An antibody to a low-incidence antigen was identified in the serum of a nontransfused male patient. The antibody was subsequently identified as anti-Wra and was only detectable at the antihuman globulin (AHG) phase of the crossmatch. Instances of severe hemolytic transfusion reactions have been reported following the transfusion of red blood cells containing low-incidence antigens in patients with antibodies directed toward these antigens (e.g., anti-Wra, -Co, -Js, etc.). Elimination of the AHG phase of the crossmatch can result in either risks or benefits. Since patients seen at this facility primarily have been multitransfused or are multiparous females, the AHG phase of the crossmatch has been maintained. Immunohematology 1997;13:20–22.

Anti-Wra was first described in 1953 in three generations of an English family, in which it had been the cause of hemolytic disease of the newborn (HDN). Since then, many examples of anti-Wra have been identified and Wra has become a well-investigated low-incidence antigen. There are few documented cases of hemolytic transfusion reactions (HTRs) due to anti-Wra. The first case of an HTR was reported by Van Loghem et al. in 1955. A second case was reported in a sickle cell anemia patient that resulted in oliguria that lasted 16 days. The antibody reacted only at the antihuman globulin (AHG) phase of testing. Other low-incidence antigens have been implicated in cases of HTRs. In 1984, Squires et al. reported a case in which a sickle cell anemia patient suffered a delayed HTR due to anti-Co. Although the incidence of HTRs due to low-incidence antigens is very low, the potential for sensitization in certain patients (i.e., the multitransfused) is higher.

Case Report
A 42-year-old Caucasian male was admitted to the Medical College of Georgia Hospital and Clinics for elective surgery. A pretransfusion workup two months earlier revealed no serologic abnormalities. On the day of admission, a sample was sent to the blood bank for crossmatch of two units of blood. The patient’s red blood cells (RBCs) typed as group A, D+ and the antibody screen was negative. One of the two units crossmatched was incompatible (2+) at the AHG phase. The immediate spin and the 37°C phases were negative. A routine antibody panel was negative. The patient’s serum was tested against selected panels of RBCs containing low-incidence antigens and the antibody specificity was identified as anti-Wra. An additional unit of blood was crossmatched and found to be compatible. There was no history of previous transfusions and the patient did not require transfusion.

Materials and Methods
The patient’s RBCs were typed for ABO and D antigens and a direct antiglobulin test (DAT) was performed on the incompatible donor unit using standard serologic techniques. The patient’s RBCs were also tested for the presence of the A1 antigen using the lectin Dolichos biflorus (Organon Teknika, Durham, NC). The patient’s serum was screened for alloantibodies using an LISS-AHG technique and a two-cell screen (Gamma Biologicals, Inc., Houston, TX). The patient’s serum was also tested against an 11-cell antibody panel (Gamma) and other selected cells (Gamma; Organon Teknika; Immucor Inc., Norcross, GA; and Baxter Diagnostics, Miami, FL). All antibody panel testing was performed using an LISS-AHG technique.
A survey of alloimmune antibodies detected and identified from September 1993 to September 1996 was undertaken in order to determine the prevalence of antibodies to low-incidence antigens in our hospital population.

Results

A DAT performed on the incompatible unit was negative. Antibody panel results were negative, but the patient’s serum reacted with two of five RBCs known to be positive for certain low-incidence antigens. Both of the reactive RBCs were Wr(a+). An additional Wr(a+) RBC from liquid nitrogen storage was also reactive. Due to a lack of reagent, the incompatible unit was not tested for Wra.

Table 1 summarizes the number of antibodies to low-incidence antigens reported from September 1993 to September 1996 at our facility from a total of 370 cases in which an alloimmune antibody was identified.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Number reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Wra</td>
<td>3</td>
</tr>
<tr>
<td>Anti-Jsa</td>
<td>1</td>
</tr>
<tr>
<td>Anti-Coa</td>
<td>1</td>
</tr>
<tr>
<td>Anti-Kpa</td>
<td>7</td>
</tr>
<tr>
<td>Anti-Goa</td>
<td>1</td>
</tr>
</tbody>
</table>

Discussion

The patient in this case did not require transfusion. However, in a report by Metaxes et al., the patient experienced a severe delayed HTR after being transfused with a unit of Wr(a+) blood. The patient had undergone an operation to remove a large abdominal mass and three units of blood were transfused. No crossmatching was done. On the day following surgery the patient became jaundiced and developed prolonged oliguria that lasted for 16 days. The patient was hemodialyzed ×3 at another hospital in an attempt to reverse the effects of renal damage. Two days after the third dialysis, the urine output began to rise until it returned to normal on the 28th postoperative day. Unfortunately, the patient expired from E. coli and staphylococcal infections.

