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An antivenin resistant, IVIg-corticosteroids responsive viper induced thrombocytopenia

Matteo Turetta [a], Fabio Del Ben [b], Donatella Londero [c], Agostino Steffan [a,*], Pierpaolo Pillinini [d]

[a] Immunopathology and Cancer Biomarkers, Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, Aviano, Italy
[b] Department of Medical Area, University of Udine, Italy
[c] Immunohematology Laboratory, Azienda Sanitaria Universitaria Friuli Centrale, Udine, Italy
[d] Department of Emergency Medicine, Azienda Sanitaria Universitaria Friuli Centrale, Tolmezzo, Italy

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ABSTRACT

In this case report the hospital management of an acute, severe thrombocytopenia in a 57-year-old man in the north-east of Italy is reported. Thrombocytopenia developed immediately after the viper bite, despite the absence of clinical signs of envenomation. No hemorrhage, ecchymoses or other signs of coagulopathy developed during the hospitalization; two doses of antivenin FAB–Fragments had no effect on thrombocytopenia, which instead responded promptly to intravenous immunoglobulins (IVIg) and glucocorticoids. Direct and indirect anti-platelet antibodies against anti-GP IIb/IIIa and Ia/IIa were detected during the treatment and turned negative after 20 weeks. The rationale of such off-label treatment is the interpretation of the thrombocytopenia as a venom-induced immune thrombocytopenia which led to splenic sequestration of platelets. To our knowledge, there is no literature about venom-induced immune thrombocytopenia against GP IIb/IIIa and Ia/IIa protein in European countries and subsequent response to IVIg and corticosteroids.

1. Introduction

Several species of the Vipera genus (Viperidae family) coexist in Northeast Italy: from a clinical perspective, the most relevant are Ammodytes (Vaa) (the European most dangerous one, which is mostly represented in our region and broadly-distributed from Balkans to Turkey), Berus (Vbb) (widespread from Western Europe to China), and Aspis (present from Pyrenees area to Germany). Snakebite is an underestimated medical emergency [1], and the description of its epidemiology in Europe is challenging, cause of a deficiency in reporting by National Health Systems: snakebite cases have been estimated in about 1 per 100,000 inhabitants every year with a mortality of 0.05%[2]; deaths commonly occur in children or patients affected by multiple comorbidities. Most relevant effects of snake envenomation are neurotoxicity and disturbances of hemostasis, being the latter the most clinically relevant for Viperae species present in our region. Venom contains a mixture of proteins and active molecules interacting with a plethora of components of the coagulation cascade, endothelial matrix and platelets, both directly (to GPI- GPIIb-IIIa -Ib-IX-V-VI) and through von Willebrand factor and collagen [3,4]. Viperae pro-coagulation enzymes lead also to fibrinogen consumption and to consumption coagulopathy [5], while haemorrhagins damage the endothelium walls of blood vessels [4] and a number of other hemorrhagic metalloproteinases display strong fibrinolytic activity as well as factor X activation [6].

Envenomation symptoms and clinical presentation are highly variable among Viperae species and even within the same species. Moreover, it has been recognized that, even if administration of anti-venom Fab fragments is considered the standard treatment in the event of a viper envenoming due to their ability to neutralize multiple venom components[7], their clinical effects are variable and unpredictable [8,9]. Anti-venoms are commonly used for any moderate-to-severe envenoming in European countries even in the absence of standardized protocols for their administration[10].

An important effect of snakebites is an asymptomatic venom-induced thrombocytopenia (VIT) [11–13], that has been widely described in the genus Crotalidae of the Viperidae family, and seems less common in European countries than in the rest of the world [6]. In this case-report, we describe a VIT characterized by immediate onset, resistance to anti-venom and rapid resolution after administration of intravenous immunoglobulins (IVIg) and glucocorticoids.

* Corresponding author.
E-mail address: asteffan@cro.it (A. Steffan).

