CASE REPORTS

UDC: 616.411-056.7:616.155.2
https://doi.org/10.2298/VSP170110168M

Ultrastructural and morphometric analysis of enlarged platelets in congenital isolated asplenia

Ultrastrukturna i morfometrijska analiza uvećanih trombocita kod bolesnika sa izolovanom kongenitalnom asplenijom

Olivera Marković*†, Tamara Martinović‡, Darko Cirić‡, Dušan Trpinac‡, Vesna Čemerikić Martinović§, Vladimir Bumbaširević‡, Jelena Bila†‖, Dragomir Marisavljević*†, Tamara Kravic- Stevović‡

***Clinical Hospital Center "Bežanijska kosa", Belgrade, Serbia; University of Belgrade, †Faculty of Medicine, Belgrade, Serbia; University of Belgrade, Faculty of Medicine, ‡Institute of Histology and Embryology, Belgrade, Serbia; §Beolab, Belgrade, Serbia; Clinical Center of Serbia, ‖Clinic for Hematology, Belgrade, Sebia**

Abstract

Introduction. Congenital asplenia is an extremely rare condition that can be separate entity due to a specific defect of spleen development or may occur in the context of a malformation syndrome. The patients with asplenia have thrombocytosis and susceptibility to life-threatening infections. **Case report.** We report a 52-years-old female patient with isolated congenital asplenia with pseudothrombocytopenia and giant platelets. Estimation of platelets life with radioactive indium showed normal lenght of platelets life (9 days). Flow cytometric analysis of platelets showed normal expression of CD41 and CD42b antigens. The mean platelet diameter of asplenic patient measured on the ultrathin sections by the transmission electron microscope was significantly higher than in the healthy individuals (3.81 ± 1.16 μm vs. 2.37 ± 0.61 μm, $p < 0.05$). There were very few platelets of diameter more than 4 μm found in healthy individuals (around 1%) in comparison to > 40% of the patient's platelets. The ultrastructural studies revealed normal morphology of megakaryocytes. The platelets were uniformly spheroid in shape with conspicuous pseudopodia and the centralization of granules. There were no marginal bands of microtubules inside the platelets. **Conclusion.** The first case of congenital asplenia with the pseudothrombocytopenia and giant platelets is presented. We discussed the pathogenesis of giant platelets and possible relation of observed ultrastructural changes of platelets with the severe three-vessel coronary artery disease in our patient.

Key words:
blood platelet; congenital abnormalities; microscopy, electron; myh9-related disorders; spleen.

Apstrakt

Uvod. Kongenitalna asplenija je izuzetno retka. Može se javiti izolovano (specifični defekt u razvoju slezine) ili u sklopu malformacionog sindroma. Bolest se najčešće dijagnostikuje u dečjem dobu, a odlikuje je nalaz trombocitoze sa malim tromboctima i infekcije inkapsuliranim mikroorganizmima koje mogu ugroziti život bolesnika. **Prikaz bolesnika.** Prikazali smo 52-godišnju bolesnicu sa izolovanom kongenitalnom asplenijom i pseudotrombocitopenijom sa gigantskim trombocitima. Ispitivanje radioaktivnim indijumom pokazalo je normalnu dužinu života trombocita (9 dana), a protočna citometrija normalnu ekspresiju CD41 i CD42b antigena na trombocitima bolesnice. Srednji dijametar trombocita meren transmisionom elektronskom mikroskopijom (TEM) bio je značajno veći nego kod zdravih osoba (3,81 ± 1,16 μm vs. 2,37 ± 0,61 μm, $p < 0.05$). Kod zdravih osoba bilo je prisutno samo nekoliko trombocita dijametra većeg od 4 μm, (oko 1%), a kod bolesnice je takvih trombocita bilo > 40%. Ultrastrukturna analiza (TEM) pokazala je normalnu morfologiju megakariocita. Trombociti u perifernoj krvi i kostnoj srži bili su uniformno sferoidnog oblika sa vidljivim pseudopodijama, centralizacijom granula i bez vidljive ivične spirale mikrotubula. **Zaključak.** U dostupnoj literaturi nema objavljenih slučajeva kongenitalne asplenije sa pseudotrombocitopenijom i gigantskim trombocitima. Diskutovana je patogeneza gigantskih trombocita i moguća povezanost uočenih ultrastrukturnih promena trombocita sa teškim oblikom trosudovne koronarne bolesti kod prikazane bolesnice.

