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Approach to the Diagnosis and Management of Drug-Induced Immune Thrombocytopenia

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Abstract

Drug-induced immune thrombocytopenia (DITP) is a challenging clinical problem that is under-recognized, difficult to diagnose and associated with severe bleeding complications. DITP may be caused by classic drug-dependent platelet antibodies (eg, quinine); haptens (eg, penicillin); fiban-dependent antibodies (eg, tirofiban); monoclonal antibodies (eg, abciximab); autoantibody formation (eg, gold); and immune complex formation (eg, heparin). A thorough clinical history is essential in establishing the diagnosis of DITP and should include exposures to prescription medications, herbal preparations and even certain foods and beverages. Clinical and laboratory criteria have been established to determine the likelihood of a drug being the cause of thrombocytopenia, but these criteria can only be applied retrospectively. The most commonly implicated drugs include quinine, quinidine, trimethoprim/sulfamethoxazole and vancomycin. We propose a practical approach to the diagnosis of the patient with suspected DITP. Key features are: the presence of severe thrombocytopenia (platelet nadir $<20 \times 10^9$/L); bleeding complications; onset 5 to 10 days after first drug exposure, or within hours of subsequent exposures or after first exposure to fibans or abciximab; and exposure to drugs that have been previously implicated in DITP reactions. Treatment involves stopping the drug(s), administering platelet transfusions or other therapies if bleeding is present and counselling on future drug avoidance. The diagnosis can be confirmed by a positive drug re-challenge, which is often impractical, or by demonstrating drug-dependent platelet reactive antibodies in vitro. Current test methods, which are mostly flow cytometry-based, must show drug-dependence, immunoglobulin binding, platelet specificity and

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ideally should be reproducible across laboratories. Improved standardization and accessibility of laboratory testing should be a focus of future research.

Thrombocytopenia caused by drugs is a particularly vexing clinical problem. It is common, yet under-recognized, difficult to diagnose and associated with severe bleeding complications. Many drugs have been associated with the development of thrombocytopenia; however, the syndrome of drug-induced immune thrombocytopenia (DITP) is an idiosyncratic drug reaction that occurs with only several drugs. DITP is caused by drug-dependent platelet antibodies that produce platelet clearance by the reticuloendothelial system, often resulting in severe thrombocytopenia and mucocutaneous bleeding.

DITP typically presents 5 to 10 days after beginning daily exposure to a drug, or within hours after re-exposure to a drug that has been taken occasionally for a period of time. Platelet counts are usually less than 20×10^9/L, the onset of thrombocytopenia is rapid, and bleeding symptoms frequently occur. DITP can resemble primary immune thrombocytopenia (ITP) [1]; however, differentiating these syndromes is important to avoid unnecessary treatments and prevent future exposures to the drug [2]. Recent studies have better defined the characteristics of drug-dependent platelet antibodies, which have led to a better understanding of the pathogenesis of this disorder [3]. DITP frequently occurs in hospitalized patients who are taking multiple medications and have a number of other comorbidities; thus relating the thrombocytopenia to a particular drug is often challenging. The lack of an accessible diagnostic test for DITP means that timely laboratory confirmation is generally not possible and providers must rely on a careful clinical to make the diagnosis. In this review, we summarize the mechanisms of DITP, the clinical features and laboratory tests, and we present a practical approach to the management of the patient with suspected DITP. We highlight areas that require further research starting with a case.

Case Presentation

A 66-year-old woman presented with seizures secondary to prosthetic mechanical mitral valve endocarditis complicated by atrial fibrillation and cerebral microabscesses. She underwent emergency mitral valve replacement, and was started on multiple medications on the day of surgery: carbamazepine, phenytoin, gentamicin, vancomycin, ranitidine, digoxin; unfractionated heparin (UFH) prophylaxis was started on postoperative day 3 [4]. On postoperative day 9, severe thrombocytopenia occurred (platelet count, 4×10^9/L), with petechiae. Review of the serial platelet counts revealed that the platelet count began to fall on postoperative day 6 (from 220×10^9/L on day 5 to 190×10^9/L on day 6). This was compatible with DITP that could be explained by any of the following six drugs: carbamazepine, phenytoin, gentamicin, vancomycin, ranitidine, digoxin, with 2 of them (carbamazepine, vancomycin) meeting criteria for drugs that were known to have been implicated in DITP reactions by clinical and laboratory features (drug-dependent antibodies) [5]. In contrast, heparin-induced thrombocytopenia (HIT) was not a plausible diagnosis, for several reasons: (a) the onset of thrombocytopenia began only 3 days after starting UFH (eg, too soon to be explained by heparin-induced immunization), and (b) HIT is not characterized

by such severe thrombocytopenia and mucocutaneous bleeding (rather, HIT is a prothrombotic syndrome). The management of this patient was to substitute potentially implicated drugs with non-cross reactive alternatives (eg, teicoplanin instead of gentamicin/vancomycin; omeprazole instead of ranitidine) as well as discontinuation of carbamazepine and phenytoin; in contrast, digoxin (judged most unlikely to cause DITP) was continued. In addition, high-dose intravenous immune globulin (IVIG) 2 g/kg administered over 2 days and platelet transfusions were administered. Within 1 day, the platelet count rose to 20×10^9/L and continued to rise steadily (Fig 1). Testing the patient's serum for drug-dependent antibodies by flow cytometry revealed the presence of vancomycin-dependent antibodies, and thus carbamazepine was restarted along with valproic acid. The platelet count recovered completely. Based upon clinical and laboratory features, the diagnosis was vancomycin-induced DITP.

