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BRIEF COMMUNICATION

Difference in megakaryocyte expression of GATA-1, IL-6, and IL-8 associated with maintenance of platelet counts in patients with plasma cell neoplasm with dysmegakaryopoiesis

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Dysmegakaryopoiesis is a diagnostic criterion for myelodysplastic syndrome (MDS), which accompanies thrombocytopenia. Recently, dysmegakaryopoiesis was reported in patients with plasma cell neoplasm (PCN). Although these patients maintained normal platelet counts, disease progressed, with most bone marrow cells being replaced by plasma cells. Several studies reported that dysmegakaryopoiesis was induced by inflammatory mechanisms; however, the exact mechanism underlying dysmegakaryopoiesis remains unknown. This study aimed to investigate whether changes in the megakaryocytic expression of GATA-1, pro-inflammatory cytokines (interleukin [IL]-6 and IL-8), and CD9 affect platelet counts and dysmegakaryopoiesis in patients with PCN and MDS. A total 114 patients were examined and categorized into four groups: MDS with dysmegakaryopoiesis (34 patients), PCN without dysmegakaryopoiesis (36 patients), PCN with dysmegakaryopoiesis (19 patients), and lymphoma without bone marrow infiltration (25 patients). Expression of GATA-1, IL-6, IL-8, and CD9 in megakaryocytes was assessed by immunohistochemical (IHC) staining of paraffin-embedded bone marrow sections. Localized expression of transcription factor and cytokines was observed in megakaryocytes. Furthermore, expression of GATA-1, IL-6, and IL-8 significantly differed (all p values < 0.05). Decreased GATA-1 expression was identified in MDS. Decreased IL-6 expression was observed in PCN and MDS. Moreover, decreased IL-8 expression was associated with dysmegakaryopoiesis, regardless of whether platelet counts were maintained. In conclusion, PCN patients with dysmegakaryopoiesis had normal platelet counts, and their megakaryocytes showed decreased IL-6 and IL-8 expression and normal GATA-1 expression. The differences in the megakaryocytic expression of cytokines in PCN and MDS with dysmegakaryopoiesis may be applicable to future therapeutic strategies. © 2019 Published by Elsevier Inc. on behalf of ISEH – Society for Hematology and Stem Cells.

Abnormal proliferation and maturation of megakaryocytes lead to dysmegakaryopoiesis, which subsequently results in the abnormal morphology of megakaryocytes. The mechanism underlying these effects is unknown. Dysmegakaryopoiesis is a key feature in diagnosing myelodysplastic syndrome (MDS), which is accompanied by peripheral thrombocytopenia. Moreover, dysmegakaryopoiesis is associated with plasma cell neoplasm (PCN), which is not accompanied by thrombocytopenia [1].

PCN differs from other diseases with respect to the microenvironment of bone marrow (BM). Malignant plasma cells bind to BM accessory cells, leading to the secretion of various cytokines [2]. Various cytokines are involved in the development of dysmegakaryopoiesis in

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patients with PCN, and platelets produced during this process may be retained in peripheral blood, unlike what occurs in patients with MDS. Therefore, to identify megakaryocytes, we selected CD61 and the following transcription factors and cytokines that may be associated with dysmegakaryopoiesis and platelet count: GATA-1, interleukin (IL)-6, IL-8, and CD9.

GATA-1 is a zinc finger transcription factor that belongs to the GATA family and is expressed in various cells, including hematopoietic lineages such as erythrocytes, megakaryocytes, mast cells, and eosinophils. It is related to the proper maturation of red blood cells and megakaryocytes [3,4]. Loss-of-function mutations of GATA-1 inhibit the differentiation and proliferation of hematopoietic cells, resulting in severe anemia, thrombocytopenia, and splenomegaly [3].

IL-6 is associated with megakaryopoiesis and increases the number of immature megakaryocytes, promotes maturation of megakaryocytes, and increases platelet counts in peripheral blood [5].

IL-8 is a well-known pro-inflammatory cytokine that functions by binding to G-protein-coupled receptors, CXCR1 and CXCR2. In contrast to normal individuals, patients with MDS and acute myeloid leukemia overexpress IL-8 and CXCR2 receptors [6].

CD9 is an early upregulator of hematopoietic progenitor cells and plays a role in megakaryocytopoiesis by participating in the membrane remodeling process [7]. CD9 expression in patients with PCN is decreased by epigenetic silencing of the gene by methylation [8] and is also decreased in CD34+ hematopoietic progenitor cells of patients with MDS [9].

