Original Articles

Relapse of aplastic anemia in children after immunosuppressive therapy: a report from the Japan Childhood Aplastic Anemia Study Group

Takuya Kamio,[1] Etsuro Ito,[1] Akira Ohara,[2] Yoshiyuki Kosaka,[3] Masahiro Tsuchida,[4] Hiroshi Yagasaki,[5] Hideo Mugishima,[5] Hiromasa Yabe,[6] Akira Morimoto,[7] Shouichi Ohga,[8] Hideki Muramatsu,[9] Asahito Hama,[9] Takashi Kaneko,[10] Masayuki Nagasawa,[11] Atsushi Kikuta,[12] Yuko Osugi,[13] Fumio Bessho,[14] Tatsutoshi Nakahata,[15] Ichiro Tsukimoto,[2] and Seiji Kojima,[9] on behalf of the Japan Childhood Aplastic Anemia Study Group

[1]Department of Pediatrics, Hirosaki University Graduate School of Medicine, Hirosaki; [2]Department of Pediatrics, Toho University, Tokyo; [3]Department of Hematology and Oncology, Hyogo Children's Hospital; [4]Department of Pediatrics, Ibaraki Children's Hospital, Mito; [5]Department of Pediatrics, Nihon University School of Medicine, Tokyo; [6]Department of Cell Transplantation, Tokai University School of Medicine, Isehara; [7]Department of Pediatrics, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, Kyoto; [8]Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, Fukuoka; [9]Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya; [10]Department of Hematology/Oncology, Tokyo Metropolitan Children's Medical Center, Tokyo; [11]Department of Pediatrics, Graduate Medical School, Tokyo Medical and Dental University, Tokyo; [12]Department of Pediatrics, Fukushima Medical University, Fukushima, [13]Department of Pediatric Hematology/Oncology, Osaka City General Hospital, Osaka; [14]Department of Pediatrics, Kyorin University School of Medicine, Tokyo; [15]Department of Pediatrics, Graduate School of Medicine, Kyoto University, Kyoto, Japan

ABSTRACT

Background
Although the therapeutic outcome of acquired aplastic anemia has improved markedly with the introduction of immunosuppressive therapy using antithymocyte globulin and cyclosporine, a significant proportion of patients subsequently relapse and require second-line therapy. However, detailed analyses of relapses in aplastic anemia children are limited.

Design and Methods
We previously conducted two prospective multicenter trials of immunosuppressive therapy for children with aplastic anemia: AA-92 and AA-97, which began in 1992 and 1997, respectively. In this study, we assessed the relapse rate, risk factors for relapse, and the response to second-line treatment in children with aplastic anemia treated with antithymocyte globulin and cyclosporine.

Results
From 1992 to 2007, we treated 441 children with aplastic anemia with standard immunosuppressive therapy. Among the 264 patients who responded to immunosuppressive therapy, 42 (15.9%) relapsed. The cumulative incidence of relapse was 11.9% at 10 years. Multivariate analysis revealed that relapse risk was significantly associated with an immunosuppressive therapy regimen using danazol (relative risk, 3.15; P=0.001) and non-severe aplastic anemia (relative risk, 2.51; P=0.02). Seventeen relapsed patients received additional immunosuppressive therapy with antithymocyte globulin and cyclosporine. Eight patients responded within 6 months. Seven of nine non-responders to second immunosuppressive therapy received hematopoietic stem cell transplantation and five are alive. Eleven patients underwent hematopoietic stem cell transplantation directly and seven are alive.

Conclusions
In the present study, the cumulative incidence of relapse at 10 years was relatively low compared to that in other studies mainly involving adult patients. A multicenter prospective study is warranted to establish optimal therapy for children with aplastic anemia.

Key words: children, aplastic anemia, relapse, risk factors, immunosuppressive therapy.

Citation: Kamio T, Ito E, Ohara A, Kosaka Y, Tsuchida M, Yagasaki H, Mugishima H, Yabe H, Morimoto A, Ohga S, Muramatsu H, Hama A, Kaneko T, Nagasawa M, Kikuta A, Osugi Y, Bessho F, Nakahata T, Tsukimoto I, and Kojima S, on behalf of the Japan Childhood Aplastic Anemia Study Group. Relapse of aplastic anemia in children after immunosuppressive therapy: a report from the Japan Childhood Aplastic Anemia Study Group. Haematologica 2011;96(6):814-819. doi:10.3324/haematol.2010.035600

— 61 —

Introduction

Aplastic anemia (AA) is thought to be an immune-mediated bone marrow disease, characterized by bone marrow aplasia and peripheral blood pancytopenia. Currently, two effective treatments are available for this disorder: allogeneic bone marrow transplantation and immunosuppressive therapy. Bone marrow transplantation from a human leukocyte antigen (HLA)-matched sibling donor can cure the majority of transplanted patients with severe AA.[1] The outcome after bone marrow transplantation has been markedly better in children than in adults, with less frequent and severe graft-*versus*-host disease and better overall survival.[2,3] However, most children with severe AA have no matched sibling donor and rely on immunosuppressive therapy as first-line treatment.