Thrombocytopenia Caused by Drugs

Many drugs can cause thrombocytopenia either by non-immune or immune mechanisms. Non-immune thrombocytopenia results in suppression of platelet production by general myelotoxicity (eg, chemotherapy), dose-dependent myelosuppression (eg, linezolid) or interference with specific megakaryocyte function (eg, bortezomib). Immune-mediated thrombocytopenia results in accelerated platelet destruction by drug-dependent platelet antibodies that cause platelet clearance (eg, quinine and quinidine) or platelet activation (eg, heparin). Drug-dependent megakaryocyte antibodies may also cause immune-mediated suppression of platelet production.

Non-Immune Suppression of Platelet Production

Platelet production depends on functional megakaryocytes in the bone marrow. Non-immune thrombocytopenia caused by drugs results from a loss of cellularity within the bone marrow and an impairment of megakaryocyte proliferation and maturation, thereby leading to a decrease in platelet production. The myelosuppression is dose-dependent and often occurs slowly over the course of several weeks [6]. Chemotherapeutic compounds, including antimetabolites, cytotoxic agents, and alkylating agents are directly toxic to hematopoietic cells.

Reversible dose-dependent myelosuppression is associated with certain antibiotics including linezolid, a synthetic oxazolidinone with activity against vancomycin and penicillin resistant gram positive bacteria. Linezolid-induced thrombocytopenia tends to occur with prolonged treatment (>2 weeks) [7–9] and at high serum concentrations [10]. Platelets are typically more severely affected than other cell lines. Neither linezolid-dependent anti-platelet antibodies nor a specific megakaryocyte effect of the drug has been demonstrated [7].

Bortezomib is a potent and reversible proteasome inhibitor used for the treatment of multiple myeloma. It is commonly associated with the development of transient thrombocytopenia [11]. Although the precise mechanism remains uncertain, bortezomib may interfere with platelet release from megakaryocytes, rather than causing a general cytotoxic effect on hematopoietic progenitor cells [12]. Thiazide diuretics, tolbutamide, and antivirals have also been associated thrombocytopenia which may be due to suppression of megakaryocytes.

Immune-Mediated Platelet Destruction

DITP is a unique clinical syndrome characterized by severe thrombocytopenia, bleeding and drug-dependent platelet antibodies. DITP can be caused by prescription medications, herbal products and certain foods and beverages [13]. Quinine, and its structural isomer quinidine, is classically associated with severe DITP (platelet count nadir, $<10 \times 10^9$/L) [14,15]. It is approved for the treatment of uncomplicated malaria caused by the parasite *Plasmodium falciparum*, and is also used off label for the treatment of leg cramps. A review of reports submitted to the Food and Drug Administration Adverse Event Reporting System from April 2005 to October 1, 2008, found 38 US cases of serious adverse events associated with quinine. As a result, the Food and Drug Administration has issued warnings against unapproved uses of quinine (http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm218202.htm). Quinine is also found in certain beverages including tonic water and Dubonnet aperitif [13].

Several theories have been proposed to explain the development of immune thrombocytopenia in the presence of certain drugs (Table 1): (1) Classic drug-dependent platelet antibodies (quinine-type); (2) hapten-induced antibodies (eg, penicillin); (3) fiban-dependent antibodies (eg, tirofiban); (4) Fab-binding monoclonal antibodies (eg, abciximab); (5) drug-induced autoantibody formation (eg, gold); and (6) immune complex formation (eg, heparin).

Drug-Dependent Antibodies

Severe thrombocytopenia that occurs within 5 to 10 days of beginning daily administration of a new drug may be the result of classic drug-dependent antibodies. Quinine binds to platelet glycoproteins (GPs) allowing platelet-reactive antibodies to bind tightly to circulating platelets only when soluble drug is present. The targets of these antibodies are often on platelet GPIIbIIIa or GPIbIX. Many quinine-dependent antibodies appear to be naturally-occurring; normally, these antibodies have a weak affinity for platelet epitopes, but in the presence of the drug their affinity for platelet targets increases leading to immune-mediated destruction [16]. Alternatively, the association of quinine with a target platelet proteins can induce a conformational neoepitope in the GP that can provoke an immune response. The fiexible nature of the target plexin/semaphorin/integrin domain of GPIIIa may conform to a denatured state [17] allowing quinine to bind to and stabilize the denatured conformation of the platelet receptor [18]. Antibody-bound platelets are rapidly cleared by the reticuloendothelial system.

