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TRANSLATIONAL BIOMEDICAL RESEARCH

Platelet Changes in Acute Leukemia

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Abstract Acute leukemia is a hematopoietic stem cell malignant disease, with abnormal proliferation of leukemic and immature cells that suppress the production of normal blood cells and extensively invade peripheral tissues. The bleeding complications are very common in acute leukemia and often lead to death. One major cause for hemorrhage is thrombocytopenia, which is caused by the replacement of normal bone marrow cells with leukemic cells and the inhibition of megakaryocytes functions. Declines in platelet count as well as in function in acute leukemia have been reported in many studies. Here, we reviewed the literatures concerning platelet changes in acute leukemia.

Keywords Changes of platelet · Acute leukemia · Research progress

Introduction

Platelets are produced by specialized bone marrow cells called megakaryocytes and are the smallest component of the blood. Under physiological conditions, platelets are involved in homeostasis by releasing granules, activating adjacent platelets, their adhesion and aggregation. They are also involved in some pathological conditions including thrombosis, tumor metastasis, inflammation, and immune response [1].

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Acute leukemia is a commonly seen malignant tumor in the hematopoietic system. It often causes abnormal bleeding and subsequently leads to death. The mechanism of bleeding causes by leukemia is complex and involves leukemic cells infiltration in the vessel wall, reduction in platelet production, and coagulation/anticoagulation dysfunction. A quantitative reduction and qualitative dysfunctioning of platelets are the leading causes of bleeding in AL [2, 3]. In this article, we reviewed platelet changes in pathological conditions of acute leukemia and in the course of treatment.

Platelet Parameters Change in Acute Leukemia

The platelet parameters can be monitored with non-invasive approaches over time. They help in disease diagnosis in early phases, monitor disease progression, and determine therapeutic outcomes. The platelet parameters include: platelet count (PLT), mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW). PLT reflects the platelet metabolic dynamics in the peripheral blood and often found low in acute leukemia due to many reasons like: (1) Malignant growth of primitive and immature cells in bone marrow inhibits megakaryocyte production which account for platelet production. (2) Chemotherapeutic agents suppress the bone marrow hyperplasia, undermine platelets structural integrity, trigger excessive release of alpha and dense granules into the peripheral blood, promote platelet aggregation, and lead to its quantitative reduction. (3) Immune response induced by repeated blood transfusions, serious infections, and various inflammatory mediators accelerate platelet destruction. PCT is calculated by multiplying PLT and MPV. It changes in the direction as PLT does. MPV reflects the state of

megakaryocyte proliferation, metabolism, and platelet production in the bone marrow. To some extent, it also reflects platelet enzyme activity.

In general, newly produced platelets have large volume, higher MPV value, and more active function. They react more promptly to thrombin-induced activation and collagen-mediated aggregation. PDW reflects heterogeneity in the platelet volume, measured in CN values. When more young platelets present in the peripheral circulation, homogeneity is reduced and PDW is increased. Many studies have shown that different causes of thrombocytopenia can be diagnosed based on the change in platelet volume parameters (PDW and MPV). For example, Ntaios et al. [4] compared idiopathic thrombocytopenic purpura (ITP) with thrombocytopenia due to bone marrow suppression and found that platelet volume parameters elevated as more platelets destroyed in the peripheral blood. Therefore, PDW and MPV are reliable indicators for ITP diagnosis. There are inconsistencies with respect to early changes in platelet volume parameters and its clinical implication in acute leukemia. It has been demonstrated that children with leukemia have an abnormally high but not significantly different MPV, whereas PDW was significantly smaller. They proposed the use of PDW as an indicator of certain pathological states and to screen leukemia [5]. On the other hand, different subtypes of childhood acute leukemia cannot be differentiated by the use of platelet parameters (MPV and PDW). Lee et al. [6] reported a reduced MPV and elevated PDW in children with leukemia, and concluded that the platelet parameters are not suitable indices for screening leukemia and follow-ups.