The combination of antithymocyte globulin and cyclosporine is now considered the standard immunosuppressive regimen for children with severe AA who lack a matched sibling donor.[4] Recent large trials of combined immunosuppressive therapy for severe AA in children demonstrated that the response rate is greater than 60% and the 3- to 5-year survival rate is approximately 90%.[5-7] However, relapse and clonal evolution with transformation to myelodysplasia or acute myeloid leukemia remain significant problems after immunosuppressive therapy, and long-term, event-free survival is less impressive than after bone marrow transplantation.[4,8] We previously reported the results of a multicenter trial of immunosuppressive therapy for children with AA (AA-92 study).[5] In the AA-92 study, the response rate at 6 months was 71%, with the probability of survival at 4 years being greater than 90%. However, a significant proportion of patients subsequently relapsed and required second-line therapy. To select the optimal therapy for such patients, a detailed analysis concerning relapse after response to immunosuppressive therapy is very important; however, analyses of relapse of AA in children after the standard combined immunosuppressive regimen are very limited.[9-11] Although the European Group for Blood and Marrow Transplantation (EMBT) reported an analysis of relapse of AA after immunosuppressive therapy in a large number of patients, the study populations were primarily adults treated in the 1970s and 1980s with antithymocyte globulin monotherapy.[9] A report from the Italian Association of Pediatric Hematology and Oncology focused mainly on the response to cyclosporine and dependence after immunosuppressive therapy.[10] A single-center retrospective analysis from the National Institutes of Health showed excellent long-term survival with a 33% cumulative incidence of relapse at 10 years in children with severe AA who responded to the standard immunosuppressive therapy; however, a detailed analysis of relapse that included risk factors was not provided.[11]

We previously conducted two prospective multicenter studies: the AA-92 and AA-97, which began in November 1992 and October 1997, respectively.[5,12] From 1992 to 2007, 473 children with AA were treated with immunosuppressive therapy in these studies, and 441 of the children were treated with antithymocyte globulin plus cyclosporine. In the present study, we assessed the relapse rate, risk factors for relapse, response to second-line treatment, and prognosis after relapse in AA children treated with an antithymocyte globulin/ cyclosporine-based regimen.

Design and Methods

Patients

Two consecutive prospective studies were designed by the Japan Childhood Aplastic Anemia Study Group and involved 79 hospitals in Japan. The eligibility criteria have been described previously.[5] The severity of disease was determined according to currently used criteria.[13,14] Disease was considered severe if at least two of the following were present: (i) neutrophil count less than $0.5\times10^9/L$; (ii) platelet count less than $20\times10^9/L$; and (iii) reticulocyte count less than $20\times10^9/L$ with a hypocellular bone marrow. AA was considered very severe if the above criteria for severe disease were fulfilled and the neutrophil count was less than $20\times10^9/L$. Non-severe disease was defined by at least two of the following: (i) neutrophil count less than $1.0\times10^9/L$, (ii) platelet count less than $50\times10^9/L$; and (iii) reticulocyte count less than $60\times10^9/L$ with a hypocellular bone marrow. Allogeneic bone marrow transplantation was recommended for those patients with severe or very severe disease who had a matched sibling donor. This study was approved by the Ethic Committee of Hyogo Children Hospital.

Treatment

The details of the immunosuppressive therapy administered were described in previous reports.[5,12] Immunosuppressive therapy consisted of horse antithymocyte globulin (Lymphoglobulin; Genzyme Corp., Cambridge, MA, USA) (15 mg/kg per day, days 1 to 5), cyclosporine (6 mg/kg per day, days 1 to 180, with subsequent adjustments to maintain the whole blood cyclosporine concentration between 100 to 200 ng/mL), and methylprednisolone for prophylaxis against allergic reactions (2 mg/kg per day for 5 days, with subsequent halving of the dose every week until discontinuation on day 28). Patients with very severe AA were treated with immunosuppressive therapy plus granulocyte-colony stimulating factor (G-CSF) (Filgrastim; Kirin, Tokyo, Japan) [400 µg/m² on day 1, with responding patients (neutrophil count > $1.0\times10^9/mL$) receiving the same dose three times a week for 3 months in the AA-92 study and for 60 days in the AA-97 study]. In the AA-92 study, the addition of G-CSF to immunosuppressive therapy for patients with severe AA and non-severe AA was randomized, while in the AA-97 study, G-CSF was not given to patients with severe AA or non-severe AA except to those with documented severe infection. All patients in the AA-92 study received danazol at a dose of 5 mg/kg/day for 6 months, and danazol was discontinued without tapering.

