Relationship between Bone Marrow Megakaryocyte Morphology and the Pathophysiology of ITP

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Immune thrombocytopenia (ITP) is a hemorrhagic disease primarily caused by platelet destruction and reduced platelet production from megakaryocytes due to autoantibodies targeting platelet glycoproteins and other antigens, resulting in thrombocytopenia. While the evaluation of bone marrow megakaryocytes is important for distinguishing different causes of thrombocytopenia, recent ITP treatment guidelines state that bone marrow examination itself is not particularly useful for diagnosing ITP. However, there are only a limited number of studies focusing on ITP, particularly on megakaryocyte morphology, indicating that further research is needed.

In this study, we compared the morphology of bone marrow megakaryocytes and their platelet production patterns between ITP and control groups. Bone marrow smear specimens were analyzed to measure megakaryocyte major axis length, area, and nuclear area, as well as the number and rate of platelet attachments on megakaryocyte surfaces. The ITP group exhibited greater megakaryocyte major axis length and a larger megakaryocyte area compared to the control group. Furthermore, the number of platelet attachments on megakaryocyte surfaces was significantly lower in ITP patients.

Additionally, megakaryocytes in the pregnancy-complicated group had larger major axis length and areas than those in the non-complicated group. In refractory cases requiring thrombopoietin receptor agonists (TPO-RAs) for treatment, the cell major axis length, cell area, and nuclear area were all larger. The percentage and number of platelet attachments on megakaryocyte surfaces were also lower in patients with *Helicobacter pylori*-positive (*H. pylori*-positive) ITP.

The morphology of megakaryocytes in ITP exhibits characteristics related to its pathology. Therefore, focusing on the size of megakaryocytes and the appearance of platelet attachments on megakaryocyte surfaces in bone marrow smears may be useful for differentiating ITP. *Shinshu Med J 73 : 155—164, 2025*

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Key words : immune thrombocytopenia, megakaryocyte, *Helicobacter pylori*, thrombopoietin receptor agonists

I Introduction

Immune thrombocytopenia (ITP) is a hematologic disorder characterized by the destruction of platelets sensitized by antiplatelet antibodies[1)-5)]. These platelets are eliminated by the reticuloendothelial system at a rate that far exceeds their production by megakaryocytes, leading to a significant reduction in circulating platelet levels and various bleeding symptoms[6)7)]. In addition, platelet production may be impaired[8)-10)] due to antibodies directly targeting bone marrow megakaryocytes, resulting in cellular damage, reduced proliferation of megakaryocyte precursor cells, and decreased

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platelet production rates[11)-15)]. Antibodies against platelet membrane glycoproteins (GP Ⅱb/Ⅲa and GP Ⅰb/Ⅸ) have been identified in patients with ITP[16)17)], suggesting that both platelets and megakaryocytes may act as antigens presented to lymphocytes by antigen-presenting cells, thereby contributing to cytotoxicity. Thus, the pathogenesis of ITP is complex and not yet fully understood.

In Japan, bone marrow examination has been routinely performed to diagnose ITP. However, recent clinical guidelines state that bone marrow examination is not necessarily required for diagnosis because here are no specific findings characteristic of the condition, and there are no differences between hematopoietic cells, including megakaryocytes, in ITP and those in a normal hematopoietic state[6)18)19)]. Previous reports have suggested that autoantibodies and activated lymphocytes in ITP inhibit megakaryocyte maturation[20)21)] or cause impairments[18)22)], leading to morphological changes in these cells. However, few studies have focused specifically on the morphological characteristics of megakaryocytes in bone marrow smears from ITP patients. The specific features of megakaryocytes in such cases remain unclear. Furthermore, in other diseases that cause thrombocytopenia, such as aplastic anemia and myelodysplastic syndromes, characteristic findings like a decrease in megakaryocytes or the presence of micromegakaryocytes serve as diagnostic clues.

Regarding bone marrow examination in ITP, especially the morphology of megakaryocytes, there are few data-driven analyses, and evidence supporting the lack of difference from normal bone marrow is scarce. Therefore, in this study, we evaluated the morphological characteristics of megakaryocytes in ITP compared to those in a normal hematopoietic state without thrombocytopenia. We aimed to determine whether there are differences, and if so, what specific features are associated with ITP megakaryocytes.

Ⅱ Materials and Methods

A Subjects

We enrolled patients clinically diagnosed with ITP at Shinshu University Hospital in Japan between January 1996 and December 2020. These patients had May-Grünwald Giemsa-stained bone marrow blood smears stored prior to treatment. Cases initially diagnosed with ITP but later identified as other diseases were excluded. Subjects with malignant lymphoma, who had no significant hematological abnormalities (including platelet counts) and no evidence of lymphoma cell infiltration in the bone marrow, were designated as Control Group 1. Another control group (Control Group 2) consisted of healthy bone marrow donors for hematopoietic cell transplantation. In Control Group 2, bone marrow samples were anticoagulated with EDTA-2K prior to preparing smears. In all groups, 10 or more megakaryocytes were observed per case.