Ključne reči:
trombociti; anomalije; mikroskopija, elektronska; myh9-povezani poremećaji; slezina.

Correspondence to: Olivera Marković, University of Belgrade, Faculty of Medicine, Clinical Hospital Center "Bežanijska kosa", Bežanijska kosa bb, 11 000 Belgrade, Serbia. E-mail: dragano@ptt.rs

Introduction

Congenital asplenia is very rare condition (1 case in 20,000 live births) which occurs sporadically, but also may have family association [1]. Congenital asplenia can be a separate entity due to a specific defect of spleen development, or may occur in the context of a malformation syndrome [2]. Since the spleen is a major producer of antibodies and splenic macrophages have a major role in bacterial phagocytosis, the patients with asplenia or hyposplenia are susceptible to life-threatening or fatal septicemia caused by encapsulated pathogens [2–4]. Life-threatening infections usually occur in childhood, but they were also described in the adult patients [2–4]. All cases with isolated congenital asplenia published so far had thrombocytosis [5–7] together with a common finding of small platelets [2].

The platelet production represents the final stage of megakaryocyte development [2]. It is commonly accepted that during the final stages of differentiation, megakaryocytes extend cytoplasmic protrusions referred to as proplatelets [8]. Proplatelets branch long processes that extend from mature megakaryocytes into the sinusoidal blood vessels of the bone marrow. The proplatelet formation is dependent on the function of microtubules [9]. Microtubular coils similar to those observed in the blood platelets can be detected only at the ends of proplatelets and not within the platelet-size beads found along the length of proplatelets [10]. Thon and Italiano [11] recently identified a previously unrecognized intermediate cell, which they termed a preplatelet. Preplatelets are defined as discoid cells, anucleate platelet progenitors 3–10 μm across that retain the capacity to convert into barbell-shaped proplatelets [11, 12]. These preplatelets may be related both to the young (reticulated) platelets associated with the increased RNA content, and the large platelet commonly seen in inherited/acquired macrothrombocytopenias [11, 12]. Preplatelets reversibly convert into barbell-shaped proplatelets in the blood that divide to form two platelets [11, 12]. When the number of peripheral microtubules is increased, the spectrin-based membrane skeleton becomes disassembled and preplatelets turn out to be incapable of undergoing further barbell proplatelet conversion, resulting in the terminal platelets of a larger size [11, 12]. Macrothrombocytopenia may therefore represent a failure to convert the preplatelets to barbell-proplatelets [11, 12].

We reported a unique case of isolated congenital asplenia presented with pseudothrombocytopenia and enlarged platelets. The patient also had severe coronary heart disease, with no history of life-threatening infections, or bleeding. The particular aim of this case report was ultrastructural and morphometric analysis of enlarged platelets obtained from the patient's peripheral blood and the bone marrow.

