Normal platelet counts mask abnormal thrombopoiesis in patients with chronic myeloid leukemia

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Abstract. Increased platelet heterogeneity has been reported in myeloproliferative neoplasms (MPN) with thrombocytosis. However, whether abnormal thrombopoiesis occurs in patients with chronic myeloid leukemia (CML) who have normal platelet counts, remains unclear. In order to explore this question, 25 patients with CML with normal platelet counts (CML-N), 40 patients with CML with elevated platelet counts (CML-E) and 33 healthy adults were recruited. The association of platelet count with mean platelet volume (MPV), platelet large cell ratio (P-LCR) and platelet distribution width (PDW) was examined. Bone marrow smears were also reviewed to assess the proliferation and abnormal lobation of megakaryocytes. The results showed that the two CML groups exhibited higher MPV, P-LCR and PDW values than those of the controls (P<0.05). Furthermore, the CML-N group was more heterogeneous in terms of thrombopoiesis than the CML-E group, as demonstrated by a higher PDW (P<0.05) and higher ratio of multinucleated dysmegakaryocytes (12.17 vs. 4.69%; χ^2=29.79; P=0.000). In addition, no correlation between platelet count and MPV, P-LCR or PDW was observed in the CML-N group (r=-0.102, -0.051 and -0.049, and P=0.619, 0.828 and 0.810, respectively). The results suggested that patients in the CML-N group have more heterogeneous thrombopoiesis of megakaryocytes and platelets, and that apparently normal platelet counts may mask the abnormal thrombopoiesis in these patients.

Introduction

Chronic myeloid leukemia (CML) is a clonal stem cell disorder that is characterized by the acquisition of an oncogenic BCR/ABL fusion protein and the proliferation of myeloid cells at all stages of development (1,2). In addition to marked granulocytic predominance, pathological and morphological findings show that the number of megakaryocytes in the chronic phase is frequently increased, and that these cells exhibit abnormal features (small with abnormal lobation) (2). Megakaryocytes are the source of platelet production, and dysmegakaryopoiesis implies dysregulated platelet production, involving dysmorphology (increased size, particularly giant platelets) (3,4), abnormal counts and impaired function (5-10).

In the chronic phase, although >50% of patients with CML develop thrombocytosis, there are a number of patients with normal platelet counts (CML-N) (11). In order to assess whether normal platelet counts mask abnormal platelet production in CML, platelet parameters, including mean platelet volume (MPV), platelet large cell ratio (P-LCR), and platelet distribution width (PDW), were compared among patients with CML with elevated platelets (CML-E), CML-N and healthy controls. In addition, correlations between the platelet count and these parameters were analyzed. Furthermore, bone marrow smears were reviewed in order to examine the association between platelet production and the proportion of dysmegakaryocytes (abnormal lobation) in patients with CML. It was hypothesized that in patients with CML, normal platelet counts do not indicate normal platelet production, and that apparently normal counts may mask disordered production.

Patients and methods

Subjects. A total of 65 patients with chronic-phase CML were recruited from April 2010 to March 2014 (Anhui provincial hospital, Hefei, China). This study was conducted according to the World Medical Association Declaration of Helsinki and approved by the Institutional Ethics Committee of Anhui Provincial Hospital. Informed consent was obtained from participants and all patients with CML were diagnosed according to the WHO 2008 guideline (2), which included examination of cell morphology and the BCR-ABL fusion gene. None of the patients had been previously treated with any BCR-ABL-targeted drugs. The patients in the present study were divided into two groups: Those with normal platelet counts (100-300x10^9/l)

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(CML-N) and those with elevated platelet counts (>300x10^9/l) (CML-E). Thirty-three healthy adults were enrolled as healthy controls. Patient characteristics are provided in Table I.

Blood routine examination. Blood samples were collected in tubes containing EDTA and examined using the fully automated hematology analyzer XE-5000 (Sysmex, Kobe, Japan). In addition to routine measurements of red blood cell (RBC) and white blood cell (WBC) counts, the platelet parameters evaluated for the purpose of the current study were: Platelet count, MPV, P-LCR and PDW. A normal platelet count in the Chinese population is 100-300x10^9/l. The results are summarized in Table I.

