Hemogram and bone marrow morphology in children with chronic aplastic anemia and myelodysplastic syndrome

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Background: **Aplastic anemia (AA) and myelodysplastic syndrome (MDS) are both acquired disorders in which bone marrow fails to produce or release sufficient blood cells. Anemia, infections and thrombocytopenia are common signs of such diseases. Clinically, it is difficult to distinguish chronic aplastic anemia (CAA) from MDS, especially from MDS without splenomegaly. As prognosis and treatment of AA and MDS are different, it is extremely important to make a differential diagnosis for the two diseases.**

Methods: **The medical records of 31 patients with CAA and 17 patients with MDS were retrospectively reviewed. Hemogram, bone marrow smear and biopsy for those patients were analyzed.**

Results: **The mean counts of monocytes and platelets in the peripheral blood of the CAA patients were significantly lower than those of the MDS patients. Bone marrow smear showed a reduction of cellularity in CAA patients. The mean counts of myeloblasts+promyelocytes, myeloblasts+proerythroblasts, and megakaryocytes in the bone marrow of CAA patients were markedly lower than those in MDS patients. But the mean lymphocyte count was reversed. Bone marrow cells showed morphological abnormalities in MDS. Hematopoietic tissue in the bone marrow biopsy decreased obviously in more than 96% of the patients with CAA. Adipose tissue in the bone marrow of CAA patients increased obviously. A reduction or deficiency (<2 cell/piece) of megakaryocytes was noted in 28 patients with CAA. Fibrous tissue in the bone marrow was detected in 5 patients with CAA. Bone marrow biopsy results showed hypercellular changes in 12 MDS patients. Ten patients showed aggregated erythroblasts which were in the same stage of development, and 15 patients had abnormal localization of immature precursors (ALIP).**

Conclusions: **Blood cell counts can be decreased in addition to the reduction of cellularity in the bone marrow without dyshematopoiesis in CAA patients. Peripheral blood monocytes, fibrous tissue and cellularity in bone marrow are increased in MDS. Dyshematopoiesis and ALIP may appear characteristically in the children with MDS. Histology of bone marrow is important in the differential diagnosis of MDS and CAA.**

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Introduction

Aplastic anemia (AA) and myelodysplasia syndrome (MDS) are acquired disorders in which bone marrow fails to produce or release sufficient blood cells,[1] but in most cases the etiology is unknown.[2-4] Since there is no characteristic cell for chronic aplastic anemia (CAA), it is difficult to distinguish CAA from MDS in patients without splenomegaly. The distinction between CAA and MDS is important because there is a higher risk of progression to acute leukemia for patients with MDS than those with CAA. Meanwhile, the management of these two illnesses is also different. Bessho et al[5] reported that the number of mass cells increased in the bone marrow of CAA patients. Others[6] observed morphological changes of red cell membrane under an electron microscope to differentiate among CAA, MDS and leukemia. By means of bone marrow morphology, histology, cytogenetics and megakaryocyte counting, bone marrow smear for detecting micromegakaryocytes by immunohistochemistry[7] and the formation rate of bone marrow megakaryocyte colony assay,[8] AA and MDS can be diagnosed in the early stage.[9-17] The appearance of a cytogenetic abnormality in bone marrow cells of AA was related to evolution to MDS and leukemia.[18] The quantification of tumor necrosis factor receptor (TNFR) expression in bone marrow stem cells may

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be a useful method to distinguish AA from MDS.[19] This study was to re-examine the distinction between CAA and MDS morphologically and histologically by analyzing the data from 31 CAA patients and 17 MDS patients in the past 8 years at our hospital.

Methods

Subjects

Thirty-one patients with CAA (23 males, 8 females; aged from 3 to 14 years, mean: 8.5 years) and 17 patients with MDS (14 males, 3 females; age from 2 to 14 years, mean: 8 years) without splenomegaly diagnosed at Xi'an Children's Hospital from 1998 through 2005 were analyzed. Diagnoses of CAA were based on the criteria formulated at the Fourth National Aplastic Anemia Symposium of China.[20] For the diagnosis of CAA, the following criteria should be met: mild anemia, bleeding and infection; decreased level of hemoglobin and decreased counts of reticulocytes, leukocytes, neutrophils and platelets; two or three lineages decreased, at least one was hypocellular shown by bone marrow smear and hypercellular spicules occasionally, increased proportion of orthochromatic erythroblasts in erythrocytic lineages, and sharply reduced megakaryocytes and prominent lymphocytes, plasma cells, macrophages, and mast cells in marrow aspirate and obviously increased adipose cells; patients with AA that progressed to severe pancytopenia met the criteria of blood and bone marrow for severe aplastic anemia (SAA) were classified as SAA type II. Diagnosis and classification of MDS were made according to the French-American-British criteria.[21] MDS was classified into refractory anemia (RA), refractory anemia with ringed sideroblasts (RARS), RA with excess of blasts (RAEB), RAEB in transformation (RAEB-T), and chronic myelomonocytic leukemia. Among the 17 patients with MDS, 12 had RA and 5 had RAEB.