Case report

A 52-year-old woman referred to the Hematology Department in 2003 because of pseudothrombocytopenia. She had a 5-year history of arterial hypertension and 2-year history of depressive neurosis. There was no history of abdominal surgery, infections, thrombosis or bleeding. She had no history of prolonged menstrual bleeding. Her two sons had normal number of platelets, normal spleen on abdominal ultrasonography and absence of giant platelets in peripheral blood smear. Physical finding was normal. Full blood counts and white cell differential were in a normal range, except for the platelet number which appeared to be low (54×10^9/L) and the mean platelet volume which was high (14.1 fL). However, when the platelets were analyzed using the CD61 (GPIIIa) MoAbs (ImmunoPLT method), or when they were counted in chamber, the platelet number was found to be around 100×10^9/L. On blood smear, the platelets were unusually large and the Howell-Jolly bodies were present in the red blood cells. The platelet aggregation in response to ristocetin, adenosine diphosphate (ADP), thrombin receptor-activating peptide (TRAP) and adrenalin as well as prothrombin time, activated partial thromboplastin time (aPTT) and bleeding time were normal. The platelet survival, analyzed by radioactive indium, showed normal life span (9 days) with the decreased index of production of platelets in the bone marrow (0.24, normal 1.0). The flow cytometry analysis of platelets showed the normal expression of the CD42b and CD41 antigens. The bone marrow aspirate and trephine biopsy showed mild hypercellularity, normal number of megakaryocytes, mild reactive changes without signs of malignant infiltration. The routine biochemistry and thyroid hormones were in the reference range. The immunological tests [antinuclear antibody (ANA), rheumatoid factor (RF), antibodies against cardiolipin and β_2-glycoprotein] and lupus anticoagulant were negative. The cytogenetic analysis showed normal 46, XX karyotype. Radiography of the chest showed a normal finding. The spleen was not visible by abdominal ultrasonography and computer tomography of abdomen was unremarkable. Scintigraphy using the 99mTc-labeled red cells showed the absence of splenic tissue in abdominopelvic cavity.

The ultrastructural analysis of platelets was done by the transmission electron microscope (TEM, FEI Morgagni 268D) and showed abnormal morphology of platelets, both in peripheral blood and in the bone marrow (Figure 1A-D). The platelets were uniformly spheroid in shape with conspicuous pseudopodia and the centralization of granules (Figure 1D). There were no marginal bands of microtubules inside the platelets (Figure 1D). Megakaryocytes of normal morphology were found on the ultrathin sections of bone marrow aspirates (Figure 1E). The platelets in the bone marrow were also large, spheroid with numerous pseudopodia and the centralization of granules with no signs of dilatation of open canalicular system in the majority of them (Figure 1F-H).

Very few platelets of diameter more than 4 μm were found in healthy individuals (around 1%) in comparison to > 40% in the patient's platelets. The mean platelet diameter (MPD) of asplenic patient from the peripheral blood sample was 3.81 ± 1.16 μm (range: 1.53–6.69 μm) and was significantly higher than MPD in healthy individuals (2.37 ± 0.61 μm, range: 1.01–3.68 μm) ($p < 0.05$, Student *t*-test). The MPD of platelets from the bone marrow aspirate (3.76 ± 0.81 μm, range: 2.09–5.84 μm) was quite similar to MPD from peripheral blood.