Assessment of megakaryocytopoiesis. Bone marrow smears were stained with Wright's-Giemsa (Baso Diagnostics Inc., Zhuhai, China), and observed by light microscopy (BX41; Olympus Corporation, Tokyo, Japan). Megakaryocyte proliferation was evaluated using the following method: For each sample, 50-100 fields were examined under low power (x20 objective). The total number of megakaryocytes in each field was counted, and the average number of megakaryocytes was then calculated (number of megakaryocytes/number of fields examined). The average number of megakaryocytes was used as an indicator by which to assess the proliferation of megakaryocytes. When performing the assessment of proliferation, dysmorphic megakaryocytes were recorded and their numbers were analyzed separately. Dysmegakaryocytes were divided in to two groups, according to the pattern of abnormal lobulation: Hypolobation (mononuclear) and multinucleation (two or more round separated nuclei; Fig. 1).

Statistical analysis. Statistical analysis was performed using SPSS 12.0 software (SPSS, Inc., Chicago, IL, USA). An independent t-test was used to compare platelet parameters and megakaryocyte proliferation in the different groups. Pearson's correlation test was performed to examine the association between platelet count, and MPV, P-LCR and PDW. χ^2 test was used to compare the constituent ratio of dysmegakaryocytes within the two CML groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Elevated MPV and P-LCR in patients with CML. Analysis using an independent-samples t-test, demonstrated that the MPV and P-LCR in patients with CML were significantly higher than those of healthy controls ($P<0.05$), regardless of whether the platelet count was elevated. No significant differences were detected between the two CML groups ($P>0.05$; Table II, Fig. 2A and B).

Increased PDW in the CML-N group. Further analysis using an independent-samples t-test, demonstrated that the PDW in the CML-N group was significantly higher than that in the CML-E group ($P<0.05$), while the latter was significantly higher than that of healthy controls ($P<0.05$; Table II, Fig. 2C).

Platelet count is not correlated with MPV, P-LCR or PDW in patients with CML-N. Analysis using Pearson's correlation test, demonstrated inverse correlations between platelet count and MPV, P-LCR and PDW in the healthy control and CML-E groups, while no correlations between these parameters were observed in the CML-N group (Fig. 3).

Proliferation and lobulation of megakaryocytes is altered in patients with CML. As platelets are known to be produced by megakaryocytes, bone marrow smears were examined, and the proliferation and lobulation of megakaryocytes was analyzed. Analysis using an independent-samples t-test, demonstrated that the proliferation of megakaryocytes was higher in the CML-E group than that in the CML-N group ($P=0.032$). Furthermore, the results of the χ^2 test, demonstrated a higher constituent ratio of multinucleated megakaryocytes in the CML-N group than in the CML-E group (12.17 vs. 4.69%; $\chi^2=29.79$; $P=0.000$), while no difference in hypolobation was detected between the two CML groups (10.08 vs. 11.88%; $\chi^2=0.626$; $P=0.429$).

Discussion

Patients with CML often exhibit morphological abnormalities in megakaryocytes, which are characterized by small and abnormal lobation features. Platelet production represents the final stage of megakaryocyte development and dysmegakaryopoiesis may result in platelet disorders, involving dysmorphology, abnormal counts and impaired function (3,9,10). In hematological malignancies, such as myelodysplastic syndrome (MDS), essential thrombocythemia, and other myeloproliferative or myeloproliferative/myelodysplastic neoplasms, abnormal platelet counts usually reflect altered thrombopoiesis (12). However, normal counts in patients may not be evidence that the process of platelet production is unimpaired. In the present study, patient in the chronic phase of CML were recruited and divided into two groups: Patients with normal platelet counts (CML-N) and patients with elevated platelets (CML-E). Although each of the CML groups exhibited higher MPV, P-LCR and PDW than the healthy controls, PDW was higher in the CML-N than that in the CML-E group. Furthermore, no correlation between platelet count and MPV, P-LCR or PDW was observed in the CML-N group. These results indicated that patients with CML with a normal platelet count have a higher PDW, while the normal association between platelet count and other platelet parameters is lost. The reason for this is unclear.

