

Vancomycin-induced Immune Thrombocytopenia Proven by the Detection of Vancomycin-dependent Anti-platelet Antibody with Flow Cytometry

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Abstract

Vancomycin-induced thrombocytopenia is a rare adverse reaction that may be overlooked because no specific diagnostic test is currently available. We herein report a patient with vancomycin-induced immune thrombocytopenia who was diagnosed by the detection of vancomycin-dependent anti-platelet antibody with flow cytometry. An IgG antibody in the patient's serum reacted with platelets only in the presence of vancomycin. Severe thrombocytopenia gave rise to life-threatening gastrointestinal bleeding, which was quickly resolved after effective platelet transfusion following the cessation of vancomycin administration. This report suggests that the flow cytometric test is useful for the differential diagnosis of thrombocytopenia and platelet transfusion should be performed after the cessation of vancomycin administration.

Key words: vancomycin, thrombocytopenia, anti-platelet antibody

(Intern Med 55: 3035-3038, 2016)

(DOI: 10.2169/internalmedicine.55.6902)

Introduction

Vancomycin, a glycopeptide antibiotic, is widely used to treat infection with resistant Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA). It is well known that vancomycin has several adverse reactions including nephrotoxicity, ototoxicity, and red man syndrome (1-4). Vancomycin-induced thrombocytopenia is a rare adverse reaction, however, it may be underestimated because of the lack of a specific diagnostic test (5-7). Von Drygalski et al. recently demonstrated that vancomycin-induced thrombocytopenia was induced by drug-dependent anti-platelet antibodies that could be detected by flow cytometry (8). We herein present a case of vancomycin-induced immune thrombocytopenia followed by life-threatening gastrointestinal bleeding that quickly recovered after discontinuation of the drug. Using flow cytometry, we identified a vancomycin-dependent anti-platelet antibody in

the patient's serum and accordingly made a diagnosis of vancomycin-induced immune thrombocytopenia.

Case Report

A 72-year-old woman with a history of hypertension was admitted for dyspnea and edema. Laboratory studies showed the following results: hemoglobin, 7.5 g/dL; white blood cells, $1.4 \times 10^4/\mu\text{L}$; platelets, $27.3 \times 10^4/\mu\text{L}$; creatinine, 13.1 mg/dL; blood urea nitrogen, 137.7 mg/dL; Na, 133 mEq/L; K, 6.1 mEq/L; Cl, 103 mEq/L; total protein, 6.3 g/dL; C-reactive protein, 15.3 mg/dL; and brain natriuretic peptide (BNP), 5,930 pg/mL (Table). Chest radiography revealed an enlarged heart shadow and infiltrative shadow in the right upper lung field. Therefore, the patient was diagnosed with pneumonia, acute heart failure, and acute renal failure. Renal failure was considered to result from an antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis because tests for anti-myeloperoxidase antibodies (MPO-ANCA)

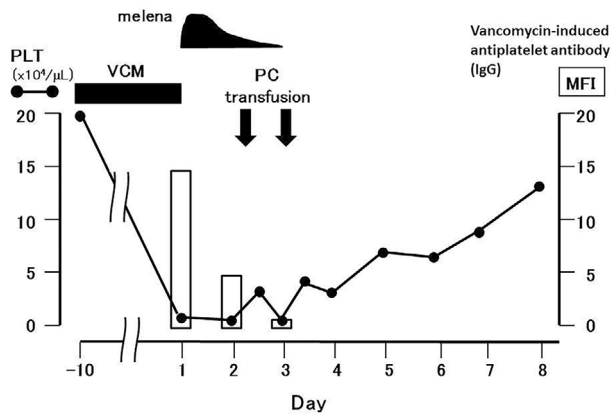
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Received for publication November 27, 2015; Accepted for publication February 24, 2016

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Table. Laboratory Data on Admission.

WBC	14,000 / μ L	T.Pro	6.3 g/dL	CRP	15.30 mg/dL
Ne	87.1 %	T.Bil	0.7 mg/dL	BNP	5930 pg/mL
Ly	7.7 %	AST	65 IU/L		
Mo	5.0 %	ALT	37 IU/L		
Eo	0.1 %	LD	497 IU/L		
Ba	0.1 %	g-GTP	25 IU/L		
RBC	260 $\times 10^4$ / μ L	ALP	413 IU/L		
Hb	7.5 g/dL	BUN	137.7 mg/dL		
Ht	22.5 %	UA	12.0 mg/dL		
PLT	27.3 $\times 10^4$ / μ L	Cr	13.1 mg/dL		
		Na	133 mEq/L		
		K	6.1 mEq/L		
		Cl	103 mEq/L		

**Figure 1. Clinical course.**

were strongly positive.

