Investigating the possibility of drug-dependent platelet antibodies

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Just about every physician has seen a case like this and then may call his or her laboratory-based colleagues for assistance.

Mabel Throckmorton, 67 years old, was admitted for treatment of community-acquired pneumonia. After obtaining cultures, two appropriate antibiotics were begun, but her respiratory status deteriorated to require intubation and ventilation. Two days later, as her pulmonary status began to improve, a routine (automated) CBC noted that her platelet count had dropped from 220,000/μL on admission to 15,000/μL. Her leukocyte count had peaked at 22,000/μL and had now declined to 10,000/μL. Her hematocrit had decreased from 38% to 33%. Her peripheral smear was unremarkable except for the cytopenias. Her medications before admission included a diuretic, digoxin, and a statin for cholesterol control. In addition to the antibiotics she was receiving, additional hospital medications included albuterol and omeprazole.

The patient had a history of spontaneous intracranial hemorrhage 30 years previously, which resolved without permanent impairment, and chemotherapy and transfusion for an aggressive lymphoma 10 years previously. Multiple transfusions coupled with five pregnancies resulted in the patient's being broadly alloimmunized to HLAs, and she required difficult-to-find matched platelets for transfusion support during that chemotherapy.

Is the sudden and dramatic thrombocytopenia related to the primary illness, previous disease reappearance, or something nosocomial? If a drug caused the platelet count drop, which one is the culprit? Because the infection appears to be under effective treatment, the patient's physician will understandably be reluctant to change antibiotics at this juncture. However, given the patient's previous history of intracranial hemorrhage, considerable anxiety would accompany the dramatic appearance of thrombocytopenia, particularly when transfusion support would be difficult to obtain. How can the laboratory assist in the evaluation and treatment of this patient?

The frequency of drug-induced thrombocytopenia is staggering. Although only 1 person in 100,000 encounters this annually in the United States,[1] some medications such as trimethoprim-sulfamethoxazole or quinine/quinidine can increase this rate 1500-fold.[2] Perhaps a quarter of all intensive care patients exhibit thrombocytopenia as a result of drugs,[3,4] and 6 percent of patients receiving the common antibiotic gentamicin make drug-dependent platelet antibodies (DDPAs).[5] Although heparin-induced thrombocytopenia (a unique form of drug-platelet interaction that will not be discussed here) is well known and often considered in the differential diagnosis of thrombocytopenia,[6] other drugs (given the frequency of their use) probably account for far more cases of drug-induced thrombocytopenia.

First Things First

However, before laying the blame for all thrombocytopenia on medications, let us first make sure that the problem is not something else (Table 1). Is the patient's platelet count *really* low? Some patients' platelets agglutinate in EDTA,[7] and some patients have autoantibodies against GPIIb/IIIa that display avidity in EDTA samples.[8] In this case, the normal platelet count on admission makes these unlikely causes. Idiopathic autoimmune thrombocytopenia

Table 1. Why is the patient thrombocytopenic?

Pseudothrombocytopenia
EDTA-induced aggregation
Glycoprotein autoantibodies avid only in EDTA
Production deficit
Primary marrow failure
Ineffective production, e.g., myelodysplasia
Primary malignancy, e.g., leukemia
Secondary malignancy (metastatic disease)
Antineoplastic medications
Accelerated peripheral clearance
Idiopathic thrombocytopenic purpura (ITP)
Posttransfusion purpura
Thrombotic thrombocytopenic purpura (TTP)
Hemolytic uremic syndrome (HUS)
HELLP: hemolytic anemia, elevated liver enzymes, low platelets*
Disseminated intravascular coagulation (DIC)
Massive hemorrhage
Drug-induced thrombocytopenia
Heparin-induced thrombocytopenia/thrombosis
Autoantibodies (ex: procainamide)
Fab_{murine} antibodies (ex: abciximab)
Hapten-induced antibodies (ex: penicillin, cephalosporins)
Glycoprotein-complexing drug (ex: quinine)
Autoantibody recognition of glycoprotein conformational change (ex:fibans)

*A variant of preeclampsia.

