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Comparison of immune manifestations between refractory cytopenia of childhood and aplastic anemia in children: A single-center retrospective study

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ABSTRACT

This retrospective single-center study assessed the incidence and clinical features of immune manifestations of refractory cytopenia of childhood (RCC) and childhood aplastic anemia (AA). We evaluated 72 children with RCC and 123 with AA between February 2008 and March 2013. RCC was associated with autoimmune disease in 4 children, including 1 case each with autoimmune hemolytic anemia, rheumatoid arthritis, systemic lupus erythematosus, and anaphylactoid purpura. No children with AA were diagnosed with autoimmune diseases. Immune abnormalities were common in both RCC and AA; the most significant reductions were in the relative numbers of CD3 – CD56+ subsets found in RCC. Despite the many similar immunologic abnormalities in AA and RCC, the rate of autoimmune disease was significantly lower in childhood AA than RCC ($p = 0.008$, $\chi^2 = 6.976$). The relative numbers of natural killer cells were significantly lower in RCC patients than AA patients. By month 6, there was no significant difference in autoimmune manifestations between RCC and AA in relation to the response to immunosuppressive therapy ($p = 0.907$, $\chi^2 = 0.014$). The large overlap of analogous immunologic abnormalities indicates that RCC and childhood AA may share the same pathogenesis.

1. Introduction

Refractory cytopenia of childhood (RCC) is the most common subtype of childhood myelodysplastic syndromes (MDS), which are heterogeneous clonal hematopoietic stem cell disorders characterized by bone marrow failure and an increased risk of acute leukemia [1]. The pathophysiology of MDS is multifactorial, complex, and poorly understood. The immune system has been implicated in the pathogenesis of MDS since Tichelli reported a patient with MDS-refractory anemia with excess blasts recovered after antithymocyte globulin (ATG) administration in 1988 [2]. Several clinical studies subsequently indicated that some patients with MDS could be responsive to ATG and cyclosporine [3,4], especially in low-risk MDS. These findings suggest that dysregulation of the immune response may be important in the pathogenesis of subtypes of MDS [5].

Some MDS patients suffer from autoimmune manifestations (AIMs), including systemic vasculitis syndrome, skin vasculitis, arthritis, Behçet syndrome, inflammatory bowel disease, and cryoglobulins [6–9]. Various immunologic abnormalities in laboratory data have also been reported, such as immunoglobulin abnormalities [10], T-cell–mediated immune attacks [11], varying numbers of CD4 + FoxP3+ regulatory T cells [12], alterations in natural killer (NK) cell function [13], abnormal cytokine profile, lymphokine production [14], and altered self-reactive antibody repertoires [15]. Increased oligoclonal T-cell expansion and suppression of hematopoietic progenitors by cytotoxic T cells have also been observed in RCC [16]. However, these studies mainly focused on adult patients. Thus, little information is available about childhood MDS with AIMs. As RCC is the most common MDS subtype in childhood, it is important to determine the incidence and clinical features of AIMs in RCC for immunosuppressive therapy, which is used to treat RCC worldwide [17].

Acquired aplastic anemia (AA) is a T-cell–mediated autoimmune disease that results in bone marrow failure [18]. Stalder et al. [19] observed clinical AIMs in approximately 10% of patients with AA. Given the similar pathophysiology of immune-mediated bone mar-

Abbreviations: AA, aplastic anemia; AIM, autoimmune manifestation; ATG, antithymocyte globulin; CR, complete remission; MDS, myelodysplastic syndromes; PR, partial remission; RCC, refractory cytopenia of childhood; TCR, T-cell receptor.

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row failure in RCC and idiopathic AA, which makes distinguishing these diseases difficult in clinical practice [20], it is important to compare the incidence and characteristics of AIMs between RCC and childhood AA.

Therefore, the present study evaluated the disease characteristics, incidence, and clinical features of AIMs in RCC. In addition, the immunological status of AA was compared with that of RCC.

2. Materials and methods

2.1. Patients (Table 1)

A total of 72 patients with RCC who were treated at the Affiliated People's Hospital of Peking University between February 2008 and March 2013 were evaluated. All patients met the minimal diagnostic criteria for MDS [21] based on the World Health Organization's morphological criteria, bone marrow biopsy, immunophenotyping, and chromosome karyotype. Children with therapy-related MDS were excluded. All analyses were performed at diagnosis and before the start of treatment.

In addition, 123 children diagnosed with acquired AA who were treated at the Affiliated People's Hospital of Peking University between January 2008 and March 2013 were evaluated. The severity of AA was defined according to the criteria described by Camitta et al. [22]. Clinical findings and chromosomal breakage/genetic testing for Fanconi anemia were performed for all patients to exclude congenital bone marrow failure, such as Fanconi anemia.

This study was approved by the Institutional Review Board of the Affiliated People's Hospital of Peking University. Written informed consent was obtained from the patients' guardians.

