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The frequency of pseudothrombocytopenia in blood donors from the central region of Poland

Częstość występowania pseudotrombocytopenii u dawców krwi z regionu Polski centralnej

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Abstract

Background: *Pseudothrombocytopenia (PTCP) is a phenomenon of falsely low platelet count associated with* in vitro *platelet agglutination due to EDTA-dependent anti-platelet antibodies. The aim of this study was to determine EDTA-dependent pseudothrombocytopenia frequency in blood donors and the characteristics of EDTA-dependent anti-platelet antibodies in PTCP donors.*

Material and methods: *Between January 2001 and August 2008, platelet count was routinely measured in 83.486 unselected Polish blood donors. EDTA-dependent anti-platelet antibodies were detected by using the platelet immunofluorescence test (PIFT).*

Results: *PTCP was diagnosed in 12 healthy donors. In EDTA samples, the average number of platelets was 66.8 × 10^9/l (SD ± 30.8 × 10^9/l) and 209.9 × 10^9/l (SD ± 46.6 x 10^9/l) in citrate samples. EDTA-dependant IgG and IgM class antibodies were found in the sera of 10 donors. In 2 donors, PTCP diagnosis was based only on the differences between platelet counts in EDTA and in citrate samples.*

Conclusion: *The PTCP frequency in blood donors was calculated at 1:6957, that is 0.014%.*

Key words: pseudothrombocytopenia, EDTA-dependent anti-platelet antibodies, blood donors

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Streszczenie

Wstęp: *Pseudotrombocytopenia (PTCP) jest zjawiskiem polegającym na fałszywym obniżeniu liczby płytek krwi* in vitro *spowodowanym aglutynacją płytek przez przeciwciała przeciwpłytkowe EDTA-zależne. Celem badań była ocena częstości występowania pseudotrombocytopenii EDTA-zależnej u dawców krwi i charakterystyka przeciwciał przeciwpłytkowych EDTA-zależnych u dawców z PTCP.*

Materiał i metody: *W okresie od stycznia 2001 do sierpnia 2008 roku zmierzono w badaniach rutynowych liczbę płytek krwi u 83 486 nieselekcjonowanych polskich dawców krwi. Przeciwciała przeciwpłytkowe EDTA-zależne były wykrywane przy użyciu testu immunofluorescencyjnego z płytkami (PIFT).*

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Wyniki: *Pseudotrombocytopenia była zdiagnozowana u 12 zdrowych dawców. W próbkach krwi pobranych na EDTA, średnia liczba płytek była 66,8 × 10^9/l (SD ± 30,8 × 10^9/l) i 209,9 × × 10^9/l (SD ± 46,6 × 10^9/l) w próbkach pobranych na cytrynian. Przeciwciała EDTA-zależne klas IgG i IgM były stwierdzone w surowicach 10 dawców. U 2 dawców, diagnostyka PTCP opierała się tylko na różnicy w liczbie płytek krwi pobranych na EDTA i cytrynian.*

Wniosek: *PTCP u dawców krwi występowała z częstością 1:6957, to znaczy u 0,014%.*

Słowa kluczowe: pseudotrombocytopenia, przeciwciała przeciwpłytkowe EDTA-zależne, dawcy krwi

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Introduction

Pseudothrombocytopenia (PTCP) is a phenomenon of spuriously falsely low platelet count associated with *in vitro* platelet agglutination. This results from the presence of anti-platelet antibodies reacting with platelets in blood drawn into EDTA. The most likely hypothesis is that such autoantibodies result from the natural elimination of aged or damaged platelets [1, 2]. These autoantibodies were shown to be directed against the GPIIb subunit of the platelet membrane [3].

The PTCP phenomenon has mainly been recognized in patients and, less commonly, in healthy subjects undergoing routine blood counts [4, 5]. So far, only two cases of PTCP in blood donors have been reported [6].

This study presents the frequency of PTCP in Polish blood donors and the characteristics of EDTA-dependent anti-platelet antibodies in their sera.

Material and methods

Between January 2001 and August 2008, we routinely measured the platelet count in 83.486 unselected Polish blood donors by using a Cell-Dyn 1700 counter, as well as blood anticoagulated with 5% K_2EDTA (Vacutainer, Sarstedt). Pseudothrombocytopenia was suspected in donors with a platelet count below 100 × 10^9/l and no evidence of bleeding or purpura. To confirm the PTCP diagnosis, the following two steps were carried out: 1/ the platelet count was simultaneously examined both in EDTA blood samples and trisodium citrate blood samples, 2/ EDTA-dependent anti-platelet antibodies were detected by using the indirect platelet immunofluorescence test (PIFT) with FITC-labeled rabbit antiglobulin conjugates against human IgG and IgM [7]. The PIFT results were evaluated by flow cytometry (FACS Calibur, CellQuest TM Software, Becton Dickinson).

To rule out essential thrombocytopenia in PTCP donors, the specific antiplatelet antibodies in MAIPA (Monoclonal Antibody Specific Immobilization of Platelet Antigen) with mouse monoclonal antibody immobilization of platelet glycoprotein (GP): GPII/IIIa (CD41), GPIb (CD42b), GP Ia/IIa (CD49b) were determined [8].

