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Original Article

Splenic megakaryocyte density in immune thrombocytopenia: insights from a histologic analysis of splenectomy specimens

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Abstract: Objectives: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by platelet destruction, for which multiple pathogenic mechanisms have been proposed. However, the spleen, as the principal site of platelet clearance, plays a pivotal role in its pathogenesis. The objective of this study was to evaluate the histopathologic features of splenectomy specimens in patients with ITP, with a particular emphasis on the presence of megakaryocytes (MKCs) in the spleen and their relationship with clinical factors. Methods: We examined spleen specimens from 16 patients with ITP and 10 controls, analyzing several aspects, including MKC density, white pulp (WP) histology, and immune cell markers. Results: There was a higher MKC density in the spleens of patients with ITP in comparison to control spleens ($P < 0.0001$). The MKC density was positively correlated with platelet transfusion requirements, indicating severe disease progression. WP atrophy was also associated with high MKC count and high platelet transfusion volume. Conclusions: These findings suggest that elevated MKC density in the spleens of patients with ITP may reflect distinct pathophysiologic pathways, possibly involving B cell-independent mechanisms. MKC density may serve as a simple and practical histologic marker for evaluating disease severity in patients with ITP.

Keywords: Immune thrombocytopenia (ITP), spleen, megakaryocyte, white pulp, extramedullary hematopoiesis, histopathology

Introduction

Immune thrombocytopenia (ITP) is an acquired form of thrombocytopenia that results from the deposition of autoantibodies or immune complexes against platelets. ITP is divided into two major groups: acute ITP, which primarily occurs in children and chronic ITP, which occurs in adults [1-3]. The annual incidence of ITP in adults and children has been reported to be 3.3 and 1.9-6.4 per 10000 population, respectively [4]. Although the two groups have different clinical characteristics, they are considered the same in terms of pathophysiology. Previous research suggests that the pathophysiology of ITP involves platelets, opsonized by anti-platelet antibodies, undergoing accelerated clearance from the circulation by the phagocytic system. This process primarily occurs via macrophages expressing Fcγ receptors, predominantly in the spleen and liver [5, 6]. Recently, the inhibition of megakaryocyte (MKC) formation by cytotoxic T cells has been proposed as another pathogenic mechanism [7, 8]. The pathology and pathophysiology are assumed to be complex, and it has been suggested that this may not be a homogeneous disease [9-11].

Steroid treatment is the most common first-line therapy and most patients show an early response. However, this therapy is ineffective for some patients with ITP during the first or middle stage [12-14]. Immunosuppressive drugs, including rituximab, are used as second-line therapies [15]. Several thrombopoietin receptor agonists (TPO-RAs) have been used as molecular targeted therapies, and tyrosine kinase inhibitors have recently been introduced [16-18].

The spleen is the main site of platelet destruction and antibody production; therefore, it is considered to play an important role in the pathophysiology of ITP [19-21]. Therefore, splenectomy is a therapeutic modality for ITP, which is performed for patients who do not respond to steroids or who require high doses of steroids [22]. Splenectomy for ITP reportedly results in sustained remission in 60-70% of patients [23, 24]. Pathologic findings in the removed spleen include hyperplasia of the white pulp (WP) and a predominance of platelet-phagocytosing macrophages [25, 26]. However, these findings only support the validity of the clinical diagnosis, and clinical relevance of histologic findings for predicting treatment efficacy and prognosis remains unclear.

Although the spleen is involved in the pathogenesis of ITP, only a few studies have evaluated the detailed histopathologic characteristics of splenectomy specimens from patients [27-31]. In addition, most of these studies were conducted before the era of TPO-RA for ITP treatment. The emergence of MKCs in the spleens of patients with ITP has been reported in several studies [31, 32]. This is thought to represent extramedullary thrombocytopoiesis. Some studies have also reported erythropoiesis or leukocytopoiesis in the spleens of patients with ITP [33-35]. Interestingly, MKCs were observed in the spleens of the patients with ITP, which is the primary site for platelet destruction.

In this study, we evaluated the histopathologic characteristics of splenectomy specimens from patients with ITP, with a particular focus on MKCs, and their correlation with clinicopathologic factors. The aforementioned studies merely described the appearance of MKC; however, the present study conducts a comparative analysis of its characteristic clinical factors and details the histopathologic changes in the spleen, in sharp contrast to the prior studies.