This study aimed to investigate whether changes in the megakaryocytic expression of GATA-1, IL-6, IL-8, and CD9 affect platelet counts and dysmegakaryopoiesis in patients with PCN and MDS. Unlike previous studies, our study assessed the expression of transcription factors and cytokines in CD61-positive megakaryocytes using immunohistochemical (IHC) staining.

Methods

Study design

From January 2004 to July 2017, patients diagnosed with PCN and MDS were selected after review of their medical records. The selected patients were divided into four groups: group 1 (MDS with dysmegakaryopoiesis), group 2 (PCN without dysmegakaryopoiesis), group 3 (PCN with dysmegakaryopoiesis), and group 4 (nondiseased control group). Among patients with MDS, only those with dysplasia of megakaryocytes were selected. Dysmegakaryopoiesis is defined as more than 10% dysplastic megakaryocytes through ≥30 megakaryocytes counted on bone marrow (BM) aspiration smears. The morphological features of dysplastic megakaryocytes are micromegakaryocytes, multiple separated nuclei, and nuclear hypolobation that is discordant between nuclear and cytoplasmic maturation [10]. In addition to MDS patients, PCN patients were also evaluated for dysmegakaryopoiesis on the basis of these morphological features. Aged-matched patients with lymphoma without BM involvement were selected for the control group. This study was approved by the institutional review board of the Chung-Ang University Hospital (IRB No. 1709-014-16107).

Immunohistochemical staining

IHC staining using paraffin-embedded BM sections was performed. After deparaffinization, the IHC staining procedure followed the manufacturer's instructions. Each IHC-stained slide was evaluated based on the diffuseness and intensity scores [11]. Positivity was determined based on whether the cytoplasm or nucleus of megakaryocytes had granular or diffuse brown staining, which was compared with that of the nondiseased control group. The diffuseness (*d*) score was determined based on the percentage of positively stained megakaryocytes. The intensity (*i*) score was assessed by dividing the average staining intensity of positively stained megakaryocytes into four levels (none, 0; weak, 1; intermediate, 2; and strong, 3) and was determined as an average of 30 megakaryocytes randomly evaluated at × 400 magnification. IHC staining results were interpreted by two independent and trained reviewers.

Statistical analysis

Statistical analysis was performed using SPSS for Windows, Version 22.0 (IBM Corp., Armonk, NY), and the statistical significance of differences between the four groups was assessed using the Kruskal–Wallis test. The Mann–Whitney *U* test was performed as a post hoc test to confirm the statistical significance among the four groups. P values < 0.05 were considered to indicate statistically significant differences, and the values of each group are expressed as median values.

Results

In this study, we investigated whether changes in the megakaryocytic expression of GATA-1, IL-6, IL-8, and CD9 affected platelet counts and dysmegakaryopoiesis in patients with PCN and MDS. This study included 114 patients who were categorized into four groups: group 1 (34 patients); group 2 (36 patients); group 3 (19 patients); and group 4 (25 patients). Table 1 outlines the characteristics of each group. As illustrated in Figure 1, various morphological features of dysplastic megakaryocytes were identified in patients with PCN. The platelet count of group 3 ranged from 53.0 to 319.0×10^9/L, with an average of 145.0×10^9/L.

Based on IHC staining, our study results revealed statistically significant differences in *d* scores for GATA-1, IL-6, and IL-8 and *i* scores for GATA-1, IL-6, IL-8, and CD9 (Supplementary Table E1, online only, available at www.exphem.org). Lower GATA-1 *i* scores (1.21 vs. 1.50, $p = 0.021$) and higher IL-6 *i* scores (0.70 vs. 0.37, $p = 0.032$) were observed in patients with MDS than in those with PCN and dysmegakaryopoiesis (Figure 2). CD9