B Methods

1 Cell morphometry of megakaryocytes

The morphology of megakaryocytes was analyzed using bone marrow smears observed under an optical microscope (OLYMPUS BX51) at 400× magnification. Photographs of megakaryocytes were captured using a Flovel Filing System FX630 (Olympus, Tokyo) and manually traced on a monitor to measure the cell major axis length, cell area, and nuclear area. An example of the examined megakaryocytes is shown in **Fig. 1a**.

2 Evaluation of platelet attachment to megakaryocytes

Platelet attachment to the megakaryocyte membrane was assessed. Platelet attachment was considered positive when at least one platelet was found in contact with a megakaryocyte **(Fig. 1b)**. The number of platelet attachments was recorded for each megakaryocyte. An ocular micrometer was used to calibrate the Flovel Filing System.

3 Statistical analysis

Statistical analyses were conducted using the Mann-Whitney U test, Fisher's exact test, and Levene test to evaluate clinical background data, cell area, nuclear area, and platelet attachment data. Statistical computations were performed using EZR and JNOVI software[23)24)]. A P value of less than 0.05 was considered statistically significant.

4 Ethical considerations

This study protocol was reviewed and approved by the Ethics Committee of the Shinshu University School

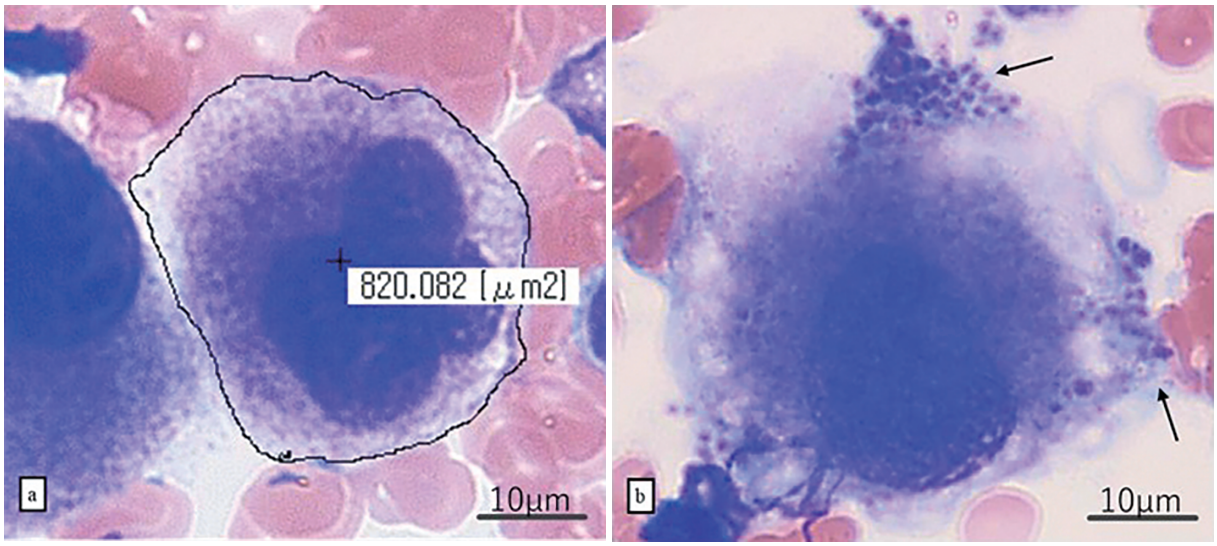


Fig. 1 Methods for evaluating megakaryocyte morphology.
a : Example of megakaryocyte area measurement. The margin of a megakaryocyte was digitally traced, and its area was calculated.
b : Observation of platelet attachment to megakaryocytes and profuse platelet production. Arrows indicate platelets attached to the surface of a megakaryocyte. The number of attached platelets was manually counted.

Table 1 Demogyaphycis of the subjects of this study

	patients N = 37	Control 1 N = 22	*P* value
Gender (Male / Female)	14 / 23	14 / 8	0.065
Age (y.o) ; median(range)	62.0 (18–87)	634 (20–83)	0.934
WBC (x10^3/μL) ; median(range)	6.1 (2.2–11.7)	5.9 (2.1–13.0)	0.839
RBC (x10^6/μL) ; median(range)	4.4 (2.5–5.6)	4.6 (2.8–5.9)	0.078
Hb (g/dL) ; median(range)	13.0 (5.8–16.8)	14.0 (9.4–16.5)	0.062
PLT (x10^3/μL) ; median(range)	1.7 (0–7.8)	23.6 (13.1–43.9)	1.86e–10
IPF (%) ; median(range)	13.8 (0–32.1)	2.4 (12–11.5)*	0.001

y.o ; years old. WBC ; white blood cell. RBC ; red blood cell. Hb ; hemoglobin. PLT ; platelet. IPF ; immature platelet fraction. * ; N = 13.

of Medicine (approval number 5,123).