(ITP) always has to be considered, but the sudden occurrence of platelet autoantibodies in this situation would be very unusual. A situation that is indistinguishable from ITP (with autoantibody formation) can occur with and well beyond the time of administration of some drugs, including L-dopa, procainamide, and gold salts, among others, although none of these appears in the history of this case.[9] The patient's lymphoma may have returned to crowd out normal hematopoietic marrow elements, but the time course would likely be much more gradual. No drugs have been administered recently that would suppress marrow function, and the patient's leukocyte response to the infection was appropriate. The story also does not fit the time course or situation for either disseminated intravascular coagulation (as the patient's infection appears to be resolving) or thrombotic thrombocytopenic purpura. The patient has not received a transfusion recently, so posttransfusion purpura is not a viable explanation.

DDPAs can also cause reduced responsiveness to platelet transfusions. Serial posttransfusion platelet counts may be of some assistance in distinguishing the effects of DDPAs and alloantibodies: although most patients with HLA- or platelet-specific alloantibodies fail to show the expected posttransfusion response in the first hour, patients with DDPAs often have the expected 1-hour corrected count increment (CCI) but accelerated removal thereafter such that a good initial response is followed by a poor CCI when measured at 18 to 24 hours after transfusion.

Thus, on first blush, considering DDPAs as the cause of the thrombocytopenia in this case seems logical. Although a primary immune response to a drug may take several weeks to much longer, an anamnestic response can occur in a few days to a week, and preformed antibodies can cause thrombocytopenia within hours.[10] Given one's multiple exposures to antibiotics throughout a lifetime and the potential for cross-reactivity of an antibody across a class of drugs, a rapid or anamnestic response may occur even without documented (or recalled) prior exposure to an antibiotic.

What's Going On?

There are multiple pathogenic mechanisms that may lie behind drug-induced thrombocytopenia, and understanding them can help explain the rationale for testing that may document the existence of DDPAs.

Knowing that some drugs (including penicillins and cephalosporins) can bind covalently to proteins on cell membranes led to the theory of DDPA stimulation through hapten, or neoantigen, formation. The drug antibody would thus actually be targeting a drug-protein structure on the surface of cells, including RBCs or platelets.[11] However, this is not the mechanism that explains the actions of most DDPAs.

Another model that has been advanced is that of antigen-antibody complex formation. Here, the antibody is directed against the soluble form of the drug (or one of its metabolites), and the platelets or RBCs involved in the process are innocent bystanders that are removed from circulation after adsorbing or binding the complex onto their surface. However, most drugs are too small to provoke an immune response, and immune complexes of drug-immunoglobulin could not be demonstrated when sought. Furthermore, interactions of DDPAs with the cells that are removed from circulation are through binding with the antibodies' (epitope-specific) Fab domains rather than the Fc domain,[12,13] suggesting that the antibodies are interacting directly with the platelets in the presence of the drug.

This theory has been supplanted by one involving (noncovalent) interaction between the drug and a surface protein, leading to a change in the conformation of the protein that would allow naturally occurring antibodies to now interact with the protein with sufficient avidity to provoke removal of the cell.[14,15] Most DDPAs appear to interact with GPIIb/IIIa, which binds fibrinogen and other ligands, or GPIb/IX, the von Willebrand factor receptor, for reasons that are not currently well understood.[16]

