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ORIGINAL ARTICLE

Investigation of Iron Metabolism for Regulating Megakaryopoiesis and Platelet Count According to the Mechanisms of Anemia

Suekyeung Kim and Sun Young Cho

Departments of Laboratory Medicine, School of Medicine, Kyung Hee University, Seoul, Korea

SUMMARY

Background: **Iron deficiency anemia (IDA) is characterized by depletion of total body iron stores or a poor supply of plasma iron. By contrast, chronic inflammation makes iron unavailable for hematopoiesis through a cytokine-mediated cascade and leads to a condition known as anemia of chronic disease (AOC). However, the laboratory data regarding the regulatory role of iron metabolism on platelet count has not been fully discussed yet. In this study, we investigated the relationship between iron status and platelet production according to different anemic mechanisms representing different iron metabolisms.**

Methods: **The study included a total of 759 specimens. Blood samples were obtained through venipuncture. The complete blood count was measured using an Advia 2120 (Siemens Healthcare Diagnostics Inc., USA). Biochemical indices including iron level were estimated using a Toshiba chemical analyzer (Toshiba, Japan).**

Results: **In the AOC group, we found a significant relationship between platelet count and serum iron level ($p < 0.27$), whereas there was no correlation in the IDA group. Moreover, when the AOC patient group was subdivided by serum iron level, a remarkable difference was observed as follows. The platelet count was significantly correlated with serum iron level only in the AOC group with decreased serum iron levels (serum iron < 50 µg/dL) ($p < 0.0001$), while there was no correlation in the AOC group with normal serum iron levels (serum iron 50 - 100 µg/dL).**

Conclusions: **Iron deficiency in AOC involves upregulated hepcidin production induced by elevated inflammatory cytokines. This can cause increased iron sequestration in macrophages and decreased iron absorption for bone marrow. The condition of decreased megakaryocytic iron supply makes megakaryocytes with higher ploidy which can release more platelets than lower ploidy. Moreover, reactive thrombocytosis in inflammatory states occurs by cytokine cascades involving interleukin 6 and thrombopoietin in AOC. These two features may enhance thrombocytosis in patients of AOC with decreased iron level. In the future, further study should be performed to elucidate regulating mechanism of iron metabolism for megakaryopoiesis in AOC patients, and guide proper supplemental therapy of iron to decrease thrombotic risk due to reactive thrombocytosis in various kinds of anemia.**

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Correspondence:
Sun Young Cho, M.D., Ph.D.
Department of Laboratory Medicine
School of Medicine
Kyung Hee University
Kyung Hee Dae Ro 23
Dongdaemun-Gu, Seoul 02447
Korea
Phone: +822 958 8674
Fax: +822 958 8609
Email: untoyou@hanmail.net

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KEY WORDS

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INTRODUCTION

Thrombocytosis can be caused by a clonal proliferative disease of the bone marrow or may be a response to various benign conditions resulting in reactive platelet increases [1]. Reactive thrombocytosis is much more fre-

quent than clonal thrombocytosis and is known to be associated with diverse pathologic conditions such as iron deficiency anemia (IDA), blood loss, and chronic inflammatory diseases [2]. In IDA, depletion of total body iron stores or a poor supply of plasma iron may induce anemia [3]. By contrast, chronic inflammation makes iron unavailable for hematopoiesis through a cytokine-mediated cascade and leads to anemia of chronic disease (AOC) [4,5]. The retention of iron within macrophages causes reduced iron availability for erythroid progenitor cells [6]. AOC is the second most common form of anemia and is characterized as a mild to moderate anemia occurring in patients with multiple infections or inflammatory disorders [7,8]. It has also been well established that there is a relationship between erythropoiesis and megakaryopoiesis under anemic conditions [9]. Certain role of iron as a component of a number of enzymes in maintaining platelet homeostasis and the platelet dysfunction as a consequence of iron deficiency had been previously suggested [10]. However, the preceding laboratory data regarding the regulatory effect of iron metabolism on thrombopoiesis has not been fully discussed. Moreover, possible association between the mechanism of anemia and iron metabolism has not been clearly demonstrated in previous studies. In this study, we investigated the relationship between iron status and platelet production according to different anemic mechanisms representing different iron metabolisms.