Assessments

A complete response was defined for all patients as a neutrophil count greater than $1.5\times10^9/L$, a platelet count greater than $100\times10^9/L$, and a hemoglobin level greater than 11.0 g/dL. For patients with severe AA and very severe AA, a partial response was defined as a neutrophil count greater than $0.5\times10^9/L$, a platelet count greater than $20\times10^9/L$, a hemoglobin level greater than 8.0 g/dL, and no requirement for blood transfusions. For patients with non-severe AA, a partial response was defined as a neutrophil count greater than $1.0\times10^9/L$, a platelet count greater than $30\times10^9/L$, a hemoglobin level greater than 8.0 g/dL, and no requirement for blood transfusions.[5] In patients with a complete response on day 180, the cyclosporine dose was tapered down slowly (10% of adjusted dose per month). In those with a partial response, cyclosporine was continued for another 6 months to allow further improvement of blood counts. Tapering of cyclosporine was started on day 360 (10% every 2 weeks) regardless of response.

The hematologic response was evaluated 6 months after the

initiation of therapy. Relapse was defined by conversion to no response from a partial or complete response and/or the requirement for blood transfusions.[5]

Statistical analysis

Failure-free survival curves were calculated by the Kaplan-Meier method, and evaluated by the log-rank test. The Cox proportional hazards model was used to assess the risk factors for relapse after immunosuppressive therapy using both univariate and multivariate analyses. The estimated magnitude of the relative risk (RR) is shown along with the 97.5% confidence interval (CI). Cumulative incidence using the competing risk method, as described by Fine and Gray,[15] was used for the assessment of factors predicting relapse. The competing events of relapse were death and transplantation.

Results

Patients' characteristics

In the AA-92 and AA-97 studies, 441 AA children were treated with antithymocyte globulin plus cyclosporine between 1992 and 2007. The characteristics of all the patients studied are summarized in Table 1. There were 112 and 329 patients in the AA-92 and AA-97 studies, respectively. The median age of all these patients was 8.3 years (range, 0 to 17 years). Patients with very severe (n=210), severe (n=149) and non-severe disease (n=82) received initial immunosuppressive therapy consisting of antithymocyte globulin and cyclosporine. Six months after the initial immunosuppressive therapy, 264 patients (59.9%) had achieved a complete response (n = 91) or partial response (n=173). Among the 264 patients who responded to immunosuppressive therapy, 42 (15.9%) subsequently relapsed. The cumulative incidence of relapse was 11.9% at 10 years and the median time from diagnosis to relapse was 21 months (range, 6 to 138 months). The median time from response to antithymocyte globulin therapy to relapse was 22 months (range, 2 to 135 months).

Risk factors for relapse

Two hundred and sixty-four patients with a total of 42 events were eligible for analyses of risk factors for relapse. In univariate analysis, two parameters, non-severe disease (RR=2.98, 97.5% CI 1.40 - 6.34, P=0.0047) and use of danazol (RR=3.44, 97.5% CI 1.78 - 6.65, P=0.00023), were statistically significant risk factors (Table 2). In contrast, the relative risk of relapse for patients with post-hepatitis AA was significantly lower than the relative risk for patients with idiopathic AA (RR=0.234, P=0.043). Gender, age, duration of AA prior to initial treatment, early response (within 90 days after immunosuppressive therapy), use of G-CSF, and HLA-DR2 could not be identified as risk factors. In multivariate analysis, two factors, non-severe AA (RR=2.51, 97.5% CI 1.15 - 5.46, P=0.02) and use of danazol (RR=3.15, 97.5% CI 1.62 - 6.12, P=0.001) remained statistically significant. Figure 1A shows the cumulative incidence of relapse of patients with non-severe AA (35.3%), severe AA (12.9%), and very severe AA (12.0%) 10 years after the first immunosuppressive therapy. The cumulative relapse rate of patients with non-severe AA was significantly higher than that of patients with severe AA (P=0.025) or very severe AA (P=0.005). Figure 1B shows the actuarial risk of relapse at 10 years

among patients treated with danazol (29.0%) and in the group not treated with danazol (9.8%) (P<0.001).

Repeated immunosuppressive therapy versus hematopoietic stem cell transplantation as second-line therapy

Among 42 relapsed patients, 17 received a second course of immunosuppressive therapy with antithymocyte globulin and cyclosporine. Eight of these 17 patients responded within 6 months and are alive. Seven of nine non-responders to second immunosuppressive therapy received hematopoietic stem cell transplantation (HSCT) as salvage therapy. The hematopoietic stem cell donors were HLA-matched unrelated bone marrow donors (n=4), unrelated cord blood donors (n=2) and one matched sibling donor. Five of seven patients are alive following HSCT. Eleven patients underwent HSCT directly from an alternative donor (unrelated bone marrow donor, n=7; unrelated cord blood donor, n=1, HLA-mismatched family donor, n=3) and seven are alive. The estimated failure-free survival from the beginning of second-line therapy was 63.6% in the HSCT group compared with 47.1% in the groups treatment with repeated immunosuppressive therapy (P=0.96).