In early stage of thrombocytopenia, platelet parameters signals hematopoietic dysfunctions and its specificity and sensitivity are subject to the influence of patient's age, processing time of the specimens and so on. In severe thrombocytopenia, erythrocyte fragmentation and cryoglobulinemia, platelet parameters limit the range of applications because of platelet histogram cannot be drawn and obstacles in parameter recording [7]. However, in clinical practice, dynamic monitoring of platelet parameter change is indispensable. For example, when chemotherapy is terminated, the bone marrow functions gradually restore. Then significant changes in the platelet parameters, PLT, MPV, and PDW will immediately follow. All this information is valuable for the assessment of medication efficacy and prognosis. In childhood acute lymphoblastic leukemia especially for non-minimal residual disease-based cases, Zeidler et al. [8] concluded that the platelet count after induction therapy can achieve a better treatment stratification. Using the platelet count recovery (up to $\geq 100 \times 10^9$/L) as the complete remission criterion, contributes to improved posttransplantation outcomes and is a significant prognostic factor for evaluating myelodysplasia (MDS) and acute myeloid leukemia (AML) patients for clinical trials and allo-SCT [9]. Increase in MPV before PLT is an early indicator during hematopoietic recovery. The unchanging PLT and MPV often indicate bone marrow failure, chemotherapy failure and poor prognosis.

Reticulated Platelet Change in Acute Leukemia

Reticulated platelets (RP) are the youngest and immature form of the platelets, come into the peripheral blood after budding off from megakaryocytes. They often contain residual RNA which has advantage for fluorescent visualization. Immature platelet fraction percentage (IPF%) and residual platelet counts (RPC) serve for measuring platelet production rate in the marrow and identifying different causes of thrombocytopenia. Psaila et al. [10] reported in AML/MSD patients that smaller platelets, lower immature platelet parameters and neonatal platelet proportion is associated with the expression of activation marker on the platelet surface. Low IPF% has been reported by Strauss et al. [11] in children with platelet production defects while it is significantly higher in acute ITP, suggesting an accelerated platelet turnover and lower absolute quantity of reticulated platelets. Moreover, IPF% has also been chosen as a diagnostic indices over platelet-associated IgG (PA-IgG) in ITP [12]. Interestingly, Strauss's group also reported an elevated IPF% in children diagnosed with acute lymphocytic leukemia (ALL), indicating that thrombopoiesis is stimulated despite virtual absence of bone marrow progenitors [11]. In addition, Cannavo et al. [13] suggested that IPF% augmentation in acute leukemia is attributed to delayed maturity of reticulated platelets.

Peripheral blood reticulated platelet (RP) count can serve as an important indicator to predict the ability of bone marrow to produce platelets. Platelet parameter changes help to evaluate therapeutic outcome of chemotherapy and stem cell transplantation and help to estimate hematopoietic recovery and its prognosis. Transfusions of platelets or megakaryocyte-stimulating factor are applied if platelet parameters suggest the necessity. RPs play pivotal roles in maintaining the coagulation. For instant, patients with higher RP count are less likely to develop serious bleeding complications [14]. In one study, in AML patients with chemotherapy-induced severe cytopenia, Ryningen et al. [15] reported 1–9 days earlier rise in RP than hematopoietic function reconstruction and predict the degree of bone marrow functional reconstruction. In another study, flow cytometric analysis of IPF% change in patients with leukemia who received allogeneic stem cell transplantation, peripheral blood platelet count recovery was observed up to 45.6×10^9/L within 3 days on average

after the transplantation, and IPF% reached at the peak even before peripheral platelet count recovery [16]. Therefore, elevated RP% reflects increased bone marrow regeneration and can be considered an additional marker of thrombopoietic recovery in the patients undergoing allogeneic stem cell transplantation.

