Characteristics of bone marrow cells in 107 patients with juvenile idiopathic arthritis: A retrospective study

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Abstract. Few studies to date have reported on the myelodysplastic features of children with juvenile idiopathic arthritis (JIA). Bone marrow specimens were collected from 107 patients aged from 7-12 years who were initially diagnosed with JIA between May 2013 and October 2015. In 107 patients with JIA, bone marrow proliferation was higher than normal and hemophagocytes were more easily observed than usual. The characteristics of bone marrow cells in 107 patients with JIA were investigated and the associations of these characteristics with the disease was discussed in the present study. It was noticed that there were similar changes in the myeloid, erythropoietic and megakaryopoietic series in the majority of bone marrow specimens; the presence of hemophagocytes was also reported. The present findings suggest that JIA is associated with specific myelodysplastic changes, and that cellular immune system dysfunction and overreactive inflammatory cytokines may contribute to the development of these myelodysplastic changes in the bone marrow.

Introduction

Juvenile idiopathic arthritis (JIA) is one of the most common immune-related diseases. We are still uncertain about the etiology of this disease, which usually presents before the age of 16, continues for at least 6 weeks, and has other potential medical causes excluded (1). The disease most commonly occurs in children between the ages of 7 and 12 years, but it may also present in adolescents up to 15 years of age and in infants. It is a subset of childhood arthritis that may be either transient and self-limiting, or chronic. The potential causes of JIA are considered to involve genetics, environmental factors, inflammatory cytokines and immune dysfunction. The clinical features include fever, joint pain (often accompanied by a skin rash), enlargement of the liver, spleen and lymph nodes, and persistent inflammation that can cause joint deformity. The younger the patient is, the more severe the symptoms are likely to be. Older patients mainly experience joint-related symptoms only (2-5).

The diagnosis of JIA is mainly based on the clinical features and the results of laboratory tests for human leukocyte antigen (HLA-B27), rheumatoid factor (RF) and matrix metalloproteinase-3 (MMP-3) (6), but hematological disorders have been seldom reported in JIA. During examination of the bone marrow of patients with JIA, we noticed a number of myelodysplastic changes. Additionally, we summarized the features of the bone marrow cells from patients with JIA, providing reference for the future clinical diagnosis and treatment of JIA.

Materials and methods

Patients. The 107 patients included in this study were initially diagnosed with JIA between May 2013 and October 2015. The age range was 2-16 years old and median age was 11 years old, including 67 boys and 40 girls. All 107 patients were diagnosed with JIA from department of pediatrics. Our study was approved by the Institutional Review Board at Ren Ji Hospital Affiliated to Shanghai Jiao Tong University in Shanghai [IRB Approval no. RJKLS (2016) 023].

Clinical features. Twenty-eight patients (26.17%) experienced a fever of variable duration, from 3 days to 3 months. Forty-eight patients (39.3%) exhibited joint swelling, which lasted between 1 month and 4 years, especially in the knee and hip joints.

Materials. Bone marrow specimens were collected from the 107 patients who were initially diagnosed and treated at the Department of Pediatrics at Renji Hospital (School of Medicine, Shanghai Jiao Tong University, Shanghai, China) from May 2013 to October 2015.

Methods. Bone marrow aspirates were obtained from the posterior iliac crest or the anterior iliac crest (7). Bone marrow slides containing particles were prepared according to ICSH guidelines, using a Wright-Giemsa Stain kit (by Baso Diagnostic Inc., Zhuhai, China), and then examined under a microscope (7). The marrow cellularity was observed under low power magnification (x100), and described as follows:

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Acellular, reduced, normal, increased, or markedly increased. The same magnification was used to determine the numbers of megakaryocytes and hemophagocytes. A differential count of nucleated cells was then performed in selected areas (Fig. 1), with ≥200 cells counted. Quantitative assessments of particular cell lineages, and of particles within hemophagocytes, were performed under x1,000 magnification using oil lens. All microscopic examinations were performed by the same researcher, in order to avoid any bias in the assessments, in a blinded manner.

Statistical analysis. The Kruskal-Wallis test was used to test whether numbers of hemophagocytes has the same distribution among the subtypes. Software SAS version 9.13 (SAS institute Inc) was used to perform the statistical analysis. And $P<0.05$ was considered to indicate a statistically significant difference.

