

HEMOLYSIS AFTER CHEMOTHERAPY: Answers
Case Study by Jim Perkins M.D. and Leon Dragon M.D. (©2010)

1. What sort of reaction to chemotherapy infusion do you think is going on?

The patient has classic symptoms of an acute hemolytic episode with fever, flank pain, chest discomfort, and red urine. This is corroborated by laboratory features of acute hemolysis including a sharp drop in her hemoglobin and haptoglobin levels and an increase in bilirubin and LDH levels. The urinalysis performed several hours after the reaction demonstrated hemoglobinuria even though the visible red color had cleared by that point. An increase in the WBC and platelet counts was consistent with a systemic inflammatory response. These symptoms and signs mimic an immediate hemolytic transfusion reaction, but the patient had not been transfused.

Medications can cause acute hemolysis by non-immune mechanisms, particularly in patients with deficiencies of the red cell enzyme G6PD, but this has not been reported for platinum compounds. Platinum compounds have rarely been associated with hemolytic-uremic syndrome, but that disorder does not cause acute hemolysis on a time scale of minutes. However, platinum compounds including carboplatin, cisplatin, and oxaliplatin have been associated with drug-induced immune hemolytic anemia (DIIHA). DIIHA can cause acute hemolysis with the signs of a systemic inflammatory response noted above.

2. What testing would you like to do?

The first test that should be done is a direct antiglobulin test (DAT). Patients with DIIHA usually have IgG, complement, or, most often, both bound to their RBCs at the time of a reaction. If IgG is present on the RBCs, an eluate should be prepared and tested. One would expect the eluate to be non-reactive with RBCs when tested untreated or in the absence of drug, even if IgG is bound to the RBCs, but this is often NOT the case (see below). Therefore, when the clinical suspicion of DIIHA is high, testing for a drug-dependent antibody (DDA) should be performed regardless of the eluate results.

“Clinical suspicion” includes a history of taking a drug associated with DIIHA in an appropriate temporal relationship to the hemolytic episode. In this case the patient had taken 4 different medications immediately prior to the carboplatin and the subsequent hemolysis. However, none of the 4 drugs taken as pre-medication are known to cause DIIHA, so they were not tested as potential precipitants. Had the test for carboplatin-dependent antibody been negative, antibodies to the other drugs could have been pursued.

DDA testing is performed in two sequences. For drugs such as beta-lactam antibiotics (penicillins and cephalosporins) that bind covalently to the RBCs, the candidate drug is incubated with red cells which are then washed to remove free drug and reacted with the patient’s serum, plasma, or eluate in an indirect antiglobulin test. For drugs that don’t bind to RBCs the patient specimen is incubated with RBCs in the presence of drug, and then an antiglobulin test is performed. The immunohematologic tests performed are typically selected according what is known about the individual candidate drug. Note however, that some DDAs can be demonstrated by either method (see “Fatal Hemolysis 5 Days Post-Caesarian Section” in this case-study section).

HEMOLYSIS AFTER CHEMOTHERAPY: Answers, pg. 2

3. What antibodies are present? What is the immunohematologic diagnosis?

Testing at the hospital demonstrated an apparent warm-reactive autoantibody on the patient's RBCs and a cold-reactive autoantibody in her serum. The immunohematology reference laboratory demonstrated a carboplatin-dependent antibody in the patient's serum reacting in the presence of free drug. Testing was not performed with cells incubated with carboplatin and then washed.

The history, laboratory evidence of hemolysis, and presence of the carboplatin-dependent antibody allow a diagnosis of carboplatin-induced immune hemolytic anemia.

4. How do you relate the positive DAT and pan-reactive eluate to the hemolytic process? Discuss this phenomenon in relation to DIIHA in general.

In cases of DIIHA if blood samples are obtained within a short time period after the hemolytic episode the DAT is typically positive (68 of 71 cases due to a DDA reported by Johnson, Fueger, and Gottschall, Transfusion, 2007), most often with both anti-IgG and anti-C3 (46 of 68 cases, Johnson, ibid). DDAs may be present in the serum and/or in the eluate. In this case since the eluate reacted with all cells in the absence of added drug it could not be used to demonstrate the DDA.