Ⅲ Results

A Patient demographics

The clinical background of each group is summarized in **Table 1**. The ITP group consisted of 37 cases, with a median age of 62.0 years and a predominance of female patients. The age distribution in the ITP group exhibited two peaks, with 62 years serving as the median boundary. Key laboratory findings included a median white blood cell count of 6.1×10^3/μL and a median hemoglobin level of 13.0 g/dL. The median platelet count and immature platelet fraction (IPF %) were 1.7×10^4/μL and 13.8 %, respectively, which were significantly different compared with the control groups.

Control Group 1 included 14 men and 8 women, with a median age of 63.5 years. The median platelet count and IPF% in Control Group 1 were 23.6×10^4/μL and 2.4 %, respectively. The breakdown of lymphoma subtype is shown in **Supplemental Table 1**.

Control Group 2 consisted of 13 men and 11 women, with a median age of 42.0 years (range : 25–60 years). The median platelet count was 26.1×10^4/μL (range :

Supplemental Table 1　Lymphoma subtype in Control 1 group

diagnosis	N
Diffuse large B-cell lymphoma	9
Follicular lymphoma	4
Mucosa-associated lymphoid tissue lymphoma	3
T-cell rich B-cell lymphoma	1
Primary cutaneous T-cell lymphoma	2
Peripheral T-cell lymphoma	1
CD20-positive B-cell non-Hodgkin's lymphoma	1
B-cell Lymphoma	1

Supplemental Table 2　Demographycis of the subjects of Control 2

	Control 2 N = 24
Gender (M/F)	13/11
Age (y.o) ; median(range)	42.0 (25-60)
WBC (x$10^3/\mu$L) ; median(range)	5.5 (4.0-12.7)
RBC (x$10^6/\mu$L) ; median(range)	4.3 (3.0-5.3)
Hb (g/dL) ; median(range)	13.4 (9.6-16.1)
PLT (x$10^3/\mu$L) ; median(range)	26.1 (16.0-37.0)

y.o ; years old. WBC ; white blood cell. RBC ; red blood cell. Hb ; hemoglobin. PLT ; platelet.

Table 2　Morphological comparison of bone marrow megakaryooncytes in ITP and Control groups

	patients	Control 1	Control 2
Megakaryocyte examined	832	458	591
Cell major axis length (μm) ; median(range)	48 (15-142)	45 (21-115)	43 (12-108)
Cell area (μm^2) ; median(range)	1,477 (237-9,811)	1,344 (340-6,601)	1,293 (227-6,472)
Nuclear area (μm^2) ; median(range)	467 (47-1,775)	456 (91-1,476)	478 (56-1,984)

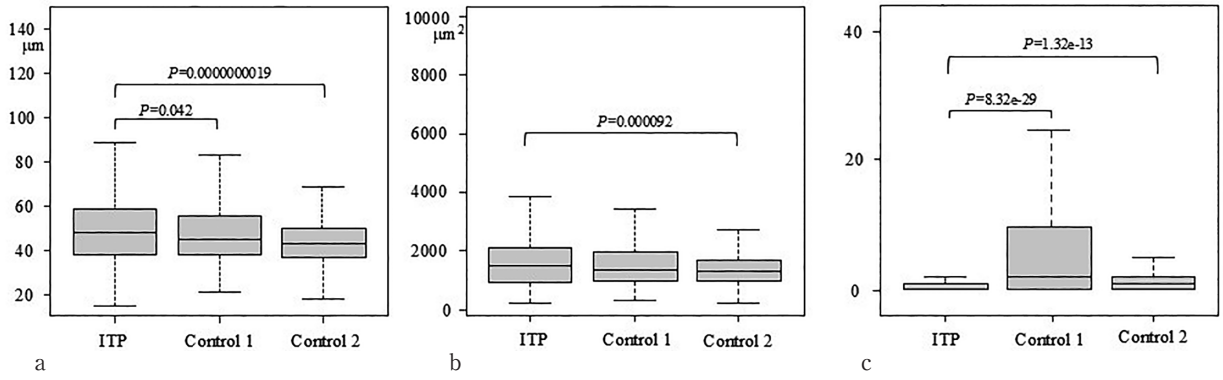


Fig. 2　Comparison of megakaryocyte morphology.
a : Megakaryocyte major axis length.
b : Megakaryocyte area.
c : Number of platelets attached to megakaryocytes.

16.0-37.0×$10^4/\mu$L, **Supplemental Table 2**).

