

CASE REPORT

Acute drug-induced immune thrombocytopenia - A work of articaine

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Abstract

Background: Drug-induced immune thrombocytopenia (DITP) is a rare, but serious complication to a wide range of medications. Upon suspicion, one should do a thorough clinical evaluation following proposed diagnostic criteria and seek laboratory confirmation. If confirmed, it is important to ensure avoidance of the drug in the future.

Study design and methods: Herein, we describe a young adult male who experienced two bouts of severe thrombocytopenia following dental treatment. The thrombocytopenia was acknowledged due to unexpected hemorrhaging during the procedures. On both occasions, he was exposed to four different drugs, none commonly associated with DITP. After the second episode of severe procedural-related thrombocytopenia, an investigation into the cause was initiated. We describe the clinical approach to elucidate which of the four implicated drugs was responsible for thrombocytopenia and the laboratory work-up done to confirm that the reaction was antibody-mediated and identify the antibody's drug: glycoprotein specificity. An alternative drug was tested both in vivo and in vitro, to identify an option for future procedures.

Results: Sequential exposure revealed the local anesthetic substance articaine to induce thrombocytopenia. Laboratory work-up confirmed drug-dependent antibodies (DDAbs) with specificity for the glycoprotein Ib/IX complex, swiftly identified by a bead-based Luminex assay. Further investigations by monoclonal antibody immobilization of platelet antigens assay (MAIPA) revealed a probable GPIb binding site. An alternative local anesthetic, lidocaine, was deemed safe for future procedures.

Conclusion: Articaine can induce rapid-onset, severe immune-mediated thrombocytopenia causing bleeding complications. A modified bead-based Luminex platelet antigen assay proved a useful addition in the DITP-investigation.

Abbreviations: DDabs, Drug-dependent antibodies; DITP, Drug-induced immune thrombocytopenia; GP, Glycoprotein; ITP, Immune thrombocytopenia; mAb, Monoclonal antibody; MAIPA, Monoclonal antibody immobilization of platelet antigens; PIFT, Platelet immunofluorescence test.

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KEYWORDS

adverse effect, articaine, drug-induced immune thrombocytopenia, GPIb/IX, MAIPA, thrombocytopenia

1 | CASE PRESENTATION

A mainly healthy 23-year-old male patient was admitted to the local hospital for dental treatment under general anesthesia. No blood tests were done on initial admission. After the extraction of two teeth, there was serious bleeding. Blood tests 4 h after initiation of the procedure revealed a platelet count of $<5 \times 10^9/L$ and immune thrombocytopenia (ITP) was suspected. He was given local hemostatic treatment and immediately transferred to a tertiary center. Upon arrival, the platelet count was $5 \times 10^9/L$ and the bleeding had stopped. The following days counts rose spontaneously to $96 \times 10^9/L$ and he was discharged. Two months later he was re-admitted for completion of the treatment. On admission, his platelet count was $261 \times 10^9/L$. Again, the procedure had to be stopped due to bleeding. The platelet count was controlled the following morning when it was $22 \times 10^9/L$.

Two episodes of procedural-related thrombocytopenia with extremely rapid onset and severity were highly suggestive of drug-induced immune thrombocytopenia (DITP), and further investigations were planned at the tertiary center. On both occasions, he had been exposed to oral acetaminophen, submucosal Septocaine (articaine with epinephrine, Septodont, France), and intravenous propofol and remifentanyl. The patient needed further dental treatment, but as the cause of the thrombocytopenia was unknown, any procedure - especially under general anesthesia, was not considered safe. After a thorough discussion with the patient, which included the possibility of bleeding complications, a diagnostic approach based on sequential exposure testing was planned at the tertiary center. Exposure to a new substance, under close monitoring of platelet counts and immediate access to platelet concentrates, was planned every second day.