Platelet Functional Changes in Acute Leukemia

Platelet Membrane Glycoprotein Expression

Under normal physiological conditions, platelets circulate with laminar flow in close proximity to the endothelium without adhesion to each other or with the endothelial cells. However, upon activation, platelet membrane glycoprotein (GP) undergoes conformation changes and then supports adhering and aggregating. Glycoprotein Ib-IX-V complex (GPIb-IX-V) and glycoprotein IIb/IIIa complex (GPIIb/IIIa) are the most important components of GP. GPIb-IX-V contains four subunits, GPIbα, GPIbβ, GPIX and GPV. Platelet adhesion is mainly mediated by GPIb (α and β) subunits via disulfide bond. The GPIbα extracellular segment (1–282) bears various important ligand binding sites, such as von Willebrand factor (vWF), a-thrombin, P-selectin and Mac-l. An intracellular segment carries binding sites for signaling molecules and actin-binding proteins. In response to the vascular injuries, vWF in plasma binds to the subendothelial matrix and subsequently to the GPIb-IX complex (Fig. 1). The binding of vWF to GPIb-IX complex triggers signaling pathways, results in calcium mobilization, cytoskeleton rearrangements, granule contents release, integrin binding affinity elevation, platelet adhesion stability rise, and thrombosis promotion. The platelet surface GPIIb/IIIa complex (CD41/CD61) is a major component of platelet GPs. It plays a central role in mediating platelet adhesion to the vessel wall. It also participates in platelet adhesion, aggregation, and content release. Under the stimulation from various factors, platelets are activated by "inside-out" signals and the GPIIb/IIIa complex is translocated to the platelet surface by associating with alpha granules and surface-connected canalicular system. The GPIIb/IIIa complex undergoes structural and spatial conformational changes in the presence of calcium and exposed the fibrinogen binding sites, which allow the platelets to bind to fibrinogen (Fig. 1). Thereby, via fibrinogens, platelets are interconnected and aggregated [17].

Qualitative and quantitative changes in platelet GPs are found in acute leukemia. It has been demonstrated in patients with MDS that the clonal stem cell defect reduced platelet surface protein expression of GPIb and GPIIb/IIIa and increased RNA expression of various GPs [18]. In another study, Psaila et al. [10] reported that AML/MDS patients compared to ITP had lower activated GPs on the platelet surface. Moreover, they also found that the platelet reactivity in AML/MDS patients with bleeding was higher than those without bleeding. In addition, increased GPIb on circulating platelets and expression of activated GPIIb-IIIa and GPIb on ex vivo activated platelets correlate with higher IPF. AML/MDS patients have lower platelet activation in in vivo and platelet reactivity in ex vivo than ITP patients. Popov et al. [19] pointed out that patients with myeloproliferative neoplasms (MPN) expressed less receptor on the platelet and increased membrane fluidity, which is attributed to the abnormal expression of GPIb receptor and changes in signaling pathway.

Platelet Aggregation

It refers to the process of platelet clumps formation. The GPIIb/IIIa complex and fibrinogen connect the dispersed platelets together. This calcium-dependent process involves adenosine diphosphate (ADP), epinephrine, collagen, thrombin, arachidonic acid, thromboxane AII (TXAII), and platelet-activating factor (PAF) (Fig. 1). It is reported in children with acute lymphoblastic leukemia that the platelet aggregation induced by various agonists was attenuated during the induction of remission therapy [20]. In another report, Girtovitis et al. [21] believed that platelet aggregation in MDS patients was impaired with respect to one or more agonists. Such impairment significantly contributed to poor prognosis. The energy status is critical for platelet aggregation. The platelet energy is mainly derived from anaerobic glycolysis. Platelet aggregation requires ATP consumption. Three main enzymes are involved in platelet energy production pathway i.e., glucose-6-phosphate dehydrogenase (G6PD), pyruvate kinase (PK), and hexokinase (HK). Functional abnormalities in one or more enzymes were found in various leukemia or in the course of its treatment [3]. Metabolic changes and enzymatic abnormalities in platelets with leukemia originate from the flawed megakaryocytes, which account for the absence of platelet functions.

