主論文の要旨

A Cytokine-Based Diagnostic Program in Pediatric Aplastic Anemia and Hypocellular Refractory Cytopenia of Childhood

（サイトカインによる小児再生不良性貧血と小児不応性血球減少症の診断システム）

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【Introduction】

Aplastic anemia (AA) is defined as an immune-mediated disorder characterized by pancytopenia accompanied by bone marrow (BM) hypoplasia. Refractory cytopenia of childhood (RCC), which is the most common subtype of pediatric myelodysplastic syndrome (MDS), was added to the pediatric modification of the World Health Organization (WHO) classification in 2008 and characterized by peripheral cytopenia, dysplasia, ineffective hematopoiesis, and a risk of progression to acute myeloid leukemia. In contrast to adult refractory anemia, the majority of patients with RCC show a marked decrease of BM cellularity. Therefore, the histological distinction between AA and hypocellular RCC is challenging and requires the collaboration of experienced hematologists and hematopathologists. The pathobiology of AA and a part of MDS is thought to involve immune-mediated processes and the overproduction of circulating soluble cytokines. Among these, thrombopoietin (TPO) and interleukin (IL)-17 are the two major cytokines that contribute to dysregulated hematopoiesis and immune processes in AA and MDS. The aim of this study was to measure the cytokine levels and evaluate whether the plasma levels of TPO and IL-17 can be used to distinguish between AA and hypocellular RCC in children.

【Materials and methods】

A total of 63 Japanese children were subjected to a central review of peripheral blood (PB) and BM morphology. Two pediatric hematologists and one hematopathologist reviewed PB/BM smears and BM trephine biopsies and classified them according to the diagnostic criteria proposed by the WHO in 2008. Age-matched healthy individuals were chosen as controls. The severity of the disease was classified according to an internationally accepted criterion. The characteristics of the patients are summarized in Table 1. Patients with abnormal karyotypes at time of diagnosis were excluded. Written informed consent was obtained from all parents. The ethics committee of Nagoya University Hospital approved the study. The plasma TPO and IL-17 levels were measured in 63 patients and 31 healthy control children using a sensitive sandwich enzyme-linked immunosorbent assay (ELISA). The plasma TPO and IL-17 levels were measured using the Human TPO Quantikine R ELISA kit (Cat. No. DTP00B, R&D systems, Minneapolis, MN, USA) and the Human IL-17 Platinum ELISA kit (Cat. No. BMS2017, eBiosience, San Diego, CA, USA), respectively, according to the manufacturer's instructions. Samples absorbance was measured at 450/570 nm using a PowerScan4 microplate reader (DS Pharma Medical Co., Japan). Corrected TPO values were calculated by dividing TPO concentration by platelet count ($\times 10^9$/L).

【Results】

Patient characteristics: The median age of the patients at the time of diagnosis was 9.5 years (range, 9.0–16.0 years). Among the 63 patients, 29 patients had AA and 34 had hypocellular

RCC. In total, 21 had very severe disease, 20 had severe disease and 22 had moderate disease severity; all cases were idiopathic. Patient characteristics are summarized in Table 1.

Plasma TPO levels: The median plasma TPO level was significantly higher in the patients than in the normal controls (1331.1 vs. 23.7 pg/mL, respectively; $P < 0.001$) and in patients with AA than hypocellular RCC patients (1892.17 vs. 1050 pg/mL, respectively; $P < 0.001$) (Fig. 1A). The correlation between plasma TPO levels and platelet counts was non-significant. The ROC curve analysis identified the threshold between AA and hypocellular RCC of 1369.8 pg/mL for TPO levels and of 1105.60 pg/mL × 10^9/L for the corrected TPO values (Fig. 2A, Fig. 2B). A total of 31 patients were assigned to TPO-high group (≥1369.8 pg/mL) and 32 patients were assigned to TPO-low group (<1369.8 pg/mL). The patients diagnosed as having hypocellular RCC were more prone to be included in the TPO-low group (25/32, 78.1%) than in the TPO-high group (9/31, 29%) ($P < 0.001$). In contrast, the likelihood of having a diagnosis of AA was significantly higher in the TPO-high group than in the TPO-low group (71% vs. 21.8%, respectively; $P < 0.001$) (Fig. 3A).

