application to bone-marrow stem-cell disorders. We believe the speed and convenience of cation-exchange microchromatography make it the method of choice in such cases. Alkali denaturation by the method of Betke et al. (5) is laborious and requires a relatively large sample volume; radial immunodiffusion is slow and subject to dilutional error at high Hb F content; densitometry is inaccurate. Liquid chromatography offers many advantages but is both expensive and complex. We propose that samples be screened by densitometry; if the proportion of Hb F exceeds 30%, then use cation-exchange microchromatography for the Hb F determination.

References

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