Laboratory examinations

All patients were subjected to bone marrow aspiration and biopsy of the posterior superior iliac spine or breastbone at diagnosis. Acid hemolysis test was performed on the patients with CAA. The results of examinations in the patients with CAA were compared with those in the patients with MDS.

Statistical analysis

The data were expressed by means±SD. Student's *t* test was used to evaluate the probability of significant differences between the two groups. The data were analyzed using SPSS10.0 software package. A value of $P<0.05$ was considered statistically significant.

Results

Hemogram in CAA and MDS patients

No differences were observed in the mean counts of hemoglobin, white blood cells, neutrophils and lymphocytes between the two groups ($P>0.05$). The mean counts of monocytes and platelets in the MDS patients were significantly higher than those in the CAA ($P<0.05$) (Table 1). In the patients with MDS, 15 showed abnormal erythrocytes such as stomatocytes, elliptocytes, dacryocytes and cell fragments. Microcytes, macrocytes and megalocytes existed in the differential blood cell of 11 patients and orthochromatic normoblasts in 3. Granules decreased in myeloid cells in 16 patients and in platelets in 12 but hypersegmental myeloid cells appeared in 10. Chromatin condensation appeared in 8 patients. Myeloblasts existed in the peripheral blood of 1 patient.

Table 1. Analysis of hemogram in the CAA and MDS groups (means ± SD)

Group	*n*	Hb (g/L)	WBC ($\times10^9$/L)	ANC ($\times10^9$/L)	M ($\times10^9$/L)	L ($\times10^9$/L)	PLt ($\times10^9$/L)
CAA	31	66.6±16.33	3.39±1.00	1.282±0.418	0.07±0.045	2.02±0.409	32.74±13.75
MDS	17	77.0±18.63	5.13±5.18	1.948±0.698	0.38±0.233	2.80±0.662	61.35±55.91
t		1.99	1.82	0.03	6.0	1.40	2.72
P		>0.05	>0.05	>0.05	<0.001	>0.05	<0.05

Hb: hemoglobin; WBC: white blood cell; ANC: absolute neutrophil count; M: monocyte; L: lymphocyte; PLt: platelet.

Table 2. Bone marrow smear results in the CAA and MDS groups (means ± SD)

Group	*n*	Stage of proliferation			Myeloblast + promyelocyte (%)	Proerythro-basophilic + proerythroblast (%)	Lymphocyte (%)	Megakaryocyte (%)
		Hypercellularity	Normal cellularity	Hypocellularity				
CAA	31	3	10	18	0.91±0.61	1.31±0.93	35.54±13.7	2.74±1.81
MDS	17	11	6	0	3.79±2.26	2.49±1.37	18.72±4.77	169.1±216.25
t					6.78	3.56	4.88	4.32
P					<0.001	<0.01	<0.001	<0.001

Table 3. Dyshematopoiesis of bone marrow in the CAA and MDS groups

Group	*n*	Micromega-karyocyte (%)	Single-round-nucleus megakaryocyte (%)	Multi-round-nucleus megakaryocyte (%)	Pelger-Huet abnormality (%)	Megaloblastic changes in granulocytic lineage (%)	Megaloblastic changes in erythrocytic lineage (%)
CAA	31	0	0	0	0	0	0
MDS	17	94.1	29.4	64.7	17.6	47.0	88.29

Table 4. Bone marrow biopsy results in the CAA and MDS groups

Group	*n*	Stage of proliferation				Hemotopoietic tissue	Adipose tissue	Existence of megakaryocyte
		Hypercellularity	Normal cellularity	Hypocellularity	Extreme hypocellularity			
CAA	31	0/31	1/31	23/31	7/31	Obvious reduction	Obvious induction	3/31
MDS	17	2/17	10/17	5/17	0	Normal range	Normal range	17/17

Table 5. Bone marrow biopsy results in the CAA and MDS groups

Group	*n*	Erythroblasts in the same developing stage	ALIP	Proliferation of fibrin tissue	Micro-megakaryocyte	Single-round-nucleus megakaryocyte	Multi-round-nucleus megakaryocyte
CAA	31	0	0	5/31	0	0	0
MDS	17	7/17	15/17	17/17	17/17	9/17	13/17

ALIP: abnormal localization of immature precursors.