Materials and methods

Case selection

We selected 16 of splenectomy cases with ITP for which clinical data were available from three hospitals. Spleen specimens from 10 patients who had undergone splenectomy for inflammatory and neoplastic diseases without splenic involvement were selected as controls. Ethical approval was obtained from the relevant institutional review board.

Immunohistochemical staining

Tissue samples were fixed in formalin and embedded in paraffin. A representative section was evaluated for each case. Tissue sections (thickness: 2 μm) were subjected to standard immunohistochemical staining for CD61 (1:1 dilution; clone 2f2; Leica Biosystems, Nussloch, Germany), CD3 (1:1 dilution; clone LN10; Leica Biosystems), CD68 (1:100 dilution; clone PG-M1; DAKO, Glostrup, Denmark), CD138 (1:1 dilution; clone B-A38; Nichirei Biosciences, Tokyo, Japan), and PD-1 (1:100 dilution; clone NAT105; Abcam, Cambridge, UK) using a Ventana Discovery XT autostainer (Ventana Medical Systems Inc., Tucson, AZ, USA), or manual protocol. Specimens subjected to H&E and immunohistochemical staining were converted to whole-slide images using a NanoZoomer S60 Digital Pathology slide scanner (Hamamatsu Photonics K.K., Hamamatsu, Japan) and were subsequently analyzed.

Histological analysis of the spleen

The dimensions of the monitor utilized in the study were 473.8 mm × 296.1 mm, which equates to a screen area of 0.14029218 m^2. A single field of view at 40× magnification on the screen was equivalent to 0.0913 mm^2, and was referred to as a high-power field (HPF).

The following histopathologic findings were examined with reference to the results of immunohistochemical staining of the respective blood cells: (a) the area of the representative section (mm^2); (b) MKC density (i.e., number of MKCs in the red pulp (RP) of the whole section per mm^2); (c) histologic pattern of the WP; hyperplastic WP (HWP) pattern, non-activated WP (nAWP) pattern or WP-depleted (WPD) pattern; (d) lymphoid follicle (LF) density (i.e., LF area per square mm^2); (e) marginal zone (MZ) density (i.e., MZ area per square mm^2); (f) LF/MZ ratio (i.e., LF width/ MZ width); (g) perivascular T-cell sheaths (i.e., the width of perivascular T-cell sheaths μm); (h) T follicular helper cell (Tfh) density (i.e., the number of PD-1 positive T-cells in LF per HPF); (i) plasma cell density (i.e., the number of CD138 positive plasma cells per HPF); and (j) macrophage density (i.e., the number of CD68 positive cells in RP per HPF) [27, 28].

Clinical factors of the cases

Information on age, sex, serum platelets (10^4/μL), the dosage ofsteroids equivalent to prednisolone (PSL) (mg/kg/day), intravenous immunoglobulin (IvIg) and platelet transfusion (U) in the hospital was obtained from medical records. The steroid dosage and platelet transfusion volume were calculated by dividing the total quantity administered throughout the entire preoperative hospitalization period for surgery by the number of days steroids were administered. The outcome of splenectomy was assessed according to the International Working Group (IWG) criteria: CR (i.e., platelet count ≥ 10 × 10^4/μL and absence of bleeding), R (i.e., platelet count ≥ 3 × 10^4/μL and at least 2-fold increase in the baseline count and absence of bleeding), and NR (i.e., platelet count < 3 × 10^4/μL or a < 2-fold increase in the baseline count or bleeding) [36].

Statistical analysis

First, the number of MKCs in the ITP and control groups was compared. We divided the cases into two groups: high MKC (≥ 10/100 mm^2) (n = 3) and low MKC (< 10/100 mm^2) (n = 13) groups based on the number of MKCs in the spleen. We assessed the correlations between the number of MKCs in the spleen and clinicopathologic factors. Correlations between histologic changes in the spleen and clinicopathologic factors were also assessed. Continuous variables were compared between the ITP and control groups using the Mann-Whitney U test. Correlations among MKC density, platelet transfusion volume, and histologic findings were assessed using Spearman's rank correlation. Categorical variables were analyzed using Fisher's exact test. All analyses were performed using GraphPad PRISM (ver. 6.0; GraphPad Software Inc., La Jolla, CA, USA). *P* values of < 0.05 were considered significant.

