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Transfusion Medicine | LETTER TO THE EDITOR

Anticoagulant-induced pseudothrombocytopenia in blood donors

Dear Sir,

Pseudothrombocytopenia (PTCP) is a phenomenon of falsely low platelet count determined on haematology analysers. As the ethylenediaminetetraacetic acid (EDTA) is a widely used anticoagulant in blood samples for determination of complete blood count, EDTA-dependent PTCP is a commonly observed phenomenon. In about 20% of PTCP cases, falsely low platelet count is also present in sodium citrate anticoagulated blood, i.e. PTCP is multicoagulant-dependent (Bizzaro, 1995). While PTCP dependent only on EDTA anticoagulant has no clinical significance for blood donors or blood product quality, additional evaluation is needed when PTCP is also citrate-dependent.

The phenomenon is present in both healthy subjects and patients with various diseases. EDTA-PTCP occurs with a frequency of approximately 0·1% in the general population, 0·15% in hospitalised patients and is more common in outpatients with isolated thrombocytopenia (7·5–17%). (Garcia Suarez *et al.*, 1991; Silvestri *et al.*, 1995). Máslanka *et al.* (2008) report on the PTCP frequency of 0·013% in 76 489 unselected Polish blood donors and Sweeney *et al.* (1995) report on the PTCP frequency of 0·2% in 945 platelet apheresis donors.

In EDTA-PTCP, platelet autoantibodies directed to the platelet membrane GPIIb/IIIa complex modified by EDTA induce *in vitro* platelet aggregate or agglutinate formation, which finally leads to a falsely decreased platelet count. EDTA-PTCP is characterised by abnormal platelet count, typically $<100 \times 10^9$ L^{-1} in EDTA-anticoagulated samples at room temperature, time-dependent fall of platelet count, evidence of platelet aggregates and clumps on either automated cell counting or microscopic analysis, and lack of signs or symptoms of platelet disorders. In the leucocyte histogram provided by automated cell counters, platelet clumps often appear as particles of less than 35 fl (Wu *et al.*, 2011; Schuff-Werner *et al.*, 2013).

According to available evidence, the most suitable and practical approach for most clinical laboratories so far is recollection of blood samples using sodium citrate, citrate/theophylline/adenosine/dipyridamole, calcium chloride/heparin or magnesium sulphate as additives, keeping the specimen at 37 °C until platelet count has been completed.

PTCP should always be excluded in platelet apheresis donors with newly discovered thrombocytopenia to avoid erroneous interpretation of platelet and leucocyte count, unnecessary workup and needless deferral of blood donors with this form of *in vitro* artefact. PTCP dependent only on the EDTA anticoagulant has no significance for blood products because due to its toxicity EDTA is used only as *in vitro* anticoagulant. Contrary, citrate-dependent PTCP might have potential adverse effects on blood components because sodium citrate is a standard anticoagulant in the blood collection bags and apheresis procedures.

To assess the incidence and characteristics of PTCP in platelet apheresis donors, retrospective analysis of reports on complete blood count along with leucocyte histogram evaluation was conducted at Department of Quality Assurance, Croatian Institute of Transfusion Medicine (CITM) for all apheresis donors during the 3-year period. Laboratory testing for PTCP was performed in all donors with platelet count less than 150×10^9 L^{-1} and deviations in leucocyte histogram yielded by the Abbott Cell Dyn 3200 or Cell Dyn Ruby (Abbott Diagnostics, Wiesbaden-Delkenheim, Germany) automated cell counter. Laboratory testing to confirm PTCP was performed at Department of Platelet and Leukocyte Diagnostics and Hemostasis. It included platelet count determination in EDTA and citrated blood samples immediately after blood sample collection (within 10 min) and 60 to 120 min later, along with evaluation of leucocyte histogram obtained on the Sysmex KXN 21 (Sysmex, Norderstedt, Germany) and Abbott Cell Dyn 3200 or Cell Dyn Ruby (Abbott Diagnostics, Wiesbaden-Delkenheim, Grmany) automated haematology cell counters. EDTA-induced PTCP is diagnosed when platelet number decreases together with the characteristic leucocyte histogram and microscopic detection of platelet aggregates in blood smear.

PTCP dependent on EDTA was demonstrated in 3 of 1987 (0·15%) blood donors undergoing platelet apheresis procedure from January 1, 2011 to 31 December 2013. All three donors were male, two whole blood donors presenting for platelet apheresis for the first time and one regular platelet apheresis donor. In all three donors, PTCP was also citrate-dependent. In all donors, aggregate formation in EDTA blood sample was time- and temperature-dependent, and normal platelet count was obtained in EDTA and citrate blood immediately after withdrawal. In citrated blood samples, platelet count was corrected for the dilution using correction factor 1·1. The mean platelet count in EDTA blood 10 min after collection was 164×10^9 L^{-1} (min 150 and max 185) and in citrate blood 188×10^9 L^{-1} (min 187 and max 190). The mean platelet count in EDTA blood 60 min after collection was 28×10^9 L^{-1} (min 13 and max 46) and in citrate blood 122×10^9 L^{-1} (min 114 and max 132). The mean platelet count in EDTA blood 120 min after collection

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was 31×10^9 L^{-1} (min 10 and max 58) and in citrate blood 89×10^9 L^{-1} (min 48 and max 110).