MATERIALS AND METHODS

The study included a total of 759 specimens from patients at Kyung Hee University Hospital, a tertiary teaching hospital, between January and December 2015. The patients were collected from various departments of internal medicine including nephrology, cardiology, hematology/oncology and endocrinology. The clinical data were reviewed from medical charts and patients who were diagnosed with chronic kidney disease were the most common. Blood samples were obtained through venipuncture. The changes in platelet counts, level of hemoglobin (Hb), white blood cell count (WBC), mean corpuscular volume (MCV), and mean platelet volume (MPV) were analyzed using an Advia 2120 (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). Serum iron and unbound-iron binding capacity was measured using a Toshiba chemical analyzer (Toshiba, Nasushiobara, Japan). Total iron binding capacity (TIBC) and transferrin saturation (TfS) were calculated based on the above measurement values. Statistical analyses were performed with MedCalc v11.6 (MedCalc Software, Mariakerke, Belgium), IMB SPSS statistical software package, version 20.0 (IBM Corporation, Armonk, NY, USA), and Excel 2007 (Microsoft Corporation, Redmond, WA, USA). A value of $p < 0.05$ was considered statistically significant. Institutional Review Board (IRB) approval was obtained for this study (IRB approval number 2014-04-203).

RESULTS

Total patients were divided by the inclusion criteria shown in Figure 1. The data of patients in each group shown in terms of platelet count, serum iron level, MPV, WBC, Hb, TIBC, TfS, and MCV are shown in Table 1 (n = 303). Among our data, relatively decreased iron parameters in the elderly subjects may be caused by various factors, including inadequate absorption of iron, nutritional status, medications, and higher prevalence of chronic disease [11].

First, we grouped the IDA and AOC patients based on the parameters of TIBC, TfS, MCV, and Hb. Patients with values of TIBC > 400 μg/dL, TfS < 20%, MCV < 80, and Hb < 11.0 g/dL were categorized as the IDA group, while the patients with TIBC < 200 μg/dL, TfS > 20%, MCV 80 - 100, and Hb < 11.0 g/dL were classified into the AOC group. In the AOC patients, we noticed a significant relationship between platelet count and serum iron level ($p < 0.27$), whereas no correlation between these two factors was shown in the IDA group. Moreover, when the AOC patient group was subdivided by serum iron level, a remarkable difference was observed. There was no correlation between platelet count and serum iron level in AOC patients with normal serum iron level (serum iron 50 - 100 μg/dL), while an elevated platelet count was correlated with serum iron level only for the group of AOC patients with low serum iron level (serum iron < 50 μg/dL) with statistical significance ($p < 0.0001$).

DISCUSSION

AOC usually develops in patients with chronic immune activation and can be combined with true iron deficiency arising from chronic blood loss [6,7]. Several inflammatory agents and cytokines are known to impair erythropoiesis and iron metabolism in AOC [5]. In addition, the cytokine cascade is reported to interfere in certain stages of platelet differentiation in the bone marrow and cause reactive thrombocytosis [5].

According to our data, the group of AOC patients with low serum iron level exclusively demonstrated a correlation between the platelet count and the iron level compared with both simple IDA patients and AOC patients with normal serum iron level. This result suggests that iron metabolism might be closely linked to thrombopoiesis in AOC patients. Abnormal iron metabolism of AOC can be differentiated from typical IDA in terms of its pathophysiology. Iron deficiency in AOC involves upregulated hepcidin production induced by increased inflammatory cytokines, which cause iron sequestration in macrophages and decreased intestinal iron absorption [7]. Kaser et al. proposed that reactive thrombocytosis in inflammatory states might occur by cytokine cascades including interleukin (IL)-6 and thrombopoietin (TPO), each of which is a prominent determinant of circulating platelets [12]. The fact that IL-6 is responsible

Table 1. Laboratory characteristics of patient groups according to anemic mechanisms and serum iron levels.

	IDA (n = 45)		AOC (normal serum iron) (n = 137)		AOC (low serum iron) (n = 121)	
	M	F	M	F	M	F
Age, mean (SD)	43.4 (27.3)	42.1 (17.4)	43.5 (25.6)	41.6 (18.1)	68.7 (10.0)	71.7 (25.4)
Platelet (10^9/L), mean (SD)	327.3 (157)	342.7 (128)	179.6 (94.8)	204 (114.5)	213.2 (102)	240 (120)
Iron (µg/dL), mean (SD)	13.4 (6.1)	11.55 (4.5)	66.0 (32.5)	62.7 (28.8)	39.8 (6.3)	39.9 (5.8)
MPV (fL), mean (SD)	7.3 (1.2)	7.8 (1.1)	8.2 (1.0)	8.3 (1.2)	8.2 (1.1)	8.0 (1.0)
WBC (10^9/L), mean (SD)	5.3 (1.7)	5.9 (1.7)	6.7 (5.0)	6.8 (4.5)	7.6 (3.8)	7.7 (4.3)
Hb (g/dL), mean (SD)	8.3 (1.6)	8.4 (1.9)	9.5 (1.0)	9.2 (1.1)	8.9 (1.3)	9.3 (1.1)
TIBC (µg/dL), mean (SD)	423 (12.1)	449 (44.0)	164 (34.3)	167 (32.5)	148 (34.7)	148.8 (35.7)
TfS (%), mean (SD)	4.9 (2.6)	3.7 (1.6)	43.0 (15.4)	43.8 (14.4)	28.8 (11.3)	29.3 (13.7)
MCV (fL), mean (SD)	70.1 (5.5)	69.4 (6.2)	62.7 (4.0)	93.6 (4.1)	91.9 (3.9)	92.6 (4.2)