Results

Number of MKCs in the spleen and statistical analyses

Details of the clinical data for the ITP cases and control cases included in the study are presented in **Table 1A** and **1B**. The study included 4 males and 9 females. Two of these patients were < 20 years of age. Steroid therapy and IVIg were administered in most but not all cases. Platelet transfusion was administered during hospitalization in three cases. TPO-RAs were introduced in 7 cases. MKCs were mainly detected in the RP of both ITP and control cases (**Figures 1**, **2**; **Table 2**). Immature MKCs with small nuclei and minimal cytoplasm were also abundant. The appearance of erythroblasts and myelocytes was unclear. The median MKC density of the ITP group was 1.23/100 mm^2 and that of the control group was 0. Significantly more MKCs were found in the spleen of the ITP group (P < 0.0001). Moreover, 3 patients in the ITP group showed a markedly increased MKC density. Thus, the MKC density in splenectomy specimens showed a bimodal distribution in ITP cases. We divided the ITP group into a high MKCs group (≥ 10/100 mm^2, n = 3) and a low MKCs (< 10/100 mm^2, 13 cases) based on the MKC density. There were no significant differences in the clinical factors (age, sex, and administration of TPO-RAs) between the two groups. The differences in steroid dosage (prednisolone equivalent), immunoglobulin

Table 1A. Clinical data of ITP patients who underwent splenectomy

	Age (year old)	Sex	Serum platelet count (just before surgery) (10^4/μL)	Serum platelet count (nadir) (10^4/μL)	PSL (mg/kg/day)	IvIg (mg/kg/day)	Platelet transfusion (U)	Thrombopoietin receptor agonists (/day)	Splenectomy outcome
Case 1	71	Female	23.6	0.6	0.42	300	PC 10×14 days	Romiplostim 480 μg	CR
Case 2	47	Male	3.5	2.9	0	330	0	0	CR
Case 3	8	Female	14.6	5.3	0	470	0	Elrombopag 25 mg	CR
Case 4	57	Male	4.0	0.6	0.14	340	PC 10×6 days	Romiplostim 250 μg	CR
Case 5	15	Male	16.0	11.9	0	1000	0	0	CR
Case 6	32	Female	13.2	5.0	0.22	300	0	0	R
Case 7	74	Female	6.9	2.0	0.52	330	PC 10×3 days	0	NR
Case 8	41	Female	19.2	19.6	0.39	0	0	0	CR
Case 9	66	Female	14.3	1.5	0.37	430	0	0	CR
Case 10	41	Female	11.1	11.5	0.51	230	0	0	CR
Case 11	50	Female	32.2	8.9	0.7	0	0	0	CR
Case 12	39	Female	8.9	0.3	0.8	340	0	0	NR
Case 13	24	Male	28.8	0.5	0.07	70	0	Romiplostim 300 μg	CR
Case 14	66	Female	6.7	4.8	0.39	0	0	Eltrombopag 12.5 mg	OCD
Case 15	70	Female	22	4.7	1.29	360	0	Eltrombopag 50 mg	CR
Case 16	51	Female	36.2	3.9	0.41	400	0	Elrombopag 50mg	CR

Abbreviations: nadir, minimum value within 30 days prior to splenectomy; PSL, prednisolone; IvIg, intravenous immunoglobulin; PC, platelet concentrate; OCD, other cause of death.

Table 1B. Clinical data of control cases Steroids and IVIg were not administered

	Age (year old)	Sex	Serum platelet count (10^4/μL)	Disease	Surgical procedure
Control Case 1	70	Female	25.1	Gastric carcinoma	Total gastrectomy and splenectomy
Control Case 2	56	Male	24.3	Pancreatic inflammatory myofibroblastic tumor	Distal pancreatectomy
Control Case 3	68	Male	26.9	Pancreatic pseudocyst	Distal pancreatectomy
Control Case 4	71	Female	20	Pancreatic body carcinoma	Distal pancreatectomy
Control Case 5	76	Male	19.6	Pancreatic intraductal papillary mucinous adenoma	Distal pancreatectomy
Control Case 6	79	Female	34.7	Gastric carcinoma	Total gastrectomy and splenectomy
Control Case 7	67	Male	26.8	Gastric carcinoma	Total gastrectomy, splenectomy and cholecystectomy
Control Case 8	77	Male	19.3	Pancreatic body carcinoma	Distal pancreatectomy with en bloc celiac axis resection
Control Case 9	71	Male	39.4	Gastric carcinoma	Total gastrectomy, splenectomy, left adrenalectomy and cholecystectomy
Control Case 10	73	Female	18.1	Pancreatic body carcinoma	Distal pancreatectomy and left adrenalectomy

