Complications of plasma exchange in thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: a study of 78 additional patients

The frequency of patients treated with plasma exchange (PE) for thrombotic thrombocytopenic purpura-hemolytic uremic syndrome (TTP-HUS) increased sevenfold from 1981 to 1997.1 Therefore, the morbidity and mortality due to PE is an increasingly important consideration in management decisions for patients with clinically suspected TTP-HUS. Some studies have described few complications associated with PE,2 but our previous report on 71 consecutive patients with clinically suspected TTP-HUS treated with PE from 1996 to 1999 demonstrated a major complication rate of 30 percent, including two deaths.3 This report describes our experience during the subsequent 3 years, 1999 to 2002, with 78 consecutive patients.

From June 25, 1999, to June 25, 2002, a total of 81 consecutive patients were referred to the Oklahoma Blood Institute for PE treatment of their first episode of clinically suspected TTP-HUS. Three patients were excluded because they died before a central venous catheter for PE was inserted.

Twenty-one of 78 patients (27 percent) had 27 major complications (Table 1). One patient died immediately after percutaneous insertion of a subclavian central venous catheter from pneumothorax and hemorrhage. Two patients suffered cardiac arrest with pulseless electrical activity: one from an anaphylactic reaction to plasma and the other from pericardial hemorrhage and tamponade, presumably due to cardiac perforation by an internal jugular catheter insertion guidewire.

Other major catheter-related complications included one patient with a retroperitoneal hemorrhage following femoral catheter insertion and seven patients with catheter thrombosis that prevented PE and/or required placement of a new central venous catheter; two of these seven patients had catheter-related venous thrombosis requiring systemic anticoagulation. Ten patients developed systemic infection: eight had blood cultures positive for the presence of bacteria (Staphylococcus aureus [five], Staphylococcus epidermidis [three]); the two patients with negative blood cultures were treated with parenteral antibiotics for presumed sepsis.

Other major plasma-related complications included hypotension in two patients requiring dopamine, acute hypoxia in two patients, serum sickness in one patient requiring systemic glucocorticoids, and severe vomiting in one patient that prevented completion of PE.

Twenty-seven (35 percent) patients developed minor complications, including 10 patients who also had major complications. The majority of the minor complications were urticaria (22 patients); other minor complications included vomiting, tetany, and hypotension responding to intravenous fluids.

These data confirm and extend our previous report.3 In summary, over 6 years we have observed 54 major complications, including 3 deaths, related to PE in 42 of 149 (28 percent) consecutive patients treated for clinically suspected TTP-HUS. These observations are essential to understand the risks of PE when evaluating the management of patients who may have TTP-HUS.

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REFERENCES
Quantitation and phenotyping of fetal RBCs in maternal blood by flow cytometry

Flow cytometry may be used to detect and quantitate fetal D+ RBCs in fetomaternal hemorrhage (FMH) to calculate the dose of Rho (D) immune globulin required to prevent maternal alloimmunization and protect against subsequent HDN. Fetal RBCs may be detected with antibodies to fetal Hb (anti-HbF) or to the D antigen (anti-D), either by indirect labeling or, more recently, by using directly conjugated MoAbs. Antibodies to additional RBC blood group antigens have been used to phenotype RBCs after transfusion and to monitor chimerism after marrow transplantation.

We report the case of a woman whose fetus died in utero at 35 weeks of gestation following trauma. The woman’s RBCs were Group A, D+, and the antibody screen was negative. The Kleihauer-Betke test demonstrated a 50-mL FMH. Cord blood was not available for phenotyping. It was therefore not known whether she could be immunized to antigens (other than D) expressed on fetal RBCs. Fetal and maternal RBCs were phenotyped by flow cytometry, the large FMH was confirmed, and an antigen mismatch was identified.