Steroid therapy, carbapenem, mechanical ventilation, and continuous hemodiafiltration (CHDF) were subsequently initiated. MRSA was ultimately isolated from the sputum, and vancomycin was administered at 1,000 mg intravenously (IV) on the first day and at 500 mg IV every 24 hours after the second day. Ten days after initiating vancomycin therapy, the patient developed massive melena followed by hypovolemic shock. At that time, her platelet count was 0.6×10^4 / μ L. Her D-dimer and fibrinogen levels were normal. According to the sudden onset of thrombocytopenia without coagulopathy, drug-induced thrombocytopenia was suspected. Because she received heparin for CHDF, we initially suspected heparin-induced thrombocytopenia (HIT) and switched from heparin to nafamostat mesilate. However, visible blood clotting in the hemodialysis circuit, the most prominent feature of HIT in dialysis patients, was not observed, and the test for HIT antibody was negative, suggesting a decreased possibility of HIT. The administration of other drugs including vancomycin and meropenem, which might be responsible for drug-induced thrombocytopenia, was discontinued. On the next day after the cessation of the administered drugs, the patient received platelet transfusion, which increased her platelet count from 0.2×10^4 / μ L to 3.4×10^4 / μ L. Her platelet count gradually increased and was 13.3×10^4 / μ L at 8 days after drug discontinuation (Fig. 1). Her melena resolved as her platelet count increased. This rapid

recovery of the platelet count after cessation of the administered drugs was consistent with drug-induced thrombocytopenia. Although the causative drug remained unknown, the clinical course of this patient was quite similar to that of patients with vancomycin-induced thrombocytopenia regarding the period between drug initiation and the onset of thrombocytopenia, severe thrombocytopenia with life-threatening bleeding, and rapid recovery after drug discontinuation (8).

Detection of vancomycin-dependent anti-platelet antibody

We attempted to identify vancomycin-dependent anti-platelet antibody, which is responsible for vancomycin-induced immune thrombocytopenia. This antibody can bind to platelets only in the presence of vancomycin (8). The patient's serum was incubated for 40 minutes at room temperature with normal washed platelets in the presence or absence of 0.3 mg/mL of vancomycin. After a wash in buffer, each sample was incubated with Alexa Fluor 488-conjugated goat F(ab')₂ anti-human IgG or FITC-conjugated goat F(ab')₂ anti-human IgM (Invitrogen) for 20 minutes at room temperature. Platelet-bound fluorescein signals were detected by flow cytometry. A positive reaction was defined as a 2-fold or greater increase in the mean fluorescence intensity of platelets compared with control serum samples.

The result of the flow cytometric analysis is shown in Fig. 2. The patient had an anti-platelet IgG antibody that was detected only in the presence of vancomycin. No IgM antibody was detected (data not shown). The antibody titer (mean fluorescence intensity) was decreased after cessation of vancomycin and undetectable as the platelet count raised (Fig. 1).

The platelet antigen recognized by this antibody was further examined using an ELISA assay (PakAuto assay, Immucor GTI Diagnostics, Waukesha, USA). The patient or normal serum was added to wells immobilized with platelet membrane glycoprotein (GP) IIb/IIIa, Ib/IX, and Ia/IIa, allowing antibody, if present, to bind. Following incubation for 30 minutes at 37°C, unbound immunoglobulins were washed away, and an alkaline phosphatase-labeled anti-human Ig antibody was added to the wells, followed by the

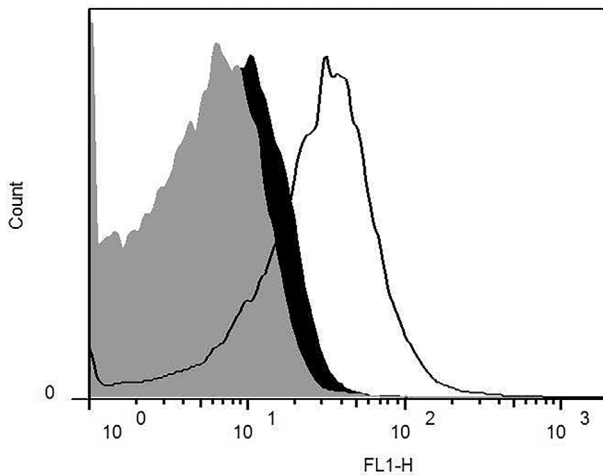


Figure 2. Histograms of fluorescence intensities of platelet-bound IgG antibody. Black: The patient's serum was tested against normal washed platelets in the absence of vancomycin. White: The patient's serum was tested against normal washed platelets in the presence of vancomycin. Gray: Normal serum was tested against normal washed platelets in the presence of vancomycin as a negative control.

enzymatic substrate p-nitrophenyl phosphate. The reaction was evaluated by reading the optical density of the wells at an absorbance of 405 nm. We found that the antibody in the patient's serum specifically recognized GPIb/IX (Fig. 3).