Hapten-Induced Antibodies

Haptens are drugs and other small molecules (<2–5 kDa) that are typically not immunogenic on their own but induce an immune response when linked covalently to a larger carrier protein [19]. The resulting antibodies recognize the protein where the “hapten” is attached. Penicillin can form a covalent linkage with proteins on red blood cells and platelets through its reactive β-lactam ring. The antibodies observed in patients treated with large doses of penicillin often caused immune hemolytic anemia [20] but may also cause immune thrombocytopenia when the hapten links to platelet membrane GPs [21].

Fiban-Dependent Antibodies

Fibans (tirofiban and eptifibatide) are medications that bind to the arginine-glycine-aspartic acid (RGD) recognition site on GPIIbIIIa and prevent the formation of platelet thrombi by competitively inhibiting fibrinogen binding. These agents are commonly during percutaneous coronary interventions. Severe thrombocytopenia occurs in 0.1% to 2% of patients treated with fibans often within hours of the first exposure to the drug [22,23]. The mechanism of thrombocytopenia is thought to be the formation of a neoepitope on GPIIbIIIa, which becomes the target of naturally-occurring antibodies or antibodies induced by prior exposure to the drug [23]. In addition to causing platelet clearance, eptifibatide-dependent anti-platelet antibodies have been shown to induce platelet activation through platelet FcγRIIa receptor binding, which may explain the occurrence of thrombosis in some patients with eptifibatide-thrombocytopenia [24].

Fab-Binding Monoclonal Antibodies

The antiplatelet agent abciximab is a chimeric Fab fragment (human-murine) that is specific for GPIIIa. It blocks the binding of fibrinogen to the RGD sequence on GPIIbIIIa [25]. It is commonly used in high-risk coronary angioplasty procedures [26]. Abciximab itself does not cause thrombocytopenia because it lacks an Fc domain; however, thrombocytopenia can occur in up to 12% of patients after repeat exposure and 2% of patients on first exposure [27] due to the presence of naturally-occurring antibodies that recognize the murine elements of the drug [28].

Severe acute thrombocytopenia has been reported following the administration of rituximab, a chimeric anti-CD20 antibody that targets B. Rituximab is commonly used to treat B cell lymphoproliferative diseases and autoimmune conditions including ITP [29]. Possible mechanisms include the formation of circulating immune complexes causing platelet lysis by complement activation [30–32] or direct binding to the CD20 antigen on the surface of platelets [30,32,33]. Thrombocytopenia is often preceded by infusion-related symptoms and cytokine secretion and may present with features of disseminated intravascular coagulation.

Drug-Induced Autoantibody Formation

Certain drugs can induce the formation of drug-*independent* platelet autoantibodies. These autoantibodies can bind to platelet antigens even in the absence of the drug and the resultant thrombocytopenia can persist after the drug is discontinued. Gold, L-dopa, procainamide and sulphonamides have been associated with this type of reaction [16]. Recently, alemtuzumab, a humanized anti-CD52 monoclonal antibody was associated with the development of ITP in 6 (2.8%) of 216 patients with multiple sclerosis [34]. Thrombocytopenia developed between 19 and 39 months from first exposure and serological studies for anti-platelet antibodies were inconclusive. The mechanism for autoantibody production is unknown, but may be caused by altered processing of platelet GPs.

Formation of Immune Complexes

Immune complex formation is well described following treatment with UFH and less commonly, low molecular weight heparin in the syndrome called heparin-induced thrombocytopenia (HIT). While in circulation, heparin binds to platelet factor 4 (PF4) and

forms an antigenic structure. Antibodies bind to the PF4/heparin complex by their Fab portion and to the surface of platelets and monocytes by their Fc portion causing intense platelet activation and release of procoagulant microparticles [35,36]. A similar syndrome has recently been described following exposure to protamine used to reverse the effects of heparin after cardiac surgery [37–39]. Protamine binds heparin to form antigenic complexes that are immunogenic in mice and humans. These complexes bind IgG antibodies which activate platelets via FcγRIIa leading to thrombocytopenia and potentially thrombosis [38,39]. The mechanism underlying HIT is distinct from other forms of DITP and the clinical presentation is characterized by thrombosis rather than bleeding [40] (Table 2).

Immune-mediated suppression of platelet production

Immune thrombocytopenia in DITP may result from antibodies that bind to megakaryocytes resulting in impaired platelet production. Eptifibatide-dependent platelet antibodies have been shown to impair viability of megakaryopoietic cells in an in vitro culture assay [41]. Similarly, quinine-dependent antibodies can bind megakaryocytes leading to the induction of apoptosis and reduced proplatelet formation [42]. These findings suggest that drug-dependent antibodies may affect the number of functional megakaryocytes available for platelet release and interfere with their capacity to produce platelets.