B　Morphological analysis of megakaryocytes

The numbers of megakaryocytes examined were 832, 458, and 591 in the ITP group, Control 1 group, and Control 2 group, respectively. The results of the analysis of the major axis length, cell area, and nuclear area of megakaryocytes are presented in **Table 2** and **Fig. 2**.

A comparison of megakaryocyte major axis length, area, and nuclear area between the ITP group and Control Group 1 demonstrated that the cell major axis length was significantly larger in the ITP group. Additionally, a comparative analysis between the ITP group and Control Group 2, which included healthy individuals, revealed that the megakaryocyte major axis length and area were significantly larger in the

Table 3　megakaryocytes morphology in relation to age among ITP patients

	patients (≦62 y.o)	patients (>63 y.o.)	*P* value
Megakaryocyte examined	433	418	
Cell major axis length (μm) ; median(range)	50 (15–125)	46 (17–142)	0.056
Cell area (μm^2) ; median(range)	1,553 (304–9,030)	1,366 (237–9,811)	0.037
Nuclear area (μm^2) ; median(range)	486 (99–1,716)	457 (47–1,775)	0.078

y.o ; years old.

ITP group (*P* = 1.90e−9 and *P* = 1.32e−13, respectively). Comparison of nuclear area showed no significant differences between the ITP group and control 1 group and control 2 group (*P* = 0.400 and *P* = 0.730, respectively, **Table 2**).

Next, we evaluated the relationships between the morphological features of megakaryocytes and clinical factors in ITP, including age, *Helicobacter pylori* (*H. pylori*) infection status, and pregnancy in female patients. When patients were divided into two groups by age (≤62 years vs. >62 years), megakaryocytes in the younger group (≤62 years) were significantly larger in terms of megakaryocyte area compared to the older group **(Table 3)**. Eight patients were positive for *H. pylori*, but their megakaryocytes did not differ significantly from those without *H. pylori* infection **(Table 4a)**. Among female patients, pregnancy was significantly associated with larger megakaryocytes **(Table 4b)**.

In terms of treatments, thrombopoietin receptor agonists (TPO-RAs) have been widely utilized for refractory or relapsed ITP patients as a second-line therapy or later. Five patients who received TPO-RA treatment **(Supplemental Table 3)** had significantly larger megakaryocytes at presentation compared to those who did not require TPO-RA for their treatments **(Table 4c)**.

No significant differences were observed in the variations of megakaryocyte major axis length, area, and nuclear area between the ITP group and the Control1 group (*P* = 0.751, 0.967, and 0.327, respectively, **Supplemental Fig. 1**).

C　Platelet attachment to megakaryocytes

Platelets observed on the surface of megakaryocytes or in close attachment to them were considered to have been produced and recently released from the nearest megakaryocytes. This process may be altered in ITP.

To analyze the relationship between megakaryocytes and proximal platelets in ITP, a platelet-attached megakaryocyte was defined as one with at least one platelet observed on its surface, with no visible space between the platelet and the megakaryocyte **(Fig. 1b)**.

Among 832 megakaryocytes analyzed in the ITP group, 322 (39 %) were platelet-attached. In contrast, 271 out of 458 megakaryocytes (59 %) in Control Group 1 and 355 out of 591 megakaryocytes (60 %) in Control Group 2 had platelet attachments (*P* = 2.01e−12 and *P* = 1.91e−15, respectively, **Fig. 3a, b**).

The number of attached platelets on platelet-attached megakaryocytes was significantly lower in the ITP group compared to Control Group 1. However, no significant difference was observed between the ITP group and Control Group 2 **(Fig. 2c)**. Platelet adhesion and aggregation, which are calcium (Ca^{2+})-dependent processes, may have been inhibited in Control Group 2 specimens due to the absence of Ca^{2+} in anticoagulated samples.

In the ITP group, most platelet-attached megakaryocytes exhibited a small number of attached platelets. Specifically, 44 % of platelet-attached megakaryocytes in the ITP group had only one platelet, compared to 14 % in Control Group 1. Conversely, megakaryocytes with more than 10 attached platelets accounted for only 5 % in the ITP group, compared to 38 % in Control Group 1 **(Fig. 3c, d)**.

Among ITP subgroups, both the proportion of platelet-attached megakaryocytes and the number of attached platelets were significantly reduced in *H. pylori*-positive ITP cases (*P* = 0.039 and *P* = 0.004,

Table 4a Bone marrow megakaryocytes in terms of *Helicobacter pylori* infection

	H.P. negative	H.P. positive	*P* value
Megakaryocyte examined	650	182	
Cell major axis length (μm) median(range)	49 (15–142)	45 (19–89)	0.368
Cell area (μm^2) median(range)	1,509 (237–9,811)	1,377 (278–4,542)	0.279
Nuclear area (μm^2) median(range)	463 (47–1,716)	475 (66–1,775)	0.325
PLT attached rate (%)	264 (40.6 %)	58 (31.9 %)	0.039
PLT attached number median(range)	0 (0–36)	0 (0–6)	0.004

H.P. ; *Helicobacter pylori*. PLT ; platelet.