Steroids and IVIg were not administered.

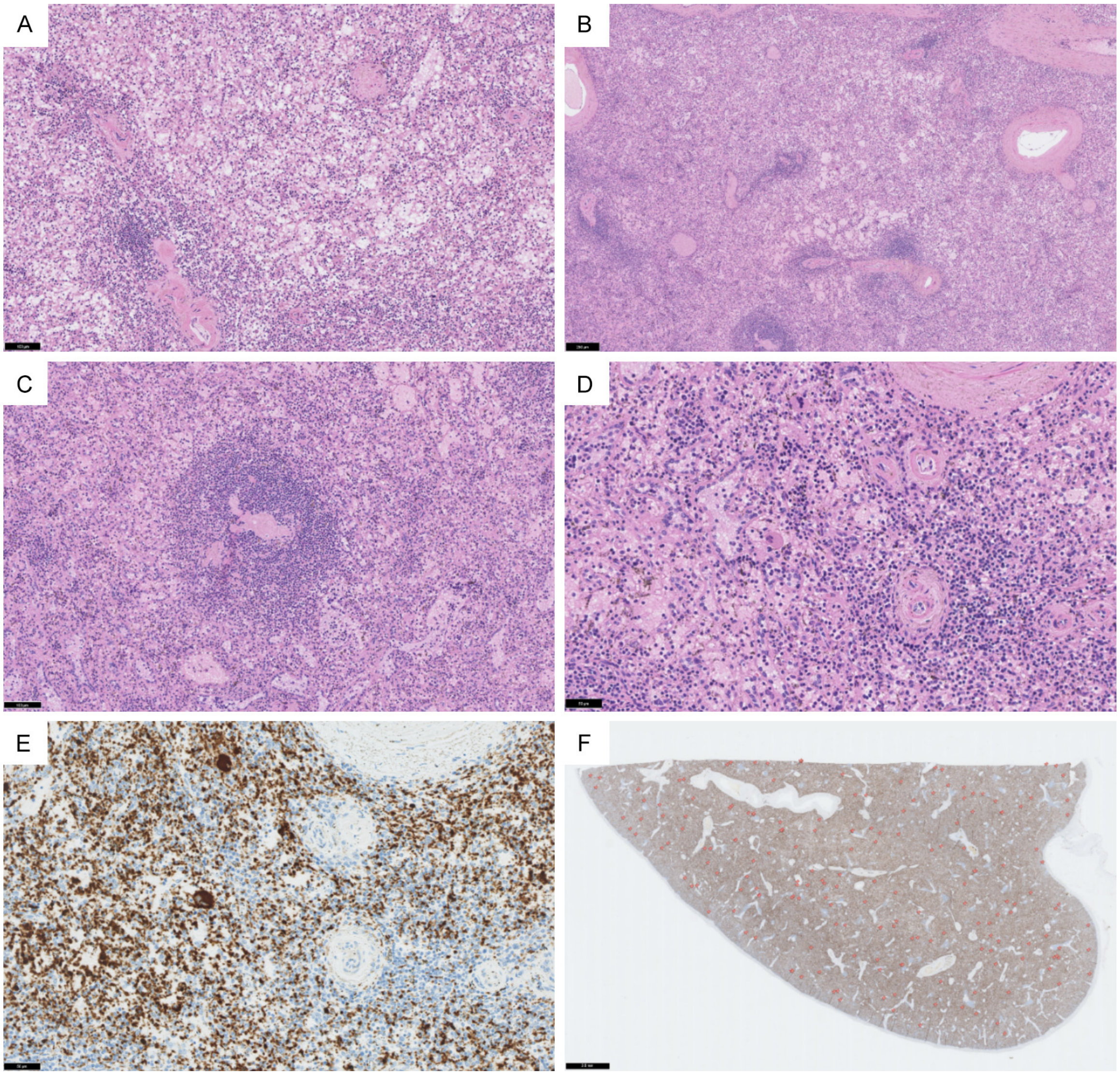

Figure 1. Histopathology image of Case 1. A: Hemotoxylin and eosin (H&E) staining of the spleen (×3.89). Atrophic white pulp (WP) and red pulp (RP) with a prominent increase in histiocytes were observed. B: H&E staining. Close-up view of the RP (×9.72). Histiocytes phagocytose phospholipids with clear, abundant cytoplasm. C: H&E staining. Close-up view of the WP (×9.75). Lymphoid follicles were atrophied. D: H&E staining. Megakaryocytes (MKCs) were observed in the RP (×22.04). Nuclear lobulation was not conspicuous and contained little cytoplasm, which is indicative of immature MKCs. E: Immunohistochemical staining for CD61 (×36.87). Cytoplasmic staining was observed, but nuclear staining was absent. F: Loupe view of immunohistochemical staining for CD61 (×0.48). Pins indicate the respective MKC.

dosage, and platelet transfusion volume during hospitalization in the high and low MKCs groups, and the correlation between MKC density and PSL dosage, immunoglobulin dosage, and platelet transfusion during hospitalization are shown in **Table 3**. The high MKC group had a significantly higher number of platelet transfusions during hospitalization, and a positive correlation was observed between the number of MKCs in the spleen and the platelet transfusion volume (**Table 4**).

Histologic change in the spleen and statistical analysis

The results of the histologic analysis of ITP cases are shown in **Table 5**. Two cases showed the HWP pattern, 7 showed the nAWP pattern, and 7 showed the WPD pattern. The correlations between MKC density and histological changes is shown in **Table 4**. The MKC density and LF/MKC ratio showed a negative correlation (P = 0.0128). There was no correlation