Plasma IL-17 levels: The median plasma IL-17 level was significantly higher in the patients than in the normal controls (22.5 vs. 5.83 pg/mL, respectively; $P < 0.001$) and in patients with AA than those with hypocellular RCC (median, 30.97 and 17.07 pg/mL, respectively; $P = 0.007$) (Fig. 1B). The ROC curve analysis identified the IL-17 level threshold between the patients with AA and hypocellular RCC to be 22.2 pg/mL (Fig. 2C). Thirty patients had IL-17 levels of ≥22.2 pg/mL (IL-17-high group) and 33 patients had IL-17 levels of <22.2 pg/mL (IL-17-low group). The number of patients with hypocellular RCC was significantly higher in the IL-17-low group (25/33, 75.8%) than in the IL-17-high group (9/30, 30%) ($P = 0.001$). The number of patients with AA was significantly higher in the IL-17-high group than in the IL-17-low group (70% vs. 24.2%, respectively; $P = 0.001$) (Fig. 3B).

Multivariate analysis of factors affecting the diagnosis of AA and hypocellular RCC: The multivariate logistic regression analysis identified the moderate disease severity (OR, 0.068; 95%CI, 0.010–0.441; $P=0.005$), TPO levels of <1369.8 pg/mL (OR, 13.40; 95%CI, 3.001–51.254; $P<0.001$), and IL-17 levels of <22.2 pg/mL (OR, 4.11; 95%CI, 1.033–19.404; $P=0.031$) as significant independent factors for discriminating patients with hypocellular RCC from patients with AA as described in Table 2.

Combination of TPO and IL17 for distinguishing hypocellular RCC from AA: According to these cytokine findings, we classified the patients to three groups described in Table 3. The number of patients with hypocellular RCC in the group 1 was 85.7% (18/21), which was significantly higher than that in the group 2 (10.5%, 2/19; $P < 0.001$). Conversely, the number of patients with AA in the group 2 was 89.5% (17/19), which was significantly higher than that in the group 1 (14.3%, 3/21; $P < 0.001$). However, the combination of TPO and IL-17 in the group 3 could not significantly distinguish between the two diseases.

【Discussion】

Distinguishing hypocellular RCC from AA remains a clinical dilemma because there is considerable similarity between the two diseases with regard to clinical characteristics, laboratory and histopathological findings, particularly in the absence of cytogenetic abnormalities. Moreover, Forester and colleagues recently showed that even using the methodology set forth by Bauman et al., pathologists show low concordance in term of differentiating between AA and hypocellular RCC. However, accurate diagnosis is critical to the proper management of these patients. Because the percentage of hypoplastic MDS is higher in childhood MDS than in adults, methods for distinguishing these diseases are important in the pediatric field. We found that TPO and IL-17 are useful in distinguishing hypocellular RCC from AA in children.

Our study revealed higher levels of TPO in patients with AA than in patients with hypocellular RCC. As TPO is passively regulated by binding to its receptor (c-Mpl) on megakaryocytes, the increased TPO plasma levels in patients with AA likely represent a compensatory physiological counteraction to the severe reduction in BM megakaryocytes and circulating platelets. In contrast, the relatively low TPO levels in patients with hypocellular RCC despite severe thrombocytopenia are caused by the presence of dysplastic BM megakaryocytes, which express c-Mpl on their cell surfaces.

Previous studies reported that $CD4^+$ Th17-cells immune responses play an important role in the pathogenesis of AA. In our study, IL-17 plasma levels were significantly higher in patients with AA than in patients with hypocellular RCC. The lower IL-17 levels in patients with hypocellular RCC than patients with AA could be explained by RCC being a subcategory of MDS, in which not all cases represent primarily immune-mediated processes and it instead has a clonal stem cell defect. Additionally, the response rate to immunosuppressive therapy and failure-free-survival has been reported to be less favorable in patients with RCC compared with patients with severe AA.

【Conclusions】

In our study, distinct TPO and IL-17 levels were observed between AA and hypocellular RCC, which reflect pathophysiological differences between these two diseases. The positive predictive impact of TPO and IL-17 thresholds was confirmed by a multivariate logistic regression analysis. We found a correlation between patients with hypocellular RCC and moderate severity of the disease. The combination of these two cytokines could more accurately predict a diagnosis of AA versus hypocellular RCC. Prospective studies are required to confirm our findings.