Transfusion Problems in Hemolytic Anemias*

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All hemolytic anemias feature shortened red cell survival due to premature hemolysis of the cell. For the purposes of this presentation, we may classify the hemolytic anemias, most broadly, according to the mechanisms leading to hemolysis.

Intrinsic Mechanisms

Hemolytic anemias due to intrinsically defective erythrocytes are essentially of three types. First are those anemias in which the red cells are defective due to lack of an essential factor, eg, pernicious anemia in relapse. The second type includes those in which the red cells have an abnormal shape because of an inherited error in the chemical makeup of the hemoglobin molecules; eg, sickle cells, elliptocytes, spherocytes, and the target cells. Last are those anemias in which the red cells are defective in their basic enzyme systems, as in paroxysmal nocturnal hemoglobinuria (PNH) (bithermal auto-hemolysins) with acetylcholinesterase deficiency, or primaquine sensitivity with glucose-6-phosphate dehydrogenase (G6PD) deficiency.

Such anemias due to intrinsically defective erythrocytes, including those in which the red cells are defective in their basic enzyme systems as in PNH, pose no problem for the Blood Bank. In these cases there is no antibody involved, and compatible group and type specific blood is abundantly available and effective in raising the hemoglobin level of these patients. However, should the reticulocyte count exceed five percent, a direct Coombs test (DCT) may be obtained which complicates matters for the Blood Bank, unless the reticulocytosis is brought to the attention of the Blood Bank staff.

Another complication may occur if the specimen sent for blood group, type, and crossmatch is obtained shortly after hemolysis has occurred. Should the serum contain hemolysins, they might not be detected in the crossmatch unless certain precautions are taken. If missed, incompatible blood may be issued —adding insult to injury.

Extrinsic Mechanisms

Those hemolytic anemias which are due to extrinsic factors may be classified, further, as non-immune or immune. Non-immune mechanisms include a) drugs and chemicals (phenylhydrazine, naphthalene, lead, snake venoms); b) physical agents (heat); c) bacteria and parasites (hemolytic streptococci, Clostridium welchii, Bartonella, plasmodia); and d) acquired sensitivity to penicillin, methyldopa, Kelfin®, or fava plant as examples. Some of the agents in this last group serve to lyse the cells, either through direct action or by formation of antibodies.

These hemolytic anemias due to extrinsic factors of the non-immune variety present no transfusion problem for the Blood Bank. However, it must be realized that in those conditions where a positive direct or indirect Coombs test is obtained (as in acquired sensitivity to methyldopa, penicillin, phenacetin) a situation may arise whereby blood may be needed in an emergency; because of this incompatibility the release of blood may be delayed, to the detriment of the patient. Therefore, it is essential that such conditions be recognized within a short period of time. The administration of blood thereafter becomes as easy as the transfusion of any patient in the previous category.

In drug sensitivity cases, however, it must be realized that the hemolytic anemia will cease once the drug administration is stopped, and the DCT usually reverts to negative. Worledge, Carstairs, and Dacie (1966) studied 30 patients who developed hemolytic anemia while taking methyldopa (Al-domet®). Cessation of therapy led to immediate hematological remission. Peculiarly, the auto-antibodies had Rh specificity, instead of being directed against methyldopa or one of its derivatives. In patients on methyldopa, in which the DCT becomes positive, warm auto-antibodies may be detected in the serum so that all donors appear to be incompatible. However, despite the presence of such incompatibility, infused red cells show normal survival. Reversion to a negative Coombs test enables the Blood Bank to obtain and prepare compatible blood for the patient without difficulty or worry. It is essential, therefore,
that the drug causing the hemolytic anemia be withheld.

Sodium cephalothin (Keflin®) is another drug which sometimes causes neutropenia and often a DCT which appears to be causally related to hemolytic anemia. The clinical significance of the DCT in these patients is primarily one of its complicating blood banking—as high as 75 percent of such patients, depending on the dose administered, may give an incompatible minor crossmatch on a non-immunologic basis.