A four year follow-up was performed on 2 donors. A trace back was performed in 7 recipients of FFP from PTCP donors.

Results and discussion

Pseudothrombocytopenia was diagnosed in 12 healthy donors; 11 males and 1 female (median age 36, range 18-57 years) with eleven of them being multiple donors. PTCP donor characteristics are presented in Table 1. The average number of platelets in EDTA samples was 66.8 × 10^9/l (SD ± 30.8 × 10^9/l) and 209 × 10^9/l (SD ± 46.6 × 10^9/l) in citrate samples.

Our study based on 83,486 routine blood counts, indicates that the PTCP frequency in blood donors was 1: 6957, *i.e.* 0.014%.

Although EDTA-dependent IgG and IgM class antibodies were determined in the sera of 10 donors, in 2 of them only IgM antibodies were detected four years ago (tab. 2). No platelet antibodies were observed in two donors (nos. 11 and 12) while the PTCP diagnosis was based on the differences between platelet counts in EDTA and in citrate samples. However, in four donors (nos. 1, 2, 9 and 10) the anti-platelet antibodies were found also to agglutinate platelets in citrate-anticoagulated blood. In such cases, the real platelet count should be determined in blood collected into another anticoagulant, such as heparin. Trisodium citrate is known to have a chelating effect on calcium ions, but to a lesser extent than EDTA [9].

Bizzaro *et al.* [2] reported that the antibodies that clump platelets in citrate are almost always of IgM

Table 1. Characteristics of PTCP blood donors

Tabela 1. Charakterystyka dawców krwi z PTCP

Donor no.	Sex	Age (years)	Platelet count (× 10^9/l) EDTA	Citrate	WBC count (× 10^9/l)	Number of blood donations
1	M	46	30	203	Normal range	6
2	M	18	82	190	Normal range	1
3	M	48	67	162	Normal range	27
4	F	33	63	127	Normal range	4
5	M	24	92	231	Normal range	5
6	M	45	126	310	12.5	19
7	M	33	81	189	17.7	27
8	M	24	66	231	Normal range	27
9	M	38	79	263	12.6	3
10	M	41	19	206	10.5	32
11	M	57	25	206	Normal range	33
12	M	32	48	201	10.8	3

Table 2. Characteristics of EDTA-dependent anti-platelet antibodies

Tabela 2. Charakterystyka przeciwciał przeciwpłytkowych EDTA-zależnych

Donor no.	Class of anti-platelet antibodies	Temperature of reactivity		Agglutination in anticoagulants	
		37°C	4°C	EDTA	Citrate
1	IgM *after 4 years* IgG + IgM	+ –	+ +	+ +	+ –
2	IgM *after 4 years* IgG + IgM	– –	+ +	+ +	+ +
3	IgG + IgM	–	+	+	–
4	IgG + IgM	–	+	+	–
5	IgG + IgM	–	+	+	–
6	IgG + IgM	+	+	+	–
7	IgG + IgM	–	+	+	–
8	IgG + IgM	+	+	+	–
9	IgG + IgM	+	+	+	+
10	IgG + IgM	+	+	+	+
11	Not detected				
12	Not detected				

type. All our PTCP donors had IgM antibodies but only in four cases the antibodies reacted in citrate.

In all donors, EDTA-dependent anti-platelet antibodies agglutinated the platelet at 4°C; in five donors however (nos. 1, 6, 8, 9, 10), the antibodies also reacted at 37°C. The follow-up study revealed that after 4 years, EDTA-dependant antibodies were still present in two donors (no. 1, no. 2).

No platelet specific antibodies (anti-HPA) were found in the donor sera.

Pseudoleukocytosis was observed in five donors (nos. 6, 7, 9, 10, 12); the white blood cells were from 10.5–17.7 × 10^9/l. In all these cases, a false increase of the white cell count was caused by clumps of platelets identified as white cells by automated instruments [10]. In clinical practice, it is crucial to know that pseudothrombocytopenia donors may have a higher leukocyte count which is of no clinical significance.

All the PTCP donors had donated blood 187 times. No post-transfusion reactions were reported for the recipients of blood from PTCP donors. A trace back was performed in 7 recipients of FFP from donors with EDTA-dependant antibodies. No

decrease of platelet count was observed in any of them. It is worth noting that at the same time, all recipients were transfused FFP not only from PTCP donors but also from non PTCP donors (2–4 units of FFP). Thus, the EDTA-dependent antibodies may have been diluted in the recipient's blood. In the literature, we found only one report concerning the transfusion of a single HLA-matched platelet from a PTCP donor to a recipient performed by Sweeney *et al.* [6]. The platelet increment was counted using EDTA and citrate samples from the recipient and no differences were observed when these post-transfusion samples remained for 2 hours at room temperature.

Conclusions

Pseudothrombocytopenia frequency in Polish blood donors was determined at 0.014%. EDTA--dependent antibodies were of IgG and IgM type.

Transfusion of FFP containing these antibodies had no effect on the recipient platelet count, as revealed in the trace back.

In PTCP donors, the platelet count should be examined in citrate blood or another anticoagulant.

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