Autoimmune Thrombocytopenia Complicated by EDTA- and/or Citrate-Dependent Pseudothrombocytopenia

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Summary
Background: Pseudothrombocytopenia (PTCP) is a well-known phenomenon. However, confusion may occur due to unusual characteristics. **Case Reports:** Two patients with autoimmune thrombocytopenia (ITP) and long-lasting PTCP are described. Initially, only the diagnosis of ITP was confirmed. During observation, discrepancies were recognized between clinical findings and platelet counts. Re-examination resulted in the additional diagnosis of EDTA-dependent PTCP. Subsequently, the latter diagnosis was changed to citrate-dependent PTCP in both cases. Interestingly, PTCP was observed to change again and became recognizable in citrate or heparin, and only during the first 20–30 min following phlebotomy in EDTA specimens. **Conclusion:** The incidence of concomitant ITP with PTCP might be higher than previously reported, and PTCP may have variable dynamics and characteristics.

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Introduction

Pseudothrombocytopenia (PTCP) is a well-known phenomenon which is most commonly reflected by clumping of platelets in EDTA and rarely in citrate, or in the presence of IgM autoantibodies to platelets [1–4]. Its pathogenesis, incidence, and association with diseases have been extensively discussed [5–8].

Though the phenomenon per se is harmless, its occurrence may lead to confusion and inadequate interventions including extensive laboratory testing and/or diagnostics, false diagnosis, and consequently false treatment [9–15]. True thrombocytopenia might also rarely be confused [15] or associated with PTCP [16–18]. Recently, PTCP has also been described in a patient receiving romiplostim for ITP [19]. Here, we report on long-lasting PTCP complicated by differential characteristics in 2 patients with ITP.

Case Reports

Case 1

A 55-year-old women with a long-term history of psoriasis, thrombocytopenia (platelet counts ranging between 19,000/µl and 99.000/µl), and an infrequent bleeding tendency was admitted to the hospital in the year 2000 due to ITP and recurrent infections. IgG autoantibodies to the platelet glycoprotein IIb/IIIa were detected on patients' platelets by MAIPA (monoclonal antibody-specific immobilization of platelet antigens), but not in the serum. In addition, a third diagnosis of IgG deficiency was confirmed. The patient was under treatment with low doses of prednisolone, and sulfasalazine for psoriatic arthritis. During observation, sulfasalazine was replaced by methotrexate and intravenous IgG (IVIgG) treatment which was administered every 6–8 weeks. In 2002, platelet counts decreased to 2,000/µl, although the patient felt well and there was no evidence for any bleeding tendency (table 1). In citrate blood, platelet counts were found to be ≥100,000/µl, and PTCP related to EDTA was confirmed by microscopy. One year later, the PTCP was found to be associated with citrate rather than EDTA. The platelet count was observed to be infrequently less than 10,000/µl, independent of the anticoagulant used. It remained a matter of speculation whether PTCP was abolished or could not be recognized due to the very low platelet counts. It is worth mentioning that platelet counting was immediately performed following phlebotomy at that time. In 2015 we recognized by a chance, time-dependent PTCP. The patient felt well, and there was no evidence for the presence of true thrombocytopenia. In fact, repeated analysis of platelet counts by using the same blood samples revealed an increase from 3,000/µl to 81,000/µl in EDTA, but not in citrate and heparin (fig. 1).

Case 2

An 18-year-old female, who had been diagnosed with ITP in 2004, was admitted to the hospital in 2009. The patient felt well, and the platelet count was

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Table 1. Course of thrombocytopenia and PTCP in patient no. 1; all blood samples were withdrawn into vacutainers

Year(s)	Pltr × 10³/μl/aggregates			Blt	Diagnosis	Plab	Treatment
	EDTA	citrate	heparin				
1964–1999	19–99 / n.t	n.t	n.t	mild	PS, Arth, Thrp	n.t.	Diclo, Pred, Sul, L-Thyr
2000	112 / n.t	n.t	n.t	mild	Hyp, ITP	GP IIb/IIIa	MTX, Simva, Meto, Rami, L-Thyr
2001	2 / +	110 / –	n.t	mild	PTCP		MTX, Simva, Meto, Rami
2002	2–85 / +	>100 / –	n.t	–			
2003	13–76 / (+)	1–21 / +	n.t	–			
2004	40–86 / (+)	2–19 / +	n.t	–		IgM, IgG***	
2005	2–96 / (+)	2–30 / +	n.t	–	BrCa		MTX, IVIgG, Simva, L-Thyr, Anast, Ena
2006	77–90 / (+)	n.t	n.t	mild			
2007	2–126 / +	< 10 / +	<10 / +	–			MTX, IVIgG, Simva, L-Thyr, Ena, Verap
2008	18–80 / (+)	< 10 / +	<10 / +				MTX, Simva, L-Thyr, Ena, Verap, Anast
2009–2014	7–82 / (+)	n.t	n.t	mild			Verap, L-Thyr, Simva, Anast, Neb, HCT
2015*	<10 / +	<10 /+	<10 / +	–			L-Thyr, Verap, Simva
2015**	80 /–	<10 /+	<10 /+	–			Anast, Cand