Table 1. Demographics of the 123 patients

	MDS ($n = 34$)	PCN without dysmegakaryopoiesis ($n = 36$)	PCN with dysmegakaryopoiesis ($n = 19$)	Non-diseased control ($n = 25$)	p value
Sex, male/female	28/6	23/13	12/7	14/11	
Age, median (range), years	71 (29–94)	75 (43–91)	71 (53–89)	56 (25–81)	
Dysmegakaryopoiesis, median (range), %	26.7 (10.0–85.0)	3.3 (0.0–6.7)	12.5 (10.0–100.0)	0.0 (0.0–0.0)	<0.0001
Plasma cells, median (range), %	0.5 (0.2–2.2)	11.2 (2.6–88.4)	21.8 (2.6–66.8)	1.0 (0.0–2.2)	<0.0001
WBC, median (range), $\times 10^9$/L	2.43 (0.77–9.11)	5.95 (2.12–17.20)	4.61 (0.98–9.20)	5.17 (4.4–8.62)	<0.0001
Hemoglobin, median (range), g/L	85 (70–154)	90 (23–144)	89 (65–122)	132 (113–146)	<0.0001
Platelets, median (range), $\times 10^9$/L	74.0 (21.0–176.0)	168.0 (42.0–546.0)	145.0 (53.0–319.0)	222.0 (166.0–364.0)	<0.0001

Hb=Hemoglobin; *MDS*=myelodysplastic syndrome; *PCN*=plasma cell neoplasm; *WBC*=white blood cell

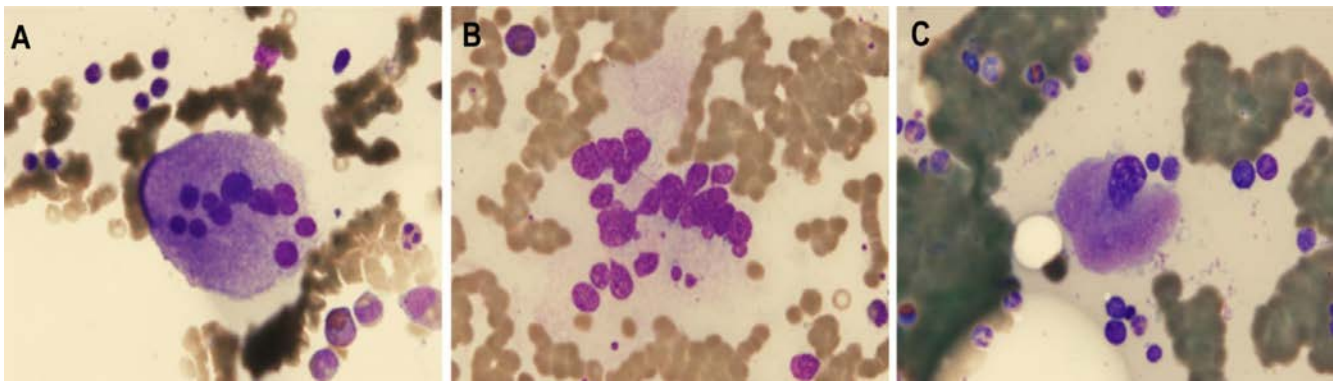

Figure 1. Morphologic manifestation of dysmegakaryopoiesis *i* score group 3 (plasma cell neoplasm with dysmegakaryopoiesis). **(A, B)** Indicated multinucleation. **(C)** Nuclear hypolobation. (Light microscope, Olympus, 100 × magnification)

i scores increased in patients with MDS and PCN compared with the nondiseased control group. IL-6 *i* scores and IL-8 *d* and *i* scores were statistically significant ($p = 0.002$, $p = 0.009$, and $p = 0.020$, respectively) when patients with PCN with and without dysmegakaryopoiesis were compared (Supplementary Figure E1, online only, available at www.exphem.org).

Discussion

The diffuseness and intensity scores of GATA-1 decreased in patients with MDS compared with those in the nondiseased control group and patients with PCN. The role of GATA-1 in vivo is unclear; however, it is known to affect the proliferation and differentiation of erythroid and megakaryocytic cells [3,12–14]. Previous studies [15] have reported reduced GATA-1 expression in MDS that has been associated with dyserythropoiesis. In another study, GATA-1–deficient megakaryocytes exhibited irregular morphologic characteristics of primary myelofibrosis (PMF) [12]. Our results are similar to those of previous studies, and thus, we can deduce that the decrease in human GATA-1 expression is closely related to the irregular morphology and faulty terminal differentiation of megakaryocytes in patients with MDS. In our study, the megakaryocytes of patients with PCN and dysmegakaryopoiesis showed a level of GATA-1 staining similar to that observed in the nondiseased control group, suggesting that GATA-1 does not play an important role in the mechanism of dysmegakaryopoiesis in patients with PCN.