Table 1. **Patients' pretreatment characteristics.**

	Very severe AA	Severe AA	Non-severe AA
Registered	210	149	82
Sex (male/female)	115/95	83/66	47/35
Median age, years (range)	8.1 (0-17)	8.3 (1-17)	8.5 (2-16)
Etiology of AA			
Ideopathic	168	125	74
Hepatitis	37	21	7
Viral infection	2	1	0
Drug	3	2	1
Median days from diagnosis to treatment (range)	20.4 (1-146)	30.6 (1-180)	44.8 (3-180)
Study (AA-92/AA-97)	46/164	38/111	28/54
Response (complete/ partial) (%)	128 (40/88) (61.0%)	91 (38/53) (61.1%)	45 (13/32) (54.9%)
Relapse (AA-92/AA-97)	6/8	9/5	11/3

Table 2. **Risk factors for relapse in patients with aplastic anemia by univariate analysis.**

| Variable	Relative risk (97.5% CI)	P
Sex, male	0.977 (0.514-1.86)	0.94
Age	1.01 (0.947-1.08)	0.78
Etiology of AA		
Ideopathic	4.97 (1.22-20.2)	0.025
Hepatitis	0.234 (0.0577-0.952)	0.043
Duration of AA prior to initial treatment	1.01 (0.998-1.02)	0.11
Response at 90 days	1.07 (0.517-2.21)	0.86
Severity of disease		
Non-severe	2.98(1.40-6.34)	0.0047
Severe	1.21(0.561-2.63)	0.62
Very severe	1	
Study, AA-92 (Danazol+)	3.44 (1.78-6.65)	0.00023
G-CSF (+)	0.915 (0.363-2.31)	0.85
HLA-DR2	0.905 (0.307-2.67)	0.86
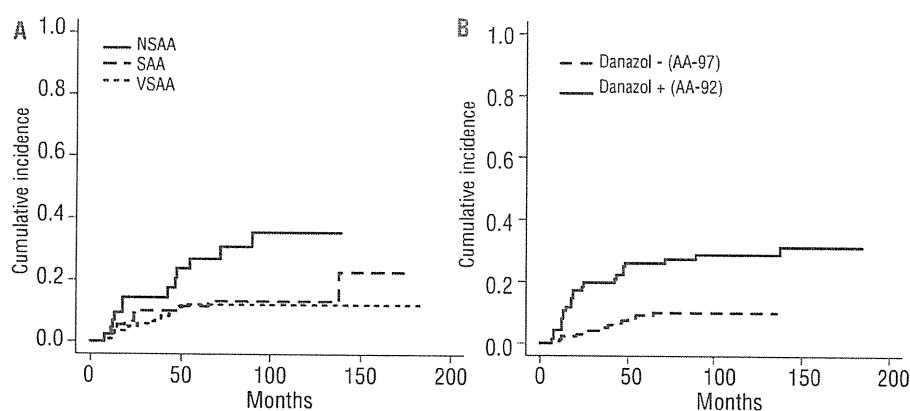
Figure 1. Cumulative incidence of relapse after immunosuppressive therapy in children with aplastic anemia. (A) The cumulative relapse rate of patients with non-severe aplastic anemia (NSAA) was significantly higher than that of patients with severe aplastic anemia (SAA) (P=0.025) and very severe aplastic anemia (VSAA) (P=0.005) 10 years after the first immunosuppressive therapy. (B) The actuarial risk of relapse at 10 years was significantly higher in the group treated with danazol (29.0%) than in the group not treated with danazol (9.8%) (P<0.001).

The overall survival rate did not differ between the immunosuppressive therapy group (84.7%) and the HSCT group (63.6%) after second-line treatment (P=0.07). Other patients were treated with cyclosporine alone (n=6) or bone marrow transplantation from a matched sibling donor (n=6). Two patients did not receive second-line treatments. One patient developed clonal evolution to myelodysplastic syndrome after 65 months, and the second developed acute myeloid leukemia after 37 months. Two patients showed clonal evolution to paroxysmal nocturnal hemoglobinuria after 138 months and 55 months. There were seven deaths among the 42 patients who initially relapsed. The causes of death were HSCT-related complications (n=5), acute myeloid leukemia (n=1) and bacteremia (n=1). The overall 10-year survival rates for patients with very severe AA, severe AA, and non-severe AA were 82.2±3.3%, 82.1±4.7% and 98.2±1.8%, respectively.