Anti-Wra occurs in approximately 1 percent of the population. It is sometimes found in the sera of male patients who have never been transfused, in patients who have formed Rh or Kell antibodies, and in patients with acquired autoimmune hemolytic anemia. The ability of anti-Wra to cause HTRs is best predicted by the immunoglobulin class of the antibody. Anti-Wra has been shown to be IgM, partly IgM, and/or IgG. Most examples of anti-Wra reactive only at the AHG phase have been shown to be IgG1. Both IgG1 and IgG3 are known to bind complement and can bring about rapid destruction of RBCs in vivo. Survival studies have shown that when small test doses of Wr(a+) RBCs are injected into individuals whose sera contain anti-Wra, half of the RBCs are cleared in 80 minutes. Rate of clearance depends on the subclass of the antibody involved and its ability to activate complement. Transfusion of RBCs containing low-incidence antigens into patients known to have the corresponding antibody must be approached with caution and consultation with the transfusion service medical director or supervisor should always be considered.

Controversies Surrounding Elimination of the AHG Crossmatch

There has been much controversy regarding the elimination of the AHG crossmatch provided that the antibody screen is negative. These differences in opinion are largely the result of documented cases of HTRs in patients who had developed antibodies to low-incidence antigens that were not detected because the AHG phase of the crossmatch had been omitted. In the literature there have been attempts to assess the frequency of incompatible crossmatches following a negative antibody screen. In a study conducted from January 1975 through November 1981, the frequency was found to be 0.00087 (1 in 1,151 patients) for an incompatibility at any phase of the crossmatch, and 0.00048 (1 in 2,076 patients) for an incompatibility only after 37°C incubation and/or after the AHG phase. However, the authors caution that it is impossible to calculate an absolute frequency due to many variables involved, such as sample size and selection. Other variables include the frequencies of the antigens on donor and test cells, recipient antibody frequency, and technical proficiency and procedures.

Risks and Benefits Associated With Elimination of the AHG Crossmatch

Both risks and benefits must be considered when deciding to eliminate the AHG crossmatch. The most obvious transfusion risk is that a severe HTR may occur when a patient has a clinically significant antibody directed against a low-incidence antigen that has not been detected because the AHG crossmatch was omit-
Most facilities use either a two- or three-cell antibody screen procedure; however, low-incidence antigens are most often not present on standard screening cells.

One transfusion service has incorporated an additional cell containing the Js<sup>a</sup> antigen as part of their routine antibody screening procedure because of a severe transfusion reaction that occurred in a patient whose serum contained anti-Js<sup>a</sup> detectable only at the AHG phase (personal communication). At that time the laboratory was performing an immediate-spin crossmatch only.

Typing reagents for low-incidence antigens are in short supply and most hospitals do not have ready access to RBCs known to be positive for low-incidence antigens. However, most reference laboratories maintain a frozen inventory of RBCs containing low-incidence antigens that may be used in antibody identification studies.

The benefits of elimination of the AHG crossmatch in lieu of an immediate-spin crossmatch would include improved rapid turnaround time for release of blood components, reduced technical time, decrease in reagent usage and cost, increased technical time for other workload functions, and better utilization of blood component inventory.

**Conclusion**

If a facility is considering discontinuing the AHG crossmatch in favor of the immediate-spin crossmatch, the number of clinically significant antibodies and sensitization rate in the patient population should be examined. Based on the large number of clinically significant antibodies (370) encountered at our tertiary care facility over 3 years (of which 3.5% were antibodies to low-incidence antigens), the AHG phase of the crossmatch has been maintained to ensure that compatible blood is being transfused and to reduce the possibility of HTRs.

**References**

2. van Loghem JJ, van der Hart M, Bok J, Brinkerink PC. Two further examples of the antibody anti-Wr<sup>a</sup>. Vox Sang 1995;5:130-4.