Failure of Microchromatographic Measurement of Fetal Hemoglobin in $\beta^0$ Thalassemia—Hereditary Persistence of Fetal Hemoglobin

J. S. Krauss, 1 M. H. Jonah, 1 L. D. Devoe, 2 and C. G. Pantazis 1

We report microchromatographic measurement of fetal hemoglobin (HbF) proportions in a 36-year-old African-American multigravida woman. At 34 weeks she delivered a 630-g male infant who subsequently did well. Hemoglobin electrophoresis of the hemolysate revealed nearly 100% HbF without HbA, an extremely unusual naturally occurring sample. Family studies revealed a combination of hereditary persistence of fetal hemoglobin (HPFH) and $\beta^0$ thalassemia minor. Southern blot technique confirmed heterozygous $\alpha_2$ thalassemia and HPFH but failed to identify the $\beta$ thalassemic lesion. The absence of HbA and the very high amounts of HbF led us to measure HbF by several methods to confirm the accuracy of microchromatography of HbF at values approaching 100%. HPLC revealed a 14% F, suggestive of microchromatographic underestimation due to glycated HbF. We conclude that cation-exchange microchromatography and the Betke method of alkali denaturation underestimate HbF values as they approach 100% and do not recommend these procedures in this rare situation.

Cation-exchange microchromatography has been used to quantify fetal hemoglobin (HbF) concentrations in sickle-cell disease (1) as well as hereditary persistence of fetal hemoglobin (HPFH) with hemoglobin C-associated conditions (2). These columns are both accurate and precise and more convenient than the labor-intensive alkali-denaturation procedure. This technique is also considerably less expensive and technical than high-performance liquid chromatography (HPLC) (3).

We report here the microchromatographic measurement of HbF in the rare condition HPFH with $\beta^0/\alpha_2$ thalassemia (4). We performed a family study to confirm HPFH/$\beta^0$ thalassemia. Because HbF will coelute with HbA1 by microcolumn cation-exchange chromatography, HbA1 microcolumns can be used to measure HbF in the absence of HbA. Patients with the rare combination of HPFH and $\beta^0$ thalassemia have virtually 100% HbF, with a small component of HbA2. Naturally occurring samples such as this are very uncommon, even in the fetus, whose hemoglobin at term commonly has a substantial HbA component.

Materials and Methods

We performed hemoglobin electrophoresis at basic pH on cellulose acetate and at acid pH on citrate agar (Helena Labs, Beaumont, TX); for densitometry we used cleared cellulose acetate plates (Beckman Instruments Corp., Brea, CA) (5). Solubility testing for HbS was performed by the method of Itano (6). Microchromatography was used to quantify HbA2 and also HbF in the absence of HbA (Sickle-Cell-F Test and Quik-Sep HbA2 Test Systems; Isolab, Inc., Akron, OH). To determine HbF by cation-exchange microchromatography, we used the method of Abraham et al. (1), which involves Bio-Rex 70 cation-exchange resin in a microcolumn. In this method “fast” hemoglobin is eluted first by low-ionic-strength buffer; the remainder of bound hemoglobin is eluted by high-ionic-strength buffer. HbF was also quantified by alkali denaturation according to the methods of Singer et al. (7) and Betke et al. (8). Kleihauer smears (fetal-cell preparations) were used to evaluate the distribution of HbF (Boehringer Mannheim Diagnostics, Indianapolis, IN) (9). Whole-blood hemograms were performed with a Coulter STKR cell counter (Coulter Electronics, Hialeah, FL) (10). Reticulocyte counts were performed with Hematrak 450 and 590 (Geometric Data-SKF, Wayne, PA). DNA analysis for thalassemia and HPFH was by the Southern blot technique (4).

Case History

Our patient was a 36-year-old African-American woman who had delivered two previous small-for-gestational-age infants and a preterm stillborn fetus. During her current pregnancy, ultrasonographic studies showed that this child was significantly growth restricted, with an estimated fetal weight below the fifth percentile for gestational age. Pregnancy was otherwise uneventful and she delivered, at 34 weeks, a 630-g male infant who subsequently did well.

The mother's hemoglobin was 95 g/L, mean cell volume 74.7 fl, corrected leukocyte count 8.9 \times 10^9/L with 49 nucleated erythrocytes, and erythrocyte distribution width 28.8; the fetal-cell preparation (Kleihauer smear) had an even pattern (100%) consistent with HPFH; the reticulocyte count was 6.6%; and HbA2 was 3.1% (Figure 1, Table 1). Electrophoresis of the mother's hemoglobin showed an HbFA2 pattern consistent with a combination of $\beta^0$ thalassemia minor and HPFH (see Figure 2). Her 34-year-old husband had a mean cell volume of 79.7 fl, a hemoglobin value of 162 g/L, an...
HbA2 value of 2.36%, and <1% fetal cells (Figure 1, Table 1). Her 9-year-old son had a hemoglobin value of 119 g/L, a mean cell volume of 63.0 fl, an erythrocyte distribution width of 17.1, an HbA2 value of 5.0%, and a fetal-cell preparation of 20% with HbF 3.6% (Figure 1, Table 1). His 4-year-old brother (d in Figure 2) had a hemoglobin electrophoretic pattern consistent with HPPH; HbA2 was 1.38%, hemoglobin was 102 g/L, and mean cell volume was 68.2 fl with 100% fetal cells (Figures 1 and 2, Table 1). A Southern blot of the mother’s DNA was consistent with heterozygous α thalassemia caused by the deletion of a single α-chain gene as well as G, A, HPFH caused by a large deletion; however, the lesion associated with β thalassemia was not identified.