2.2. Treatment

The patients with AA received ATG and/or cyclosporine as first-line therapy. Patients with AA were administered rabbit ATG (Genzyme, Batiments, France) 3–5 mg kg^{-1} day^{-1} for 5 days, in combination with oral cyclosporine 3–8 mg kg^{-1} day^{-1}, which was continued for 180 days. Transfusional support, antibiotics to treat infections, and growth factors to treat neutropenia were administered as needed. The patients with RCC received cyclosporine and androgen as first-line therapy. Eleven RCC children with an abnormal karyotype or a poor response to immunosuppressive therapy underwent bone marrow transplantation.

The primary endpoint of this study was the hematologic response rate at 6 months, including complete remission and partial remission. CR (complete remission) was defined as normalization of peripheral-blood counts, and PR (partial remission) was defined as transfusion independence for more than 60 days.

2.3. Serologic evaluation of immunologic abnormalities

Serologic evaluations of immunologic disturbances were screened routinely in all patients, regardless of the presence of clinical signs of autoimmune disease. Serologic immunologic abnormalities, including auto-red cell antibodies (i.e., Coombs' test) as well as rheumatoid factor, antinuclear antibody, anti-mitochondrial antibody, anti-histone antibody, ribosomal antibodies, nuclear ribonucleoprotein, anti-SSA antibody, and anti-SSB antibody positivity, were evaluated. C3 and C4 complements, as well as immunoglobulin levels (i.e., IgA, IgM, IgG, IgD, and IgE), were measured by turbidimetric immunoassay. Cutoff values were defined according to normal values in Chinese children and local laboratory ranges [23].

2.4. Immunophenotype studies

Fresh peripheral blood samples were collected into AC Caliber (Becton Dickinson, CA, USA) and analyzed using Cellquest software (Becton Dickinson). For each tube, 10,000 events were collected in a gate created around the viable lymphocyte population using forward scatter/side scatter anti-CD3 antibody conjugated with fluorescein isothiocyanate, anti-CD8 conjugated with R-phycoerythrin, anti-CD45 conjugated with peridinin chlorophyll protein, anti-CD4 conjugated with allophycocyanin, and anti-CD56 conjugated with R-phycoerythrin. Mixtures were incubated at room temperature for 20 min in the dark. Erythrocytes were subsequently lysed using lysing solution (Beckman Coulter, CA, USA). The relative numbers of CD3-, CD4-, CD8-, CD56-, and CD19-positive lymphocytes were presented in quadrant charts.

2.5. Detection of T-cell receptor (TCR) δ rearrangement in bone marrow

Bone marrow samples were obtained from all patients. EDTA-anticoagulated bone marrow samples taken at the time of diagnosis were subjected to Ficoll-Hypaque (1.077 g/mL; Pharmacia, Uppsala, Sweden) density gradient centrifugation, and the mononuclear cell fraction was cryopreserved. DNA was extracted using a modified phenol/chloroform method and dissolved in nuclease-free water. The outer primers were 5′-AAA TGC TAG CTA TTT CAC CCA-3′ and 5′-TCA TCC ATC TCT CTC TCT TC-3′; the inner primers were 5′-TTG TAG CAC TGT GCG GAT CC- 3′ and 5′-GCA CCA TCA GAG AGA GAT GA-3′.

The bone marrows of 6 healthy donors served as negative controls, while the bone marrows of 6 patients with acute lymphoblastic leukemia accompanied by the clonal rearrangement of TCR δ were used as positive controls. Nested PCR was performed as described previously Schmidt et al. [24]. The PCR product was observed in polyacrylamide gel electrophoresis.

2.6. Statistical analysis

Patient characteristics were evaluated by descriptive statistics. Rates were compared by Fisher's exact test. Qualitative variables were assessed by Student's *t*-test. All *p*-values were two-sided, and the level of significance was set at $p < 0.05$. All statistical analyses were performed using SPSS software version 14 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Clinical evidence of immunologic abnormalities (Table 2)

RCC was associated with autoimmune disease in 4/72 patients, including 1 case each with autoimmune hemolytic anemia, rheumatoid arthritis, systemic lupus erythematosus, and anaphylactoid purpura. In contrast, none of the 123 patients with AA was diagnosed with an autoimmune disease. The rate of autoimmune disease was significantly lower in childhood AA than in children with MDS ($p = 0.008$, $\chi^2 = 6.976$).

3.2. Laboratory evidence of immunologic manifestation (Table 3)

Lymphocytopenia (i.e., $<1 \times 10^9$ L^{-1}) was found in 9 patients with RCC and 11 patients with severe AA.

3.3. Gamma-globulin abnormalities

Among the patients with RCC, 9 had hypogammaglobulinemia, and 1 had elevated IgG.. In contrast, 8 patients with AA had