There was no drop in platelet count following oral exposure to acetaminophen. 70 min after exposure to subcutaneous Septocaine 40 mg/mL, he experienced shivers and nausea. At 90 min, a fever of $38.3^\circ C$ was recorded and at 140 min the platelet count was $<5 \times 10^9/L$, contrasting $270 \times 10^9/L$ before exposure. He received a platelet transfusion, elevating the count to $24 \times 10^9/L$ (Figure 1). After this, platelet count rose spontaneously and there was remission of systemic symptoms. There are four documented time points

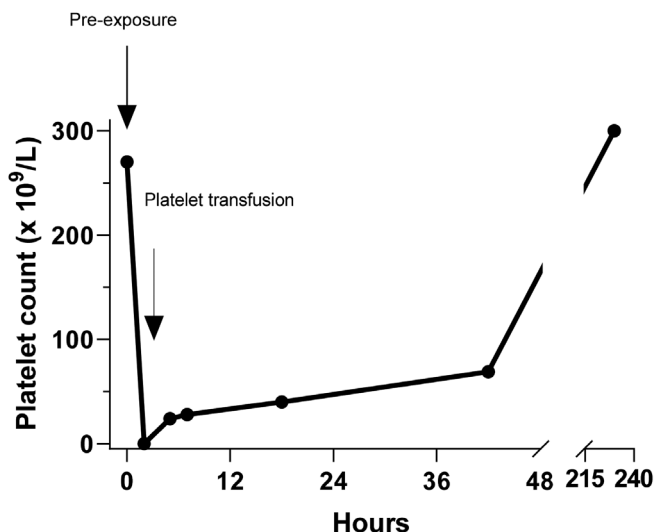


FIGURE 1 Platelet count development during clinical provocation. There was a rapid decrease in platelet numbers following subcutaneous injection of Septocaine. Platelet transfusion gave a satisfactory increase in platelet count

(2004–2013) of previous exposure providing possible sensitization events. On one occasion he experienced a severe headache.

Planned exposure to intravenous propofol and remifentanyl was canceled. He later underwent controlled exposure to single-agent subcutaneous epinephrine and lidocaine without a drop in platelet count. The dental work has been completed under general anesthesia without complications, utilizing lidocaine as the local analgesic drug. The patient has given written consent to exposure testing and to the publication of the case.

2 | IN VITRO INVESTIGATIONS

The presence of drug-dependent antibodies (DDAbs) was tested for by flow cytometry platelet immunofluorescence test (PIFT), monoclonal antibody immobilization of platelet antigens (MAIPA) assay, and a bead-based Luminex assay (Pak Lx, Immucor). All assays except for the initial flow cytometry test were modified by the addition of Septocaine, following guidelines from ISTH¹ and our in-house protocol for drug-dependent antibody testing.

For standardized DITP-testing patient plasma/serum, control sera, donor platelets (blood group O, HPA-1a+) and the drug are required. Our negative control is group AB donor plasma, and the positive control is an in-house validated anti-HPA-1a (mAb 26.4). Ideal control sera as described by Arnold et al¹ were not available.

Investigation during the acute episode following controlled exposure was performed using standard direct platelet antibody testing by PIFT. Three EDTA samples were tested; a sample drawn the same morning (pre-exposure), a 2-h post-exposure sample (platelet count $<5 \times 10^9/L$) and the third sample drawn after platelet transfusion. Direct testing of the earliest post-exposure sample was positive. There were no detectable antibodies bound to platelets isolated from pre-exposure or post-transfusion samples (Figure 2A).

Standardized indirect testing was done a few weeks after the incident. Briefly, 20 μL patient and control plasma were incubated with group O donor platelets in the presence and absence of Septocaine, at three concentrations, 0.02 mg/mL, 0.002 mg/L and 0.001 mg/mL, selected based on reported plasma concentrations following therapeutic dosing² in a final volume of 40 μL . There were positive reactions for the drug-spiked patient sample (Figure 2B). There was no reactivity when Septocaine was not added and there was no reactivity with control plasma at any drug concentration, ruling out unspecific binding (Figure 2C).