Arth = Arthritis; Anast = anastrozole; Blt = bleeding tendency; Cand = candesartan; Diclo = diclofenac; Ena = enalapril; HTC = hydrochlorothiazide; Hyp = hypertension; Hypo = hypothyreosis; ITP = autoimmune thrombocytopenia; IVIgG = intravenous IgG; L-Thyr = L-thyroxine; Meto = metoprolole; MTX = methotrexate; Neb = Nebivolol; n.t. = not tested or unknown; Plab = platelet antibodies; Pltr = Platelet range; Pred = prednisolone; PS = psoriasis; PTCP = pseudothrombocytopenia; Rami = ramipril; Simva = simvastatine; Sul = sulfasalazine; Thrp = thrombocytopenia; Verap = verapamil; (+) = weak aggregates; + = significant aggregates.
*Immediately after phlebotomy.
**30 min after phlebotomy (see fig. 1).
***Platelet antibodies of IgM- and of IgG-classes. The former antibodies appeared to be related to citrate (fluorescence test), and the later antibodies to platelet antigens.

Table 2. Course of thrombocytopenia and PTCP in patient no. 2, all blood samples were withdrawn into vacutainers

Year(s)	Pltr × 10³/μl/aggregates			Bleeding	Diagnosis	Plab	Treatment
	EDTA	citrate	heparin				
2004–2006	<10–>50/n.t.	n.t.	n.t.	+	ITP	n.t.	Pred, IVIgG, Cyclo
2007–2008	>50 / n.t.	n.t.	n.t.	–		n.t.	no treatment
2009	<20–61 / n.t.	n.t.	n.t.	+		n.t.	Pred
2010	1–24 / n.t.	n.t.	n.t.	+		–	Anti-D, Dexa, Pred, Methylpred
2011–2012	19–22 / n.t.	n.t.	n.t.				eltrombopag
2013	<20–32 / +	n.t.	n.t.	+	+AIHA	–	Dexa, Pred
	28–32 / –	n.t.	n.t.	–		GP IIb/IIIa	Pred, Aza
2014	17–44 / –	n.t.					
	5 / n.t.	50 / n.t.	58 / n.t.	–		–	
2015	24 / n.t.	107 / n.t.	n.t.	–			Pred
	85 / n.t.	13 / n.t.	n.t.	–			

AIHA = Autoimmune hemolytic anemia; Aza = azathioprine; Cyclo = cyclophosphamide; Dexa = dexamethasone; ITP = autoimmune thrombocytopenia; IVIgG = intravenous immunoglobulins; Methylpred = methyl-prednisolone; n.t. = not tested or unknown; Plab = platelet autoantibodies; Pltr = platelet range; Pred = prednisolone; + = significant aggregates.

observed to be 61,000/μl without treatment (table 2). In 2010, a bleeding tendency developed, and the platelet count increased from 4,000/μl to more than 100,000/μl following treatment. IgG autoantibodies to the platelet glycoprotein IIb/IIIa were detected on patients' platelets by MAIPA, but not in the serum. In 2013, the patient developed autoimmune hemolytic anemia of the warm type (Evans syndrome) and her platelet count increased from 10,000/μl to a maximum of 320,000/μl following treatment with elthrombopag, whereas low-dose prednisolone and azathioprine remained ineffective. An EDTA-dependent PTCP was unexpectedly detected in 2014. This was observed to change to citrate and time-dependent PTCP in 2015 (fig. 1). It remains unclear whether the thrombocytopenia diagnosed in 2004 is somewhat related to EDTA.