RBCs from the patient, two adult donors (controls, phenotypes O R R2 and O rr K+ k+ Fy(a+b+)), and cord blood were washed, and a mixture of cord and adult RBCs was made; 20-μL aliquots of 5 percent suspensions were labeled with 10 μL of FITC-conjugated anti-HbF (Silenus, Boronia, Victoria, Australia), 5 μL of FITC-conjugated anti-D (BRAD-3),2 200 μL of MoAb, or 50 μL of antiserum (Table 1). RBCs were incubated either at room temperature for 30 minutes (anti-HbF) or at 37°C for 1 hour (blood group antibodies). Before labeling with anti-HbF, RBCs were fixed (in 1 mL of 0.05 percent glutaraldehyde in PBS), washed, permeabilized (in 0.5 mL of 0.1% Triton X-100 in PBS with 0.1% BSA), and washed. Unlabeled primary antibodies were detected with 100 μL of 1-in-100 FITC-conjugated anti-human IgG (F(ab); Jackson ImmunoResearch, West Grove, PA). Samples were analyzed with a flow cytometer (FACSCalibur, Becton Dickinson, Oxford, UK), collecting 50,000 events and placing markers as appropriate on histograms of log-integrated FL1 fluorescence. The percentage of gated events and the median fluorescence of events under the markers were computed.

Using adult RBCs alone or with added cord RBCs, a marker was set to encompass strongly fluorescent fetal RBCs on histograms of FITC-anti-HbF–stained RBCs. The percentage of events under this marker in the adult sample (0.09%) was subtracted from that of the patient’s sample (2.59%), that is, 2.5 percent (Fig. 1A). Using the formula FMH (mL of packed fetal RBCs) = percentage of positive events × 22, the FMH in the patient’s sample was calculated to be 55 mL.

The median fluorescence of RBCs obtained with the blood group-specific antibodies is listed in Table 1. O R R2 RBCs were tested with the MoAbs and O rr RBCs with the antisera; the patient’s RBCs were tested with all antibodies. All samples, except three, exhibited fluorescence, as the patient’s RBCs were negative with anti-Jkb and anti-e did not show binding. For most antibodies, the median fluorescence of RBCs was similar between the patient’s RBCs and controls exhibiting a single dose of antigen (O R R2 or O rr) and the patient, indicating that similar amounts of antibodies were bound. Only one sample showed two populations; this occurred when the patient’s RBCs were incubated with anti-K. The major population (97.7%) was K+ (median fluorescence, 54), but a minor population of 2.2 percent of events was K– (median fluorescence, 4) (Fig. 1B). Subtracting the background of unlabeled events under this marker (0.2%) gave 2 percent K– cells or a 44-mL FMH.

Some examples of anti-C, anti-c, and anti-E gave greater fluorescence than others (Table 1). It was ob-

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**TABLE 1. Flow cytometric data of fluorescence of RBCs labeled with a panel of blood group antibodies**

<table>
<thead>
<tr>
<th>Antibody specificity</th>
<th>Clone (MoAb) or serum</th>
<th>Control RBCs (O R R2 or O rr)</th>
<th>Patient’s RBCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>FITC-BRAD-3*†</td>
<td>191</td>
<td>167</td>
</tr>
<tr>
<td>D</td>
<td>BRAD-8†</td>
<td>743</td>
<td>661</td>
</tr>
<tr>
<td>C</td>
<td>MS257*</td>
<td>57</td>
<td>36</td>
</tr>
<tr>
<td>C</td>
<td>HMR7†</td>
<td>118</td>
<td>109</td>
</tr>
<tr>
<td>c</td>
<td>MS37*</td>
<td>29</td>
<td>19</td>
</tr>
<tr>
<td>c</td>
<td>MS51*</td>
<td>85</td>
<td>93</td>
</tr>
<tr>
<td>E</td>
<td>HIRO-18*</td>
<td>79</td>
<td>76</td>
</tr>
<tr>
<td>E</td>
<td>HIRO-91†</td>
<td>34</td>
<td>32</td>
</tr>
<tr>
<td>E</td>
<td>907*</td>
<td>41</td>
<td>51</td>
</tr>
<tr>
<td>e</td>
<td>MS70*</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>K</td>
<td>Serum</td>
<td>43</td>
<td>54 and 4</td>
</tr>
<tr>
<td>k</td>
<td>Serum</td>
<td>12</td>
<td>27</td>
</tr>
<tr>
<td>Fy*</td>
<td>Serum</td>
<td>33</td>
<td>50</td>
</tr>
<tr>
<td>Fy†</td>
<td>Serum</td>
<td>19</td>
<td>44</td>
</tr>
<tr>
<td>Jk*</td>
<td>Serum</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Jk†</td>
<td>Serum</td>
<td>12</td>
<td>5</td>
</tr>
</tbody>
</table>

* The MoAbs were submitted to the Third International Workshop on Monoclonal Antibodies Against Human Red Blood Cells and Related Antigens held in 1996.
† The MoAbs were submitted to the Fourth International Workshop on Monoclonal Antibodies Against Human Red Blood Cells and Related Antigens held in 2001.
served previously that some IgG non-anti-D MoAbs gave relatively low fluorescence in flow cytometry, compared to agglutination. IgM antibodies against Rh C, c, E, and e may be more effective.