To assess glycoprotein reactivity we performed Pak Lx and MAIPA. In Pak Lx, an assay designed for alloantibody detection, each bead population displays one type of platelet glycoprotein, GPIa/IIa, GPIIb/IIIa, GPIV or GPIb/IX. Briefly, 10 μL patient or control plasma was incubated for 60 min with 40 μL bead mix in the presence or absence of Septocaine (0.002 mg/mL) before washing and addition of conjugation reagent in line with standard protocol.

The patient sample revealed strong reactivity with GPIb/IX beads in the presence of Septocaine (Figure 3A). The reactivity was markedly reduced by 1 h pre-incubation with GPIb/IX-specific mAb SZ2 prior to incubation with patient plasma and Septocaine (Figure 3B). Further, purified patient IgG from plasma (Protein G HP Spintrap) also showed reactivity to GPIb/IX in Pak Lx in the presence of Septocaine (Figure 3A). This confirms that antibody reactivity was reliant on native articaine, as opposed to metabolites formed in the presence of plasma cholinesterases.

Glycoprotein specificity was confirmed in MAIPA when utilizing mAb FMC-25 as capture antibody for the

GPIb/IX-complex (OD 1.17), but not when capturing the same complex with mAb SZ2 (OD 0.06).

3 | DISCUSSION

Drug-induced *immune* thrombocytopenia is a rare complication. The true incidence is unknown, but a few epidemiological studies estimate an annual incidence of 10/1,000,000,³ with a probable higher occurrence among critically ill and hospitalized patients. Clinically it manifests as thrombocytopenia occurring 5–10 days after exposure to a drug, earlier if the patient is previously sensitized. The thrombocytopenia is typically severe with a platelet count below $20 \times 10^9/L$ and bleeding complications have been reported in up to 74% of cases.⁴ The thrombocytopenia resolves when the drug is discontinued, with the rate of recovery depending on drug elimination kinetics. It is important to diagnose the condition to ensure avoidance of the drug in the future. When more than one drug is implicated, it is important to identify which one is the culprit, to avoid discontinuing essential treatment from the patient.

To clinically establish a diagnosis of DITP, there are certain criteria that must be met.⁵ First, therapy with the suspected drug must precede the onset of thrombocytopenia. Second, the recovery must be complete and sustained after the drug is discontinued. Third, other causes of thrombocytopenia must be ruled out. An additional criterion is thrombocytopenia upon re-exposure to the same drug. If all criteria are met, the diagnosis is definitive. A probable diagnosis can be established if criteria 1, 2 and 3 are present. Failure to meet the first criterion makes the diagnosis unlikely.^{5,6} Our patient met all four criteria, thus a definitive clinical diagnosis could be made.

In working up a DITP-suspicion, clinical provocation is usually advised against as the thrombocytopenia can be extremely severe. In this case, there were four implicated drugs, all procedural-related, thus no time-line available to elucidate which drug was the offending agent. The patient needed further dental treatment, and it was necessary to identify which drug to avoid. None of the administered substances are commonly associated with DITP. Of the four drugs, acetaminophen is the only drug appearing on the list from the Blood Center of Wisconsin (OUSHC),⁷ thus the first one clinically tested. The second drug, Septocaine, gave swift and severe thrombocytopenia with a positive direct platelet-antibody test. In retrospect, laboratory testing *before* provocation would probably have identified Septocaine as the agent provocateur, thus potentially circumventing the need for exposure testing.