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2. Results

A 57 years old, previously healthy male was admitted to hospital for a snakebite on his right knee. The patient arrived in the emergency room two hours after the bite. At presentation, his vital signs were: blood pressure 130/90 mm Hg; pulse 100 beats/min; hemoglobin oxygen saturation 97% (FiO_2 21%). His anamnesis was not significant, except for a positive serology for borreliosis treated few years before, negative for snake bites. A blood count performed several years earlier documented a platelet count of 199 × 10^3/μL (reference 150–400).

At the admission, the patient did show neither pain, nor local hemorrhage or swelling of the envenomation site: in particular, no neurotoxic symptoms (paresis, loss of sensitivity, paresthesia, hypotonia) and central effects as fever or diaphoresis were observed, with the exception of an episode of vomit. Since the first laboratory tests, performed at admission, displayed a trend to thrombocytopenia (initial platelet count of 93 ×10^3/μL), the patient was hospitalized in the emergency medicine department. No hemorrhagic or thrombotic manifestations were clinically observed during the whole period of hospitalization. Other causes of thrombocytopenia were excluded through anamnesis and physical examination, laboratory testing and peripheral blood smear analysis. Other laboratory results at the admission were within the reference ranges, including: prothrombin time (PT) 1.10 (reference, 0.85–1.15), INR 1.10 (reference, 0.80–1.20); liver function tests, renal and serum electrolyte were normal or slightly augmented; WBC count and formula had no alterations. Pseudo-thrombocytopenia or pre-analytical error due to platelet aggregation or satellitism was excluded by microscopic examination of the blood smear.

Two hours after the admission, a lymphedema progressively developed from the distal third of the thigh to the right foot; laboratory findings at this time were a constant reduction of the platelets (52 ×103/μL) and an unaltered INR of 1.15; during hospitalization the patient always maintained hemodynamic stability complaining with only slight pain in the lower right limb. The fall in platelets was not accompanied by any other significant laboratory alterations except for an expected rise in D-dimer (3686 FEUng/mL, reference 0–500) (Table S1).

After 40 h since admission the platelet count had dropped to about 5 × 103/μL without signs of bleeding, therefore an anti-venom treatment, consisting of a monospecific Vbb antivenom ovine FAB – Fragments (ViperaTAb, 200 mg in 100 mL saline isotonic solution in 2 h, IV) was administered (Fig. 1). After a transient rise in platelet count (34 ×103/μL), in a few hours the value dropped again to 5 × 103/μL. Since the platelet count remained unaltered over time, after 12 h a second dose of the same anti-venom was administered, which again caused a modest rise of platelets (9 × 103/μL) followed by a sharp decline few hours later. No other symptoms or clinical manifestations were documented during these phases; in particular, no signs of anemia of hemorrhage were observed and no fluids or inotrope infusion was needed.

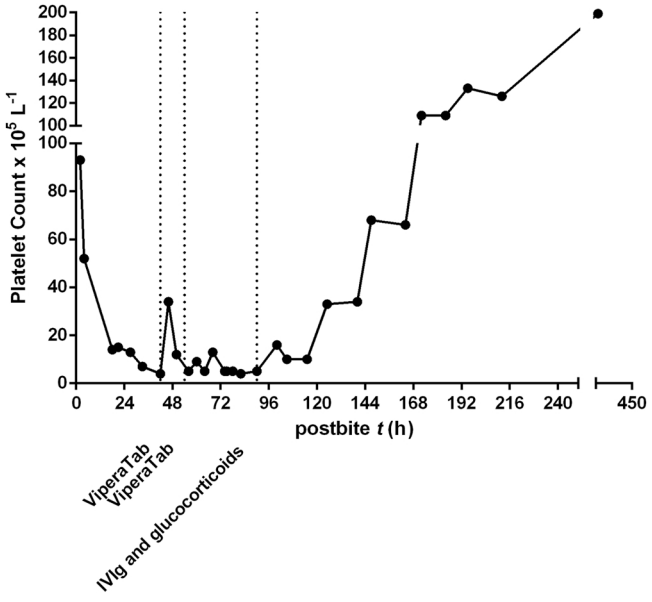

Fig. 1. Platelet count over time. Administration of ViperaTAb and combination therapy of methylprednisolone and immunoglobulins are reported.