The patient characteristics including platelet count, iron level, MPV, WBC, Hb, TIBC, TfS, and MCV are shown. Abbreviations: IDA - iron deficiency anemia, AOC - anemia of chronic disease, MPV - mean platelet volume, WBC - white blood cell count, Hb - hemoglobin, TIBC - total iron binding capacity, TfS - transferrin saturation, MCV - mean corpuscular volume.

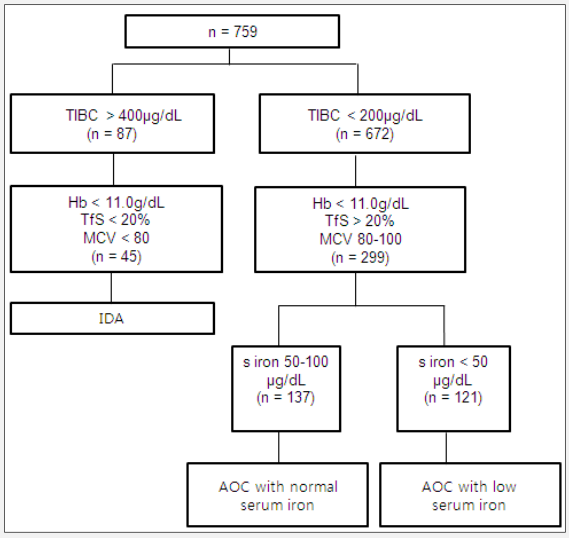

Figure 1. Classification of the patient group in this study.

Patients were grouped into IDA and AOC according to their iron parameters. Abbreviations: Hb - hemoglobin, TIBC - total iron binding capacity, TfS - transferrin saturation, MCV - mean corpuscular volume, IDA - iron deficiency anemia, AOC - anemia of chronic disease.

for reactive thrombocytosis has also been suggested by Tefferi A [13]. Also, hepcidin is a key molecule which regulates cellular iron through binding to ferroportin, leading to an interruption of iron transport from macrophages to the circulation [14]. Several cytokines and proteins mediate the expression and production of hepcidin. The inflammatory cytokine IL-6 has been widely known to trigger hepcidin expression through a pathway involving signal transducer and activator of transcription 3 during inflammatory processes [8,14]. In addition, bone morphogenetic protein plays a central role in hepcidin formation via phosphorylation of Smad proteins [14]. These unique pathways involving hepcidin are associated with maintaining the iron status in AOC unlike simple IDA. Although Akan et al. assessed the effects of several other thrombopoietic cytokines including erythropoietin, TPO, and IL-11 in IDA, no significant relationships were observed [15]. As a conse-

quence of iron deficiency, the condition of decreased megakaryocytic iron supply makes megakaryocyte cell lines with higher ploidy which can release more platelets to maintain homeostasis of platelet production [1, 16]. The unique characteristics of the megakaryocyte polyploidization process and its effects on platelet production have been demonstrated in previous studies [17, 18]. Still, additional research is required to identify a definite relationship between thrombocytosis and iron status. The most likely explanation for our results is that both iron deficient status and chronic inflammation itself seem to contribute to the linkage between serum iron and platelet count in patients of AOC with decreased iron level.

The possibility of elevated platelet count as a risk factor of thromboembolic events in certain chronic inflammatory diseases such as chronic kidney disease and inflammatory bowel disease patients was already described in a few studies [16,19]. However, the definite clinical significance of reactive thrombocytosis in patients with AOC is still uncertain. Moreover, a significant proportion of AOC patients develop concomitant true iron deficiency resulting from concurrent blood loss [6]. So far, little data has been reported on the differences of controlling mechanisms in iron homeostasis between AOC versus AOC combined with iron deficiency [6]. The limitation of this study is that we did not directly measure the levels of mediators including hepcidin, TPO, and other cytokines. Further studies are needed to verify the linkage between these mediators and megakaryopoiesis in anemic patients. In the future, further studies should be performed to elucidate the regulating mechanism of iron metabolism for megakaryopoiesis in AOC patients and to guide proper supplemental therapy of iron to decrease thrombotic risk due to reactive thrombocytosis in various kinds of anemia.

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Declaration of Interest:
The authors do not report any conflicts of interest.

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