Discussion

The PTCP described here was rather obscure in regards to various aspects. It was complicated with ITP which is per se characterized by thrombocytopenia. The question whether PTCP was already present prior to the manifestation of ITP or developed during observation independent of ITP remains speculative. However, its long-lasting nature appears to be reflected by an association rather than a coincidence. This question has previously been discussed in
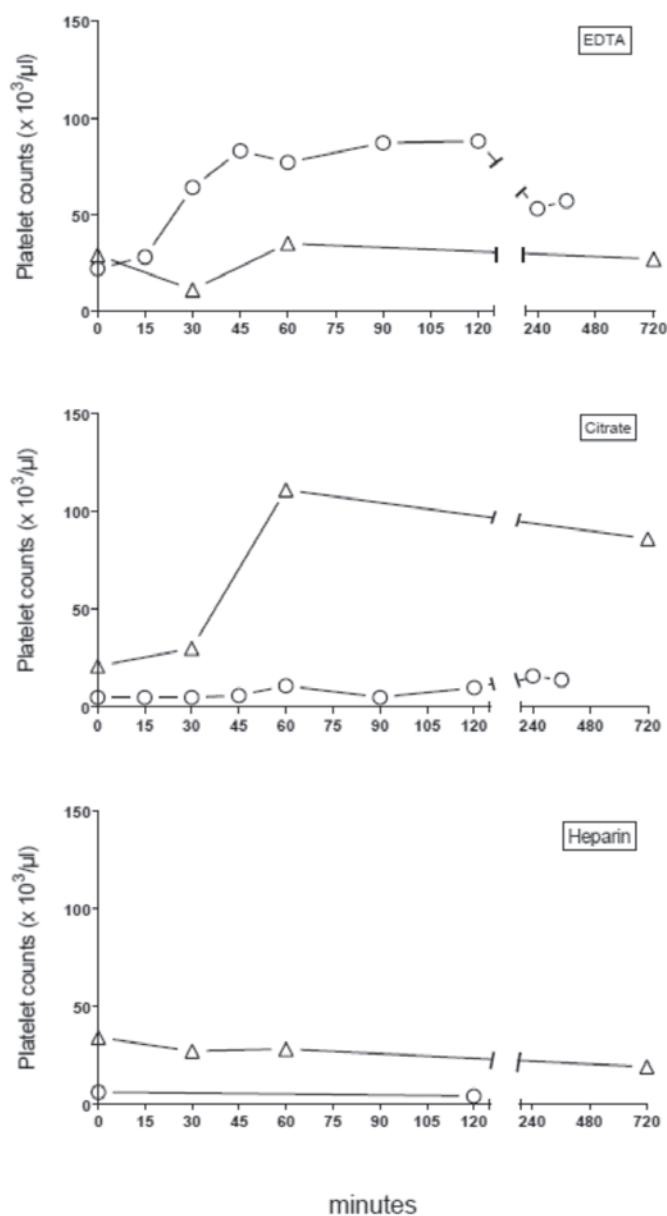
Fig. 1. Time and anticoagulant-dependent PTCP in 2 patients with ITP (O = patient no. 1, Δ = patient no. 2).

two patients who were receiving heparin and developed anti-PF4 IgM antibodies. In both cases, platelet aggregation was dependent on the antibodies, resulting in the question of whether the concomitance was reflected by a coincidence or an association [17]. In contrast, a true association could be demonstrated in two other patients who had heparin-induced thrombocytopenia and PTCP [18].

In our patients, PTCP was independent of the true platelet count and ITP. This finding may reflect rather a coincidence than an association. On the other hand, the long-lasting course of both ITP and PTCP do support rather an association than an unexpected coincidence. Another possible explanation might be that the PTCP in the first patient was related rather to the psoriasis than to the ITP. This notion is supported by the clinical course of the patient and by a previous report describing PTCP in a patient with a medical history of benign psoriasis [20]. Though no association was discussed in that report, an association cannot be excluded.

Another strange aspect is the switch from EDTA- to citrate-dependent PTCP in both patients. The mechanism by which this switch may operate remain obscure. For the most part, EDTA is responsible for the vast majority of PTCPs, and citrate is used to disclose this phenomenon. Correspondingly, this was, in fact, initially the case in our patients. The reason for the switch described here remains unclear.

Most intriguingly, the PTCP in case 1 was not only anticoagulant-dependent but also EDTA- and time-dependent. This finding could be proven by repeated platelet counting using identical blood samples. This phenomenon has not yet been described in PTCP. Usually, clumping of platelets in the presence of EDTA is inversely related to the length of time elapsed since phlebotomy [9, 20].

We recommend to consider PTCP not only in the list of ITP differential diagnosis but to also include ITP in the list of diseases associated with PTCP. In addition, PTCP may alter its appearance over time. Recently, Nagler et al. [22] have described a typical histogram of PTCP in an EDTA sample as compared to normal histogram in a citrated sample of the same patient. Ultimately the use of CTP (citrate, pyridoxal 5⃞-phosphate and Tris) as anticoagulant may be helpful in suspected cases.

Disclosure Statement

The author declares not conflict of interest.

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