Deviation in leucocyte histogram indicating platelet aggregates (NWBC flag) and falsely elevated leucocyte count demonstrated 100% sensitivity on Sysmex KXN 21 and Abbott Cell Dyn analysers.

Similar data were obtained in the study group of 1525 whole blood donors who donated blood at CITM from 1 October 2013 to 2 July 2014. The mean platelet count was $260 \pm 55 \times 10^9$ L^{-1} ($252 \pm 53 \times 10^9$ L^{-1} for male donors and $272 \pm 57 \times 10^9$ L^{-1} for female donors). Thrombocytopenia with platelet count less than 150×10^9 L^{-1} was found in nine (0·59%) donors, of which 6/942 (0·64%) were males and 3/583 (0·51%) females. PTCP dependent on EDTA was confirmed in only two (0·13%) donors, one male and one female.

These data indicate that screening for PTCP prevents unnecessary deferral of thrombocytopenic platelet apheresis donors with PTCP dependent only on EDTA. However, although the incidence of citrate-dependent PTCP is extremely low in blood donor population, it may be the reason for the preparation of platelet concentrates containing microaggregates, which are not visible on routine macroscopic examination of platelet products (van der Merr *et al.*, 2015). Platelet activation, cytokine release and microparticle formation in blood products may lead to thrombin production and activation of coagulation cascade in the recipient (Lazarus *et al.*, 2001). Post-transfusion reactions with dyspnoea such as Transfusion Related Acute Lung Injury (TRALI), Transfusion Associated Circulatory Overload (TACO) and Transfusion Associated Dyspnoea (TAD) are recently investigated also for non-immune mechanism, suggesting platelet and red blood cell microaggregates as potential mediators (Máslanka *et al.*, 2015).

Therefore, we decided to postpone donation of any blood component from donors with citrate-dependent PTCP for 1-year period and repeat laboratory testing. Upon retesting, one of three donors was allowed to donate whole blood and apheresis platelets because of normal platelet count and leucocyte histogram. In the other two donors, EDTA- and citrate-PTCP persisted and blood/blood component donation postponed for another year when testing will be repeated.

We believe that screening for PTCP in platelet apheresis donors with thrombocytopenia in EDTA samples and using sodium citrate as a second anticoagulant can significantly improve donor care, while detection of citrate-dependent PTCP can also contribute to the quality of platelet concentrates prepared by apheresis procedure.

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M.T. designed the study, collected, and analysed the data and wrote the paper. T. V. and J. G.-H. collected, analysed and interpret the data, revised the paper and assisted with editing.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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REFERENCES

Bizzaro, N. (1995) EDTA-dependent pseudothrombocytopenia: a clinical and epidemiological study of 112 cases, with 10-year follow-up. *American Journal of Hematology*, **50**, 103–109.

Garcia Suarez, J., Merino, J.L., Rodriguez, M., Velasco, A. & Moreno, M.C. (1991) Pseudothrombocytopenia: incidence, causes and methods of detection. *Sangre (Barcelona)*, **36**, 197–200.

Lazarus, E.F., Browning, J., Norman, J., Oblitas, J. & Litman, S.F. (2001) Sustained decreases in platelet count associated with multiple, regular plateletpheresis donations. *Transfusion*, **41**, 756–761.

Máslanka, K., Marciniak-Bielak, D. & Szczepinski, A. (2008) Pseudothrombocytopenia in blood donors. *Vox Sanguinis*, **95**, 349.

Máslanka, K., Uhrynowska, M., Łopez, P., Wróbel, A., Smoleńska-Sym, K., Ostas, A. & Brojer, E. (2015) Analysis of leucocyte antibodies, cytokines, lysophospholipids and cell microparticles in blood components implicated in post-transfusion reactions with dyspnoea. *Vox Sanguinis*, **108**, 27–36.

van der, Merr, P.F., Dumont, L.J., Lozano, M. *et al.* (2015) Aggregates in platelet concentrates. *Vox Sanguinis*, **108**, 96–100.

Schuff-Werner, P., Steiner, M., Fenger, S. *et al.* (2013) Effective estimation of correct platelet counts in pseudothrombocytopenia using an alternative anticoagulant based on magnesium salt. *British Journal of Haematology*, **162**, 684–692.

Silvestri, F., Virgolini, L., Savignano, C., Zaja, F., Velsig, M. & Baccarani, M. (1995) Incidence and diagnosis of EDTA-dependent pseudothrombocytopenia in a consecutive outpatient population referred for isolated thrombocytopenia. *Vox Sanguinis*, **68**, 35–39.

Sweeney, J.D., Holme, S., Heaton, W.A., Campbell, D. & Bowen, M.L. (1995) Pseudothrombocytopenia in plateletpheresis donors. *Transfusion*, **35**, 46–49.

Wu, W., Guo, Y., Zhang, L., Cui, W., Li, W. & Zhang, S. (2011) Clinical utility of automated platelet clump count in the screening for the ethylene diamine tetra acetic acid-dependent pseudothrombocytopenia. *Chinese Medical Journal*, **124**, 3353–3357.