IL-6 expression was reduced in patients with MDS and PCN compared with the nondiseased control group. IL-6 is an inflammation-mediated cytokine, and its elevated levels are associated with chronic inflammation, autoimmune diseases, and cancer development [16,17]. Additionally, IL-6 induces maturation of megakaryocytes as a thrombopoietic factor in vitro [16]. In cytokine gene polymorphism studies of patients with MDS, high IL-6 levels are associated with severe anemia and thrombocytopenia, thereby affecting the severity of cytopenia in patients with MDS [18]. Our results indicated that IL-6 may be involved in the development of PCN and MDS; however, these results contradict those of previous studies, probably because serum IL-6 concentrations were not measured and only megakaryocyte localization of IL-6 in BM biopsy using IHC staining was assessed. One study revealed that there was no significant difference in IL-6 expression between malignant and stromal cells, although there was difference in

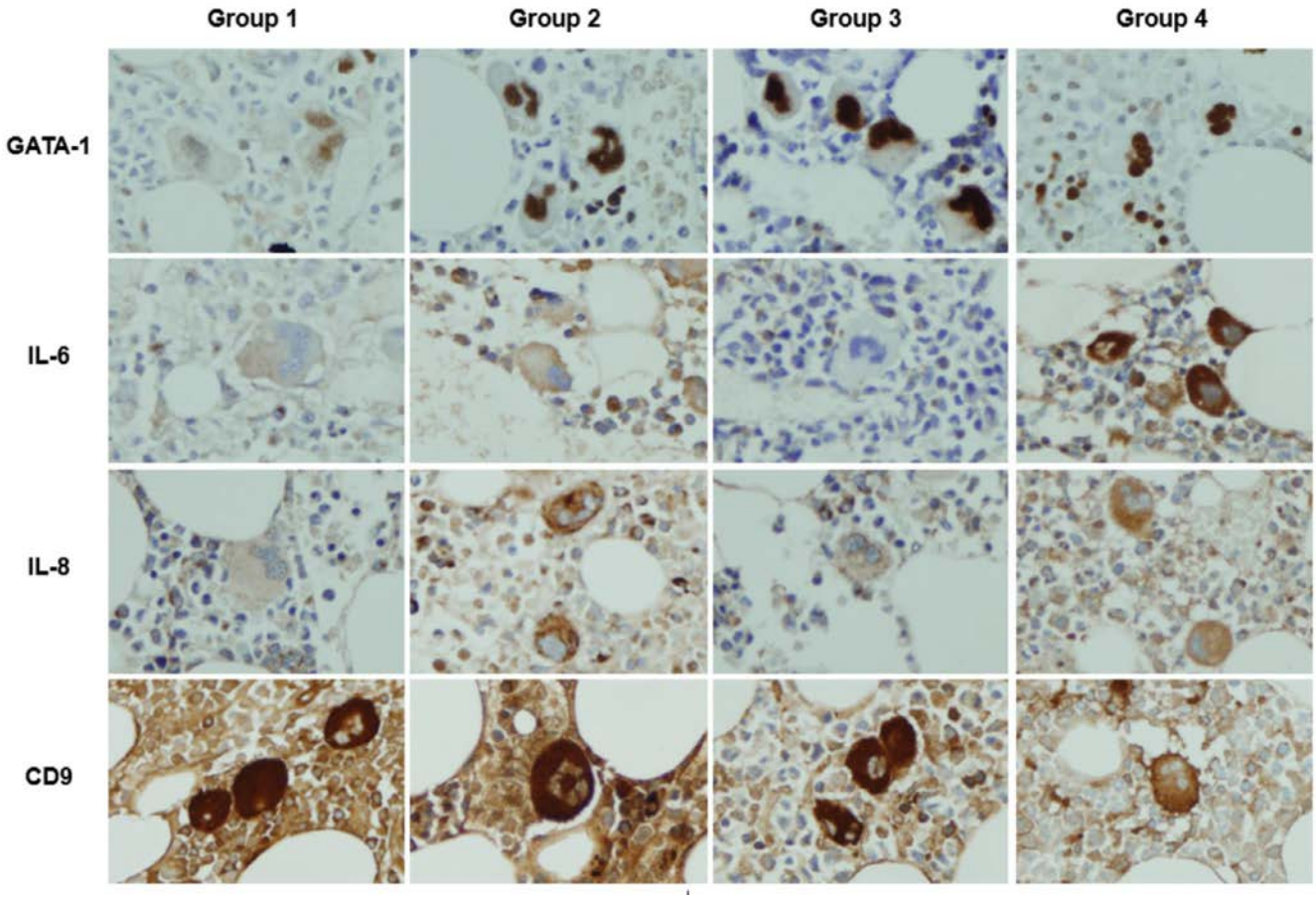

Figure 2. Megakaryocytic expression of GATA-1, IL-6, IL-8, and CD9 in each group: group 1 (myelodysplastic syndrome with dysmegakaryopoiesis), group 2 (plasma cell neoplasm without dysmegakaryopoiesis), group 3 (plasma cell neoplasm with dysmegakaryopoiesis), and group 4 (nondiseased control group). (Light microscope, Olympus, 40 × magnification)