Establishing the Diagnosis of DITP

Clinical History

A thorough clinical history is essential in establishing the diagnosis of DITP. A complete drug history should be obtained from any patient presenting with thrombocytopenia including prescription medications, herbal preparations and even certain foods and beverages. DITP is frequently overlooked as a cause of thrombocytopenia and the syndrome may be clinically indistinguishable from primary ITP. Moreover, DITP can occur because of occult drug exposures. Reddy et al describe 5 patients with persistent thrombocytopenia who were presumed to have relapsing ITP [43]. They received multiple treatments including splenectomy before the use of quinine tablets to self-treat leg cramps was uncovered. In one report, severe thrombocytopenia after knee replacement surgery was found to be caused by vancomycin that was contained in the prosthesis cement [44]. Recurrent thrombocytopenia after ingestion of walnuts has been confirmed by a walnut re-challenge and the demonstration of walnut (*Juglans regia*)–dependent platelet antibodies in vitro [45]. Other foods and natural products with definite evidence for causing DITP are cow's milk, cranberry juice, sesame seeds (in tahini), African bean (*Lupinus termis*), and Jui [13] (Table 3). These reports highlight the importance of a careful and detailed history of drug exposures including their temporal relationship with the development of the thrombocytopenia.

Certain unique clinical features can help distinguish DITP from other causes of thrombocytopenia. Particular attention should be paid to the severity of thrombocytopenia, the presence of bleeding, the timing and rapidity of the onset of thrombocytopenia and the implicated drug. Thrombocytopenia is typically severe with platelet counts typically below 20×10^9/L. Evidence of hemostatic impairment is almost always present and patients can present with petechiae, oral mucous membrane blood blisters, serious gastrointestinal

bleeding or intracranial hemorrhage. Deaths from bleeding have been reported [46]. The onset of thrombocytopenia occurs 5 to 10 days after first drug exposure with classic DITP reactions and more rapidly (within hours) following fiban and abciximab. Rarely, the development of thrombocytopenia after abciximab can be delayed for up to 8 days, since platelet-bound abciximab may persist for up to 2 weeks after treatment [47]. The most commonly implicated drugs in DITP reactions include quinine, quinidine, trimethoprim/sulfamethoxazole and vancomycin (Table 4); however, any drug should be considered if the timing and clinical presentation fits.

Clinical Criteria for DITP

To help establish the likelihood that a particular drug was the cause of the thrombocytopenia, several clinical scoring systems have been developed. In 1982 Hackett et al proposed the following criteria: (1) Thrombocytopenia developed while the patient is taking the drug, resolved once the drug is stopped and did not recur while the patient was off the drug; (2) other causes of thrombocytopenia were excluded; (3) the thrombocytopenia recurred upon re-administration of the drug; and (4) an in vitro test for drug-dependent platelet antibodies was positive [48]. A positive re-challenge or positive laboratory test was sufficient to confirm the diagnosis. The authors identified 24 drugs from published reports that were implicated in DITP using these criteria (Table 4). In 1998, George et al used similar clinical criteria to establish levels of evidence for DITP drugs [47] and identified 48 drugs with a definite association (Table 4). This list has been updated regularly and is available online (www.ouhsc.edu/platelets). These criteria have been helpful for developing lists of implicated drugs, but are less helpful for the clinician faced with a patient with suspected DITP since they can only be applied retrospectively.

Laboratory Criteria for DITP

The detection of drug-dependent platelet antibodies in vitro can confirm the diagnosis of DITP. Challenges with the implementation of DITP testing have included a wide variety of techniques used over the years, the lack of standardization, and the lack of validation across a range of drugs.

The characteristics of a positive in vitro test proposed by Hackett included one measure of sensitivity-that the patient's serum plus test drug produce a measurable effect in test platelets, and two measures of specificity-that neither non-implicated drugs nor serum from non-thrombocytopenic controls produce the effect. A variety of tests have been developed based on these principles; however, they have often been difficult to interpret due the lack of validity and reproducibility. Thus, by consensus among a group of experts, Arnold et al proposed the following criteria for the assessment of the quality of DITP laboratory test methods and results from published reports, called the "*DITP criteria*": (1) Drug (or drug metabolite) was required for the reaction in vitro; (2) Immunoglobulin binding was demonstrated; (3) Two or more laboratories obtained positive results on separate occasions; and (4) Platelets were the target of immunoglobulin binding. The laboratory diagnosis of DITP was considered *definite* when all criteria were met; and *probable* when positive results were reported by only one laboratory instead of two [5].

Test Methods That Met Laboratory Criteria

Several assays can measure the ability of antibody (in serum or plasma) to bind platelets in the presence of the drug or its metabolite. Sera from affected patients with no drug added and sera from patients who did not develop thrombocytopenia after drug exposure can be used as negative controls [50]. Test methods [51] that met validity criteria were flow cytometry, platelet suspension immunofluorescence test, enzyme immunoassays, radiolabeled anti-globulin-based assays, and GP-specific assays (Table 5). Flow cytometry is most commonly used because of its sensitivity and ability to provide a quantitative result. Some laboratories use assays that detect binding of antibodies to whole platelets using enzyme-conjugated or fluorescent-tagged secondary antibodies. A number of older tests have been used throughout the years to evaluate the effect of specific drugs on platelets, which are mainly of historical interest (Table 6).