In order to address this question, bone marrow smears were examined. A difference in the proportion of dyslobated megakaryocytes was observed between the CML groups, and a higher proliferation of megakaryocytes was observed in the CML-E group. Dyslobated megakaryocytes, including cells with hypolobation (mononuclear) and multinucleation (binucleate or more round separated nuclei) are a pathological feature of MDS (13), and hypolobated megakaryocytes are also frequently observed in patients with CML (2). Bone marrow examination in the present study, demonstrated that in addition to hypolobation, a number of multinucleated megakaryocytes were also observed in patients with CML, and the ratio of dyslobated megakaryocytes to megakaryocytes was higher in the CML-N group than that in the CML-E group.

Table I. Patient characteristics.

Characteristic	Patients with CML		Healthy controls (n=33)
	With normal platelet count (n=25)	With elevated platelet count (n=40)	
Age, years	42 (15-82)	43 (16-81)	46 (17-74)
Gender, M/F	14/11	31/9	18/15
WBC (10^9/l)	198.44 (129.36)	179.37 (80.55)	5.99 (1.31)
PLT (10^9/l)	188.88 (69.39)	541.70 (222.28)	224.88 (37.52)
MPV (fl)	11.60 (1.06)	11.22 (1.05)	10.66 (0.78)
PDW (fl)	15.54 (2.99)	13.93 (2.72)	13.07 (1.74)
P-LCR (%)	37.79 (8.35)	34.19 (7.91)	30.23 (6.7)
BCR-ABL1 fusion gene	Positive	Positive	NA

Values for age are given as median (range). Values for WBC, PLT, MPV, P-LCR and PDW, are given as mean (standard deviation). M/F, male/female; WBC, white blood cell count; PLT, platelet count; MPV, mean platelet volume; PDW, platelet distribution width; fl, femtoliters; P-LCR, platelet large cell ratio; CML, chronic myeloid leukemia; NA, not applicable.

Table II. P-values from independent-samples t-test analysis in the different groups.

Groups	Platelet parameter		
	MPV	P-LCR	PDW
CML-N vs. CML-E	0.173	0.109	0.032[a]
CML-N vs. Controls	0.001[a]	0.001[a]	0.000[b]
CML-E vs. Controls	0.015[a]	0.028[a]	0.037[a]

[a]P<0.05, [b]P<0.001. MPV, mean platelet volume; P-LCR, platelet large cell ratio; PDW, platelet distribution width; CML, chronic myeloid leukemia; CML-N, patients with CML with a normal platelet count; CML-E, patients with CML with an elevated platelet count.

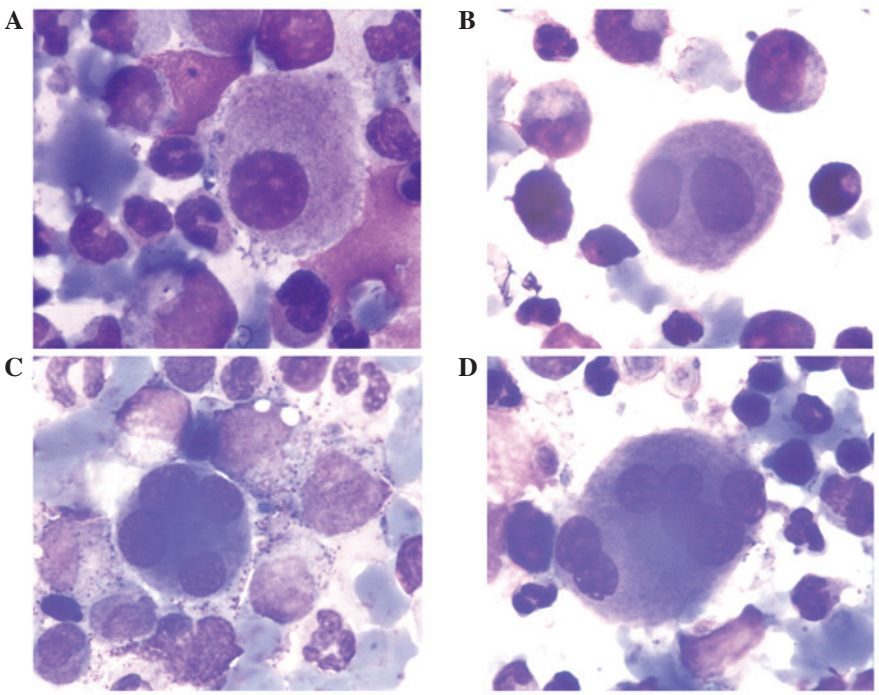

Figure 1. Representative images showing abnormal lobation in megakaryocytes (Wright-Giemsa; magnification, x1000). (A) Hypolobation (mononuclear). (B-D) Multinucleation with different morphologies (two or more round separated nuclei).