Discussion

Analysis of relapse in children with AA responding to immunosuppressive therapy will provide valuable information for the management of childhood AA. Here, we present the results of a comprehensive analysis of the largest consecutive series of AA children treated with standard immunosuppressive therapy. Relapse of AA after immunosuppressive therapy is relatively common, with actuarial risks of 30 - 40% having been reported.[16-18] In the present study, the cumulative incidence of relapse at 10 years was 11.9%, which is relatively low compared with that found in other studies that primarily involved adult patients.[16-18] Differences in the study populations may explain the discrepancy between the results of our current study and those of the other studies. A recent Italian study of childhood AA showed a 16% cumulative incidence of relapse, which is comparable with that found in our study.[10]

Multivariate analysis of the data from this retrospective multicenter study shows that the use of danazol was the most statistically significant risk factor for relapse. From 1992 to 2007, 441 children with newly diagnosed AA were treated with immunosuppressive therapy consisting of antithymocyte globulin and cyclosporine with (the AA-92 study) or without danazol (the AA-97 study). There are several reports of the efficacy of anabolic steroids in the treatment of AA. A randomized trial from the EBMT SAA working party demonstrated that the addition of an ana-

bolic steroid (oxymetholone) to antithymocyte globulin treatment improved the response rate of patients with treated AA.[14] In our study, consistent with that report, the response rate at 6 months was higher in the patients who received immunosuppressive therapy with danazol (67.9%) than in the group of patients who received immunosuppressive therapy without danazol (57.1%). Furthermore, our results also showed that the cumulative relapse rate was significantly higher in the patients treated with immunosuppressive therapy plus danazol (Figure 1B). The reason danazol has an impact on relapse is unknown. However, it is possible that a number of cases with an androgen-responsive congenital bone marrow failure syndrome such as dyskeratosis congenita were hidden in our series of AA patients, and discontinuation of danazol was responsible for relapse. Recent reports have shown that a bone marrow failure syndrome of variable severity due to dyskeratosis congenita may be present in otherwise phenotypically normal individuals, and can masquerade as acquired AA.[19-22] We found mutations in the telomerase reverse transcriptase (*TERT*) gene, which is one of the genes causing dyskeratosis congenita, in two of 96 Japanese children with acquired AA.[23] Recently, more dyskeratosis congenita genes have been discovered. It is possible that more cases with an androgen-responsive dyskeratosis congenita were hidden in our series of AA patients. Alternatively, danazol may inhibit complete eradication of pathological T-cell clones by antithymocyte globulin through an unknown mechanism. Understanding the effects of androgens and developing androgen-mimetic drugs could be of significant benefit.

In our cohort of patients with non-severe AA, most patients were transfusion-dependent. In the AA-92 and AA-97 studies, 82 patients with non-severe AA were treated with the standard immunosuppressive regimen consisting of antithymocyte globulin and cyclosporine. Six months after the initial immunosuppressive therapy, 13 patients had achieved a complete response and 32 patients achieved a partial response. Among the 32 patients who achieved a partial response, 14 patients later relapsed. However, 18 patients with non-severe AA patients who achieved a partial response maintained their hematologic response, and 12 of them subsequently achieved a complete response. When childhood non-severe AA is treated with supportive care, 67% of patients progress to develop severe AA, suggesting that it is important to consider early immunosuppressive therapy.[24] Our data indicate that

— 64 —

immunosuppressive therapy is beneficial for some patients with non-severe AA.

A previous Japanese study showed that the addition of G-CSF to immunosuppressive therapy increased the hematologic response rate after 6 months and reduced the relapse rate in adult patients with severe AA.[25] Recently, Gurion et al. conducted a systematic review and meta-analysis of randomized controlled trials comparing treatments with immunosuppressive therapy with or without hematopoietic growth factors in patients with AA. The addition of hematopoietic growth factors did not affect mortality, response rate, or occurrence of infections, but did significantly decrease the risk of relapse.[26] The data from our AA-92 trial were included in this meta-analysis. In contrast to the other five studies in the meta-analysis, only our study included patients with non-severe AA, who had a significantly higher relapse rate than that of patients with either severe AA or very severe AA. Differences in the study populations may explain the discrepancy between the results of our current study and those of the other studies in the meta-analysis. To compare our results with the other studies, we excluded patients with non-severe AA from the statistical analysis, and compared the risk of relapse between patients who did or did not receive G-CSF. The results again showed no significant differences in the relative risk between them (RR=2.71, 97.5% CI 0.614 - 12.0, P=0.19).

The majority of patients who experienced relapse responded to reintroduction of immunosuppressive agents.[27] Our present study also demonstrates that a second course of immunosuppressive therapy was a safe and effective treatment for the patients who relapsed after the first immunosuppressive therapy. However, an optimal second immunosuppressive therapy regimen has not yet been established. Furthermore, about half of the relapsing patients eventually received HSCT in our study. The treatment choice was based on center-related preferences or on anecdotal evidence. A multicenter prospective study is warranted to establish optimal therapy for these patients.