Another antibiotic which complicates blood banking, and may be a cause of hemolytic anemia, is penicillin. In contrast to cephalothin, penicillin produces both a DCT and indirect Coombs test (ICT). This means that the major crossmatch, in addition to the minor crossmatch, is incompatible. In a review of allergic drug-induced hemolysis, Dausset and Contu (1967) found penicillin to be the offending drug in 14/34 cases. Another difference between these two related antibiotics is that an eluate which will react with penicillin as well as cephalothin-coated cells may be prepared. No such eluates may be prepared from the sera of patients receiving cephalothin.

Another drug known to rarely cause hemolytic anemia on an immunological basis is phenacetin (acetophenetidin). This drug also produces a number of side effects: direct dose dependent hemolytic anemia, methemoglobinemia, sulfhemoglobinemia, and (rarely) a severe hemolytic anemia in G-6-PD deficient individuals. Patients who develop sensitivity to the drug produce antiphenacetin antibodies which can directly (although weakly) agglutinate red cells, and produce and ICT and an active eluate. The addition of phenacetin to the patient’s serum neutralizes the antibodies in the presence of complement after two hours of incubation. A basic difference between Keflin® and penicillin sensitivity, and phenacetin sensitivity is the failure of normal erythrocytes to absorb phenacetin.

Quinidine, quinine, p-aminosalicylic acid, stibophen, and sulfomethrazine have been cited as rare causes of hemolytic anemia.

Varying mechanisms of action have been postulated for these drugs. The cause-effect relationship of these drugs is dependent on the in vitro demonstration of enhancement of the ICT in the presence of the offending drugs. It seems that the mechanism of immunologic hemolysis induced by some drugs may be different than that produced by others. In 1965, Levin summarized the mechanism leading to drug sensitivity with antibody formation as a drug or a drug degradation product combining with tissue protein to form a complete antigen. These provoke formation of antibodies which possess structural specificity toward either the drug hapten, the aminoacid residue binding hapten to protein, or the structural configuration of the protein moiety. If the drug attaches to the red cell membrane proteins first, the formed antibodies sensitize the red cells and lead to their destruction. According to Ackroyd (1952), this is the mechanism of immune hemolysis in penicillin sensitivity. A variation of this scheme has been proposed which suggests that cephalothin causes a positive DCT by binding pre-existing plasma proteins to erythrocytes. Another mechanism of cell destruction has been advanced by Miescher and Cooper (1960) who suggest that drug and antibody combine in the plasma with secondary and non-specific fixation to the cell.

The hemolytic anemias of immune origin may be the result of the presence of a) iso-antibodies or iso-hemolysins, or b) auto-antibodies or autohemolysins. Hemolytic anemias caused by auto-antibodies are acquired. From the standpoint of mechanism they may be classified as primary idiopathic (occurs in patients in whom no primary disease can be demonstrated), or secondary symptomatic (is associated with some other disease; eg, malignant lymphomas, leukemia, virus pneumonia, infectious mononucleosis, or collagen disease). From the standpoint of serology, the auto-antibodies may be classified as warm antibodies, with best reactivity at 37 C; and cold antibodies, with maximum reactivity at lower temperature. In turn, the cold and warm antibodies may be subclassified into normal and pathogenic. The presence of both of these kinds of antibodies is shown best by the DCT, which is the distinguishing feature, and by auto-agglutination. Patients with the pathogenic type of either the cold or warm type are said to have auto-immune hemolytic disease or anemia. Most antibodies eluted from the cells of patients with the warm type of auto-immune hemolytic anemia detect an antigen associated with the Rh agglutinin complex.

Hemolytic anemias due to iso-antibodies and iso-hemolysins are, in the majority of cases, easy to deal with; cells lacking the corresponding antigen are usually relatively easy to find, and compatible blood may therefore be transfused. However, certain iso-antibodies are directed against an antigen that is present in a high percentage of the population; should blood be requested, particularly in large amounts, it would be very difficult to obtain the required number of units of compatible blood. For example, in order to find two units of compatible blood for a patient with anti-k antibody, the blood of 1,000 donors must be crossmatched since the percentage of Cellano negative individuals is only 0.2 percent. Other examples of this type of incompatibility may be cited. It is immensely difficult to transfuse patients with paroxysmal cold hemoglobinuria. These patients show a positive DCT, free plasma hemoglobin, and free antibodies in their serum. These antibodies are of anti-P, or anti-Ti specificity. Since the corresponding
Tj⁺ antigen is present on the red cells of more than 99.9 percent of people, it is obvious that to find a unit of compatible blood more than 1000 randomly selected donors must be crossmatched.