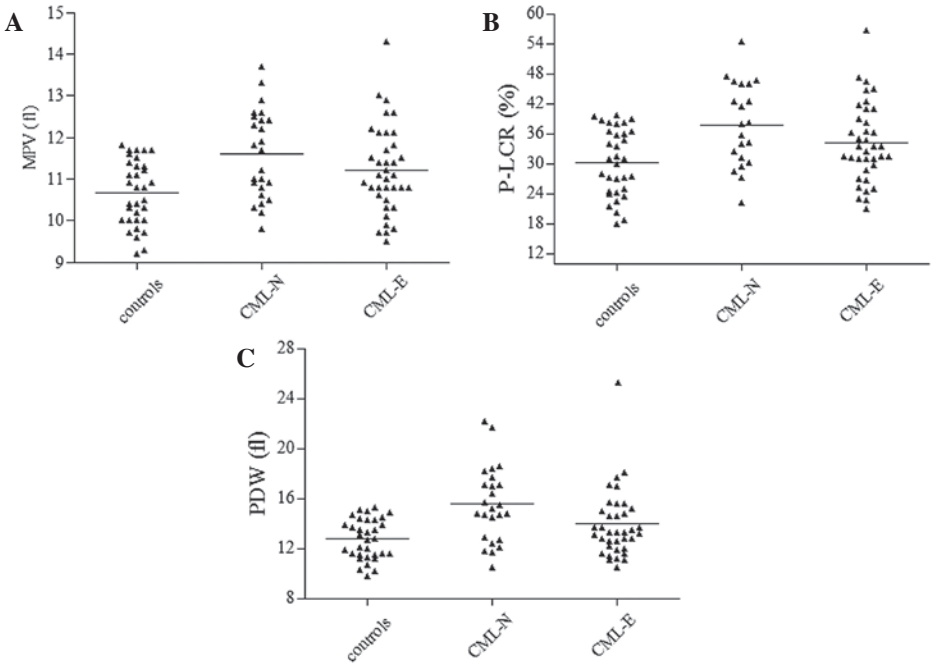

Figure 2. Platelet parameters in healthy controls, and CML-N and CML-E groups. Horizontal lines indicate the mean. (A) MPV: CML-N > healthy controls (P=0.001) and CML-E > healthy controls (P=0.015). (B) P-LCR: CML-N > healthy controls (P=0.001) and CML-E > healthy controls (P=0.028). (C) PDW: CML-N > healthy controls (P=0.000), CML-E > healthy controls (P=0.037) and CML-N > CML-E (P=0.032). CML, chronic myeloid leukemia; CML-N, patients with CML with a normal platelet count; CML-E, patients with CML with an elevated platelet count; MPV, mean platelet volume; P-LCR, platelet large cell ratio; PDW, platelet distribution width.