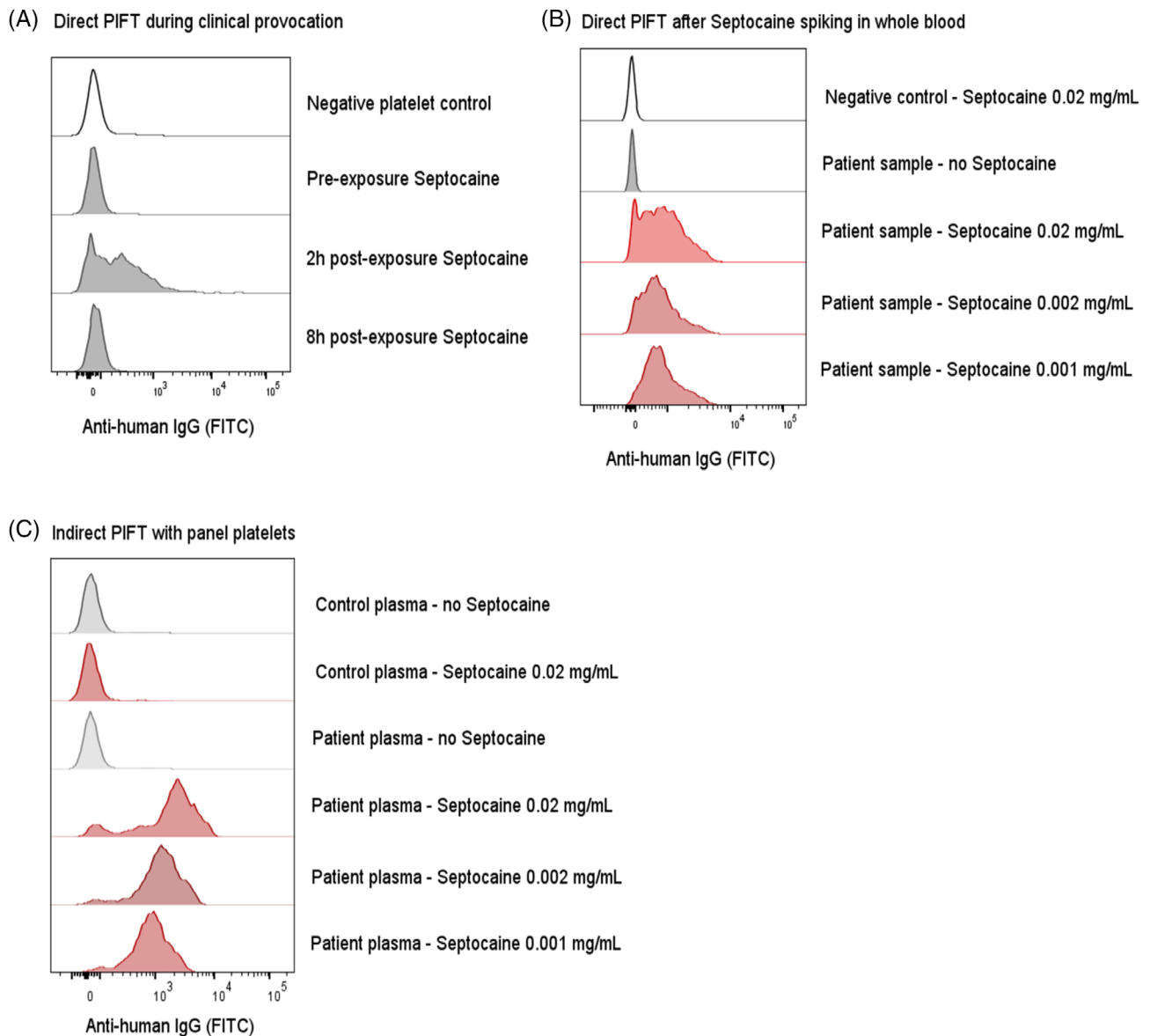


FIGURE 2 Antibody testing by flow cytometry platelet immunofluorescence test (PIFT). (A) Direct testing of patient platelets pre-exposure, 2 h after exposure and 8 h post-exposure (after platelet transfusion). The test was performed without adding Septocaine to the reaction, but still positive for the 2-h sample (fluorescence ratio 3.6). (B) Patient whole blood spiked with Septocaine at three different concentrations before isolating platelets for 'in vitro' direct testing. Results similar to the in vivo sensitized platelets. (C) Patient and control plasma incubated with donor platelets for 45 min in the presence and absence of Septocaine, washed three times and incubated with FITC-conjugated anti-human IgG. All drug concentrations gave a positive reaction for the patient sample, there was no reactivity when Septocaine was not added and there was no reactivity with control plasma at any drug concentration, ruling out unspecific binding [Color figure can be viewed at wileyonlinelibrary.com]