serum IL-6 concentrations [19]. More research is necessary to determine whether there are any differences in serum and tissue IL-6 concentrations. IL-8 is reduced in patients with MDS and those with PCN and dysmegakaryopoiesis compared with that in patients with PCN but without dysmegakaryopoiesis and the nondiseased control group. Tefferi et al. [20] reported that plasma levels of IL-8 in PMF patients were higher than normal, and plasma IL-8 levels were associated with poor prognosis; however, the difference from our results is thought to be due to the difference in the sample used in the study, such as IL-6. Our results revealed a significant association between decreased IL-8 expression in megakaryocytes and dysmegakaryopoiesis. Our findings conclusively revealed that inflammatory cytokines IL-6 and IL-8 are downregulated in dysmegakaryopoiesis patients with or without thrombocytopenia, although this expression only identified megakaryocyte localization in BM biopsy using IHC staining.

CD9 is involved in hematopoietic cell differentiation and in the process of membrane remodeling of megakaryocytes [7] and may be different in MDS. CD9 gene expression differs between patients with PCN and MDS [8,9]; however, there was no significant difference between PCN and MDS in our study, suggesting the need for further investigation of CD9 in both diseases.

Our study has limitations. First, we could not compare serum cytokine concentrations. However, because we wanted to estimate the gene expression of megakaryocyte localization, we assessed the diffuseness and intensity of cytokine expression using IHC staining. Second, many cytokines influence dysmegakaryopoiesis and platelet counts; however, we selected only one transcription factor and three cytokines. Third, the number of patients included in each disease group was small.

Conclusions

Megakaryocytes in patients with PCN and dysmegakaryopoiesis exhibited decreased IL-6 and IL-8 expression but normal GATA-1 expression. The differences in the expression of cytokines in PCN and MDS with

dysmegakaryopoiesis may be helpful in illuminating the pathophysiology of dysmegakaryopoiesis and are applicable to future therapeutic strategies.

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Conflict of interest disclosure

The authors declare that there is no conflict of interest in relation to the publication of this article.

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Supplementary Files

Supplementary Table E1

Supplementary Table E1. Statistical comparison of IHC scoring between groups

		Mean rank	Median value	IQR*	χ^2	*p value*
GATA-1 d†	Group 1	42.44	0.74	0.22	11.095	0.011
	Group 2	68.03	0.87	0.15		
	Group 3	60.50	0.77	0.25		
	Group 4	60.54	0.80	0.32		
GATA-1 i‡	Group 1	40.47	1.21	0.59	13.299	0.004
	Group 2	67.28	1.66	0.76		
	Group 3	63.84	1.50	0.48		
	Group 4	61.76	1.50	0.59		
IL-6 d†	Group 1	55.15	0.49	0.80	20.409	<0.0001
	Group 2	51.49	0.45	0.91		
	Group 3	40.74	0.30	0.50		
	Group 4	82.10	0.86	0.14		
IL-6 i‡	Group 1	52.00	0.70	0.71	25.326	<0.0001
	Group 2	59.60	0.87	1.17		
	Group 3	32.05	0.37	0.53		
	Group 4	81.30	1.23	0.67		
IL-8 d†	Group 1	54.72	0.87	0.02	9.489	0.023
	Group 2	62.65	0.98	0.00		
	Group 3	44.24	0.90	0.07		
	Group 4	63.94	0.99	0.00		
IL-8 i‡	Group 1	44.62	0.97	0.17	13.623	0.003
	Group 2	71.10	1.34	0.55		
	Group 3	47.82	0.98	0.83		
	Group 4	62.80	1.20	0.29		
CD9 d†	Group 1	56.12	1.00	0.00	1.734	0.629
	Group 2	57.96	1.00	0.00		
	Group 3	56.47	1.00	0.00		
	Group 4	59.50	1.00	0.00		
CD9 i‡	Group 1	74.71	2.97	0.14	45.550	<0.0001
	Group 2	62.57	2.91	0.28		
	Group 3	66.92	2.97	0.40		
	Group 4	19.64	2.13	0.45		