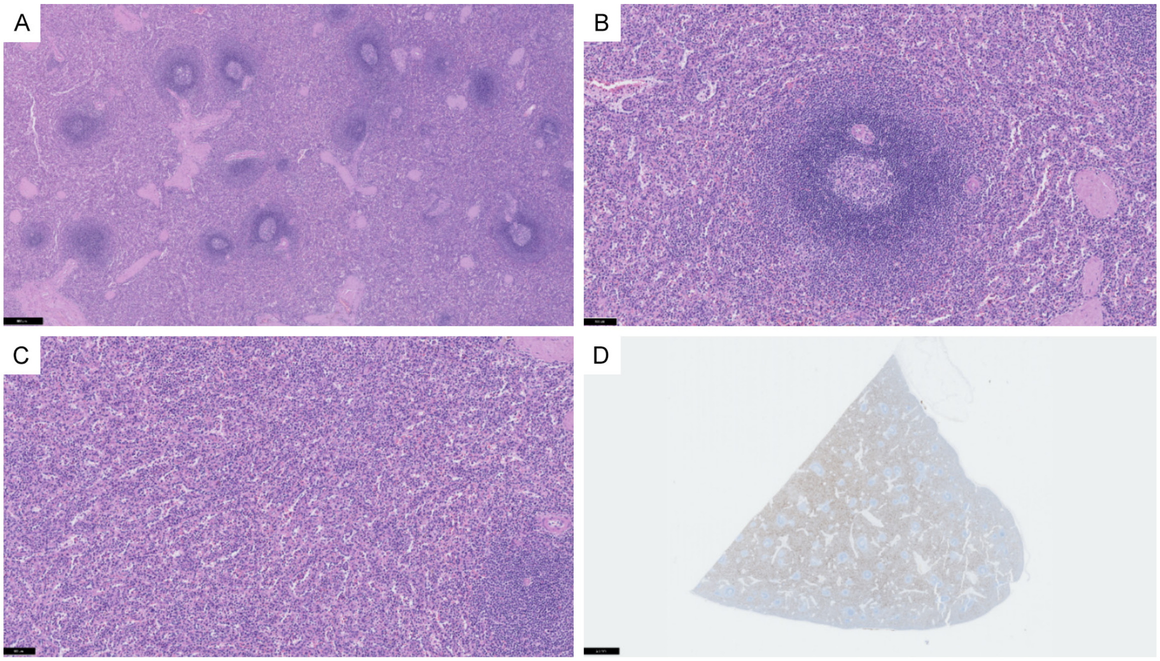

Figure 2. Histopathologic image of Case 12. A: Hemotoxylin and eosin (H&E) staining of the spleen (×1.69). Lymphoid follicles are prominent in white pulp (WP). B: H&E staining. Close-up view of the WP (×9.58). Active lymphoid follicles and abundant marginal zones were observed. C: H&E staining. Close-up view of the red pulp (×11.4). Mild congestion is also observed. Megakaryocytes (MKCs) was not evident on H&E staining. D: Loupe view of immunohistochemical staining for CD61 (×0.42). MKCs were not present. Note: Prominent white pulp with germinal centers can be physiologic in children; interpretation reflects age-appropriate histology.

Table 2. MKC density of the ITP and control cases

	Number of MKCs	Area of the section (mm^2)	MKC density (/100 mm^2)		Number of MKCs	Area of the section (mm^2)	MKC density (/100 mm^2)
Case 1	173	348	49.7	Control Case 1	0	489	0
Case 2	7	360	1.94	Control Case 2	0	308	0
Case 3	4	278	1.44	Control Case 3	0	429	0
Case 4	155	374	41.4	Control Case 4	1	366	0.273
Case 5	3	382	0.785	Control Case 5	0	352	0
Case 6	2	410	0.488	Control Case 6	0	273	0
Case 7	27	381	7.09	Control Case 7	0	332	0
Case 8	2	294	0.680	Control Case 8	0	327	0
Case 9	3	421	0.713	Control Case 9	3	421	0.713
Case 10	4	278	1.44	Control Case 10	0	401	0
Case 11	144	447	32.2				
Case 12	0	246	0				
Case 13	9	408	2.21				
Case 14	4	392	1.02				
Case 15	1	309	0.32				
Case 16	0	312	0				

between MKC density and other histologic findings. An analysis of the platelet transfusion volume during hospitalization and histologic changes in the spleen showed that the platelet transfusion volume had a strong negative correlation with LF density, MZ density, and the LF/MZ ratio ($P < 0.0001$), as well significant negative correlations with the perivascular T-cell

Table 3. Clinical features and statistical analysis of each group

	High MKC group	Low MKC group	*p*-value
Male (case)	1	3	
Female (case)	2	10	P = 1.0
Administration of TPO-RA			
(+) (case)	2	5	
(-) (case)	1	8	P = 0.55
Age (year)	57	41	P = 0.2321
Steroid dosage (mg/kg/day)	0.420	0.390	P = 0.6089
Immunoglobulin dosage (mg/kg/day)	300	330	P = 0.4357
Platelet transfusion volume (U)	140	0	P = 0.025

Steroid dosage, immunoglobulin dosage, and platelet transfusion volume are shown as the median values. The steroid dosage was the prednisolone equivalent.

sheath and plasma cell density (P = 0.0321 and P = 0.044, respectively).