Platelet Activation

Resting platelets scatter in vessels and activate in response to the vascular injury, changes in blood flow and/or chemical stimulations. Platelet activation process involves a series of changes such as release of dense granules, alpha granules and lysosomes into the plasma and activation of markers on the platelet surface (Fig. 1). Numerous studies have been reported abnormal activation of platelets in acute leukemia. Mechanisms leading to such activation are

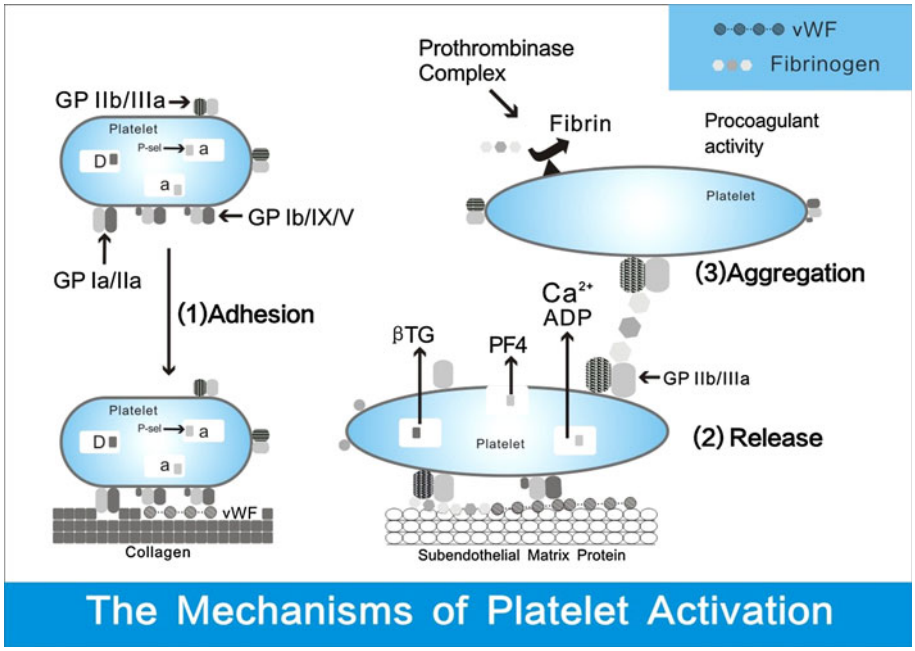

Fig. 1 The mechanism of platelet activation. The figure outlines the three phases of platelet activation including adhesion, secretion, and aggregation. *(1) Adhesion:* Following blood vessel injury, platelet adhere to the exposed subendothelial extracellular matrix; collagen, proteoglycans, and fibronectin. vWF acts as an adhesion bridge between the platelet glycoprotein (GP) Ib-IX-V complex and exposed collagen, also GPIIb/IIIa and GPIa/IIa adhere to fibrinogen. Platelet adhesion enhances interaction between adjacent platelets and induces a series of metabolic reactions; platelet release reaction, shape change and aggregation. *(2) Release:* Platelet adhesion stimulates intracellular signaling, leading to the secretion of platelet granule contents which include adenosine diphosphate (ADP), serotonin, fibrinogen, β-thromboglobulin (βTG), platelet factor-4 (PF4), etc., and phospholipids reorganization with formation of coagulation complex and fibrin. Released ADP and thromboxane A2, and encourage the platelet aggregation to the site of vascular injury. *(3) Aggregation:* Platelet aggregation via fibrinogen bound to GPIIb/IIIa receptor catalyzed by Ca^{2+} dependent prothrombinase complex and starts procoagulant activity. ADP induces a conformation change in GPIIb/IIIa receptor allow fibrinogen binding. Auto-catalytic reaction activating other platelets. The whole process results in the formation of primary hemostatic plug