On the basis of the median fluorescence values, the most likely phenotypes of maternal and fetal RBCs in the patient’s sample were maternal, C+ , c+ , D+ , E+ , K+ , k+ , Fy(a+b+), Jk(a+b–); and fetal, C+ , c+ , D+ , E+ , K–, k+, Fy(a+b+), Jk(a+b–). The only difference detected between maternal and fetal RBCs was that fetal RBCs were K–.

Thus, using this panel of antibodies, there were no blood group antigens detected on fetal RBCs that could immunize the mother.

Flow cytometry can reliably detect minor RBC populations of 1 percent or less, depending on the antibody used for identification, and has advantages over other techniques such as differential agglutination (rapid but less sensitive) and microscopic enumeration of RBC-binding fluorescent microspheres (sensitive but tedious). Reports of flow cytometric detection and quantification of minor cell populations when using one to three antibodies have been extensively reviewed. The case study detailed here demonstrates the utility and rapidity of flow cytometry, both for quantification of FMH and for extensive phenotyping of mixed RBC populations.

REFERENCES

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LETTERS TO THE EDITOR
Ten-year survival of transfusion recipients identified by hepatitis C lookback

Empirical data from population-based studies on the long-term (≥10-year posttransfusion) probability of survival of transfusion recipients are needed for public policy decisions, but no such data currently exist for patients transfused after 1981. The 10-year probabilities of survival of Olmsted County, Minnesota residents transfused in 1981 were often used previously, but these figures most likely do not reflect the survival of patients transfused in the 1990s. Five- to 10-year posttransfusion probabilities of survival that range from 26% to 41 percent can be inferred from HCV lookback studies. These studies were not designed to calculate posttransfusion survival, however, and it is difficult to infer the length of recipient follow-up from the presented data. The New York University Medical Center (NYUMC) HCV lookback study was designed to calculate posttransfusion survival, and it reported 4-year posttransfusion probabilities of survival that were 13 percent lower than those reported from Olmsted County, and 40-month probabilities of survival that were 20 percent lower than the Olmsted County figures. For patients transfused in 1994 in northern England, Wells et al. reported 2- and 5-year probabilities of survival that were, respectively, 15 percent and 22 percent lower than the Olmsted County figures. Thus, when data on posttransfusion survival are needed as input for future studies, posttransfusion survival probabilities of 66, 60, 47, and 40 percent, respectively, at 1, 2, 5, and 10 years posttransfusion can be used until definitive empirical data become available. A population-based study that includes several US counties has to be conducted to generate the information needed for public policy decisions in the future.

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REFERENCES

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>227</td>
</tr>
<tr>
<td>Men</td>
<td>140</td>
</tr>
<tr>
<td>Women</td>
<td>87</td>
</tr>
<tr>
<td>Young (under 41)</td>
<td>39</td>
</tr>
<tr>
<td>Middle-aged (41–65)</td>
<td>73</td>
</tr>
<tr>
<td>Senior (over 65)</td>
<td>115</td>
</tr>
<tr>
<td>Medical*</td>
<td>107</td>
</tr>
<tr>
<td>Surgical*</td>
<td>120</td>
</tr>
<tr>
<td>Recipients of ≤10 RBC units</td>
<td>83</td>
</tr>
<tr>
<td>Recipients of &gt;10 RBC units</td>
<td>144</td>
</tr>
</tbody>
</table>

* Patients transfused for treatment of a “medical” or “surgical” disease.2,5,6
SUBMISSION OF LETTERS

Instructions for submission of letters can be found in the Detailed Instructions for Authors published on pages 128 to 133 of the January issue. Submit letters to:

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