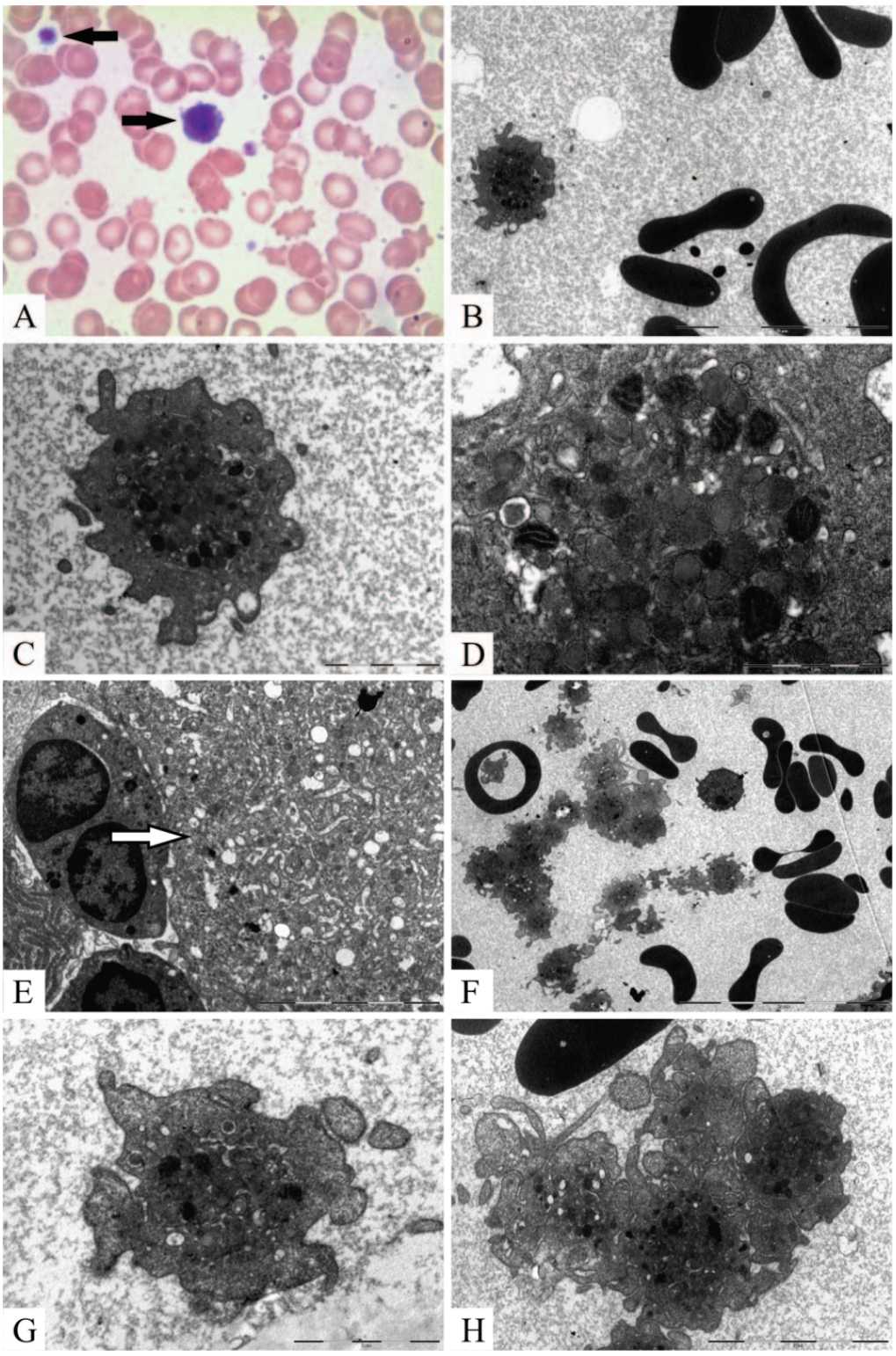

Fig. 1 – Morphology of platelets seen on light microscope (A), and the transmission electron microscope (B-H) in peripheral blood (A-D) and the bone marrow (E-H). (A) Blood smear of the patient studied with the unusually large platelets (arrows) and Howell-Jolly bodies in erythrocytes (May Grünwald Giemsa, x100) (B, C). The spheroid forms of large platelets with pseudopodia without obvious dilatation of open canalicular system. The vacuoles and darker inclusions could be seen in erythrocytes B) x4,400; C) x11,000). (D) A large magnification of platelet shows centralization of granules in peripheral blood and the lack of the visible marginal band of microtubules (x28,000). (E) The ultrastructural appearance of the cytoplasm of megakaryocyte found on ultrathin sections of bone marrow aspirates (x7,100). (F-H) The large, spheroid platelets in the bone marrow with numerous pseudopodia and the centralization of granules although no sign of dilatation of open canalicular system could be spotted [F) x2200; G x14,000; H) x7,100].

In 2009, a pacemaker was implanted in the patient because of tachybrady form of atrial fibrillation. In July 2012, she complained on chest pain and fatigue. Coronarography revealed the three-vessel coronary artery disease. The urgent surgical revascularization of the heart was successfully done, followed by the anticoagulant therapy. On her last follow-up (April 2016) she was in a good clinical condition and without complains.

Discussion

Isolated congenital asplenia is a rare condition. Literature review [2] only yielded about 50 cases since the first report of Myerson and Koelle [13] in 1956. This report described 24 sporadic and 26 family cases of isolated congenital asplenia, a majority in children. Our patient did not have other somatic anomalies beside asplenia and this condition was not found in any family member. Therefore, we concluded that our patient had sporadic isolated congenital asplenia.

