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ORIGINAL ARTICLE

Diagnosis of acquired bone marrow failure syndrome during childhood using the 2008 World Health Organization classification system

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Abstract Distinguishing hypoplastic myelodysplastic syndrome from aplastic anemia (AA) is challenging. In the present study, Japanese and Chinese pediatric hematologists and pathologists conducted a joint review of bone marrow (BM) smears and trephine biopsies in 100 children with acquired BM failure syndrome, using the criteria proposed in the 2008 edition of the World Health Organization classification of hematopoietic and lymphoid tissues. The final consensus for the diagnoses of 100 children was AA in 29 patients, refractory cytopenia of childhood (RCC) in 58 patients, and refractory cytopenia with multilineage dysplasia (RCMD) in 13 patients. No significant differences between Japanese and Chinese children were found with regards to clinical and laboratory findings, or the distribution of diagnoses. Patients with RCC/RCMD showed milder disease severity and BM hypocellularity than those with AA. To establish the provisional entities for RCC, it is essential to prospectively compare the clinical outcomes between AA and RCC groups in a large number of patients.

Keywords Diagnosis · Bone marrow failure syndrome · Childhood · Classification system · 2008 world health organization

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Introduction

The incidence of aplastic anemia (AA) is approximately 3-fold more common in East Asia than in Europe and United States where yearly incidence rates are approximately $2/10^6$. Geographical variations may play a role in this discrepancy, which partly may be due to genetic disposition and/or environmental factors [1]. In addition, the difference in diagnostic criteria between Eastern and Western countries may be responsible for the variation in outcomes. Previously, bone marrow (BM) trephine biopsies were not common in East Asia. Moreover, most patients with relative erythroid hyperplasia and dysplasia were diagnosed as AA [2].

Childhood myelodysplastic syndrome (MDS) is very rare. In addition, hypocellularity of the BM is more common in childhood MDS. Thus, it is often difficult to distinguish hypoplastic MDS from AA, especially in cases without cytogenetic abnormalities. The new edition of the World Health Organization (WHO) classification for myeloid neoplasms outlines a provisional entity for refractory cytopenia for childhood (RCC) in which the diagnostic criteria for distinguishing RCC from AA are proposed [3].

The present study reviewed and classified the slides of BM smears and trephine biopsies in 100 children with acquired bone marrow failure syndrome(BMFS) in Japan

and China according to the strict criteria proposed by the WHO classification system (2008 edition).

Design and methods

A total of 100 children with cytopenia and hypocellular BM (50 cases from Japan and 50 cases from China between 2009 and 2011) were included in our study. Chinese cases were diagnosed at the Blood Disease Hospital, Chinese Academy of Medical Sciences (CAMS). Japanese cases were registered to the central review system of the Japanese Society of Pediatric Hematology (JSPH).

Individuals were eligible if the following criteria were satisfied: patients had to be 18 years old or younger, and with hypocellular BM. To obtain a diagnosis for cytopenia, at least two of the following must be present: (1) neutrophil $<1.5 \times 10^9$/l; (2) hemoglobin <10 g/dl; and (3) platelet $<50 \times 10^9$/l. Patients with clinical signs of inherited BMFS and/or positive chromosome fragility tests were excluded. Also, patients who had previously been treated with anticancer drugs and radiation were not eligible to participate. All patients had both bone marrow aspirate cytology and trephine biopsy samples.

The severity of the disease was classified according to internationally accepted criteria [4, 5]. AA patients exhibited no morphological changes in their hematopoietic cell lineages. RCC was defined as persistent cytopenia with <5 % blasts in the BM and <2 % blasts in the peripheral blood (PB). In addition, RCC patients had <10 % dysplastic changes in more than two cell lineages, or >10 % in one cell lineage. Refractory cytopenia with multilineage dysplasia (RCMD) exhibited >10 % of the dysplastic changes in more than two cell lineages. Dysplastic features of BM aspirate cytology and trephine biopsies sampled were evaluated according to recommendations by the French–American–British (FAB) Cooperative Leukemia Working Group and the morphology group of the European Working Group MDS in children (EWOG-MDS) [6, 7].

Bone marrow hypocellularity was classified as mild to moderate (5–50 % of the normal age-matched controls) and as severe (<5 % of the normal age-matched controls) according to the results obtained from the trephine biopsies. Cytogenetic examinations were performed with trypsin–Giemsa banding techniques. Twenty metaphases were analyzed at each examination. A cytogenetic clone was thought to exist when two or more cells had the same structural chromosome changes or extra chromosome. At least three cells with the same missing chromosome were considered to constitute a clone.