Results

Bone marrow examination. In 107 patients with JIA, twenty-seven (25.23%) had normal cellularity of the bone marrow, fifty-four (50.47%) had increased cellularity, and twenty-six (24.30%) had markedly increased cellularity (Table I). With respect to the megakaryocyte count, we noted (0-5) per slide in 1 case (0.93%), (5-25) in 25 cases (23.36%), and (>25) in 81 cases (75.70%), which is a mild-moderate increase. Regarding classification results, the myeloid series accounted for (30.5-85%), including 25 cases >60% (23.36%), with a medium percentage of 53%, and in 39 (36.45%) cases toxic granulation can be seen (Fig. 2). The erythroid series accounted for (6-44.0%), with a medium percentage of 22.5%. Two patients had mild erythro-dysplastic hematopoiesis, including megaloblastoid changes and nuclear dysmorphism. The lymphatic series accounted for (3-42.5%). No obvious morphological changes were observed.

In all 107 patients with JIA, hemophagocytes were observed (0-2/per slide) in 60 cases (56.07%), swallowing platelets (Fig. 3) in 40 cases (66.67%), erythrocytes in 22 cases (36.67%), neutrophil granulocytes in 4 cases (6.67%), lymphocytes in 1 case (1.67%), orthochromatic normoblasts in 8 cases (13.33%), and impurities in 29 cases (48.33%). Hemophagocytes were rarely seen (3-5/per slide) in 10 cases (9.35%), swallowing platelets in 8 cases (80.00%), erythrocytes in 8 cases (80.00%), neutrophil granulocytes in 3 cases (30.00%), orthochromatic normoblasts in 2 cases (1.87%), and impurities in 4 cases (3.74%). Hemophagocytes were clearly seen (6-15/per slide) in 13 cases (12.15%), swallowing platelets in 10 cases (9.35%), erythrocytes in 8 cases (7.48%), neutrophil granulocytes in 3 cases (2.80%), orthochromatic normoblasts in 3 cases (2.80%), and impurities in 9 cases (8.41%). Additionally, we listed the number of hemophagocytes in the samples from each of the 107 patients with JIA (Table II), but found no significant differences among the subtypes ($P>0.05$).

In our study, 4 patients were received MAS in their discharge diagnosis, with 3 sJIA and 1 undifferentiated JIA. Hemophagocytes were occasionally seen (0-2/per slide) in the 3 sJIA cases, and clearly seen (6-15/per slide) in the 1 case of undifferentiated JIA.

Table I. Cellularity of bone marrow.

Cellularity	Myeloid:erythroid (M:E) ratio	Number of cases
Markedly increased	1:1	26 (24.30%)
Increased	1:10	54 (50.47%)
Normal	1:20	27 (25.23%)
Reduced	1:50	0
Acellular	1:300	0

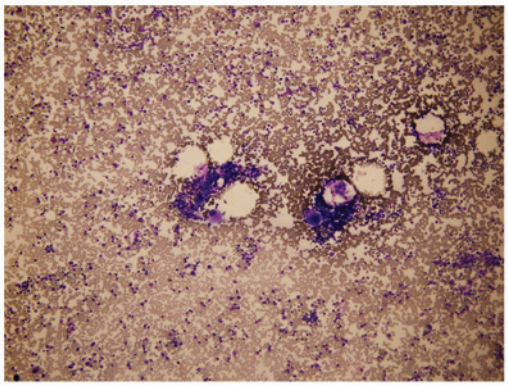

Figure 1. Increased proliferation of bone marrow cells (Wright-Giemsa stain; original magnification, x100).

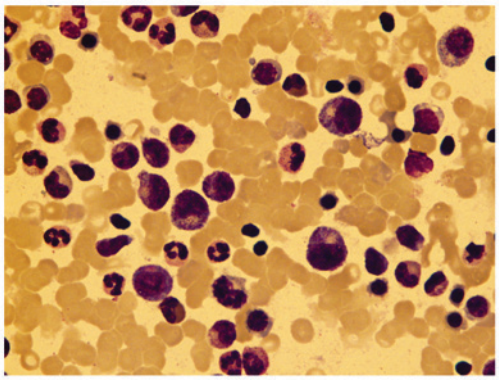

Figure 2. Toxic granulation in the myeloid series (Wright-Giemsa stain; original magnification, x1,000).