The transfusion of a patient with the warm antibody type of auto-immune hemolytic anemia is also a formidable problem. The cells of such patients give a positive DCT and, infrequently, an ICT. A positive ICT in these patients is associated with a poor prognosis.

**Anti-e Antibodies**

Anti-e is one of the most disliked antibodies when found in a patient with auto-immune hemolytic anemia who is in need of blood transfusion, for three main reasons:

1. It is a warm antibody; the administration of incompatible blood is certain to be detrimental to the patient who obviously can do without an additional load of plasma hemoglobin.

2. This antibody is directed against a very common antigen. After crossmatching 50 units of blood, one may find one unit of compatible blood if lucky. For each additional unit requested, 50 units must be crossmatched.

3. The adverse effect of the antibodies on the supposedly compatible red cells is often demonstrated clinically by the frequency of hemolytic transfusion reactions in patients having auto-immune hemolytic anemia in general; and in patients having auto-immune hemolytic anemias due to anti-e reacting not only with e-positive cells but also, although much more weakly, with e-negative cells. Consequently, although e-negative cells may have a near normal life span following initial transfusion, subsequent transfusions of these patients result in the appearance of a higher titer of antibodies. This complicates future crossmatching tests. One other result of failure to apply extreme restraint on the use of blood in such cases is the exhaustion of the limited supply of the rare R,R_e blood. Hence, when such blood is needed as a life-saving measure, it is often not available. The first case study—auto-immune hemolytic anemia due to anti-e, secondary to chronic lymphocytic leukemia (CLL)—typically illustrates this point.

L.C, a Negro male diagnosed as having CLL in January, 1966, was taking chlorambucil until January, 1968. In October, 1968, he was found to have anemia with a hemoglobin of 4.5 gm percent. A sample of blood sent to the Blood Bank showed the following results:

<table>
<thead>
<tr>
<th>Blood Group:</th>
<th>O Rh_(a) (Pos.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Coomb's Test:</td>
<td>Positive (1+)</td>
</tr>
<tr>
<td>Most Probable Genotype:</td>
<td>R₁R₂ (CDe/cDE)</td>
</tr>
<tr>
<td>Antibody Identification:</td>
<td>Anti-e. Detectable only by the Coomb's technique</td>
</tr>
</tbody>
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Blood was released, and three units of R₂R₂ (cDE/cDE) were released and administered.

It is obvious that prior to transfusion the direct and indirect Coombs tests were weak. Although e-negative blood was transfused, both the DCT and ICT became progressively stronger—as evidenced by a rise in antibody titers from one in pre-transfusion to eight in post-transfusion blood sample. As the number of transfusions increased, the patient’s serum became increasingly incompatible not only with the blood of e-positive donors, but also with that of e-negative donors. Just before his demise, L.C. received the last two units of R,R_e, which we were able to obtain from the rare bloods stored in a frozen state at the New York Blood Center.

**Anti-I Antibodies**

A patient with auto-immune hemolytic anemia of the strictly cold antibody type, which does not agglutinate cells at 37 C, presents a lesser problem. Usually the antibody involved is of anti-I specificity. Dacie reported finding cold auto-antibodies rather than warm ones in about 20 percent of cases of idiopathic acquired hemolytic anemia and in about 30 percent of cases secondary to such conditions as CLL. Further, an upper respiratory tract infection associated with a group of organisms known as pleuro-pneumonia-like organisms was found in all cases in which acquired hemolytic anemia followed an attack of primary atypical pneumonia. Anti-I antibodies are directed against an antigen which is much more prevalent than the e antigen. In order to find a unit of compatible blood, the Blood Bank would have to crossmatch the serum of the patient against the blood of 4,400 randomly selected people. However, as mentioned earlier, such patients are a lesser problem in transfusion mainly because the majority of the patients show an antibody which reacts below body temperature only; therefore, when absolutely essential such patients may be safely transfused, provided transfusion is given conservatively.