Before the joint meeting between Japan and China, slides from 50 cases were reviewed by two pediatric

Table 1 Comparison of patients' characteristics and laboratory findings in Japanese and Chinese children with bone marrow failure syndrome

	Japan	China	*p* value
No. of patients	50	50	
Median age at diagnosis, years (range)	10 (1–18)	9 (3–16)	0.422
Gender, male/female	25/25	20/30	0.315
Severity of cytopenia			
Very severe	6	4	
Severe	17	9	0.109
Non-severe	27	37	
Peripheral blood data at diagnosis			
Median			
WBC, $\times 10^9$/l (range)	2.8 (0.4–5.0)	3.9 (0.17–7.0)	0.009
Neutrophil, $\times 10^9$/l (range)	0.58 (0–2.7)	0.68 (0–2.1)	0.269
Platelet, $\times 10^9$/l (range)	20 (2–83)	25 (4–64)	0.423
Hemoglobin, g/dl (range)	7.4 (4.1–14.7)	7.8 (4.5–12.0)	0.689
Reticulocyte, $\times 10^9$/l (range)	31.8 (1.5–70.7)	42.8 (1.2–123.2)	0.920
Mean corpuscular volume, fl (range)	98 (76–112)	99 (75–118)	0.764
Days from onset to diagnosis			
≤30	25	24	
30–180	11	8	0.608
≥180	14	18	
Final diagnosis			
AA	12	17	
RCC	33	25	0.265
RCMD	5	8	

AA aplastic anemia, *RCC* refractory cytopenia of childhood, *RCMD* refractory cytopenia with multilineage dysplasia, *WBC* white blood cell count

hematologists and one pathologist from each country. The joint review meeting was held for 3 days in March 2011 at Blood Disease Hospital, CAMS in China. In the first step, the slides of each country were exchanged and reviewed by observers separately. In the second step, the consensus review for all cases was performed by four hematologists and two pathologists from both countries. The materials included PB, BM aspirate smears and BM trephine biopsies. The present study was approved by the ethics committee of Blood Disease Hospital, CAMS and Nagoya University Hospital. Chi-square tests and independent-samples *T* tests were used to compare the difference between two groups. *p* values of <0.05 were considered to be statistically significant.

Results

Comparisons of patient characteristics and laboratory findings at diagnosis between China and Japan are presented in Table 1. Patient characteristics were comparable between the two countries. Although the median WBC count was significantly higher in Chinese children than Japanese children ($p = 0.009$), other PB counts at diagnosis were not different. The days from onset to diagnosis widely varied from 1 to 4165 days. 32 patients required more than 180 days. 7 patients presented with isolated thrombocytopenia, which had gradually proceeded to pancytopenia. They required 47–3040 days from onset to diagnosis.

There were 9 cases whose status of diagnosis was in question between hematologists and pathologists. 4 cases were diagnosed as AA by hematologists, but as RCC by pathologists, the final diagnoses were all RCC. In contrast, 2 cases were diagnosed as RCC by hematologists, but as AA by pathologists, the final diagnoses were RCC. Among the 3 cases who were diagnosed as RCMD by hematologists, 2 cases were diagnosed as RCC and one case was diagnosed as AA by pathologists, by the joint review committee, the diagnoses of 2 cases were consistent with pathologists, the other one was diagnosed RCMD.

Final consensus for the diagnoses of 100 patients was as follows: AA in 29 cases, RCC in 58 cases and RCMD in 13 cases. The distribution of diagnoses was not different between Japanese and Chinese: 12:17 in AA, 33:25 in RCC and 5:8 in RCMD, respectively.

Table 2 displays patient characteristics and laboratory data for the AA, RCC and RCMD groups. Among the three

Table 2 Comparison of patients' characteristics and laboratory findings among children with AA, RCC and RCMD