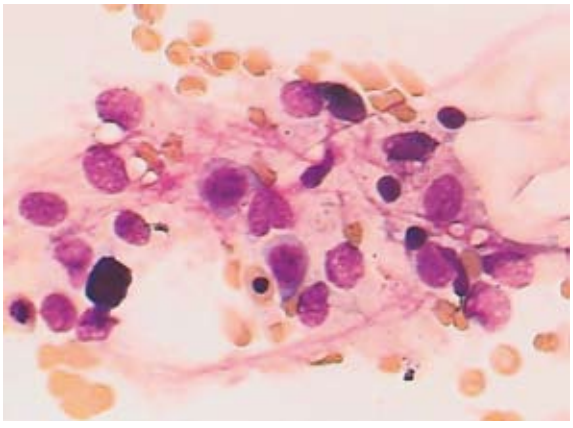

Fig. 1. Bone marrow smear in the CAA group showing hypocellularity and normal morphology (Wright, 10×100).

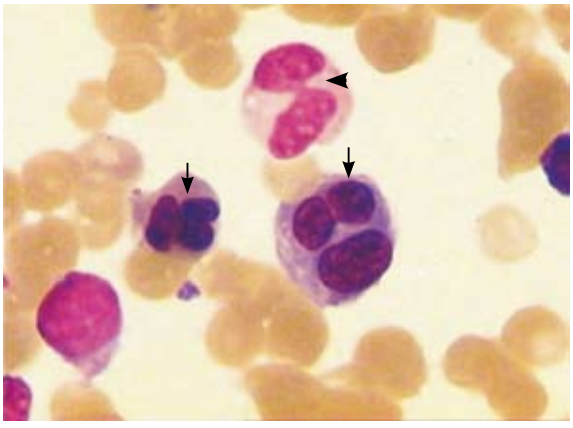

Fig. 2. Dyshematopoiesis in the MDS group shown by bone marrow smear. Arrow: erythroblasts dysplasia; arrowhead: Pegler-Hunt (Wright, 10×100).

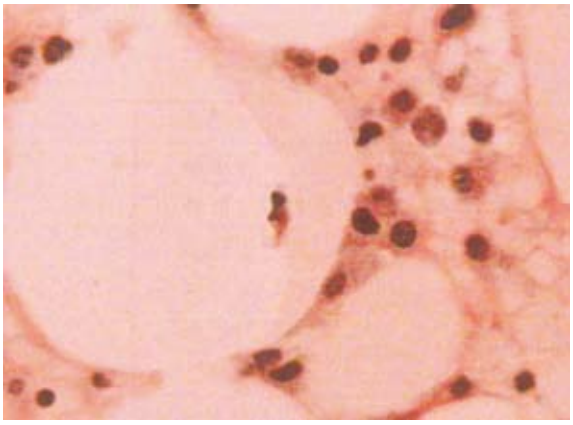

Fig. 3. Bone marrow biopsy in the CAA group showing hypocelluarity and normal morphology (HGE, 10×40).

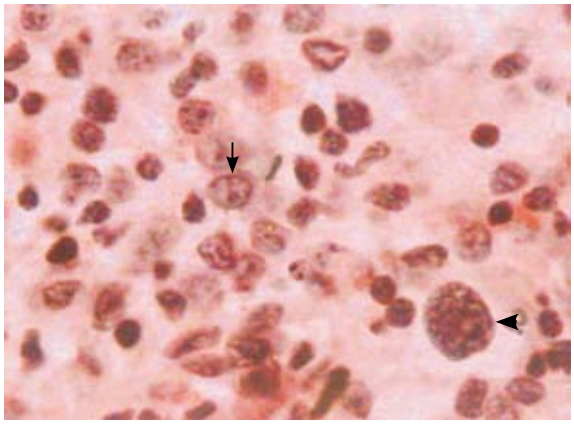

Fig. 4. MDS in bone marrow biopsy. Arrow: abnormal localization of immature precursors; arrowhead: single-round-nucleus megakaryocytes (HGE, 10×40).

Bone marrow smear in CAA and MDS patients

Bone marrow of the MDS patients showed active or marked proliferation. The counts of myeloblasts, promyelocytes, proerythroblasts, basophilic erythroblasts and megakaryocytes in the bone marrow of the MDS patients were significantly higher than those of the CAA patients, and the count of lymphocytes was lower than that in the CAA patients ($P<0.01$). Dyshematopoiesis in the bone marrow of the MDS patients was characterized by megaloblastic changes in erythrocytic or granulocytic lineage, single-round-nucleus and multi-round-nucleus megakaryocytes, micromegakaryocytes,[22] and the Pegler-Hunt in myeloid cells (Tables 2, 3) (Figs. 1, 2).