The advisability of transfusing these patients, conservatively and only when absolutely essential, is based on the fact that continued transfusion of such patients may eventually result in production of the antibodies which are reactive at body temperature. Blood administered to these patients has a reduced life span and may invoke a hemolytic transfusion reaction which, in turn, may produce warm reacting antibodies. Such patients have an abnormal immune response and the antibodies, although cold, are pathogenic.

A few of the patients with auto-immune hemolytic anemia due to anti-I have antibodies which react initially not only in the cold but also at 37 C. Transfusion of such patients with incompatible blood must be avoided unless it is to alleviate debilitating symp-
toms or is a life saving procedure. Such patients are susceptible to antibody production when repeatedly challenged with the corresponding antigen.

Therefore, the sooner and more frequently these patients are transfused, the sooner the stage is reached when transfusion of incompatible blood is no longer a life saving measure. One can be more liberal in the use of blood for such patients if the very rare ii genotype blood is available. To our knowledge no individual living in the Richmond, Virginia area belongs to this phenotype. Since these patients are kept relatively comfortable with the judicious use of immunosuppressive therapy and avoidance of exposure to cold, the use of hemotherapy may be avoided for a considerable period of time. The successful blood management of such a patient is represented in the second case study.

D.O. was a 60-year-old white female who had been in good health until ten days prior to hospital admission on October 25, 1965. At that time she noted severe headaches and weakness of her lower extremities with ataxia; she developed nausea, vomiting, and fever. She was diagnosed as having bronchopneumonia and was treated accordingly. She was also found to have severe anemia with a hemoglobin of 5.4 gm percent. History showed no meleina, hematuria, or epistaxis and she had noted bruising only during the two weeks prior to admission. She had no history of jaundice. Family history was non-contributor. Later she was referred to William T. Dabney of the Medical College of Virginia, who found her to have a moderately enlarged spleen. Cr61 red cell survival time and studies showed a T 1/2 of 14 days (normal 25-35 days), and a slight increase in the spleen to liver ratio with normal splenic localization index. A blood sample sent to the Blood Bank on December 29, 1966 showed the following:

| Blood Group: | O Rh0 (Neg.) |
| Direct Coombs Test: | Weakly Positive |
| Indirect Coombs Test: | Positive, Titer—1:4 |
| Antibody Specificity: | Anti-I |

The anti-i antibodies were found to react not only in the cold but also at 37 C. The Blood Bank accordingly advised withholding transfusing until absolutely essential. This patient was treated and kept on a maintenance dose of prednisone. The transfusion of blood has been avoided purposefully until the present, despite the fact that her hemoglobin has been 6-7 gm percent. Dr. Dabney saw her again in January, 1970 and found her active despite her anemia. The DCT was repeated 15 times between December, 1966 and January, 1970. Reactivity ranged between negative and 1+ strong with the last test, like the first one, being only a weak positive. The failure of the Coombs test to become stronger after four and one-half years may be the result of non-transfusion. This, as suggested previously, indicates the value of withholding blood transfusion in such cases.

Anti-i Antibodies

Another case exemplifying a transfusion problem in hemolytic anemia is that caused by a high titer of anti-i antibodies in patients with IM. Last year we reported the fourth case of IM complicated by severe, but transient, auto-immune hemolytic anemia due to anti-i, as seen in a third case study.