A form of this mechanism has been invoked to explain why first exposure to fibans can cause rapid and profound thrombocytopenia in a small proportion of patients. Drugs of this class, such as tirofiban and eptifibatide, are administered after percutaneous transluminal coronary angioplasty in an attempt to maintain arterial patency. These drugs have been engineered to bind tightly to a specific sequence of amino acids (arginine-glycine-aspartic acid) on GPIIb/IIIa to block platelet activation. In doing so, slightly altered conformations occur in the glycoprotein complex to allow the binding of preexisting auto-antibodies, which would not have a target in the absence of the drug's effect.[17,18] Interestingly, although both tirofiban and eptifibatide achieve their pharmacologic presence through the same mechanism, the conformational changes they induce in GPIIb/IIIa appear to be different because antibodies capable of clearing platelets that have been treated with one of these drugs usually do not bind to platelets treated with the other.[19]

Another drug in common use to inactivate circulating platelets is abciximab. This is a chimeric monoclonal antibody, the murine portion of which is targeted to GPIIb/IIIa and blocks binding with fibrinogen. Some individuals have naturally occurring antibodies to the murine portion of the antibody, and administration of the drug can lead to rapid and profound thrombocytopenia as the anti-mouse antibody bound to platelets clears the drug-platelet complex.[20] Other patients form such an antibody about a week after initial exposure, and thrombocytopenia can develop at this time as well.

Identifying the Culprit

How can the laboratory help identify the presence of a DDPA that might be causing the thrombocytopenia in this case? A review to ensure that other laboratory results and clinical information do not point toward a nondrug cause

should be performed first. Reviewing lists of most commonly encountered causative agents of DDPAs would be a good next step, as many clinicians may not be aware of the substantial frequency with which some drugs induce DDPAs and thrombocytopenia. Certainly heparin should be on this list,[6] but the list extends far beyond this and includes some often-implicated antibiotics (Table 2).

Table 2. Partial listing of drugs causing immunologically mediated thrombocytopenia*

Abciximab	Digoxin	Omeprazole
Acetaminophen	Dipyridamole	Orbofiban
Acyclovir	Enoxaparin	Phenytoin
Amikacin	Eptifibatide	Piperacillin
Amlodipine	Famotidine	Procainamide
Amoxicillin	Fluconazole	Propranolol
Amphotericin B	Furosemide	Quinidine
Ampicillin	Gentamicin	Quinine
Argatroban	Glyburide	Rifampin
Atenolol	Gold salts	Salicylates
Bortezomib	Heparin	Sulfonamides
Carbamazepine	Hydrochlorothiazide	Sulfonylureas
Cefazolin	Imipenem	Suramin
Cefotetan	Interferon	Teicoplanin
Ceftazidime	Linezolid	Tirofiban
Ceftriaxone	Lotrafiban	Tobramycin
Cimetidine	Metoprolol	Trimethoprim/ sulfamethoxazole
Ciprofloxacin	Metronidazole	Valganciclovir
Clarithromycin	Naproxen	Valproate
Clindamycin	Nizatidine	Vancomycin
Clopidogrel		

*This listing includes drugs reported in the literature as causes of immune drug-induced thrombocytopenia or encountered in the authors' experience.[10] The reader is referred to a Web site provided by Dr. James George, University of Oklahoma, for an updated, well-characterized compendium of reports of drugs inducing thrombocytopenia: www.ouhsc.edu/platelets. Consideration should also be given to thrombocytopenia arising after ingestion of folk or herbal remedies.

There are several types of assays that are applied by reference laboratories to detect DDPAs.[3,21,22] Flow cytometry is commonly used to detect an increase in binding of IgG to platelets after incubation with drug and the patient's serum. (The order of addition—incubation of platelet with drug followed by addition of serum versus the incubation of drug with serum followed by addition of this mixture to platelets—may depend on the pathophysiologic mechanism at work, and the alternative can be tried if the initial approach is not positive, because variations are seen among patients and certainly among drugs.) A similar approach of incubating normal platelets with drug and patient serum can be followed with detection of IgG bound to platelets using goat anti-human IgG conjugated to alkaline phosphatase to detect the DDPA via an enzyme immunoassay; the DDPA is thus detected by conversion of a substrate to a colored product. Because standardized kits are not available in the United States for such testing, referral of specimens to a reference laboratory usually is required. The resulting turnaround time renders the information useful only in retrospect usually, because clinical decisions about drug discontinuation often have to be made in a much shorter time frame.