Table 4b Comparison of Megakaryocyte Morphology Between Pregnant and Non-Pregnant Females With ITP

	non-pregnant	pregnant	*P* value
Megakaryocyte examined	445	119	
Cell major axis length (μm) median(range)	46 (15–142)	53 (26–93)	0.0025
Cell area (μm^2) median(range)	1334 (237–9511)	1742 (357–3939)	0.000372
Nuclear area (μm^2) median(range)	453 (54–1775)	493 (99–1591)	0.0844
PLT attached rate (%)	162/445 (36.4 %)	50/119 (42.0 %)	0.463
PLT attached number median(range)	0 (0–19)	0 (0–9)	0.268

PLT ; platelet.

Table 4c Comparison of Megakaryocyte Morphology Based on Indication of Thrombopoietin Receptor Agonist

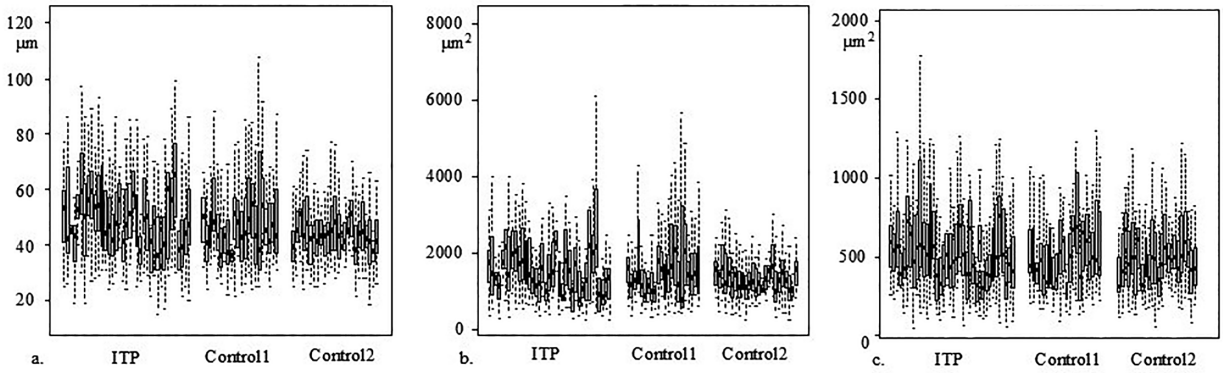
	patients treated with TPO-RA	patients without TPO-RA	*P* value
Megakaryocyte examined	131	701	
Cell major axis length (μm) ; median(range)	50 (25–142)	47 (15–125)	0.012
Cell area (μm^2) ; median(range)	1,632 (348–9,811)	1,416 (237–9,030)	0.007
Nuclear area (μm^2) ; median(range)	557 (66–1,471)	456 (47–1,775)	0.0008
PLT attached MK (%)	46 (35.1 %)	276 (39.4 %)	0.375
PLT attached number ; median(range)	0 (0–36)	0 (0–8)	0.151

TPO-RA ; thrombopoietin receptor agonist. PLT ; platelet. MK ; megakaryocyte.

Supplemental Table 3 Therapies and their Efficacies for the patients with ITP

Treatment	N	Effective	No response
corticosteroid	19	11	8
H.P. eradication	8	4	4
splenectomy	4	3	1
High dose intravenous immunoglobulin	5	3	2
TPO-RA	5	2	3

H.P. ; *Helicobacter pylori*, TPO-RA ; thrombopoietin receptor agonist.



Supplemental Fig. 1　Comparison of variance in megakaryocyte morphology in individual patient with ITP and control subjects.

The abscissa indicates each patient or control subject, while the ordinate represents the median value and the interquartile range (1Q to 3Q) for each parameter.

a ; megakaryocyte major axis length, b ; megakaryocyte area, c ; nuclear area of megakaryocyte.