Discussion

Splenectomy is highly effective in the treatment of refractory ITP because the spleen plays an important role in the pathophysiology of ITP [5, 6, 19, 23, 24]. The removal of platelets from peripheral blood, which is the main pathology of ITP, is primarily carried out by macrophages in the spleen. Platelets are opsonized by disease-specific anti-platelet antibodies, and phagocytosis by macrophages is enhanced by Fc receptors. Macrophages then present platelet-derived antigens and activate CD4+ T-cells. Activated T cells activate B cells, which become autoreactive B cells, and B cells further differentiate into plasma cells and produce anti-platelet antibodies [10, 37]. Antibody-producing cells are found in various locations, including the peripheral blood and bone marrow; however, the spleen is thought to be the main site of antibody production [19]. The presence of MKCs in the spleen, as reported in this study, suggests extramedullary hematopoiesis. In adults, extramedullary hematopoiesis can occur in multiple organs, including the liver and spleen. However, in the pathophysiology of ITP, the spleen is primarily responsible for antiplatelet antibodies and platelet phagocytosis. Therefore, it is noteworthy that extramedullary hematopoiesis was observed in the spleen. In the present study, the number of MKCs in splenectomy specimens was microscopically counted. Significantly more MKCs cells were found in patients with ITP than in non-ITP cases. The presence of MKCs in the spleens of patients with ITP has been reported in several studies. To the best of our knowledge, in 1919, Kaznelson was the first to report the presence of MKCs in the spleens of patients with ITP [38]. In each report, MKCs without nuclear atypia were observed in RP. Bowman measured the number of MKCs and reported an average of 13/100 mm^2 in 45 patients with ITP [31]. Although the variance in MKC counts of individual cases in their study was unknown, the average of 13/100 mm^2 was similar to that in this study (8.84/100 mm^2). The number of cases in their study may have been either higher or lower than reported. They also studied a similar group of patients who died from trauma, brain tumors, and pneumonia with average MKC counts of 5/100 mm^2, 8/100 mm^2, and 19/100 mm^2, respectively. In cases of pneumonia, extramedullary hematopoiesis due to hemopoiesis caused by severe infections is likely to be considered; however, the large number of MKCs seen in cases of external causes or brain tumors is contradictory to this study and is very interesting. Hayse et al. and Delia et al. also mentioned megakaryopoiesis