A commonly used approach for the detection of HLA- and platelet-specific antibodies can be adapted for the detection of drug antibodies. The solid-phase red cell adherence assay (SPRCA) technique can be adapted to detect DDPAs stimulated by a wide variety of drug classes.[23,24] This technique can be learned by any skilled technologist and applied to yield clinically useful results in just a couple of hours, removing the guesswork from changing a patient's drug regimen. Validating such an assay should include comparison of results with a laboratory having an established reputation in the field. Performance of the technique is aided by close cooperation with the hospital pharmacy in acquiring the needed "reagent" drug supply and choosing the appropriate drug concentration to use in the system. Any assay for DDPAs will be hampered, of course, by the lack of ability to include drug metabolites in the test system (as these may, in some cases, be the chemicals that are the true culprits[14]) and by aqueous solubility limitations. If the patient also happens to be alloimmunized to HLA- or platelet-specific determinants, performance of this assay will require use of reagent platelets that lack the corresponding epitopes. This assay offers a nice complement to a laboratory's ability to identify antibodies causing heparin-induced thrombocytopenia through a solid-phase anti-platelet factor 4 (anti-PF4/heparin) enzyme immunoassay.[25]

In cases in which the drug has caused a perturbation of the immune system and the generation of a true autoantibody, laboratory testing will be unable to identify the culprit drug because the autoantibody will bind with all platelets. Such antibodies usually target a structure in the GPIIb/IIIa complex and are indistinguishable from the autoantibodies that arise in ITP. Further complicating assessment (and treatment) is the recognition that these autoantibodies may persist long after discontinuation of the drug. Recognition of the exact nature of the situation thus depends on identifying one of the drugs known to cause this kind of problem on the patient's medication list and demonstration of an ITP-like autoantibody (not dependent on the presence of drug) by flow cytometry or SPRCA.

What Then?

If the drug that caused the thrombocytopenia as a result of a DDPA can be identified and removed from the patient's regimen, an increase in the platelet count should be seen relatively quickly. The longer the serum half-life of the drug, of course, the slower will be the recovery. The patient's platelet count usually will begin to recover (or responses to platelet

transfusions return to normal) within 2 to 5 days. Although antibodies to drugs can decline or disappear despite continued administration,[26] discontinuation, especially in profound thrombocytopenia, would usually be advised, followed by avoidance of the drug in the future for fear of provoking an even stronger anamnestic response.[10] Substitution of a drug in the same class as the offending drug usually does not result in thrombocytopenia owing to cross-reactivity of the DDPA despite chemical similarities of the pharmacologic agents.[27] However, caution and close observation may be advisable.

While the issue of DDPAs is being investigated, it may also be worthwhile to examine the patient's RBCs. In the case presented above, is the new anemia related to the DDPA, to hydration status, or to a marrow-based problem? RBCs can be subject to accelerated removal from circulation through some of the same mechanisms as platelets, and, because of the different surface proteins of RBCs, the prominence of anemia versus thrombocytopenia may vary between situations. Nevertheless, recognition that drug-induced hemolysis is occurring or may occur could provide useful clinical information and impetus for a change in the patient's drug regimen. For example, in one series (preliminarily reported in Leach et al.[28]), 10 of 17 selected patients with a DDPA that had been identified by SPRCA also had a positive direct antiglobulin test; the eluates from all 10 patients failed to react in an RBC antibody panel, but 7 of these contained the same DDPA that had been identified in the patient's serum and reacted in a DDPA SPRCA.

In conclusion, the very real possibility that a drug is causing a patient's thrombocytopenia deserves investigation and, if substantiated, concerted action. Although not all situations will be able to be resolved through laboratory testing, there is clearly assistance the laboratory can provide to help guide possible changes in the patient's drug regimen.

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