Combining Clinical and Laboratory Criteria to Identify Implicated Drugs

To narrow the list of drugs implicated in DITP, Reese et al proposed a classification system that incorporated clinical criteria and results of laboratory testing [49]. The authors cross-referenced an online list of drugs with a US national voluntary-reporting database and DITP test results from a reference laboratory database. Using this strategy, the authors identified 24 drugs (Table 4). More recently, Arnold et al identified 16 drugs that met both clinical criteria and laboratory criteria from published reports which were felt to have the strongest evidence for causing DITP [4]. Quinine, quinidine, trimethoprim/sulfamethoxazole, vancomycin, penicillin, rifampin, carbamazepine, ceftriaxone, ibuprofen, mirtazapine and suramin; the GPIIbIIIa inhibitors abciximab, tirofiban and eptifibatide; and heparin (Table 4).

Approach to management of the patient with suspected DITP

Diagnosis

We propose an approach to the diagnosis and management of the patient with new-onset thrombocytopenia in whom DITP is suspected (Fig 2). The diagnosis should be considered in any patient who develops severe thrombocytopenia (nadir platelet count less than 20×10^9/L) with bleeding complications that begins 5 to 10 days after starting daily administration of a new drug, or within hours of the first exposure to certain drugs such as fibans and abciximab or within hours of subsequent drug exposures. Other causes of thrombocytopenia should be sought in patients without these clinical features. If the implicated drug has been previously shown to be associated with DITP (Table 4), then the diagnosis is more likely; however, DITP should be considered with any drug. Positive laboratory testing demonstrating drug-dependent platelet reactive antibodies can confirm the diagnosis, but a negative test does not rule it out. Recurrence of thrombocytopenia with drug re-exposure can also confirm the diagnosis. A drug re-challenge, starting with low doses of drug, may be considered when common drugs such as acetaminophen [62] are implicated and results of laboratory testing are negative or unavailable. This approach may expose patients to bleeding risk and thus should only be done under careful supervision.

Management

The most important aspect of management is to immediately stop the drug. Platelet counts typically begin to recover within 1 to 2 days. Platelet transfusions may be required to treat patients with severe thrombocytopenia and bleeding and other supportive measures including high dose IVIG and a brief course of corticosteroids may be reasonable. Drug-dependent platelet antibodies can persist for many years; thus, patients with a confirmed diagnosis should be counselled to avoid future exposures to the drug [63]. Counselling on drug avoidance is less certain for patients in whom the diagnosis cannot be confirmed.

Future directions

Accessible and reliable laboratory testing would help establish the diagnosis of DITP and provide guidance for clinicians and patients on the need for drug avoidance. However, testing is only available in a few reference laboratories and test methods have not been standardized [64]. Reasons for this are: (1) no single method has been universally accepted, although flow cytometry is commonly used; (2) assays must be specifically developed for each drug, some of which cannot be solubilized at neutral pH; (3) DITP may be due to metabolites of the drug which are not always available for use as a reagent in the test; and (4) other platelet antibodies can confound the measurement of drug-dependent antibodies. The International Society of Thrombosis and Hemostasis is working towards standardizing laboratory testing for the detection of drug-dependent platelet antibodies for the most commonly implicated drugs.