in the spleen [26, 32], but no quantitative evaluation was performed, and its relationship with clinical factors is not known. To the best of our knowledge, no other report has focused on MKCs and examined its correlation with the clinicopathologic features of ITP cases in as much detail as we did in this case. Few studies have examined the relationship between MKCs and more recently used TPO-RAs. At a glance, this fact seems to be merely extramedullary hematopoiesis induced by thrombocytopenia; however, we were able to divide ITP cases into two groups based on the presence or absence of MKCs. A clinicopathologic analysis was performed, and the number of MKCs in ITP cases correlated with platelet transfusion volume. Histologic changes in the spleen associated with platelet transfusion have not been previously reported. Therefore, the possibility of MKCs emerging in the spleen as a result of transfusion cannot be completely ruled out.

Table 4. Correlation of MKC density and platelet transfusion volume with treatments and histologic changes of the spleen

		Platelet in serum	PSL	IvIg	PC	LF density	MZ density	LF/MZ ratio	Perivascular T-cell sheaths	Tfh density	Plasma cell density	Macrophage density
MKC density	Spearman r	0.1237	0.1758	-0.1021	0.6481	-0.4516	-0.374	-0.5957	-0.09165	-0.1184	-0.1559	-0.04963
	p value	0.642	0.501	0.6918	0.0048	0.0663	0.1467	0.0128	0.7179	0.6453	0.5345	0.8414
Platelet transfusion volume	Spearman r					-0.3328	-0.4832	-0.4248	-0.1467	-0.05401	-0.1213	0.1903
	p value					< 0.0001	< 0.0001	< 0.0001	0.0321	0.0857	0.044	0.4661

Table 5. Analysis of the histologic change in ITP cases

	MKC density (/100 mm^2)	Histological pattern of WP	LF density (/mm^2)	MZ density (/mm^2)	LF/MZ ratio	Perivascular T-cell sheaths (μm)	Tfh density (/HPF)	Plasma cell density (/HPF)	Macrophage density (/HPF)
Case 1	49.7	WPD	0	0.0129	0	204.2	5	21	78
Case 2	1.94	nAWP	0.00595	0.0965	0.346	135.6	26	14	84
Case 3	1.44	nAWP	0.00155	0.0911	0.473	275.3	10	19	93
Case 4	41.4	WPD	0	0.0252	0	130.1	5	16	122
Case 5	0.785	nAWP	0.000188	0.0506	0.435	250.9	1	17	79
Case 6	0.488	nAWP	0.00430	0.0590	0.437	237.9	20	27	108
Case 7	7.09	WPD	0.00202	0.0361	0.388	147.1	43	21	78
Case 8	0.680	nAWP	0.00437	0.0518	0.482	141.3	16	15	98
Case 9	0.713	WPD	0.00143	0.0278	0.480	136.0	28	18	84
Case 10	1.44	nAWP	0.000166	0.0661	0.382	247.1	39	23	47
Case 11	32.2	WPD	0	0.00707	0	104.9	2	26	58
Case 12	0	HWP	0.0184	0.0534	0.575	177.8	65	19	40
Case 13	2.21	nAWP	0.00144	0.0510	0.418	271.2	69	19	68
Case 14	1.02	WPD	0	0.0495	0	146.6	3	25	68
Case 15	0.32	HWP	0.00215	0.0677	0.439	218.9	26	22	69
Case 16	0	WPD	0	0.0233	0	114.8	4	27	88