After 90 h since admission, considering the persistence of a very low thrombocytopenic count (4–5 ×103/μL) in stable clinical conditions and unaltered INR and aPTT, the cause of thrombocytopenia was judged to be autoimmune, thus an off-label treatment of IVIg and glucocorticoids was started and a blood sample was sent for research of anti-platelets antibodies (APA). A 4-days treatment course of methylprednisolone (60 mg IV) and immunoglobulin (50 g in 6 h on the first day; 30 g in 4 h the remaining 3 days) led to an immediate, constant increase in platelet count. Laboratory tests revealed the presence of both direct and indirect APA against anti-GP IIb/IIIa and Ia/IIa.

At day 10, having reached a platelet count of 126 × 10^3/μL, the patient was discharged; treatment at discharge was Prednisone 25 mg and gastric protection with Lansoprazole 30 mg. At day 18, the patient was re-evaluated and a clear improvement in lymphedema together with a platelet count of 199 × 10^3/μL elements were observed. New laboratory tests revealed the absence of APA.

3. Discussion

Coagulopathy after viper bite is common and is a significant cause of morbidity [5]; different types of coagulopathy have been described, according to the multitude of toxins that can be present in the venoms (targeting coagulation factors, directly damaging vessels or acting as platelet inducers\ inhibitors leading to consumption thrombocytopenia [14]) with a known variability among different families [15]. Venoms can induce thrombocytopenia by direct destruction of platelet precursors and platelets depletion in either disseminated intravascular coagulation (DIC) or tissue sequestration at the wound site [12], that can act singularly or in combination. VIT is thus a well-known complication which remains of unclear etiology in North America, and even less described in the European countries; such discrepancy can be due to both intrinsic differences among venoms and different antivenin treatments. The course of VIT is known to be extremely variable: rattlesnake VIT is known to usually improve immediately after AV administration [16,17], but with an incomplete and variable effect [18]; a retrospective review of all patients admitted for rattlesnake envenomation in California over a period of about 20 years concluded that usually an immediate antivenom treatment improves VIT in a dosage independent manner and that the clinical significance remains uncertain in absence of bleeding [11]. In preliminary studies, thrombocytopenia improved immediately also after Fab antivenom administration, but recurred, necessitating repeat dosing [19], posing the question of its efficacy in improving VIT. In Europe, a case of severe VIT has been recently reported in a Slovenian patient promptly treated with ViperaTAb: in that case, after immediate onset of thrombocytopenia (about 20 × 10–3/μL), the administration of ViperaTAb led to a transient 7-folds increase of platelets in two hours that rapidly returned and stabilized to pre-administration values. At 72 h after bite, a second administration of the TAb led to a second transient increase of platelet count, that soon decreased and maintained at about 70 × 103/μL; a slow increase in platelet count led to platelets normalization 7 days after the bite [20].

The Antivenom used in our case consists of Fab fragments derived from antibodies present on ovins immunized against Vbb venum. This antivenom is particularly useful in Great Britain [21] or in Scandinavian regions because of the exclusive presence of the Vbb [22]; the widespread presence of Vbb in the northern and eastern European countries together with its good safety profile makes it a good choice in case of shortage of polyvalent antivenoms [23].

Although cross-efficacy of the antivenom among different species is controversial and poorly predictable [8], ViperaTAb demonstrated cross-reactive capabilities against Vaa, V. aspis, and V. latastei [24] in in-vitro immunological experiments and in vivo preclinical efficacy studies [20].

It has been reported that this mono-specific Vbb antivenom may be insufficient in contrasting the intrinsic variability in venom composition of European viper subspecies and, in particular, against Vaa, which have more complex venoms with regards to Vbb [25]. Moreover, it is known that Vaa venoms can vary in both biochemical properties and biological activities among snakes coming from different geographical regions [26] and during the seasons [27].

In our case, two doses of ViperaTAb were administered at 40 and 52 h after the bite; the motivation of such approach was due to the discrepancy between the clinical condition of the patient and the laboratory findings in terms of thrombocytopenia; it is impossible to know whether an earlier administration might have given a better outcome [28].