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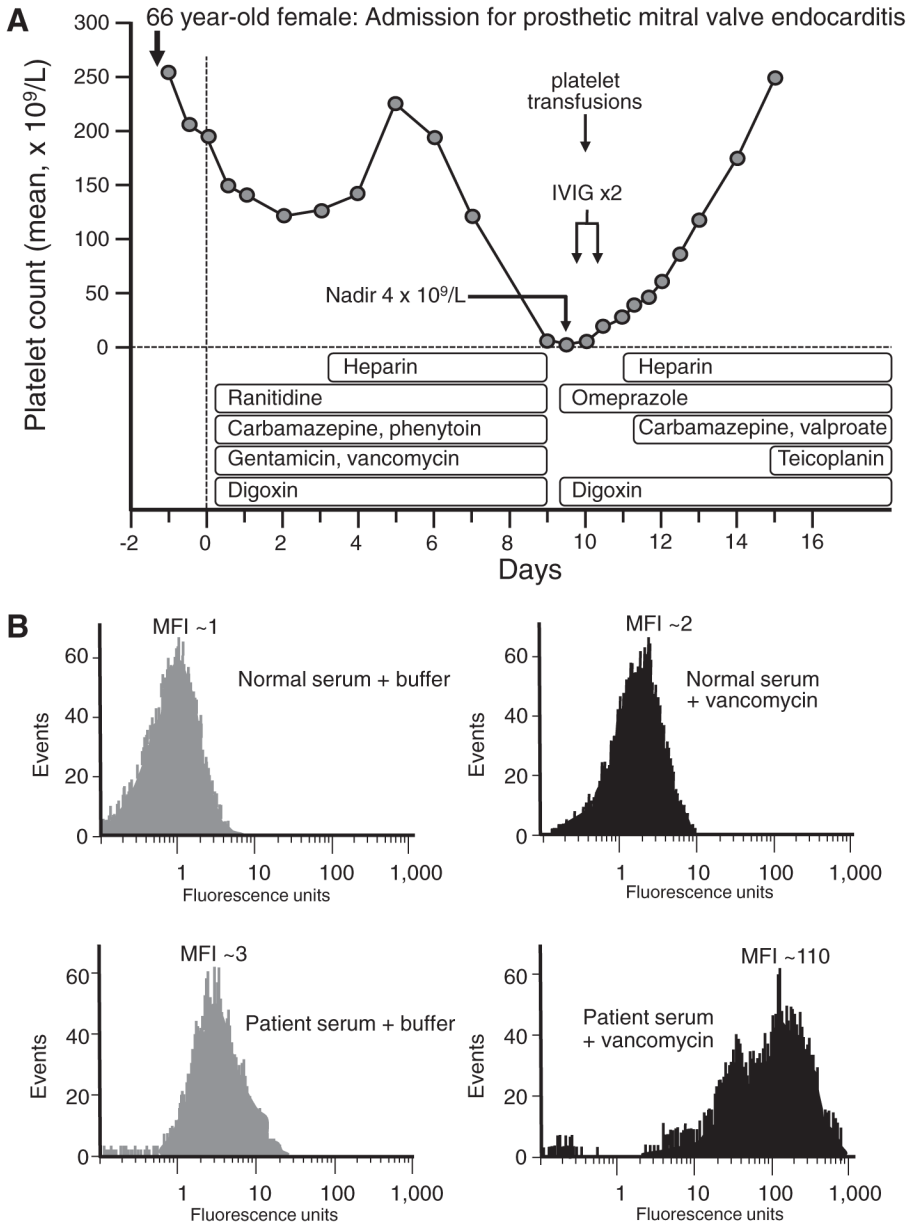

Fig 1.

Illustrative case presentation of drug-induced immune thrombocytopenia. A, Clinical picture (See text for details). B, Results of patient serum and drug-dependent antibody binding by flow cytometry. IVIG = intravenous immune globulin. MFI, mean fluorescence intensity. Reproduced with permission from: Warkentin TE. Thrombocytopenia caused by platelet destruction, hypersplenism, or hemodilution. Adapted from *Elsevier* 2013: 1895–1912.

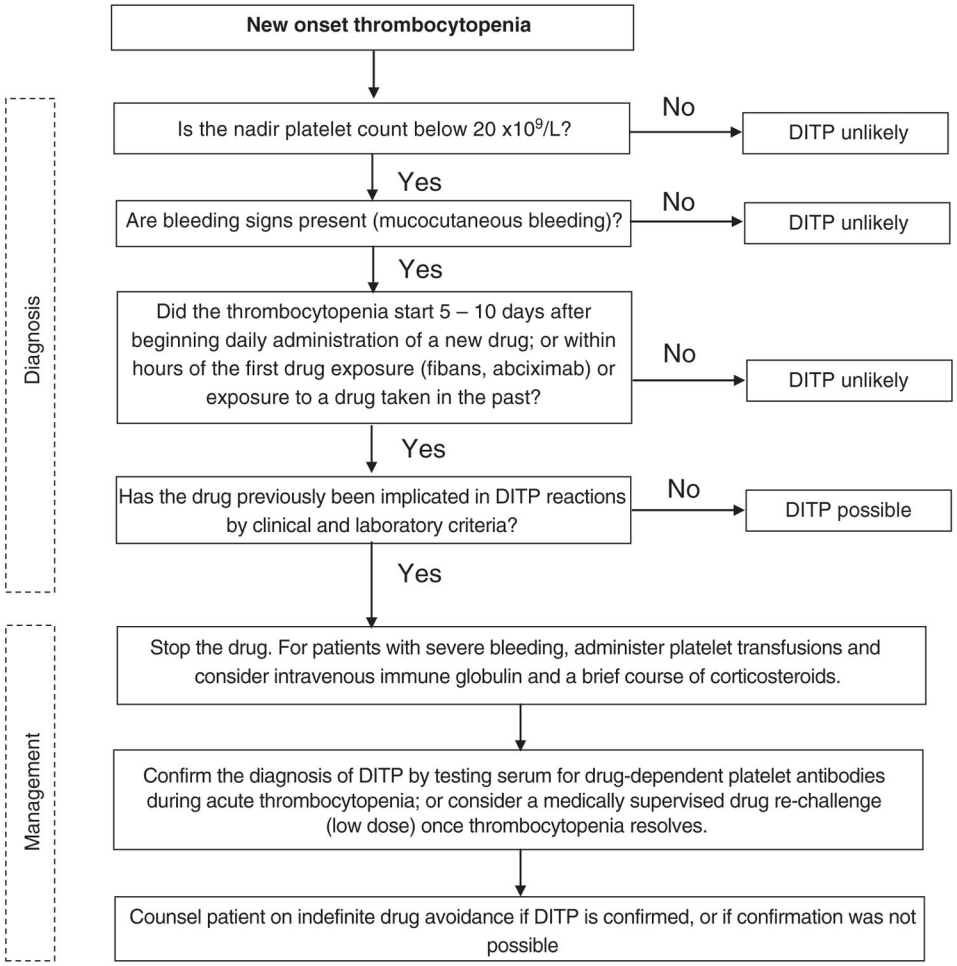

Fig 2.