**Results**

HbF was measured five different ways. HbF by HPLC was 97.6% (T.H.J. Huisman, personal communication), of which ~14% was the early-eluting F1 (Figure 3); HbF values by the Betke method, by the Singer method, by microchromatography, and by subtraction (%HbF = 100 − %HbA2) were 89.5%, 98.6%, 88.9 ± 0.5% (n = 5), and 96.9%, respectively (Table 2). The CV for microchromatography was 0.8%. The mean percent HbF for the Betke and microchromatographic methods was 89.0% ± 0.5% (n = 6); the mean percent HbF for the Singer, HPLC, and subtraction methods was 97.7% ± 0.7% (n = 3; Table 2). The standard error of the difference of mean between the high and low HbF values was 19.3%, which corresponds to P < 0.001. We were unable to determine HbF in the HPFH son because of insufficient sample volume. Serum iron stores (II) were above-normal in the mother and normal in the son with β thalassemia minor.

**Discussion**

The proband exhibits a form of β thalassemia in which β-chain production is absent [β0] rather than decreased [β+]. The related disorder HPFH is associated

---

**Table 1. Family Hematologic Studies**

<table>
<thead>
<tr>
<th></th>
<th>Hb, g/L</th>
<th>Mean cell volume, fl.</th>
<th>Erythrocyte distribution width</th>
<th>Reticulocytes, %</th>
<th>Fetal cell, %</th>
<th>HbF, %</th>
<th>HbA2, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td>95</td>
<td>74.7</td>
<td>28.8</td>
<td>6.8</td>
<td>100</td>
<td>98.8</td>
<td>3.10</td>
</tr>
<tr>
<td>Father</td>
<td>152</td>
<td>79.7</td>
<td>13.2</td>
<td>1.2</td>
<td>&lt;1.0</td>
<td>—</td>
<td>2.36</td>
</tr>
<tr>
<td>Son</td>
<td>119</td>
<td>63.0</td>
<td>17.1</td>
<td>1.5</td>
<td>20</td>
<td>3.6</td>
<td>5.02</td>
</tr>
<tr>
<td>Son d (Fig. 2)</td>
<td>102</td>
<td>68.2</td>
<td>13.9</td>
<td>0.2</td>
<td>100</td>
<td>—</td>
<td>1.38</td>
</tr>
<tr>
<td>( \bar{x} \pm SD )</td>
<td>117 ± 22</td>
<td>71.4 ± 6.3</td>
<td>18.2 ± 6.2</td>
<td>2.4 ± 2.5</td>
<td>55 ± 45</td>
<td>51.1 ± 47.5</td>
<td>2.96 ± 1.3</td>
</tr>
<tr>
<td>Normal range</td>
<td>120–172</td>
<td>76–100</td>
<td>11.5–14.0</td>
<td>0.1–2.4</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>1.5–3.0</td>
</tr>
</tbody>
</table>
with a large deletion of the δ and β loci, which is confirmed by Southern blotting and occurs in ~0.1% of African-Americans. HbF concentrations are increased in HPFH and can be increased in β thalassemia. HbA₂ concentrations can be decreased in HPFH because of decreased δ-chain synthesis. The loci of the β-like genes are found on chromosome 11.

The family study confirmed these two lesions of this patient’s β locus (12). Thus, the patient produced α, γ, and δ chains, but not β chains because of deletions of both β loci. The family study also confirmed the maternal double heterozygosity at the β locus for HPFH, which was passed on to one son, and for β0 thalassemia, which was passed on to the other son. Her HbA₂ concentration was not increased despite β0 thalassemia because of a combination of α₂ thalassemia and HPFH, which lowers α- and δ-chain synthesis, respectively. The mother is incapable of bearing progeny with a normal β locus. The presence of maternal α₀ thalassemia, identified by Southern blotting, lessens α-chain synthesis, which ameliorates the deleterious effects of excess α-chain production for β thalassemia by balancing α- and γ-chain synthesis (4).

This is the first use of microchromatography to determine HbF proportions in the rare combination of β₀ thalassemia/HPFH. Cation-exchange microchromatography uses negative charges to bind the more-positively charged molecules and competitively elutes the less-positively charged molecules when exposed to positively charged small ions. Given that HbA₁ columns could be adapted for HbF measurement when HbA was <5% (1), as may occur in sickle-cell disease, we attempted to measure HbF in our β₀ thalassemia/HPFH patient.

Our data are consistent with microchromatographic underestimation of HbF caused by column retention or, more likely, nonbinding of glycated HbF as well as saturation of binding sites. The 14% HbF, by HPLC is supportive of nonbinding of glycated HbF. Clearly, subtraction of HbA₂ from 100%, the Singer method of alkalai denaturation, and HPLC produced higher, more accurate HbF values than did microchromatography and the Betke method of alkalai denaturation, which underestimates HbF concentrations when HbF is >40%.

Accurate measurement of HbF at birth and during early life is important because of evidence of delay in activation of the HbF switch in infants of diabetic mothers (13), conflicting data concerning the correlation of HbF concentrations with sudden infant death syndrome (14, 15), correlation of HbF/weight with fetal maturity (16), and HbF increase as a marker of erythroleukemia in adults (17). Our results contrast with microchromatographic HbF data generated by Abraham et al. (1) for theoretical mixing of HbF and HbS suggestive of strict linearity even at 100% HbF. We believe that microcolumns underestimate HbF when HbF is >80%; therefore, we do not recommend microchromatographic measurement of HbF in β₀ thalassemia/HPFH.

We thank P.F. Milner and H.H. Kazazian, Jr., for DNA analysis and T. H. J. Huisman for the HPLC data.

References