Appendix

The following centers and persons participated in the Japan Childhood Aplastic Anemia Study Group: Japanese Red Cross Nagoya First Hospital (K. Kato); Kyoto Prefectural University of Medicine (S. Morimoto); Kobe University School of Medicine (Y. Takeshima); Hyogo College of Medicine (Y. Ohtsuka); Tokai University (H. Yabe); Shizuoka Children's Hospital (J. Mimaya); Fukushima Medical University (A. Kikuta); Tokyo Metropolitan Children's Medical Center, Tokyo (T. Kaneko); Osaka City General Hospital (J. Hara); Nagoya University (S. Kojima) ; Jichi Medical School (T. Yamauchi); Kagoshima University (Y. Kawano); Okayama University (M. Oda); Hokkaido University (R. Kobayashi); Hiroshima University (S. Nishimura); Kanazawa University (S. Koizumi); Keio University (T. Mori); Hiroshima Red Cross Atomic Bomb Hospital (K. Hamamoto); Chiba University (T. Sato); Hirosaki University (E. Ito); Teikyo University School of Medicine (F. Ohta); Tottori University (T. Kawakami); Dokkyo University School of Medicine (K. Sugita); Kumamoto National Hospital (K. Takagi); Seirei Hamamatsu Hospital (T. Matsubayashi); Hyogo Children's Hospital (Y. Kosaka); Yokohama City University (K. Ikuta); Yamaguchi University (H. Ayukawa); Kanagawa Children's Medical Center (T. Kigasawa); Hirakata City Hospital (C. Kawakami); Nakadohri General Hospital (A. Watanabe); Gumma Children's Hospital (T. Shitara); National Defence Medical College (I. Sekine); Gifu University School of Medicine (K. Isogai); Kumamoto University School of Medicine (S. Morinaga); University of Ryukyu (N. Hyakuna); Narita Red Cross Hospital (K. Sunami); Asahikawa Medical College (M. Yoshida); Nagoya City University (Y. Ito).

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

References

1. Storb R. Bone marrow transplantation for aplastic anemia. Cell Transplant. 1993;2(5):365-79.
2. Horowitz MM. Current status of allogeneic bone marrow transplantation in acquired aplastic anemia. Semin Hematol. 2000;37(1):30-42.
3. Ades L, Mary JY, Robin M, Ferry C, Porcher R, Esperou H, et al. Long-term outcome after bone marrow transplantation for severe aplastic anemia. Blood. 2004;103(7):2490-7.
4. Davies JK, Guinan EC. An update on the management of severe idiopathic aplastic anaemia in children. Br J Haematol. 2007;136(4):549-64.
5. Kojima S, Hibi S, Kosaka Y, Yamamoto M, Tsuchida M, Mugishima H, et al. Immunosuppressive therapy using antithymocyte globulin, cyclosporine, and danazol with or without human granulocyte colony-stimulating factor in children with acquired aplastic anemia. Blood. 2000;96(6):2049-54.
6. Bacigalupo A, Bruno B, Saracco P, Di Bona E, Locasciulli A, Locatelli F, et al. Anti-lymphocyte globulin, cyclosporine, prednisolone, and granulocyte colony-stimulating factor for severe aplastic anemia: an update of the GITMO/EBMT study on 100 patients. European Group for Blood and Marrow Transplantation (EBMT) Working Party on Severe Aplastic Anemia and the Gruppo Italiano Trapianti di Midollo Osseo (GITMO). Blood. 2000;95(6):1931-4.
7. Führer M, Rampf U, Baumann I, Faldum A, Niemeyer C, Janka-Schaub G, et al. Immunosuppressive therapy for aplastic anemia in children: a more severe disease predicts better survival. Blood. 2005;106(6): 2102-4.
8. Kojima S, Ohara A, Tsuchida M, Kudoh T, Hanada R, Okimoto Y, et al. Risk factors for evolution of acquired aplastic anemia into myelodysplastic syndrome and acute myeloid leukemia after immunosuppressive therapy in children. Blood. 2002;100(3):786-90.
9. Schrezenmeier H, Marin P, Raghavachar A, McCann S, Hows J, Gluckman E, et al. Relapse of aplastic anaemia after immunosuppressive treatment: a report from the European Bone Marrow Transplantation Group SAA Working Party. Br J Haematol. 1993;85(2):371-7.
10. Saracco P, Quarello P, Iori AP, Zecca M, Longoni D, Svahn J, et al. Cyclosporin A response and dependence in children with acquired aplastic anaemia: a multicentre retrospective study with long-term observation follow-up. Br J Haematol. 2008;140(2):197-205.
11. Scheinberg P, Wu CO, Nunez O, Young NS. Long-term outcome of pediatric patients with severe aplastic anemia treated with antithymocyte globulin and cyclosporine. J Pediatr. 2008;153(6):814-9.
12. Kosaka Y, Yagasaki H, Sano K, Kobayashi R, Ayukawa H, Kaneko T, et al. Prospective multicenter trial comparing repeated immunosuppressive therapy with stem-cell transplantation from an alternative donor as second-line treatment for children with severe and very severe aplastic anemia. Blood. 2008;111(3):1054-9.
13. Camitta BM, Thomas ED, Nathan DG, Gale RP, Kopecky KJ, Rappeport JM, et al. A prospective study of androgens and bone marrow transplantation for treatment of

— 65 —

severe aplastic anemia. Blood. 1979;53(3): 504-14.