Approach to the diagnosis and management of a patient with new-onset thrombocytopenia in whom drug-induced immune thrombocytopenia is suspected.

Table 1

Mechanisms of the development of drug-dependent platelet antibodies

Mechanism	Description	Examples of Drugs
Classic drug-dependent platelet antibodies	Non-covalent binding of drug to platelet glycoproteins creates a neoepitope and allows antibody binding that marks the platelets for destruction by the reticuloendothelial system	Quinine, quinidine
Hapten-induced antibodies	Covalent binding of drug to platelet proteins induced an immune response and antibody production	Penicillin
Fiban-dependent antibodies	Binding of drug to the RGD sequence of GPIIbIIIa creates a neoepitope that enhances the affinity of anti-platelet antibodies	Tirobifan, eptifibatide
Fab-binding monoclonal antibodies	Monoclonal antibodies bind to platelet surface proteins and become targets of naturally occurring antibodies that lead to platelet destruction	Abciximab
Autoantibody production	Drug exposure leads to autoantibody production, which is independent of the drug.	Gold
Platelet-localizing immune complexes	Drug binds PF4, forming large complexes on platelet surfaces to which IgG binds via its Fab moieties, with subsequent interactions of IgG Fc with platelet Fcγ, leading to platelet activation and release of procoagulant microparticles (similar mechanisms result in monocyte activation)	Heparin

RGD sequence = arginine-glycine-aspartic acid. PF4, platelet factor 4.

Table 2

Differences between classic (quinine-type) DITP and HIT

	DITP	HIT
Onset of thrombocytopenia	5–10 days	5–10 days (classic)
Median platelet count nadir	10×10^9/L	60×10^9/L
Clinical manifestation	Bleeding	Thrombosis
In vitro diagnostic testing	Flow cytometry (or other method) demonstrating patient IgG binding to washed platelets only in the presence of the drug	Functional test demonstrating activation of washed platelets by patient serum in the presence of pharmacologic heparin concentrations (peak platelet activation at 0.1– 0.3 IU/mL).
Treatment	Stop the drug	Stop the heparin and start an alternate anticoagulant
Persistence of drug-induced platelet antibodies	Indefinitely	Median detectability, 50 to 80 days
Re-exposure after confirmed diagnosis	Never	Possible (pending non- detectability of platelet- activating antibodies)

Table 3

Foods, beverages and natural products associated with definite evidence * for the development of drug-induced immune thrombocytopenia [13]

Substance	Platelet nadir	Bleeding	Confirmation
Cow's milk	5×10^9/L	Yes	Re-challenge
Cranberry juice	1×10^9/L	Yes	DDAbs
Jui	0	Yes	Re-challenge
Lupinus termis bean	10×10^9/L	Not reported	Re-challenge
Sesame seeds	6×10^9/L	Yes	Re-challenge
Walnut (*Juglans regia*) [46]	4×10^9/L	Yes	Re-challenge and DDAbs

* Definite evidence = Ingestion of the substance preceded the thrombocytopenia and platelet count recovered once the substance was stopped; candidate substance was the only agent ingested or other agents were continued or re-introduced and platelet count remained normal; other etiologies of thrombocytopenia were excluded; confirmation in vivo with a re-challenge or in vitro with the demonstration of drug-dependent platelet antibodies (DDAbs).

Table 4

List of drugs implicated in drug-induced immune thrombocytopenia reactions from various sources (definite evidence)