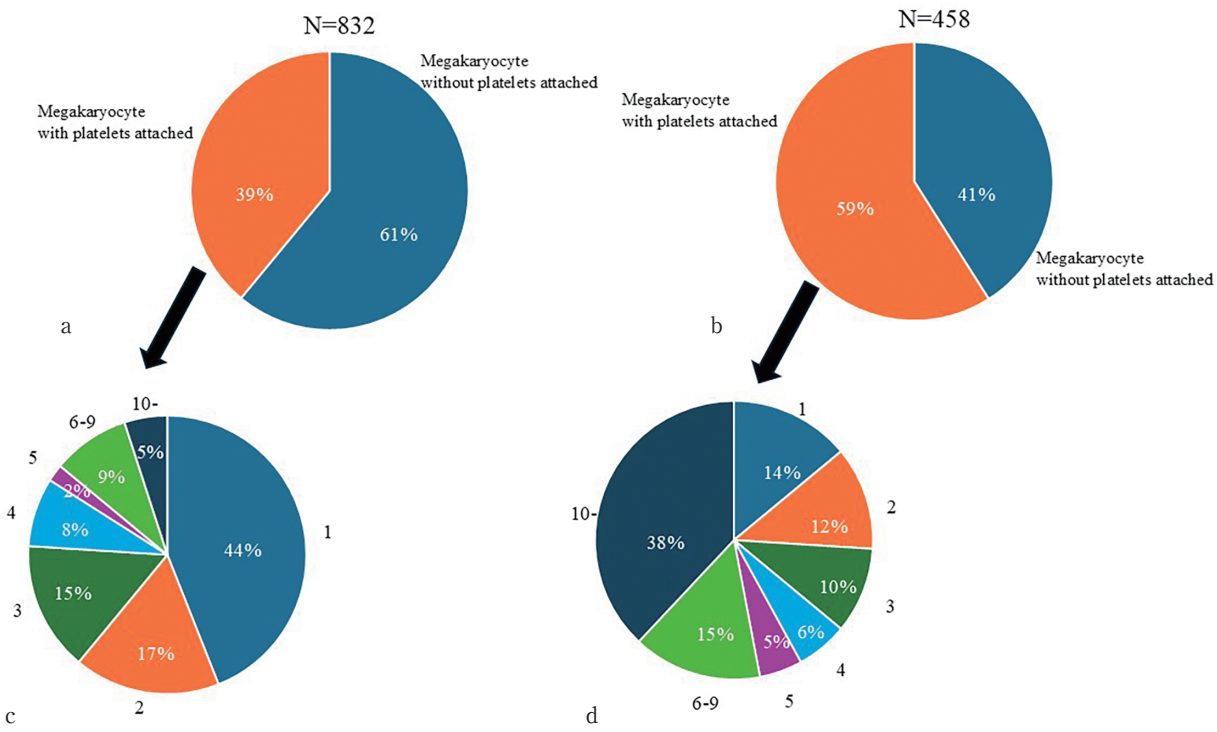


Fig. 3　Distribution of attached platelet numbers to each megakaryocyte.

Megakaryocytes with or without platelets attached to their surfaces in the ITP group (a) and Control Group 1 (b) and their breakdown of the number of attached platelets among megakaryocytes with platelet attachments in the ITP group (c) and Control Group 1 (d).

Among the megakaryocyte with platelets on their surface, 86 % of megakaryocytes in the ITP group exhibited five or fewer platelet attachments, while only 5 % had 10 or more platelet attachments. In contrast, in Control Group 1, 47 % of the megakaryocytes with platelet attached had five or fewer platelet attachments, and 38 % had 10 or more platelet attachments.

respectively, **Table 4a**). Other factors, such as pregnancy status or the need for TPO-RA treatment, did not significantly influence these parameters **(Table 4b, c)**.

Ⅳ Discussion

In this study, we found that megakaryocytes in ITP patients had a longer major axis length compared to both control groups and a larger size compared to one control group. Larger megakaryocytes were also observed in the younger population with ITP, suggesting that age might influence megakaryocyte size. Measuring the precise size of megakaryocytes in routine clinical practice is challenging due to the cumbersome procedures and time required to evaluate multiple cells. Furthermore, the differences in the major axis length or area between ITP and controls are often too subtle to assess visually.

Branehög et al. reported that megakaryocyte area and volume in ITP patients were larger than in healthy and control groups, along with a higher proportion of immature megakaryocytes in the ITP group[25]. Our findings are generally consistent with their study. However, their study analyzed tissue specimens, targeted a relatively younger population of ITP patients, and employed different analytical methods.

Previous reports have described immature morphology in ITP megakaryocytes, including degenerative nuclear changes, vacuole formation, and reduced cytoplasmic granules, indicative of cellular damage[26][27]. Plasma from ITP patients administered to healthy recipients showed decreased platelets and similar organelle changes, suggesting antiplatelet antibody-mediated damage to megakaryocytes[27]. However, our study did not assess qualitative morphological details due to the limited quality of available bone marrow smears. Future studies using higher-quality samples from healthy controls without anticoagulants and employing advanced morphological analyses are warranted.

One limitation of our study is the lack of comparisons between ITP and other thrombocytopenic diseases. For example, micromegakaryocytes are characteristic of dysmegakaryopoiesis in myelodysplastic syndromes. The size of megakaryocytes could potentially serve as a marker to differentiate these diseases.