14. Bacigalupo A, Chaple M, Hows J, Van Lint MT, McCann S, Milligan D, et al. Treatment of aplastic anaemia (AA) with antilymphocyte globulin (ALG) and methylprednisolone (MPred) with or without androgens: a randomized trial from the EBMT SAA working party. Br J Haematol. 1993; 83(1):145-51.

15. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc. 1999;94(446): 496-509.

16. Schrezenmeier H, Marin P, Raghavachar A, McCann S, Hows J, Gluckman E, et al. Relapse of aplastic anaemia after immunosuppressive treatment: a report from the European Bone Marrow Transplantation Group SAA Working Party. Br J Haematol. 1993;85(2):371-7.

17. Frickhofen N, Heimpel H, Kaltwasser JP, Schrezenmeier H. Antithymocyte globulin with or without cyclosporin A: 11-year follow-up of a randomized trial comparing treatments of aplastic anemia. Blood. 2003;101(4):1236-42.

18. Rosenfeld S, Follmann D, Nunez O, Young NS. Antithymocyte globulin and cyclosporine for severe aplastic anemia; association between hematologic response and long-term outcome. JAMA. 2003;289 (3): 1130-5.

19. Vulliamy T, Marrone A, Dokal I, Mason PJ. Association between aplastic anaemia and mutations in telomerase RNA. Lancet. 2002;359(9324):2168-70.

20. Yamaguchi H, Baerlocher GM, Lansdorp PM, Chanock SJ, Nunez O, Sloand E, et al. Mutations of the human telomerase RNA gene (TERC) in aplastic anemia and myelodysplastic syndrome. Blood. 2003;102 (3):916-8.

21. Yamaguchi H, Calado RT, Ly H, Kajigaya S, Baerlocher GM, Chanock SJ, et al. Mutations in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia. N Engl J Med. 2005;352(14):1413-24.

22. Fogarty PF, Yamaguchi H, Wiestner A, Baerlocher GM, Sloand E, Zeng WS, et al. Late presentation of dyskeratosis congenita as apparently acquired aplastic anaemia due to mutations in telomerase RNA. Lancet. 2003;362(9396):1628-30.

23. Liang J, Yagasaki H, Kamachi Y, Hama A, Matsumoto K, Kato K, et al. Mutations in telomerase catalytic protein in Japanese children with aplastic anemia. Haematologica. 2006;91(5):656-8.

24. Howard SC, Naidu PE, Hu XJ, Jeng MR, Rodriguez-Galindo C, Rieman MD, et al. Natural history of moderate aplastic anemia in children. Pediatr Blood Cancer. 2004; 43(5):545-51.

25. Teramura M, Kimura A, Iwase S, Yonemura Y, Nakao S, Urabe A, et al. Treatment of severe aplastic anemia with antithymocyte globulin and cyclosporin A with or without G-CSF in adults: a multicenter randomized study in Japan. Blood. 2007;110(6):1756-61.

26. Gurion R, Gafter-Gvili A, Paul M, Vidal L, Ben-Bassat I, Yeshurun M, et al. Hematopoietic growth factors in aplastic anemia patients treated with immunosuppressive therapy-systematic review and meta-analysis. Haematologica. 2009;94(5): 712-9.

27. Scheinberg P, Nunez O, Young NS. Retreatment with rabbit anti-thymocyte globulin and cyclosporin for patients with relapsed or refractory severe aplastic anaemia. Br J Haematol. 2006;133(6):622-7.
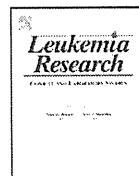
Letter to the Editor

CD7-positive acute myelomonocytic leukemia with trisomy 21 as a sole acquired chromosomal abnormality in two adolescents

1. Introduction

Trisomy 21 is one of the most common acquired chromosomal abnormalities in myeloid malignancies, but is rarely found as a sole anomaly. It has been reported that only 0.3% of adult patients with acute myeloblastic leukemia (AML) or myelodysplastic syndrome have trisomy 21 as a sole acquired abnormality [1]. Although several reports indicate that this karyotypic abnormality is associated with the French–American–British (FAB) AML subtypes M2, M4 and M5 [2,3] and with a poor prognosis [3,4], its clinical and prognostic implications have not been fully evaluated. Some investigators have focused on the association between this chromosomal abnormality and CD7 expression, and 4 adult patients with CD7-positive AML have been reported so far [5–8]. Although one report includes 2 adolescent AML cases with trisomy 21 as a sole acquired abnormality [3], there have been no reports on pediatric CD7-positive AML patients with this anomaly. Here, we report 2 unique adolescent cases of CD7-positive acute myelomonocytic leukemia with trisomy 21 as a minor clone and as a sole acquired chromosomal abnormality.