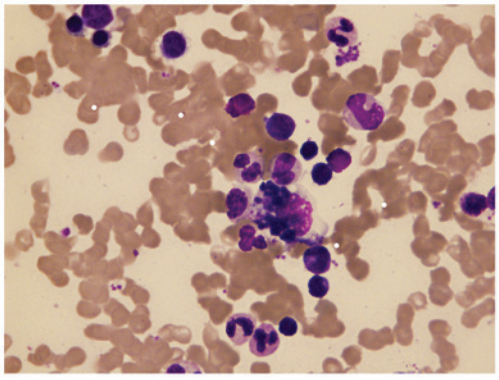

Figure 3. Hemophagocytes swallowing platelets and impurities (hematoxylin and eosin stain; original magnification, x1,000).

Table II. Number of hemophagocytes in each category of juvenile idiopathic arthritis.

Number of Hemophagocytes/per slide	Systemic arthritis	Enthesitis-related arthritis	Polyarthritis RF-positive	Polyarthritis RF-negative	Oligoarthrtitis	Undifferentiated arthritis	Total
0	4	9	0	1	1	15	30
0-2	9	13	4	0	3	29	58
3-5	0	3	0	0	0	4	7
6-15	1	3	1	0	1	6	12
Total	14	28	5	1	5	54	107

Discussion

We found abnormal quantitative myelodysplastic changes in our 107 patients with JIA. An increase in the cellularity and a mild-moderate increase in the megakaryocytic series had been found in most cases, in addition to other qualitative changes. The most common morphological change was toxic granulation in the myeloid series. For the erythroid series, megaloblastic changes and nuclear dysmorphism were seen in 2/107 cases. Furthermore, 77.57% of the patients had hemophagocytes present in bone marrow smears. As bone marrow aspiration is an invasive examination, no healthy, age-matched volunteers were included in this study.

None of the patients had any indication of certain infections at the time of bone marrow examination, according to their clinical history; thus, abnormal smear findings, such as toxic granulation and the presence of hemophagocytes, may be related to the disease itself. The pathophysiological mechanism underlying the hematopoietic changes in JIA remains uncertain. Cellular immune system dysfunction has been implicated, and may lead to alterations in the microenvironment of the bone marrow, through the effects of dysregulated cytokines and other local intracellular messengers (8,9).

Mellins *et al* (8) reported that systemic JIA (sJIA), currently classified as a subtype of JIA (1), appears to be driven by the continuous activation of innate immune pathways with abnormally regulated production of innate proinflammatory cytokines, suggesting that sJIA is an autoinflammatory disorder. It is recognized that a proportion (10-30%) of patients with sJIA develop macrophage activation syndrome (MAS). MAS is a syndrome characterized as overreactive inflammation driven by the excessive activation and expansion of T cells (mainly $CD8^+$), leading to the activation of hemophagocytic macrophages (9,10); it is also known as a complication of pediatric rheumatic disorders, particularly JIA (11) and systemic lupus erythematosus (SLE) (12,13). It is recognized as a severe, potentially life-threatening complication, the same disorder as the secondary or 'reactive' form of hemophagocytic lymphohistiocytosis (HLH) (14). Both conditions have high mortality rates (15). Even with appropriate and timely treatment, one English study reported mortality rates was 2/9 in 2001 (16), and one Iranian study reported mortality rates was 2/5 in 2011 (17).

Abnormal quantitative and qualitative features in bone marrow smears, especially the presence of hematopoietic cells, and their phagocytosis by macrophages, is common. The frequent presence of hemophagocytes indicates that bone marrow examinations are one of the most important auxiliary examinations for the diagnosis of JIA, in order to exclude or make the diagnose of MAS.

In conclusion, we investigated the characteristics of bone marrow cells in 107 patients with JIA. And we noticed increased cellularity; increased megakaryocyte count and the presence of hemophagocytes were in the majority of bone marrow specimens. We propose that JIA is associated with specific myelodysplastic changes, and that cellular immune system dysfunction and overreactive inflammatory cytokines may contribute to the development of these myelodysplastic changes in the bone marrow. In order to exclude or make the diagnose of MAS, bone marrow examination should be considered as an important auxiliary examination.

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Availability of data and materials

The dataset used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DZ wrote the manuscript. JZ and FC designed the study. DZ and YZ analyzed and interpreted the patient data. FC gave advice about the study and gave final approval of the version to be published. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board at Ren Ji Hospital Affiliated to Shanghai Jiao Tong University in Shanghai [IRB Approval no. RJKLS (2016) 023]. Patient consent was waived by the ethics committee.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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