A previous study describing biphasic VIT postulated the early VIT as caused by venom-induced platelet aggregation and the delayed VIT as a consequence of local platelet sequestration in areas of vascular damage near the wound site [13]; in this work, crotaline-specific antivenom was able to reverse early VIT by releasing platelets aggregated to venom proteins, but it was useless in the resolution of delayed VIT. In our patient, early VIT appeared immediately after the bite and caused hospitalization, but both the time lapsed and the weak effect of the antivenom led us to conclude that VIT in our patient was caused by neither aggregated nor adhered platelets.

A possible further motivation of poor response to ViperaTAb of our patient could be the antivenom clearance: in a case of Crotalus atrox envenomation, authors postulated that thrombocytopenia was likely due “in part” to renewed venom antigenemia after clearance of Fab antivenom [19]; in our patient, a difference in pharmacokinetic and pharmacodynamic between venom and ViperaTAB might be possible, but unlikely, since at every administration the effect of the antivenom had been very weak in contrasting VIT.

A study focusing on venin components affecting hemostasis found that in Viperidae, and Crotalidae, snaclecs (snake C-type lectins) can be responsible for the occurrence of thrombocytopenia: in this view, VIT is seen as the result of platelets activation through the interaction with platelet GPIb receptor or their adhesion to blood vessel walls [29]. In accordance to this, it should be noted that anti-snaclecs Fab are scarcely present in ViperaTAb, since it is produced by using Vbb venom that contains lower amounts of snaclecs [25]. In our case, both ViperaTAb administrations led to a very slight and transient increase in platelet counts, followed by rapid falls to critical values. We judged the VIT as a consequence of splenic sequestration and destruction because of an immune response against platelets, possibly due to snaclecs presence, that we treated with an off-label administration of IVIg infusion and corticosteroids.

It has been described, in fact, that some snake toxins express their hemorrhagic effect by binding GPVI, inducing structural changes of the protein[30]. Autoimmunity test results, arrived after having started IVIg and corticosteroids therapies, demonstrated the presence of both direct and indirect anti-platelet antibodies against anti-GP IIb/IIIa and Ia/IIa. The severe thrombocytopenia and the presence of APA confirmed our clinical suspicion of an immune-mediated mechanism. The major benefit of IVIg and corticosteroid treatment was accompanied by a prompt recover of the thrombocytopenia to non-pathological platelet levels.

At follow-up, 20 weeks later, APA turned negative.

To our knowledge, there is no literature about venom induced immune thrombocytopenia against GP IIb/IIIa and Ia/IIa protein in European countries and subsequent treatment based on IVIg and corticosteroids.

4. Materials and methods

This is a case report of a patient envenomed by snakebite in the NorthEast of Italy and treated with ViperaTAb at the Emergency Department of The Clinical Hospital of Tolmezzo (Udine). The patient expressed informed consent for the divulgation of the report. Ethical approval was obtained from the Institutional Ethical Committee of the Hospital. ViperaTAb was supplied by MicroPharm Ltd., Newcastle Emlyn, UK. Direct platelet antibody testing to detect autoantibodies attached to patient platelets was performed with a solid phase system for the detection of IgG antibodies (Capture P, Immmucor); Indirect method to identify platelet glycoprotein specific antibodies in patient serum or plasma was performed using an ELISA kit (GTI-Pak Plus-Immucor).

Key Contribution

The use of IVIg against Vipera induced thrombocytopenia has never been reported and might represent a potential therapeutic approach.

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CRediT authorship contribution statement

Turetta Matteo: Writing – original draft, Writing – review & editing, Formal analysis, Visualization. **Del Ben Fabio:** Writing – review & editing. **Londero Donatella:** Resources. **Pillinini Pierpaolo:** Investigation, Data Curation, Resources. **Agostino Steffan:** Conceptualization, Writing – Review & Editing, Funding acquisition.

Declaration of Competing Interest

There is no conflict of interest. Table S1. Coagulation tests (Platelet count, INR, aPTT).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2022.03.033.

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