R.P. was a 20-year-old white male who had been in good health until six days prior to admission to the Medical College of Virginia Hospital. On that day, he was involved in an automobile accident. Examination of the patient at the referring hospital emergency room immediately following the accident revealed no serious injury, with negative X-rays, and he was released. Four days later he began to have some vomiting and pain in the right side of the abdomen, which continued until the day of admission. The next day he developed dark urine and a mustard-colored stool. At this time, one day prior to admission, he was found jaundiced with a bilirubin level of 7.6 mg percent, a hemoglobin of 8.0 gm percent, and a hematocrit of 22 percent. There was marked tenderness in the right upper quadrant and a possible epigastric mass. He was referred to the Medical College of Virginia Hospital for further evaluation and admitted on April 20, 1968. The past medical history was not remarkable except for a prior appendectomy. Examination showed a pulse of 120/min and a blood pressure of 130/90. He was jaundiced with some abdominal distress. Eyes showed marked icterus. The abdomen showed tenderness in the right upper quadrant and there was a small, ill-defined, tender epigastric mass. The spleen was barely palpable. Prior to admission the patient had never received blood, blood components, antibiotics, or other drugs. He denied exposure to any chemicals, and there was no history of allergy to drugs or chemicals. The rest of the accessory history was not remarkable. A tentative diagnosis of liver hematoma was made, and surgery to relieve it was entertained. For this purpose eight units of blood were requested. Because of difficulties encountered in blood grouping, typing, and crossmatching, blood was withheld until studies were completed to clarify the discrepancies. The difficulties consisted of a discrepancy between cell grouping which showed him to be group AB and serum grouping which showed him to be group O. The DCT using broad spectrum Coombs serum was strongly positive; his serum agglutinated 60 randomly selected group A bloods and all members of two commercial panels of cells. Using appropriate techniques, the blood group of the patient was found to be A,B. Initial laboratory studies on admission showed a hemoglobin of 5.2 gm percent, and a
hematocrit of 15 percent which steadily fell to 4.5 gm percent and 12 percent respectively over the next eight hours. The white cell count was 13,500/mm³; the differential showed 54 percent polymorphonuclear leukocytes, 31 percent lymphocytes and 14 percent considered monocytes. Nucleated red cells were also present. Blood chemistry studies included amylase-70 (normal 150) Somogyi units, alkaline phosphatase 18 (normal 0.8 to 2.9) Bessay-Lowry units, LDH 570 (normal 20 to 68) International units, and SGOT 225 (normal 0 to 40) Karmen units. Total protein was 6.9 gm percent with 56 percent albumin and A/G of 1.27:1. Plasma hemoglobin was 210 mg percent, and the urine was strongly positive for bile and contained 160 mg of hemoglobin. On the second day of admission, a liver radiosotopic scan revealed a moderately increased size and decreased uptake in both lobes, and increased concentration in the spleen which was enlarged. Because of the immunologic reaction which is indicated by the DCT, hemoglobinemia and bilirubinemia, he was started on prednisone. The red cell count on this day was 1.92 mil/cu mm, and the platelet count was 364,000/cu mm. The peripheral blood smear showed nucleated red cells, shift to the left, and 50 percent lymphocytes with one-half appearing as pathological lymphocytes. The bone marrow showed good myeloid, erythroid, and megakaryocytic cellularity with moderate increase in erythroid/myeloid ratio and a slight increase in reticuloendothelial cells. The stain for iron was strongly positive. On this day he received two and one-half units of whole blood without untoward reaction. Permission to release blood was based on compatibility tests with patient's serum after absorption with own cells. Blood was released which showed incompatibility no stronger than a selfcontrol by the saline and albumin methods, and which was negative by the Coombs test. The following day, April 22, the patient showed a clear urine but the urine became hemoglobinuric almost immediately after the administration of two more units of blood. A heterophile agglutination test was performed, and the unabsorbed serum showed a 1:448 titer which remained unchanged after guinea pig kidney absorption, and became negative after beef cell absorption. Further laboratory studies using rare cells of the phenotype ii and absorption-elution techniques indicated that the antibody causing the incompatibilities was of anti-i specificity. This antibody occurs rather commonly in patients with IM, although in low titer. As such it reacts strongly with cord blood and only weakly, if at all, with adult blood at 4 C only. Therefore, it is usually of no clinical significance and does not present crossmatching difficulties. However, when it does, it is important to identify the antibody within a short period of time. This shows the immunohematologist that it is advisable to release blood for these patients, since transfusion problems which may occur may be outweighed by the complications of withholding blood.