Septocaine is a combination drug. It consists of articaine, an amide group local anesthetic, with epinephrine added to extend tissue availability. Its' main application is local anesthesia during dental procedures. With combination drugs, one cannot be sure which component causes the adverse reaction. In this case, we strongly suspected articaine, as epinephrine is an endogenous substance and thus unlikely to cause the acute effect seen in this case. Confirming this assumption, the clinical test, injecting epinephrine as a single agent a

week after the Septocaine provocation, did not cause thrombocytopenia.

For DITP, laboratory testing criteria have been suggested as follows⁸; Dissolved drug or drug metabolite must be added to the test in vitro, the methods must be able to quantify antibody binding, proper controls with normal serum must be used to exclude non-specific binding and starting material must be washed platelets, PRP or purified platelet antigens. All these criteria are met by our investigations. Preferably, another lab should verify

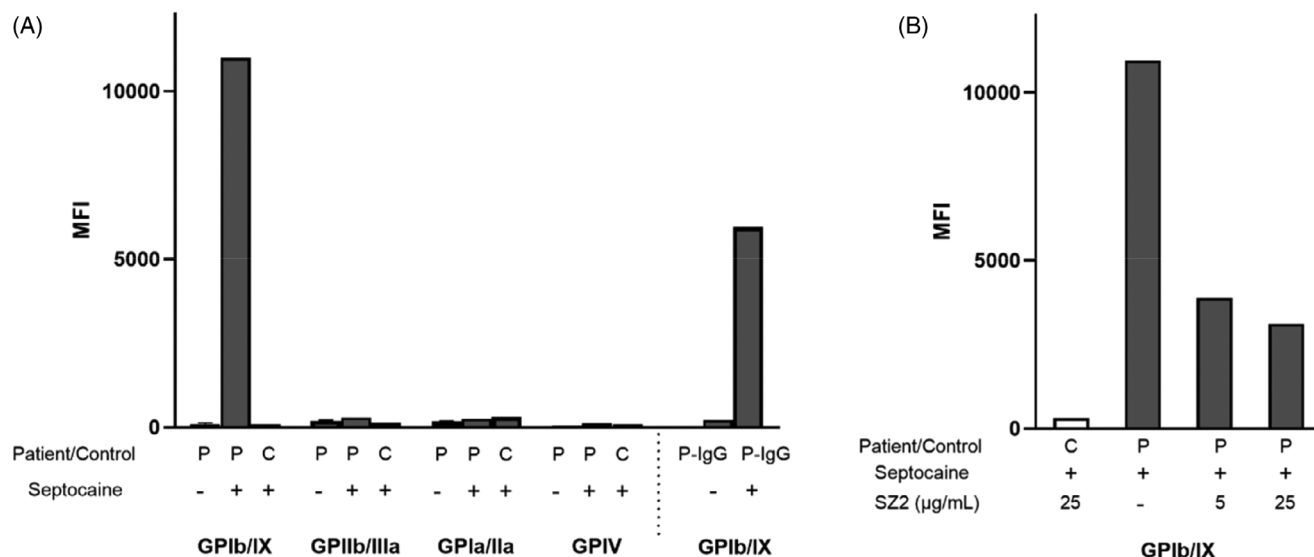


FIGURE 3 Pak Ix assay. (A) Patient plasma with Septocaine (0.002 mg/mL) shows strong positivity with all beads coated with GPIb/IX. There is no reactivity with other glycoprotein complexes. Control plasma with Septocaine is also negative. In a separate experiment, (indicated by the dashed line), purified IgG from patient plasma was also showed antibody binding exclusively in the presence Septocaine in Pak Ix. Bars represent mean value. (B) GPIb/IX-reactivity of patient plasma in the presence of Septocaine is reduced by SZ2 in increasing concentrations

the results, but based on the substantial complimentary data in this investigation, the sample was not sent to confirmatory testing.

