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Invited critical review

Clinical applicability of reticulated platelets

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ABSTRACT

Background: Reticulated platelets (RPs), immature platelets newly released from the bone marrow into the circulation, have a high content of ribonucleic acid and are larger and more active in thrombus formation.
Objective: This review compiles articles that evaluated RP in order to establish their clinical significance.
Discussion: RPs increase when platelet production rises and decrease when production falls. As such, the measurement of circulating RPs allows the assessment of thrombocytopenia, i.e., bone marrow production or peripheral destruction.
Conclusion: RPs are a promising laboratory tool for evaluation of idiopathic thrombocytopenia (differentiating hypoproduction from accelerated platelet destruction), chemotherapy and after stem cell transplantation (predicting platelet recovery) and thrombocytosis (estimating platelet turnover). Additional randomized and well controlled clinical studies are required to clearly establish the significance of circulating RPs in other clinical conditions.

Contents

1. Introduction

Reticulated platelets (RPs) or immature platelets are similar to reticulocytes newly released by the bone marrow into the circulation [1,2]. RPs were identified in 1969 by Ingram and Coopersmith who observed increased circulating immature platelets in dogs after acute

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blood loss [3,4]. These platelets exhibited residual RNA when stained with methylene blue. During maturation, cellular RNA is unstable and degrades within 24 h [5,6].

Our understanding of the mechanisms associated with platelet production, ie, thrombopoiesis, has improved recently. Identification of the growth factor thrombopoietin (a critical hematopoietic cytokine) and its receptor c-Mpl (mainly expressed on megakaryocytes) represented an important advance in the field [7]. Further, two erythroid transcription factors, nuclear factor erythroid-2 (NF-E2) and GATA1, were found to be essential for megakaryocytes to complete maturation and platelet release [8,9]. These findings and the availability of recombinant thrombopoietin have encouraged other studies aiming to clarify fundamental aspects of thrombopoiesis [10]. Thrombopoietin is produced by the liver and it is the primary regulator of megakaryocyte progenitor expansion and differentiation. Thrombopoietin, through c-Mpl, activates several signaling pathways, promoting cellular survival and proliferation. Due to its central role in hematopoiesis, alterations of thrombopoietin or its receptor contribute to the occurrence of diseases; as congenital and acquired thrombocytosis, thrombocytopenia and aplastic anemia [11].

Circulating platelets depends on thrombopoiesis stimulation and platelet removal from the bloodstream. It is known that activation-dependent mechanisms enhance platelet turnover and contribute to the presence of RPs in circulation. Therefore, assessment of RP could differentiate peripheral destruction vs suppression of bone marrow thrombopoiesis [12].

Although described in 1969, RPs were not clinically assessed for many years. Lack of appropriate methods and inability to compare results complicated these studies. Many factors contributed to this problem analytically, ie, types and concentration of fluorescent dyes, incubation time and temperature, fixation, RNAse treatment and the flow cytometric data analysis, including gating and threshold settings [13]. A major technical problem was that platelets show non-RNA specific binding to fluorescent dye resulting in background staining that is size-dependent [14].

2. Properties of reticulated platelets

The number of circulating RPs reflect the rate of thrombopoiesis, increasing with increased synthesis and decreasing with decreased production. RP exhibits a greater mass and higher prothrombotic potential compared to smaller platelets. Their count might reflect increased platelet consumption during the evolution of thrombosis or a prelude to the thrombus development [15]. Recently, McBane et al. [16], reported that newly formed platelets synthesized various granule and membrane glycoproteins, including glycoprotein (GP) Ib-IX-V and GP IIb/IIIa (integrin $\alpha_{IIb}\beta_3$) following stimulation. These observations suggest that young platelets are preferentially recruited for thrombus participation vs older ones. These researchers have postulated that younger RPs have an increased propensity for thrombus participation under high shear conditions compared to mature platelets. An increased receptor density of integrin β_3 in younger platelets may probably justify this finding.

RP aggregates faster with collagen and have increased P-selectin and thromboxane A2 [17]. P-selectin has an essential role in the recruitment of leukocytes to the inflammatory focus [18]. It has been suggested that RPs are present in inflammation and interact with leukocytes and endothelial cells [19]. Moreover, RPs have reduced response to antiplatelet drugs, ie, aspirin, possibly due to their greater reactivity and impaired cyclooxygenase-1 and -2 inhibition [20].