As discussed above, DDAs take two forms, antibodies that react with drug coated RBCs which have been washed to remove free drug and antibodies that react in the presence of free drug as in this case. Johnson and co-workers have termed these DDAs "DTRC" for 'drug-treated RBCs' and "IPOD" for 'in the presence of drug' (Johnson, ibid), terms that have the advantage of referring to the actual immunohematologic test results, rather than to hypotheses regarding the underlying mechanisms. In many cases a DDA can be demonstrated by both techniques as illustrated in the accompanying case "Fatal Hemolysis 5 Days Post-Caesarian Section".

One would expect that if drug, either bound to the RBCs or present freely in the reaction mixture, is required to mediate the binding of a DDA to the RBCs, then the patient's serum or eluate would NOT react if drug were not added in vitro. In this case, however, the eluate reacted strongly with all panel cells. This phenomenon has several possible explanations.

First, it could simply be a coincidence. The patient could have had an autoantibody bound to her RBCs before developing the carboplatin-dependent antibody. In fact, patients with warm-reactive autoantibodies seem to develop blood group alloantibodies more readily than other individuals. Perhaps the same phenomenon could be true for DDAs.

Second, in addition to inducing DDAs, treatment with certain drugs is thought to induce formation of antibodies that react with all or most RBCs in the absence of drug and which sometimes cause immune hemolytic anemia. Such antibodies mimic warm-reactive autoantibodies, causing a positive DAT with anti-IgG, and a pan-agglutinin in the eluate or both the serum and eluate. This phenomenon is classically associated with the anti-hypertensive agent α -methyldopa, but is also seen after treatment with the chemotherapeutic agent fludarabine and other drugs. The drug is implicated in inducing the antibody and the hemolytic anemia because appearance of the antibody is temporally associated with initiation of the drug, disappears over time when the drug is stopped but recurs when the drug is re-started, and is

HEMOLYSIS AFTER CHEMOTHERAPY: Answers, pg. 3

more common at higher drug doses (Shulman and Reid, Transfusion Medicine Reviews, 1993). Disappearance of the antibody takes many months. This phenomenon has not been reported with carboplatin treatment.

Third, the patient's serum or plasma may react with all cells because there is drug present in the patient specimen that was not added in vitro. This is seen in the accompanying case of a cefotetan-dependent antibody (see "Fatal Hemolysis 5 Days Post-Caesarian Section" in this case-study section) in which the antibody screen and all crossmatches were negative prior to administration of the drug, but all became positive using specimens after drug administration. Johnson and coworkers (ibid) demonstrated this phenomenon in 5 of their 71 cases by dialyzing the patients' blood samples to remove drug administered to the patient. Routine antibody detection tests were positive before dialysis, became negative with the dialyzed samples, and reverted to positive when drug was added in vitro.

Finally, patients may have an apparent autoantibody at the time they make a significant DDA. Ahrens and co-workers (Am J Hematol 2006) reported 9 cases of IHA in which diclofenic-dependant antibodies were demonstrated. Six patients had "autoantibodies" (reactive in the absence of added drug) in their serum on presentation and 7 of the 8 tested had such antibodies in their eluates. In all cases these findings initially obscured the diagnosis. In the series of Johnson and coworkers (ibid) 16 of 71 patients had a reactive eluate in the absence of added drug, 2 of which were strongly reactive as in this case; 27 patients (38%) had a broadly reactive serum antibody. Two had "serology similar to cold agglutinin disease", but the DATs in those cases were stronger than in the present case, as were the apparent serum autoantibodies. Arndt and Garratty (Semin Hematol, 2005) distinguish such broadly reactive antibodies coincident to DDAs from "true" autoantibodies induced by methyl dopa and other drugs. They propose a mechanism by which the polyclonal antibody responses to the neo-antigen formed by the combination of RBC and drug include clones for which the epitope is largely composed of RBC structures, and which can therefore bind to the RBCs when drug is not present. Of note, a similar phenomenon may be seen in patients forming new blood group alloantibodies; specifically patients having a primary or secondary immune response with blood group specificity may transiently have antibody in their serum or eluate that disappears over a matter of weeks.

5. What is the implication of the new results?

Three months after the original presentation the DAT is negative. One would expect to see the positive DAT due to a DDA become negative over time after the drug is stopped as the antibody-bound RBCs are eliminated. This finding suggests that the apparent autoantibody detected on initial testing was not due to a pre-existing autoantibody, nor was it a drug-induced 'autoantibody' such as those provoked by α -methyl dopa, treatment. It does not help us distinguish between mechanisms three and four discussed above.