This case stands out with its' extremely early recognition of thrombocytopenia due to excessive procedural-related hemorrhage. Drugs with other indications, routes of administration, and pharmacokinetics given for non-procedural purposes rarely have such an instantly observable effect, and the thrombocytopenia might go unrecognized. The rate of platelet recovery will depend on the drug's presence in the circulation, and with a half-life of 20 min,² articaine is expected to be eliminated within a few hours. There was no need for further intervention as the platelet count normalized spontaneously over the following days (Figure 1).

Interestingly, the Pak Lx assay demonstrated GPIb/IX-specific DDABs. Although not routinely used in DITP investigations, we demonstrate its' usefulness as a supplement. Assay applicability has previously been shown for methylprednisolone-dependent antibodies to GPIIb/IIIa and GPIV.⁹

DDABs to the GPIb/IX-complex have been reported for quinine,¹⁰ ceftriaxone,¹¹ rifampicin¹² and ranitidine,¹³ for all these the antibody binding site has been mapped to GPIX. For quinine, binding to GPIb α has also been shown, pinpointed to the region between amino acid residues 283–293.¹⁴ This is close to the SZ2 binding site, mapped to amino acid residues 276–282 of the GPIb α .¹⁵ As Septocaine-dependent GPIb/IX-reactivity in MAIPA confirmed with FMC-25 capture could not be achieved when utilizing SZ2, we hypothesize that the patient's

DDABs bind in this vicinity. This assumption is further supported by the blocking effect with SZ2 on the Pak Lx beads (Figure 3B).

Concomitantly to the thrombocytopenia, there was acute monocytopenia. The mechanism behind this is unclear as monocytes do not typically express GPIb/IX. Possible explanations could be GPIb α adsorbed onto monocytes, platelets bound to monocytes, a similar neopeptide on monocyte glycoproteins in the presence of the drug or removal of platelet: DDABs: monocyte complexes in the spleen.

The immunogenicity of substances varies, and some drugs are reported to give this specific complication more frequently than others. For articaine, we have not been able to find other reported cases. Reports of thrombocytopenia following administration of other local anesthetics are also sparse in the literature. Reviewing the list from OUSHC,⁷ other commonly used amide group anesthetics do not appear, with the exception of one single case after lidocaine exposure.¹⁶ This could mean that DITP is an extremely uncommon complication with this class of drugs, or that the sporadic use and failure to identify the true cause of unexplained thrombocytopenia renders it a difficult diagnosis to make. There are exceptions that might be relevant to our case. A related amide drug, procainamide, is widely reported for various immune-related complications, including DITP.^{17,18} Cocaine was previously a commonly used local anesthetic. Reported cases of thrombocytopenia following cocaine use are in their description highly suspicious of immune mechanisms.¹⁹ Studies regarding in-class cross-reactivity of DDABs are

few, with a notable exception describing varying degrees of cross-reactivity between DDABs to different betalactams.²⁰ Without predictive tools to identify an alternative anesthetic for future procedures, lidocaine had to be tested, both clinically and in vitro. There was no thrombocytopenia following subcutaneous injection, and no antibodies to lidocaine neither upfront nor eight weeks following the exposure.

In conclusion, a DITP diagnosis relies on a combination of clinical and laboratory investigations, an approach successfully employed to diagnose the first case after articaine administration. As exposure can cause bleeding complications, we recommend that laboratory work-up, when available, is considered as the first-line approach in such investigations.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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How to cite this article: Læg Reid IJ, Olsen MI, Harr JI, Grønli RH, Mørtberg TV, Ernsts en SL, et al. Acute drug-induced immune thrombocytopenia - A work of articaine. *Transfusion*. 2022;62:1142–7. <https://doi.org/10.1111/trf.16858>