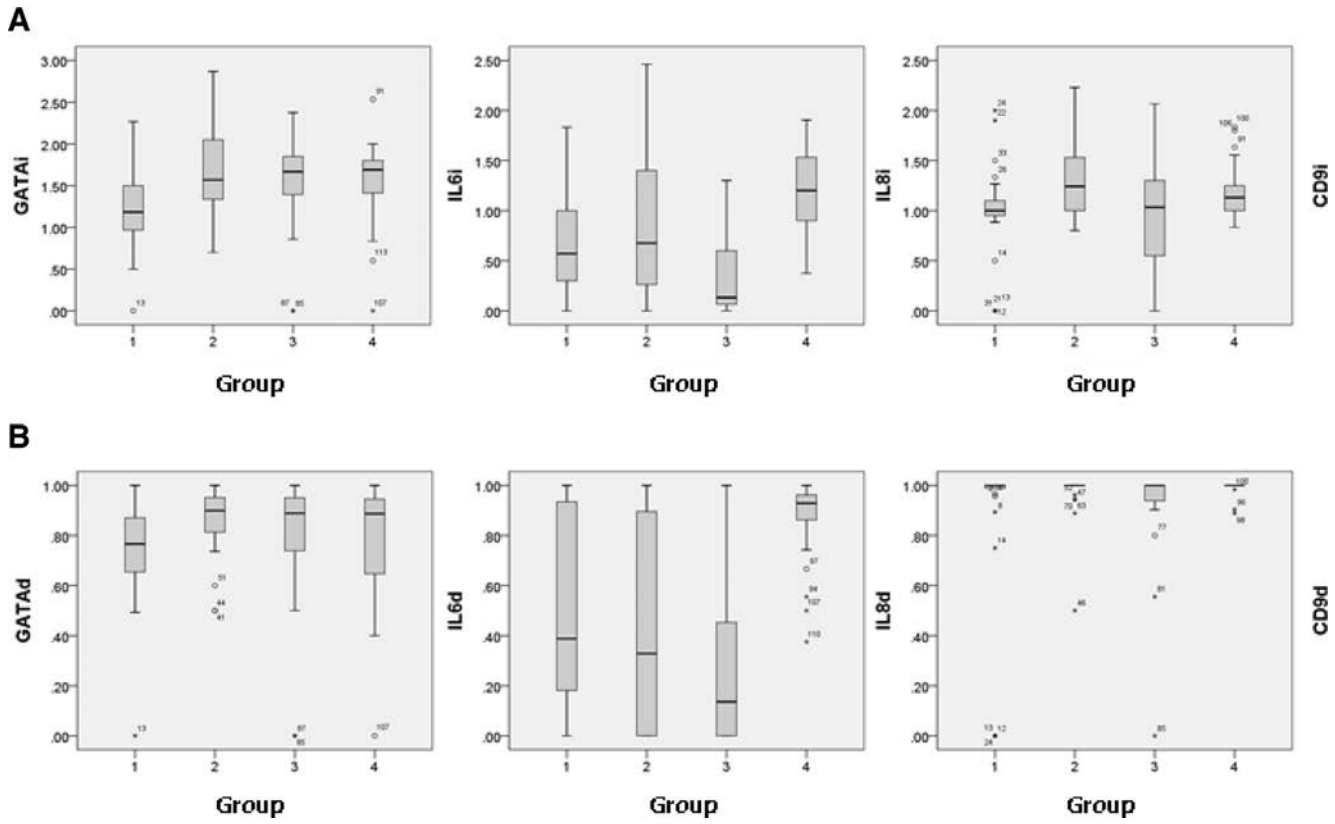
Group 1 (Myelodysplastic syndrome with dysmegakaryopoiesis), Group 2 (Plasma cell neoplasm (PCN) without dysmegakaryopoiesis), Group 3 (PCN with dysmegakaryopoiesis) and Group 4 (non-diseased controls).

*IQR, Interquartile range.

†d, diffuseness score.

‡i, intensity score.

Supplementary Figure E1



Supplementary Figure E1. Comparison of Intensity(A) and diffuseness(B) score by group. Group 1 (Myelodysplastic syndrome with dysmegakaryopoiesis), Group 2 (Plasma cell neoplasm (PCN) without dysmegakaryopoiesis), Group 3 (PCN with dysmegakaryopoiesis) and Group 4 (non-diseased controls)