A majority of the reported adult cases of congenital asplenia were presented with thrombocytosis [2]. On the contrary, our patient had near normal number of platelets which were unusually large. To our knowledge, this is the first reported case of asplenia with giant platelets. More than 40% of the measured asplenic patient platelets diameters were larger than 4 μm, while the diameters of platelets obtained from healthy individuals ranged above that value very scarcely, around 1%. The largest measured diameter in the patient's sample (6.7 μm) was almost two folds higher than the largest value of platelets found in healthy individuals.

The unusually large platelets were noticed before in macrothrombocytopenias [14]. Although the platelets seen in our case were large size, they differed from the appearance of the macrothrombocytes seen in the macrothrombocytopenias. Ultramicrographs of the previously published cases with macrothrombocytopenias revealed aggregates with large vacuoles and areas mostly devoided of dense bodies and alpha granules [14]. In the case presented here, there were no large vacuoles seen in the platelets while both dense bodies and alpha granules were apparent.

Some reported patients with congenital asplenia had thromboembolic complications. An adult case of isolated congenital spleen agenesis complicated by thrombocytosis and chronic thromboembolic pulmonary hypertension was previously described [7] as well as the patient with congenital asplenia, thrombocytosis and myocardial infarction [5]. Our patient also had the diffuse coronary artery disease but without thrombocytosis. However, her cardiovascular condition might be related to the ultrastructurally altered platelets. It was previously shown that higher-than normal mean platelet volume may be considered as a risk factor for vascular complications [15]. It was previously noticed that the platelet size correlated with the platelet reactivity and that larger platelets had greater prothrombotic potential. The elevated platelet size (MPV) was found to be associated with increased platelet aggregation, increased expression of adhesion molecules and elevated risk of cerebro- and cardiovascular diseases [15, 16].

The number of platelets in our patient, determined by automatic counter, was significantly lower than determined immunologically by CD61 or by counting in a chamber due to the inability of automatic counter to recognize large platelets. Precise determination of platelets count in patients with giant platelets requires the use of immunological method or counting platelets in a chamber. It is particularly important when a patient has indication for anticoagulant or antiplatelet therapy, as it was in our case.

It is well-known that in the resting state the platelets are discoid whereas activated platelets develop pseudopodia or extensions from the cell wall [11]. The platelet activation is also consistent with certain morphological features such as dilatation of open canalicular system [17]. However, the open canalicular system did not show any dilatation in the cells of the specimens that we studied. Both platelets seen in the bone marrow and peripheral blood were rounded and there were no prominent extensions from the cell wall and the marginal microtubule coils could not be spotted. Perhaps the large platelets seen in our patient with asplenia were not the activated cells, but rather the cells that represented, or resembled proplatelets. Namely, proplatelets have an average diameter of 2–4 μm and can be distinguished from the platelets by their diameters, (> 2 μm vs. ≤ 2 μm. respectively) [11].

The conversion from pre- to proplatelet is driven by microtubule-based forces, which are governed by two major biophysical properties: microtubule coil diameter and microtubule coil thickness [11]. Interestingly, these forces both regulate and predict the size of circulating platelets generated by proplatelets, providing an explanation for the approximately 2 μm diameter of platelets [11]. According to the Thon and Italiano [11], circular preplatelets are released into the blood, rapidly convert into barbell proplatelets, and undergo fast rounds of abscission that result in mature platelets, or alternatively, preplatelets may become trapped in the microcapillaries of the bone marrow, lung, or spleen where intravascular shear forces drive proplatelet to platelet production. In respect to this, the absence of the spleen can lead to partial absence of transition of proplatelets to platelets.

Conclusion

The case presented in this paper is very unusual due to the presence of enlarged spherical platelets in peripheral blood and the bone marrow of the patient with congenital asplenia. Peculiarity of this case is in the presence of severe form of coronary artery disease that might be in relation with the unusually large platelets that could be in persistent activation. This unusual finding might shed the light on the role of spleen in the formation of platelets.