Although analysis of peripheral RPs have emerged as a powerful tool to estimate bone marrow production, this approach has some inherent limitations. Poor analytic standardization as well as the lack of appropriate internal and external quality control makes it difficult to compare inter-laboratory results. Currently, two Hematology analyzers, Sysmex (XE- and XN-series) and Abbott (CELL-DYN Sapphire), are commercially available to measure RPs. Despite different methodologies, both have showed clinical utility [15].

Two prospective observational studies assessed RPs count for diagnosis of thrombocytopenia and concluded that this method showed high sensitivity (93%) and specificity (85%) [21,22].

In-house methods have established the normal range of RPs in healthy subjects to be 8.6 ± 2.8% [23] and 1.1–6.1% [4]. In a recent review, Hoffmann [15], reported that the normal reference range of 1–15%. Unfortunately, poor standardization makes it difficult to establish a well-defined normal range [23]. Recently, several studies have assessed RPs normal ranges using Hematology analyzers. Comparable results were obtained. For example, the Abbott (CELL-DYN Sapphire) range was 0.4–2.8% [24], or 0.4–6.0% [25,26] whereas the Sysmex (XE- and XN-series) range was 0.8–6.3% [27] or 0.70–5.50% for males and 0.90–5.30% for females [28].

Mangalpally et al. [29], investigated platelet activation patterns relative to size and compared the inhibitory effects of aspirin. These researchers found that a higher proportion of large platelets (density, >1.055; mean volume, 12 μ^3) bound fibrinogen and von Willebrand factor following stimulation. These large platelets also expressed P-selectin and integrin αIIbβ3 in absence or presence of aspirin. Similarly, Guthikonda et al. [30,31], demonstrated that in healthy subjects and patients with coronary artery disease, RPs were associated with impaired aspirin effectiveness ex vivo. These researchers concluded that this finding could be due to RPs increased activity vs senescent platelets.

Hoffmann [15], using a Sysmex Hematology analyzer, demonstrated a strong positive correlation between RP count and fraction of large platelets, except in aplastic anemia. Although the large platelet fraction appeared highly correlated to mean platelet volume (MPV), data was not provided. On the other hand, Meintker et al. [32], using Abbott CELL-DYN found that RPs and MPV were poorly correlated in relatively small study groups. They concluded, however, that these parameters reflect different aspects of thrombopoiesis and are not interchangeable. In fact, in a large group of subjects with normal platelet counts, they found a significant negative correlation between RPs and MPV effectively demonstrating that RPs are not necessarily large.

2.1. Clinical applicability of RPs in thrombopoietic disorders

2.1.1. Diagnosis of thrombocytopenias

Because thrombocytopenia can be caused by decreased bone marrow production, excessive peripheral destruction or abnormal storage, it is not always simple to define etiology. Methods currently used to evaluate thrombopoiesis are complex, expensive and invasive. These include bone marrow aspiration, quantification of IgG-bound platelets and platelet survival studies [23]. RPs count seems to be a promising alternative to assess bone marrow production [21].

The percentage of RPs in patients with idiopathic thrombocytopenic purpura (ITP) was significantly higher (8.41 ± 5.35%) than healthy subjects (1.92 ± 1.27%) [23].

Thomas-Kaskel et al. [33], evaluated RPs in ITP with respect to treatment and its usefulness in identifying complex causes of thrombocytopenia in allogeneic stem cell transplantation. They concluded that the RPs were a viable tool to improve the diagnosis and prognosis.

Thrombocytopenia frequently occurs in liver disease and even more frequently in hepatitis. Possible mechanisms of thrombocytopenia in hepatitis are immune-mediated platelet destruction (due to antiplatelet antibodies or immune complexes), hypersplenism and decreased production by the bone marrow (due to reduced hepatic thrombopoietin synthesis or viral effect on megakaryocytes). Interferon therapy for hepatitis may also induce thrombocytopenia and finding which can justify suspension of antiviral therapy. Mechanisms of thrombocytopenia vary and some have multiple causes [34]. A new thrombopoietin receptor agonist has recently been reported to increase platelet counts in hepatitis C virus related cirrhosis, thereby allowing a subset of