complex. However, they involve following steps: (1) Proliferating leukemia cells invade into the bone marrow, other body tissues and organs. The vascular endothelial cells are damaged and thus cause augmentation of vWF and increase exposure of the subendothelial matrix, finally activate the platelets. (2) Leukemia cells release procoagulant molecules, leading to elevated thrombin production and reduce anticoagulant activities, hence activate the platelets. (3) Leukemia cells express excessive adhesion molecules on the surface, thus enhance the interactions among leukemic cells and endothelium and thus induce the platelet activation. (4) Chemotherapeutic agents can activate platelets directly or indirectly via damaging the endothelium. (5) Cytokine and chemokine levels are higher in the course of leukemia. Some cytokines and chemokines might affect the spread of leukemia cells and activate platelets. In this connection, patients with acute leukemia are associated with higher platelet activation, suggesting that interactions between activated platelet and tumor cells can be one of the causes of intensive infiltration and bleeding in acute leukemia [22].

Platelet Factor-4 (PF4)

It is synthesized by the megakaryocytes and usually stored in the dense granules of megakaryocytes and platelets. It is released in the form of a complex composed of proteoglycans by the activated platelets during adhesion and aggregation. PF4 marks the differentiation and maturation of megakaryocyte, and platelet activation [23]. Some studies recommend plasma PF4 concentration as an in vivo platelet activation marker due to its better sensitivity and accuracy over other aggregometries. Shi et al. [24] found, in pediatric acute lymphoblastic leukemia (ALL), a reduced serum PF4 peak in proteomic analysis and PF4 can be used as a potential marker to differentiate ALL, AML and healthy subjects. Another report demonstrated that AML patients in complete remission had significantly higher PF4 in peripheral blood than AML patients that did not receive treatment or were in partial remission [25]. A serum PF4 >2.492 μg/mL is equivalent to a serum PF4 recovery level of $>100 \times 10^9$/L, and thus can be used as a good indicator of blood cell count recovery in complete remission.

P-selectin

The P-selectin is a trans-membrane glycoprotein, also known as granule membrane protein-140 (GMP-140) or lysosomal membrane protein CD62P. It is stored in alpha granules or Weibel–Palade bodies in resting platelets. Upon platelets or endothelial cell activation, alpha granules or Weibel–Palade bodies membrane fuses with cell membrane, then P-selectin is passed to the cell membrane surface and soluble forms of P-selectins into the plasma. P-selectin ligand recognition and interaction are calcium dependent and it can recognize a variety of ligands. One of the natural and typical ligands is P-seleetin glycoprotein ligand-1(PSGL-1), a glycoprotein that is present on the surface of leukocytes. It also interacts with other members of the selectin family, such as E-selectin, and I-selectin. PSGL-1 recruits white blood cells to the endothelium to participate in physiological and pathological events. P-selectin identifies the activated platelets by their molecular markers, mediates the attachment of platelets and granulocytes to the endothelium, and plays a role in inflammatory response, pathological thrombosis and tumor cell proliferation and metastasis. Luo et al. [22] reported that CD62P expression was upregulated in patients with acute leukemia and in complete remission before and after ADP stimulation. They also found less number of platelets and their activation abnormalities in early onset of acute leukemia. These abnormalities are due to malignant proliferation of myeloid leukemia cells, which results in a decrease of bone marrow megakaryocytes or even their dysfunction. Megakaryocyte dysfunction can cause the platelet activation disorder. Endothelial activation factors expression in pediatric ALL patients changed in a different degree [26]. It is found that, the CD62P expression was upregulated in the induction treatment while thrombin generation markers and inflammatory cytokines were downregulated. This was attributed to the detrimental effect of chemotherapy drugs on vascular endothelial cells. Leinoe et al. [27] pointed out that the low level of P-selectin is a prognostic indicator for bleeding complications in AML. P-selectin (CD62P) <36 molecules of equivalent soluble fluorochrome x 10^3 ($P < 0.0015$) and platelet count $<40 \times 10^9$/L ($P = 0.01$) were potent markers of hemorrhage.