Albumin Autoagglutination Phenomenon

The last case posing a transfusion problem which I present is a rare phenomenon which may coexist in a patient with hemolytic anemia. This phenomenon has been termed the albumin autoagglutination phenomenon. The first example was reported by Weiner (1956).

V.P. was a seven-year-old white female patient. Three weeks prior to admission to the Medical College of Virginia Hospital the patient had followed a truck spraying 30 percent dichloro-diphenyl-trichloroethane. She had also been exposed to dog spray insecticide and had been given chloromycetin in March and April, 1964 for pharyngitis. She was admitted to a hospital on July 6, 1964 with 5 gm/100 ml of hemoglobin and jaundice. She was successfully transfused with 250 ml of whole blood without crossmatching difficulties. On July 10 she was transferred to the Medical College of Virginia Hospital with a hemoglobin level of 6.8 gm/100 ml. She was diagnosed as having acquired hemolytic anemia, possibly on the basis of inhalation of an insecticide. Despite the fact that blood was urgently needed, due to difficulties in the albumin phase of the crossmatch the Blood Bank could not release the blood requested. Further studies showed that this case was an example of the non-immune autoagglutination phenomenon. On this basis, on July 17 the patient was successfully transfused with 125 ml of packed red cells compatible in the Saline and Coombs tests, although very strongly incompatible in the albumin test. Admittedly, this phenomenon is rare. Rarity, however, does not detract from its practical significance, since blood required in an emergency for such a patient may be withheld on the presumption that it contains specific high-protein active antibodies.

Transfusion Criteria

It is worth remembering that transfusion could lead to complications, particularly in patients with autoimmune hemolytic anemia. Therefore, the longer the transfusion is avoided, the longer the complications of blood transfusion are delayed and the better the prognosis. In all hemolytic anemias, the anemia per se is not an indication for transfusion, and transfusions are given only when the symptoms justify. Transfusions are not means of maintaining the hemoglobin levels in the normal range.

Does every severe anemia always demand transfusion? The answer to this vital question is NO. This answer is based on the fact that in patients with extremely marked anemia, transfusion may easily overload the circulation and precipitate cardiac failure. Therefore, it is mandatory that whenever the chance
exists that the patient will respond to some other form of treatment, transfusion of blood should be avoided.

An ideal example is the patient with pernicious anemia in relapse. It is commonly agreed that transfusion of such patients should be avoided unless (1) there is hemorrhage associated with thrombocytopenia and (2) the patient has pneumonia or some other infection which may interfere with the response to Vitamin B₁₂. Inhalation of 100 percent oxygen by such patients may effectively raise the O₂ carrying capacity of the blood, to tide them over a short period of extremely severe anemia. A blood with 3 gm Hb percent normally carries about 4 ml oxygen per 100 ml, while the inhalation of 100 percent oxygen increases the oxygen content of the plasma by an increment of 2 ml oxygen per 100 ml. This means that such treatment may effect an increase equivalent to 1.5 gm Hb percent, which in the case of the patient with 3 gm Hb means a rise to a level of 4.5 gm percent. Should these patients be transfused, the transfusion rate should not exceed 0.5 ml/lb of body wt/hr. Sharpey-Schafer (1945) suggested that it would be wise to limit the volume of transfused blood to 500 ml/48 hrs (preferably as PRC) and to take various measures to reduce right auricular pressure, such as keeping the patient propped up in a sitting position. The central venous pressure should be observed and any rise should be an indication for slowing or stopping the transfusion. Other safety measures are (1) warming of the patient and (2) administration of a diuretic.

Summary

In this presentation I have attempted to present some of the transfusion problems that face the Blood Bank and the physician treating the patient. I have briefly discussed methods of recognizing the complicating factors, interpretation of their clinical significance, and the proper hemotherapeutic management of such cases. Finally, I hope that this presentation is a convincing thesis for a better understanding of the Blood Bank and its problems, since the patient's welfare is better served when there is a rapport between the clinicians and the Blood Bank staff.

References


WILLIAMS D: Personal communications