We also observed a significantly lower proportion of platelet-attached megakaryocytes in ITP compared to the control groups. This phenomenon, often noted anecdotally in clinical practice, lacked robust evidence until now. Our findings confirmed a reduction in the number of attached platelets, consistent with morphological changes in ITP megakaryocytes. However, a significant number of megakaryocytes in the control groups also lacked platelet attachments. Without appropriate disease controls, establishing a definitive cutoff value for platelet-attached megakaryocytes in ITP remains challenging.

In ITP patients complicated by *H. pylori* infection, antibodies against the cytotoxin-associated antigen A (CagA) component of *H. pylori* may cross-react with platelet antigens, contributing to thrombocytopenia[28)-30)]. Megakaryocytes in these patients exhibited fewer attached platelets, suggesting potential targeting by anti-CagA antibodies.

Megakaryocyte area and major axis length were larger in ITP patients complicated by pregnancy compared to non-pregnant ITP patients. Within the ITP group, younger patients had significantly larger megakaryocyte areas, likely reflecting age-related differences. Pregnancy-specific changes may also influence megakaryocyte morphology. Previous studies have reported higher serum thrombopoietin levels and megakaryocyte hypoplasia in pregnant ITP patients, suggesting a distinct pathology[31)32)]. Further research is needed to elucidate the relationship between pregnancy and megakaryocyte morphology.

TPO-RAs are widely used for refractory ITP and have proven highly effective. In our study, megakaryocytes in patients requiring TPO-RAs were larger than those in patients who did not require such treatments. Emerging therapies, including SYK inhibitors and anti-FcRn-targeted agents, offer additional options for refractory ITP. Morphological evaluations of megakaryocytes may provide insights into predicting treatment response and clinical evaluation of ITP refractoriness.

This study exclusively examined bone marrow

smears. Morphological evaluations of megakaryocytes using formalin-fixed, paraffin-embedded tissue specimens are routine in clinical practice for thrombocytopenic patients. Comparative studies using different sample types are necessary to validate our findings.

Reticulated platelets have been highlighted as a useful tool for differentiating thrombocytopenia, including ITP[19]. In this study, the immature platelet fraction (IPF), measured as the reticulated ratio (RR), was significantly elevated in the ITP group. However, in one case with severe thrombocytopenia, the IPF could not be measured, highlighting the need for caution in its application.

V Conclusion

Megakaryocytes in ITP exhibit distinct morphological features, including larger size, a wider size distribution, and fewer attached platelets compared to controls. These findings highlight the potential of megakaryocyte morphology as a diagnostic marker for ITP. Integrating these morphological observations into advanced laboratory tests may enhance strategies for the differential diagnosis and management of thrombocytopenic disorders.Further research is essential to refine these diagnostic tools and deepen our understanding of the clinical significance of megakaryocyte morphology in ITP and related conditions. Such investigations may also provide insights into optimizing treatment strategies and improving patient outcomes.

References

1) Karpatkin S, Siskind GW : In vitro detection of platelet antibody in patients with idiopathic thrombocytopenic purpura and systemic lupus erythematosus. Blood 33 : 795-812, 1969
2) Karpatkin S, Strick N, Karpatkin MB, Siskind GW : Cumulative experience in the detection of antiplatelet antibody in 234 patients with idiopathic thrombocytopenic purpura, systemic lupus erythematosus and other clinical disorders. Am J Med 52 : 776-785, 1972
3) Lightsey AL Jr, McMillan R, Koenig HM, Schanberger JE, Lang JE : In vitro production of platelet-binding IgG in childhood idiopathic thrombocytopenic purpura. J Pediatr 88 : 415-418, 1976
4) Nielsen OH, Tuckuviene R, Nielsen KR, Rosthoj S : Flow cytometric measurement of platelet-associated immunoglobulin in children with newly diagnosed Immune Thrombocytopenia. Eur J Haematol 96 : 397-403, 2016
5) Arnason JE, Campigotto F, Neuberg D, Bussel JB : Abnormalities in IgA and IgM are associated with treatment-resistant ITP. Blood 119 : 5016-5020, 2012
6) Neunert C, Lim W, Crowther M, Cohen A, Solberg L Jr, Crowther M : The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia Blood 117 : 4190-4207, 2011
7) George JN, Woolf SH, Raskob GE, et al : Idiopathic thrombocytopenic purpura : a practice guideline developed by explicit methods for the American Society of Hematology. Blood 88 : 3-40, 1996
8) Grossi A, Vannucchi AM, Casprini P, et al : Different patterns of platelet turnover in chronic idiopathic thrombocytopenic purpura. Scand J Haematol 31 : 206-214, 1983
9) Ballem PJ, Segal GM, Stratton JR, Gernsheimer T, Adamson JW, Slichter SJ : Mechanisms of thrombocytopenia in chronic autoimmune thrombocytopenic purpura. J Clin Invest 80 : 33-40, 1987
10) Louwes H, Zeinali Lathori OA, Vellenga E, de Wolf JT : Platelet kinetic studies in patients with idiopathic thrombocytopenic purpura. Am J Med 106 : 430-434, 1999
11) Chow L, Aslam R, Speck ER, et al : A murine model of severe immune thrombocytopenia is induced by antibody- and CD8+ T cell-mediated responses that are differentially sensitive to therapy. Blood 115 : 1247-1253, 2010
12) McKenna JL, Pisciotta AV : Fluorescence of megakaryocytes in idiopathic thrombocytopenic purpura (ITP) stained with fluorescent antiglobulin serum. Blood 19 : 664-675, 1962