R E F E R E N C E S

1. *Mahlaoui N, Minard-Colin V, Picard C, Bolze A, Ku C, Tournilhac O*, et al. Isolated congenital asplenia: French nationwide retrospective survey of 20 cases. J Pediatr 2011; 158(1): 142– 8, 148.e1
2. *Gilbert B, Menetrey C, Belin V, Brosset P, Lumley L, Fisher A*. Familial isolated congenital asplenia: A rare, frequently hereditary dominant condition, often detected too late as a cause of overwhelming pneumococcal sepsis. Report of a new case and review of 31 others. Eur J Pediatr 2002; 161(7): 368–72.
3. *Vincentelli C, Molina EG, Robinson MJ*. Fatal pneumococcal Waterhouse-Friderichsen syndrome in a vaccinated adult with congenital asplenia. Am J Emerg Med 2009; 27(6): 751.e3–5
4. *Germing U, Perings C, Steiner S, Peters AJ, Heintzen MP, Aul C*. Congenital asplenia detected in a 60 year old patient with septicemia. Eur J Med Res 1999; 4(7): 283–5.
5. *Chanet V, Tournilhac O, Dieu-Bellamy V, Boiret N, Spitz P, Baud O,* et al. Isolated spleen agenesis: A rare cause of thrombocytosis mimicking essential thrombocythemia. Haematologica 2000; 85(11): 1211–3.
6. *Rose C, Quesnel B, Facon T, Fenaux P, Jouet JP, Bauters F*. Congenital asplenia, a differential diagnosis of essential thrombocythemia. Presse Med 1993; 22(34): 1748. (French)
7. *Takahashi F, Uchida K, Nagaoka T, Honma N, Cui R, Yoshioka M*, et al. Isolated congenital spleen agenesis: a rare cause of chronic thromboembolic pulmonary hypertension in an adult. Respirology 2008; 13(6): 913–5.
8. *Italiano JE, Lecine P, Shivdasani RA, Hartwig JH*. Blood platelets are assembled principally at the ends of proplatelet processes produced by differentiated megakaryocytes. J Cell Biol 1999; 147(6): 1299–312.
9. *Tablin F, Castro M, Leven RM*. Blood platelet formation in vitro. The role of the cytoskeleton in megakaryocyte fragmentation. J Cell Sci 1990; 97(Pt 1): 59–70.
10. *Italiano JE, Patel-Hett S, Hartwig JH*. Mechanics of proplatelet elaboration. J Thromb Haemost 2007; 5 Suppl 1: 18–23.
11. *Thon JN, Italiano JE*. Platelets: production, morphology and ultrastructure. Handb Exp Pharmacol 2012; (210): 3–22.
12. *Machlus KR, Thon JN, Italiano JE*. Interpreting the developmental dance of the megakaryocyte: A review of the cellular and molecular processes mediating platelet formation. Br J Haematol 2014; 165(2): 227–36.
13. *Myerson RM, Koelle WA*. Congenital absence of the spleen in an adult; report of a case associated with recurrent Waterhouse-Friderichsen syndrome. N Engl J Med 1956; 254(24): 1131–2.
14. *Pretorius E, Oberholzer HM, van der Spuy WJ, Meiring JH*. Macrothrombocytopenia: Investigating the ultrastructure of platelets and fibrin networks using scanning and transmission electron microscopy. Ultrastruct Pathol 2009; 33(5): 216–21.
15. *Bath P, Algert C, Chapman N, Neal B*. Association of mean platelet volume with risk of stroke among 3134 individuals with history of cerebrovascular disease.. Stroke 2004; 35(3): 622–6.
16. Slavka G, Perkmann T, Haslacher H, Greisenegger S, Marsik C, Wagner OF, Endler G. Mean platelet volume may represent a predictive parameter for overall vascular mortality and ischemic heart disease. Arterioscler Thromb Vasc Biol 2011; 31(5): 1215–8.
17. *Maurer-Spurej E, Pfeiler G, Maurer N, Lindner H, Glatter O, Devine DV*. Room temperature activates human blood platelets. Lab Invest 2001; 81(4): 581–92.

Received on January 10, 2017.
Revised on September 21, 2017.
Accepted on September 22, 2017.
Online First November, 2017.