Bone marrow biopsy in the CAA and MDS groups

Hemotopoietic tissue reduced and adipose tissue increased in 30 of the 31 CAA patients. Cells for each lineage and each stage showed no morphological abnormalities. Bone marrow biopsy of 5 patients showed proliferation of fibrin tissue. Megakaryocytes appeared in bone marrow biopsy of 3 patients with CAA (<2 cell/piece). Twelve patients with MDS showed hypercellularity of bone marrow while the other 5 patients showed hypoplasia. Ten patients showed aggregated erythroblasts which were in the same developing stage, and 15 patients showed abnormal localization of immature precursors (ALIP). In all the MDS patients, there were proliferated fibrin tissue and micromegakaryocytes. Nine patients showed megakaryocytes with single round nucleus or mutiple round nuclei (Tables 4, 5) (Figs. 3, 4).

Discussion

CAA and MDS are caused by the defect of hemopoietic stem cells. Anemia, infections and thrombocytopenia are common signs of such diseases. They can co-exist or transform to each other; moreover, 10% of MDS patients may present with low proliferation of cells.[23] It is hard to distinguish CAA from MDS clinically, especially from MDS without splenomegaly. The diversity of prognosis and medical treatment needs to clarify the difference between the two diseases. Despite the use of genetics, molecular biology and immunology for the diagnosis, routine blood examination, bone marrow aspiration and biopsy still can not be replaced and are very important in the diagnosis of hematological diseases.

Significant difference was found in hemogram monocyte counts between the CAA and MDS groups, as reported previously.[23] Platelet counts decreased in both CAA and MDS groups, but it was a little higher in the MDS group than in the CAA group. In MDS patients, different morphological abnormalities of differential blood cells existed.

Bone marrow aspiration results showed proliferation was more active in MDS than in CAA. The counts of early stage hematopoietic cells increased markedly in the MDS patients, but reduced in the CAA patients. The count of lymphocytes was increased and that of megakaryocytes was reduced in the bone marrow of the CAA patients, which showed failure of hematopoietic function. Various morphological changes appeared in the bone marrow of the MDS patients, which existed in erythrocytic, granulocytic and megakaryocytic lineages. Dyshematopoiesis is one of the important features of MDS.

Bone marrow biopsy revealed that whatever degree of the bone marrow proliferation, there was a reduction of cellularity. Hematopoietic tissue decreased, adipose and fibrin tissues increased obviously in the bone marrow in 5 of the CAA group. A reduction or deficiency of megakaryocytes other than dyshematopoiesis was noted in bone marrow of all CAA patients. All of these findings were in accordance with the pathological changes of CAA. Bone marrow hypercellularity was found and scarely hypoplasia in the MDS patients. No severe hypoplasia was found, and the condition that adipose tissue replaced hemotopoietic tissue did not appear. In the bone marrow of MDS patients, the fibrin tissue, megakaryocytes and micromegakaryocytes were easily detected. ALIP was detected in 15 (88.2%) of 17 patients. Wu et al[24] reported that ALIP was observed in only 36% of the MDS patients and increased adipose tissue existed in 71% bone marrow, which were lower than the results in our study. This was possibly due to the lack of low-proliferated MDS. Multi-round-nucleus megakaryocytes were found in bone marrow of 13 MDS patients; single-round-nucleus megakaryocytes were found in 9 and immature erythrocytes in the same stage in 10. The result suggested that examination of the morphological abnormalities by bone marrow biopsy was very important.[7-11]

In conclusion, in CAA patients, two or three lineage cells are reduced and the cellularity decreased. The count of megakaryocytes is reduced or deficient and the adipose tissue is proliferated and hemtopoietic tissue reduced obviously in bone marrow, but the proliferation of fibrin tissue exists in a few children. Morphological abnormalities do not appear in bone marrow of CAA children. For MDS children, more monocytes are seen in hemogram in addition to the increased counts of myeloblasts, promyelocytes, proerythroblasts and basophilic erythroblasts in bone marrow. The count of megakaryocytes is also higher than that in CAA

children. Bone marrow biopsy for MDS can reveal insignificant deficiency of hemotopoietic tissue[7] in contrast to fibrin tissue because of the production of megakaryocytes with no function.[25] Abnormal megakaryocytes, ALIP and dyshematopoiesis are the features of MDS.[26] Erythroblast islets in the bone marrow of MDS and CAA patients in the same developing stage have not been reported so far. Various clinical features of CAA and MDS are perhaps based on different changes of molecular biology, but hemogram, bone marrow smear and biopsy are always essential to the diagnosis of blood diseases.

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