	Hackett [48]	George [46]	Reese [49]	Arnold [5]
Implicated drugs	Acetaminophen	Quinidine	Abciximab	Quinine
	Allylisopropylcarbamide	Quinine	Acetaminophen	Quinidine
	Alprenolol	Rifampin	Amiodarone	Trimethoprim-Sulfamethoxazole
	Chlorothiazide	Trimethoprim-sulfamethoxazole	Ampicillin	Vancomycin
	Digitoxin	Methyldopa	Carbamazepine	Penicillin
	Digoxin	Acetaminophen	Eptifibatide	Rifampin
	Levamisole	Digoxin	Ethambutol	Carbamazepine
	Methicillin	Danazol	Haloperidol	Ceftriaxone
	Methyldopa	Diclofenac	Ibuprofen	Ibuprofen
	Novobiocin	Aminoglutethimide	Irinotecan	Mirtazapine
	Organic arsenicals	Amphotericin B	Naproxen	Oxaliplatin
	Oxprenolol	Aminosalicylic acid	Oxaliplatin	Suramin
	Para-aminosalicylic acid	Oxprenolol	Phenytoin	Abciximab
	Quinidine	Vancomycin	Piperacillin	Tirofiban
	Quinine	Levamisole	Quinidine	Eptifibatide
	Rifampicin	Meclofenamate	Quinine	Heparin
	Stibophen	Diatrizoate meglumine-diatrizoate sodium	Ranitidine	
	Sulfathiazole	Amiodarone	Rifampin	
	Sulfisoxazole	Nalidixic acid	Simvastatin	
	Acetylsalicylic acid	Cimetidine	Sulfisoxazole	
	Co-trimoxazole	Chlorothiazide	Tirofiban	
	Desipramine	Diatrizoate Meglumine	Trimethoprim-sulfamethoxazole	
	Diazepam	Interferon-α	Valproic acid	
	Diphenylhydantoin	Sulfasalazine	Vancomycin	
		Ethambutol		
		Iopanoic acid		
		Sulfisoxazole		
		Tamoxifen		
		Thiothixene		
		Naphazoline		
		Amrinone		
		Lithium		
		Diazepam		
		Haloperidol		
		Alprenolol		
		Tolmetin		
		Nitroglycerin		
		Minoxidil		

Hackett [48]	George [46]	Reese [49]	Arnold [5]
	Diazoxide		
	Chlorpromazine		
	Isoniazid		
	cephalothin		
	Difiuormethylornithine		
	Piperacillin		
	Diethylstilbestrol		
	Methicillin		
	Deferoxamine		
	Novobiocin		

Table 5

Test methods used to evaluate drugs suspected of causing DITP that met all validity criteria [5]

Method	Description
Flow cytometry	Quantification of patient antibody bound to platelets using fluorescent-labeled antiglobulin processed on a flow cytometer, with the ability for simultaneous detection of different antibody classes.
Platelet suspension immunofluorescence test	Semi-quantitative measurement by light microscopy of drug-dependent antibody bound to platelets using fluorescent-labeled antiglobulin.
Monoclonal antibody immobilization of platelet antigens (MAIPA)	Antibody detection is based on tri-molecular complexes formed by binding of glycoprotein- specific monoclonal antibodies to intact platelets with bound human antibody. Following lysis of the platelet membrane, glycoprotein-specific human antibody is measured using enzyme-conjugated anti-globulin in an enzyme immunoassay.
Antigen-capture ELISA (ACE; modified ACE)	Similar to MAIPA assay, but glycoprotein-specific monoclonal antibody is used to capture human antibody bound to the specific platelet protein after lysis of the platelets. This allows for routine processing and storage of lysates for future testing with various monoclonal antibodies.
Enzyme immunoassay	Enzyme-conjugated antiglobulin is used to detect platelet-bound IgG on platelets adherent to microtitre wells.
Platelet antiglobulin test	Modification of the enzyme immunoassay in which radiolabeled antiglobulin is used to detect the human antibody bound to target platelets.
Immunoprecipitation	Measurement of antibody bound to ^{125}Iodine- radiolabeled platelet proteins which are identified by their mobility following gel electrophoresis.
Immunoblot assay	Measurement of human antibody binding to platelet proteins previously separated by gel electrophoresis and transferred to a membrane. Antibody binding may be limited by destruction of epitopes on the denatured proteins during electrophoresis.

Table 6

Historical techniques previously used to evaluate the effect of drugs on platelets

Test	Description of methods
Patch test	Topical application of the drug to the patient's skin. The test was considered positive if local purpura developed [52].
Antiglobulin consumption assay (serum bindable IgG)	A fixed amount of antiglobulin was incubated with test platelets. Residual antiglobulin was measured by complement lysis of IgG-coated erythrocytes or by quantification of radiolabeled antiglobulin bound to IgG-coated beads [53].
Platelet factor 3 (PF3) test ("immune injury" test)	Normal citrated platelet rich plasma was incubated with drug and patient serum. Increased PF3 activity, indicated by a shortening of the Russell's viper venom (Stypven) time, was considered positive [54]. This assay was refined with a neutralization [55] or IgG isolation step [56].
Clot retraction inhibition test	Visual inspection of a blood clot formed in solution in the presence of test serum with and without drug. Inhibition of clot retraction was considered positive indicating the lack of functional platelets as a result of antibody interference [57].
Complement fixation test	Incubation of patient serum, drug, test platelets and a source of complement. Residual complement activity was measured by lysis of IgG-sensitized sheep red blood cells [58].
Platelet agglutination test	Patient serum was incubated with test platelets in the presence of the drug. Clumping in the test tube was assessed visually and graded semi-quantitatively from 0–4 after a 2-hour incubation at 37°C [59].
Platelet lysis test	^{51}Cr-labeled platelets were incubated with test serum (or IgG), normal platelets, and a source of complement with and without drug. Percentage platelet lysis was calculated [60,61].