Pac-1

Procaspase activating compound-1 (PAC-1) is a monoclonal antibody that recognizes fibrinogen binding sites within the GPIIb/IIIa complex on an activated platelet. In activated platelets, GPIIb and GPIIIa binds each other in 1:1 ratio in a Ca^{2+} dependant manner and interact with ligands to exert platelet functions. It has been demonstrated in one report that PAC-1 level was enhanced in patients with acute leukemia and downregulated in complete remission and further lowered in acute leukemia patients complicated with megakaryocyte dysplasia [22]. In connection, Foss et al. [28] found that anthracycline, both daunorubicin and idarubicin, significantly increased the expression of activation-associated membrane molecules by normal platelets.

Interactions Between Leukemia Cells and Platelets

The platelets usually interact with blood cells via adhering or releasing a variety of soluble mediators. These mediators are able to affect the activity of hematopoietic stem and progenitor cells. Abnormal platelets were found in the various types of malignant disease including AML and other types of leukemia. Platelets also interact with the circulating tumor cells which can be coated with platelets and thereby protected from the clearance of immune surveillance. Such platelets–coated tumor cells have an enhanced ability to attach with endothelial cells and endothelial extracellular matrix and thus promote the tumor invasion and metastasis. Besides, platelet secreted factors contribute to tumor cell growth. Foss et al. [23] suggested that AML cells and platelets change the feature of each other. Chemotherapeutic agents usually alter platelet functions, thus in the course of chemotherapy, many cytokines related to platelet activations and leukemic cells are substantially upregulated. In preclinical trials, it was shown that functional platelets are crucial to cancer cell metastasis in the hematopoietic system. Platelets depletion or pharmacological inhibition or genetic downregulation of platelet membrane proteins significantly suppress tumor metastasis. A new type of anticancer drugs are being conceived by targeting platelet surface receptors. However, mechanisms of platelet surface receptors on metastasis are not completely understood, and the effects of receptors on metastasis are far more complicated than expected [29]. Tumor cells express many surface proteins that normal cells do. In other words, they attach to platelets, trigger platelet activation, and take advantage to migrate and disseminate with platelets. Studies have shown that there exist many common characteristics between the white blood cells and tumor cells in the process of migration and dissemination. The flow cytometric results showed that AML cells also express PSGL-1, which can recruit P-selectin and E-selectin to the endothelium [30]. These findings inspire a new strategy for leukemia treatment. Walenkamp et al. [31] demonstrated that bacterial protein Staphylococcal-superantigen-like-5 (SSL5) effectively blocks the interaction between leukemia HL 60 and endothelial cells via rapid competitive binding to HL-60 cell surface PSGL-1.

Future Studies

The platelet function evaluation is critical to early diagnosis and treatment of hemorrhagic and thrombotic diseases. Current platelet function evaluation methods are limited. Numerous scientists are striving to find simple and effective evaluation methods for acute leukemia with high sensitivity and specificity. Flow cytometry, as a new technology to measure platelet activities and functions, has been widely adopted in clinical practice. It provides a path to the comprehension of complex changes in the formation of hemorrhagic and thrombotic diseases. It also gives reliable information regarding physiological and pathological conditions of acute leukemia patients in clinical examinations. Multiple parameters systemically and flexibly should be taken into consideration including changes in platelet parameters, membrane protein antibodies and activation marker proteins, and these parameters should be standardized for the disease diagnosis. It will help to the early prevention and treatment of acute leukemia and bleeding complications, and will also guide the physicians to apply the platelet transfusion and medications. To conclude, the unequivocal mechanisms of platelet changes in acute leukemia require further detailed studies.

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Conflict of interest Authors have no conflict of interest.

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