Histologic features of the spleen, previously proposed in the present study, were also examined [27, 28]. A significant negative correlation was observed between MKC density and the LF/MZ ratio. Furthermore, MKC density, LF density, MZ density, and LF/MZ ratio were found to be correlated with platelet transfusion volume. These results strongly suggested that the number of MKCs in the spleen was related to WP atrophy and ITP severity. Pizzi et al. reported a correlation between WP hyperplasia and disease activity [27, 28]. It is reasonable to posit that splenic WP plays a role in the immune response and activation of B cells, which in turn reflects the disease status of ITP. In summary, this is a visual representation of the pathophysiology of the immune system, with a particular emphasis on the role of B cells. Recent research has demonstrated that, in addition to anti-platelet antibodies, cytotoxic and regulatory T cells play a significant role in the pathogenesis of ITP [10, 37]. It is proposed that the cases exhibiting an elevated MKC density in this study may represent a cohort of highly active ITP cases undergoing a distinct pathway of B-cell-independent processes.

The present study carried several limitations. First, we were unable to examine the spleens of patients with thrombocytopenia due to causes other than ITP. Such comparisons may help identify histologic features specific to ITP. Second, bone marrow tissue could not be uniformly assessed, as biopsies were not available in all cases and some were obtained at outside institutions, making standardized evaluation difficult. Our findings demonstrated only a cross-sectional association between splenic MKC density and platelet transfusion volume during hospitalization. Because long-term outcomes were not evaluated, no conclusions could be drawn regarding the prognosis. A prospective study with standardized endpoints is necessary to address these questions. In this study, all available splenectomy specimens from patients with ITP were analyzed; nevertheless, the number of cases remained relatively small at 16. Although the cohort size was smaller than that reported by Pizzi et al. and Furudoï et al., the present investigation employed an objective whole-field counting method with area normalization (per 100 mm^2), thereby enhancing the reproducibility of measurements. Furthermore, our study systematically demonstrated cross-sectional correlations with clinical severity, as reflected by platelet transfusion volume and with WP morphology, underscoring its significance [27-29].

This study reported histologic changes in the spleens of patients with ITP and focused on MKCs. The number of MKCs was found to be significantly higher in some cases. The MKC density is correlated with platelet transfusion volume, which may reflect disease activity. Furthermore, a correlation with WP atrophy was observed, suggesting that MKCs appear in cases of increased disease activity through pathways other than B cells. In most cases, the removed spleen is subjected to a pathologic examination; however, it is challenging to identify clinically significant findings in spleen tissue. All the cases considered to have a significant number of MKCs in the present study had at least 100 MKCs per section. This density could be easily identified without the need for immunohistochemical staining.

Conclusion

We demonstrated that splenic MKC density was significantly elevated in patients with ITP in comparison to controls. A higher MKC density correlated with increased platelet transfusion requirements and atrophic changes in the WP, suggesting that MKC accumulation reflects greater severe disease activity. These findings indicate that MKC density may serve as a simple histologic marker of disease severity and could highlight alternative. B-cell-independent mechanisms in the pathophysiology of ITP. Although limited by a relatively small cohort and lack of long-term follow-up, this study provided new insights into the spectrum of the histologic findings in the spleens of ITP. Larger prospective studies are warranted to validate the prognostic value of splenic MKC density and to clarify its mechanistic role in ITP progression.

Disclosure of conflict of interest

None.

Abbreviations

ITP, immune thrombocytopenia; MKC, megakaryocyte; TPO-RA, thrombopoietin receptor agonist; WP, white pulp; HPF, high-power field; RP, red pulp; HWP, hyperplastic white pulp; nAWP, non-activated white pulp; WPD, white pulpdepleted; LF, lymphoid follicle; MZ, marginal zone; Tfh, T follicular helper cell; PSL, prednisolone; IvIg, intravenous immunoglobulin; IWG, International Working Group.

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