Discussion

This report described a patient with vancomycin-induced immune thrombocytopenia associated with life-threatening gastrointestinal bleeding. Drug-induced thrombocytopenia was suspected from her clinical course and the diagnosis was confirmed by the detection of vancomycin-dependent anti-platelet antibody with flow cytometry. This flow cytometric test made it possible to definitively diagnose vancomycin-induced immune thrombocytopenia, and our case showed the diagnostic value of this test.

The clinical features of vancomycin-induced thrombocytopenia have been reported in detail (8, 9). Patients are typically administered vancomycin for at least six days, and the platelet counts decrease by a mean of 93% from the pre-treatment values. Nadir counts averaging $1.36 \times 10^4/\mu\text{L}$ are reached approximately eight days after vancomycin treatments (ranging from 1 to 27 days). The platelet counts return to the pre-treatment values after discontinuation of the drug, and the median time required for the platelet level to return to at least $15 \times 10^4/\mu\text{L}$ is 7.5 days (ranging from 4 to 17 days). One-third of the patients have extensive ecchymosis and severe mucous hemorrhaging that may be life-threatening.

Von Drygalski et al. (8) reported that vancomycin-dependent antibody was detected in about 20% of patients who were referred for vancomycin-dependent, platelet-reactive antibody testing because of the clinical suspicion of

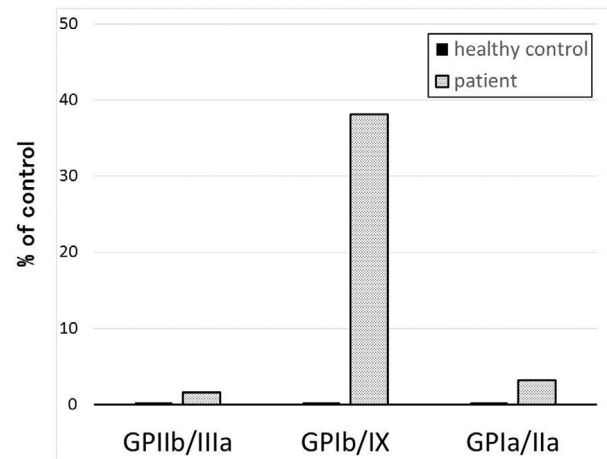


Figure 3. platelet antigen recognized by vancomycin-dependent antibody. Antibody binding in the patient's serum and normal serum to each platelet glycoprotein was measured using a pakAuto assay kit. Specific binding was expressed as % of control calculated using the following formula: % of control (%) = (sample - negative control) / (positive control - negative control) \times 100. Negative and positive controls were included in the kit.

vancomycin-induced thrombocytopenia. The majority of vancomycin-induced antibodies detected were the IgG type, with 2% of antibodies being the IgM type. No vancomycin-dependent antibodies were detected in 25 patients who had normal platelet counts after receiving vancomycin or another drug. The antibody was detected in only 1 of 451 normal subjects. These findings suggest that vancomycin-dependent antibody detected by flow cytometry is highly specific for patients with vancomycin-induced thrombocytopenia.

We identified that the antibody in the present patient recognized GPIb/IX. Previous studies have reported that vancomycin-dependent antibodies recognized either or both GPIIb/IIIa and GPIb/IX (10). This epitope profile is quite similar to that of antibodies in patients with quinine and other drug-induced thrombocytopenia (11, 12). It is therefore suggested that drug-dependent antibodies responsible for severe thrombocytopenia are directed against the common antigens GPIIb/IIIa and/or GPIb/IX.

It remains to be determined whether the vancomycin-dependent antibody exists for a limited period or permanently. A previous study reported a patient in whom the second episode of thrombocytopenia was induced by re-administration of vancomycin 6 months after the first episode (8). In our patient, the antibody was detected only for several days. However, it is possible that an undetectable level of the antibody persists in the serum. Re-administration of vancomycin may induce an anamnestic immune response and cause thrombocytopenia. Accidental re-administration of vancomycin should be avoided irrespective of the detection of the antibody.

Platelet transfusion in patients with vancomycin-induced thrombocytopenia is likely to be ineffective because of im-

immune destruction of platelets. The failure of platelet transfusion during vancomycin administration has been reported in previous studies (5, 8, 13, 14). A recent study reported that platelet transfusion failed to elevate the platelet counts in 11 of 14 patients (8). However, platelet transfusion was effective in our case. This may be explained by the biological feature of the anti-platelet antibody to react with platelets only in the presence of vancomycin. In our case, platelet transfusion was performed after discontinuation of vancomycin. Similar to our case, successful platelet transfusion was reported in a patient after discontinuation of vancomycin (15). It should be noted that platelet transfusion must be performed after discontinuation of vancomycin.

Vancomycin is a mainstay in the treatment of MRSA infection in hospitalized patients. Such patients may have many comorbidities and take many drugs, which precipitate thrombocytopenia. The flow cytometric test for vancomycin-dependent antibody may be useful in the differential diagnosis of thrombocytopenia in these patients and lead to successful treatment of patients with potentially life-threatening bleeding.

The authors state that they have no Conflict of Interest (COI).

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