13) Chang M, Nakagawa PA, Williams SA, et al : Immune thrombocytopenic purpura (ITP) plasma and purified ITP monoclonal autoantibodies inhibit megakaryocytopoiesis in vitro. Blood 102 : 887–895, 2003
14) McMillan R, Luiken GA, Levy R, Yelenosky R, Longmire RL : Antibody against megakaryocytes in idiopathic thrombocytopenic purpura. JAMA 239 : 2460–2462, 1978
15) Hoffman R, Zaknoen S, Tang HH, et al : An antibody cytotoxic to megakaryocyte progenitor cells in a patient with immune thrombocyopenic purpura. N Engl J Med 312 : 1170–1174, 1985
16) Fujisawa K, O'Toole TE, Tani P, et al : Autoantibodies to the presumptive cytoplasmic domain of platelet glycoprotein IIIa in patients with chronic immune thrombocytopenic purpura. Blood 77 : 2207–2213, 1991
17) Kuwana M, Kaburaki J, Ikeda Y : Autoreactive T cells to platelet GPIIb–IIIa in immune thrombocytopenic purpura. Role in production of anti–platelet autoantibody. J Clin Investig 102 : 1393–1402, 1998
18) Stasi R : Immune thrombocytopenia : Pathophysiologic and clinical update. Semin. Thromb Hemost 38 : 454–462, 2012
19) Kashiwagi H, Kuwana M, Murata M, et al : Diagnostic Reference Guide 2023 Edition of Adult immune thrombocytopenic purpura. Rinsho Ketsueki 64 : 1245–1257, 2023
20) McMillan R, Wang L, Tomer A, Nichol J, Pistillo J : Suppression of in vitro megakaryocyte production by antiplatelet autoantibodies from adult patients with chronic ITP. Blood 103 : 1364–1369, 2004
21) Stahl CP, Zucker–Franklin D, McDonald TP : Incomplete antigenic cross–reactivity between platelets and megakaryocytes : Relevance to ITP. Blood 67 : 421–428, 1986
22) Olsson B, Ridell B, Carlsson L, Jacobsson S, Wadenvik H : Recruitment of T cells into bone marrow of ITP patients possibly due to elevated expression of VLA–4 and CX3CR1. Blood 112 : 1078–1084, 2008
23) jamovi (Version 2.5) [Computer Software].The jamovi project (2024). (Accessed 29th Seprember, 2024, at https://www.jamovi.org)
24) Kanda Y : Investigation of the freely available easy–to–use software 'EZR' for medical statistics. Bone Marrow Transplantation 48 : 452–458, 2013
25) Branehög I, Kutti J, Ridell B, Swolin B, Weinfeld A : The relation of thrombokinetics to bone marrow megakaryocytes in idiopathic thrombocytopenic purpura (ITP). Blood 45 : 551–562, 1975
26) Diggs LW, Hewlett JS : A study of the bone marrow from 36 patients with idiopathic hemorrhagic, thrombopenic purpura. Blood 3 : 1090–1104, 1948
27) Pisciotta AV, Stefanini M, Dameshek W : Studies on platelets. X. Morphologic characteristics of megakaryocytes by phase contrast microscopy in normals and in patients with idiopathic thrombocytopenic purpura. Blood 8 : 703–723, 1953
28) Perez–Perez GI, Dworkin BM, Chodos JE, Blaser MJ : Campylobacter pylori antibodies in humans. Ann Intern Med 109 : 11–17, 1988
29) Takahashi T : Molecular mimicry by Helicobacter pylori CagA protein may be involved in the pathogenesis of H. pylori–associated chronic idiopathic thrombocytopenic purpura. Br J Hematol 124 : 91–96, 2004
30) Franceschi F, Christodoulides N, Kroll MH, Genta RM : Helicobacter pylori and Idiopathic Thrombocytopenic Purpura. Ann Int Med 140 : 766–767, 2004
31) Moreira MB, Sharma A, Bussel JB : Immune thrombocytopenia and pregnancy : challenges and opportunities in diagnosis and management. Expert Review of Hematol 17 : 595–607, 2024
32) Zhang X, Zhao Y, Li X, et al : Thrombopoietin : a potential diagnostic indicator of immune thrombocytopenia in pregnancy. Oncotarget 7 : 7489–7496, 2016

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