	AA	RCC	RCMD	*p* value
No. of patients	29	58	13	
Median age at diagnosis, years (range)	9 (3–14)	9 (1–18)	10 (5–16)	0.749
Gender, male/female	11/18	27/21	7/6	0.591
Severity of cytopenia				
Very severe	8	2	0	
Severe	12	11	3	<0.001
Non severe	9	45	10	
Peripheral blood data at diagnosis				
Median				
WBC, $\times 10^9$/l (range)	3.1 (0.17–6.3)	3.7 (1.1–7.0)	3.2 (1.4–5.5)	0.075
Neutrophil, $\times 10^9$/l (range)	0.48 (0–2.1)	0.67 (0–2.7)	0.76 (0.36–1.07)	0.164
Platelet, $\times 10^9$/l (range)	16 (2–57)	27 (6–83)	28 (2–64)	0.224
Hemoglobin, g/dl (range)	7.4 (4.1–12.0)	8.0 (4.0–14.7)	7.5 (4.2–11.6)	0.327
Reticulocyte, $\times 10^9$/l (range)	13 (1.2–42)	43 (1.5–123)	48 (10–94)	0.003
Mean corpuscular volume, fl (range)	94 (75–112)	99 (80–118)	97 (88–110)	0.061
Cellularity in the bone marrow				
Mild–moderate hypocellularity	13	54	13	
Severe hypocellularity	16	4	0	<0.001
Chromosomal abnormalities	1	3	0	0.68
Days from onset to diagnosis				
≤30	17	28	4	
30–180	4	12	3	0.537
≥180	8	18	6	

AA aplastic anemia, *RCC* refractory cytopenia of childhood, *RCMD* refractory cytopenia with multilineage dysplasia, *WBC* white blood cell count

groups, there were no significant differences with regards to median age at diagnosis, sex, or days from onset to diagnosis. While 8 out of 29 (28 %) patients in the AA group had very severe cytopenia, only 2 of the 58 patients (3 %) in the RCC group and none of the 13 patients in the RCMD group had very severe cytopenia. On the other hand, 45 of the 58 patients (78 %) in the RCC group and 10 of the 13 patients (77 %) of the RCMD group had non severe cytopenia ($p < 0.001$). In addition, 16 out of 29 AA patients (55 %) exhibited severe hypoplastic of BM cellularity, while only 4 out of 58 RCC patients (7 %) and none of the RCMD patients had severe hypoplastic BM. A number of the RCC/RCMD patients exhibited mild to moderate hypocellularity ($p < 0.001$).

Data for cytogenetic analyses were available from 75 patients. Abnormal karyotypes were detected in one patient from the AA group (47,XX,+8[10]/46,XX[10]) and in 3 patients in the RCC group (47,XX,+8[10]; 46,Y, t(x:3)(p11.2;q13)[10]; 47, XY,+8[1]/49, idem, +6,+21[3]/46, XY[16]).

Discussion

It is the first project to have a joint meeting to review BM samples from children with BMFS between Japanese and Chinese hematologists and pathologists. Our results demonstrated that the clinical and laboratory findings and the distribution of diagnosis was not different in Japanese and Chinese children. Patients with RCC/RCMD were milder in disease severity and BM hypocellularity, compared to those with AA.

According to the FAB classification, the annual report from JSPH indicated that the number of AA and RA was 71:9 in 2006 and 63:6 in 2007, respectively. The annual incidence of childhood AA in Japan was $3.7/10^6$. Using the new criteria, it is may be $<2.0/10^6$, which is comparable with the incidence of AA in the Western countries.

According to 2008 WHO classification system, the ratio of AA and RCC/RCMD in Germany is unknown, but the proportion of very severe AA among severe AA (64 %) was much higher than that of Japanese children [7]. Among 1002 Japanese children with AA, the distribution of disease severity was as follows: very severe in 246 (24.6 %) children; severe in 305 (30.4 %) children; and non-severe in 451 (45.0 %) children, respectively. These figures suggest that a considerable number of children with AA in Asia may be diagnosed as RCC using the criteria set forth from the German group. Thus, the difference in diagnostic criteria may be responsible for the high incidence of AA in Asian countries.

WHO classification system recommended that children who satisfy the criteria for RCMD should be considered as RCC until the numbers of lineages involved are fully evaluated whether it is an important prognostic discriminator in childhood MDS [3]. In our study, 13 of the 71 MDS children (18 %) were classified as RCMD. The BM samples were more cellular, and dysplasia of cell morphology was more prominent than those in RCC.

The most important aspect of the new proposal from the WHO classification system is whether the diagnosis has an impact on clinical outcomes including, response to treatment and incidence of late clonal diseases. From the German group, results from immunosuppressive therapy (IST) with anti-thymocyte globulin and cyclosporine for children with RCC were reported [8]. The response rate and 3-year overall survival rate in children with RCC were comparable to those with severe AA who received the same IST [9]. Unfortunately, due to a short follow-up period and variety of treatments, we could not define this issue. It is very important to collaborate with all Asian countries to compare the frequence of AA and RCC in children between Asian and Western countries. To establish the new entity of RCC, future studies should unravel the etiology and biological nature of both AA and RCC.

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Conflict of interest The authors reported no potential conflicts of interest.

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