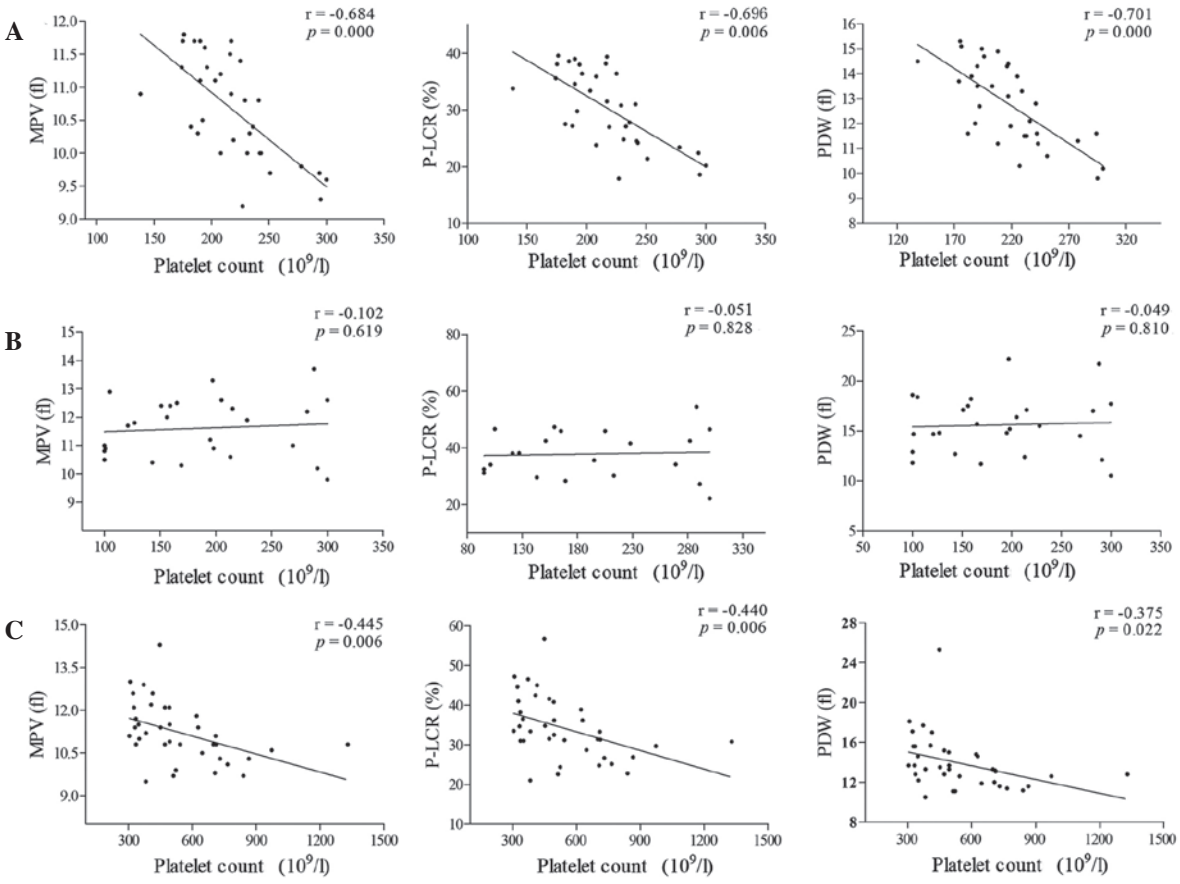

Figure 3. Correlation between platelet counts and other platelet parameters among healthy controls, CML-N and CML-E groups. (A) Healthy controls. (B) CML-N group. (C) CML-E group. Inverse correlations were observed between platelet count and MPV, P-LCR and PDW in the CML-E group and healthy controls. However, no significant correlations between platelet count and MPV, P-LCR or PDW were observed in the CML-N group. CML, chronic myeloid leukemia; CML-N, patients with CML with a normal platelet count; CML-E, patients with CML with an elevated platelet count; MPV, mean platelet volume; fl, femtoliters; P-LCR, platelet large cell ratio; PDW, platelet distribution width.

Ordinarily, the transition from megakaryocyte to platelet is under rigorous control (14-17). However, regulation of this process may be disrupted in certain diseases, particularly in neoplasms with a clonal origin (12,18-20). It was hypothesized that dysmegakaryocytes reflect disorders of megakaryocyte development and result in dysregulated platelet production, as indicated by higher MPV, P-LCR and PDW values in patients with CML than in healthy controls. Furthermore, loss of the correlation between platelet count and other parameters of platelet function in the CML-N group, may indicate dysregulation of platelet production. Patients in the CML-E group exhibited normal megakaryocytes and platelets, which masked the abnormal platelet production. However, a lower ratio of multinucleated megakaryocytes to normal megakaryocytes indicated that this population was less heterogeneous, which may be another explanation for the lower PDW in the CML-E group compared with the CML-N group.

Platelet formation following megakaryocyte development is a complex process and the mechanisms underlying this process remain unclear. Further research into abnormal megakaryocytes and thrombopoiesis may provide novel insights into hematological malignancies.

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