2. Case reports

2.1. Case 1

A 15-year-old boy was admitted to our hospital in November 2006 because of fever and gingival swelling and bleeding. Findings on physical examination included petechiae over the body and hepatomegaly (5 cm below the costal margin). Examination of the peripheral blood revealed the following: hemoglobin 8.3 g/dL, platelets 15×10^9/L, and leukocytes 227×10^9/L with 40% blasts and 8% monocytes (18.2×10^9/L). The bone marrow was hypercellular with 96% blasts, which were positive for myeloperoxidase but negative for non-specific esterase staining. Immunophenotypic analysis by flow cytometry showed that the blasts were positive for CD7, CD13, CD33, CD34, and HLA-DR expression and negative for lymphoid markers. Chromosome analysis of bone marrow cells revealed 47,XY,+21[2]/46,XY[38]. The patient had no clinical findings of Down syndrome. Interphase fluorescence in situ hybridization (FISH) analysis using a specific probe for the RUNX1 gene labeled with Spectrum-Green plus a set of 3 probes for 21q22 (D21S259, D21S341 and D21S342) labeled with Spectrum-Orange, showed that 26 out of 1000 bone marrow cells had 3 green and 3 red signals (2.6%). Serum and urine lysozyme levels were 43.4 μg/mL (reference range, 5.0–10.2 μg/mL) and 8.6 μg/mL (undetectable in normal subjects), respectively. Although morphological analysis of bone marrow cells was suggestive of a diagnosis of AML-M2 based on the FAB classification, the final diagnosis was AML-M4 because

of the increased number of peripheral blood monocytes and elevated serum and urine lysozyme levels [9]. The patient was treated with the AML-99 protocol [10] and achieved complete remission after the first course of chemotherapy. Chromosome analysis of the bone marrow cells showed a normal karyotype, 46,XY in all 20 cells analyzed. He received 5 additional courses of consolidation chemotherapy, which was finished in June 2007. Trisomy 21 was never found on chromosome analysis performed after each course of chemotherapy. He has remained in continuous complete remission for 4 years after diagnosis.

2.2. Case 2

A 14-year-old girl was referred to our hospital in April 2007 because of fever, nasal bleeding, and gingival swelling and bleeding. On admission, petechiae were observed over the body, and the liver and spleen were palpable 8 cm and 4 cm below the costal margin, respectively. Examination of the peripheral blood revealed the following: hemoglobin 5.1 g/dl, platelets 4×10^9/L, and leukocytes 73.1×10^9/L with 56% blasts and 15% monocytes (11.0×10^9/L). The bone marrow was hypercellular with 61% blasts, which were positive for myeloperoxidase but negative for non-specific esterase staining. Surface marker analysis showed that the blasts expressed CD7, CD13, CD33, CD34, and HLA-DR. Cytogenetic analysis of bone marrow cells revealed 47,XX,+21[2]/46,XX[18]. The patient did not manifest characteristics associated with Down syndrome. Interphase FISH analysis using a probe for the RUNX1 gene showed that 41 out of 1000 bone marrow cells had trisomy 21 (4.1%). The serum lysozyme level was elevated at 43.1 μg/mL, but lysozyme was undetectable in urine. A diagnosis of AML-M2 was suggested by the morphological findings of the bone marrow cells, but peripheral blood monocytosis and an elevated serum lysozyme level led to a final diagnosis of AML-M4. The patient underwent induction chemotherapy using the AML-99 protocol and achieved complete remission after a single course of chemotherapy. Cytogenetic analysis of bone marrow cells showed a normal female karyotype in all 20 metaphases analyzed. She was subsequently treated with 5 courses of consolidation chemotherapy and completed therapy in November 2007. Trisomy 21 was never detected on chromosome analysis performed after each course of chemotherapy. She has remained in continuous complete remission for 3 years after diagnosis.

The clinical characteristics and laboratory data of the 2 patients are summarized in Table 1. No mutations were detected in the RUNX1 or GATA1 genes in DNA from the patient's bone marrow cells (data not shown).

3. Discussion

We describe the first 2 pediatric CD7-positive AML cases with trisomy 21 as a sole acquired abnormality. These cases shared several unique clinical features. First, both patients were diagnosed

Table 1

Clinical characteristics and laboratory data of the patients.

	Case 1	Case 2
Age	15y	14y
Sex	Male	Female
WBC count ($\times 10^9$/L)	227	73.1
FAB classification	M4	M4
Non-specific esterase staining	Negative	Negative
Monocyte count ($\times 10^9$/L)	18.2	11.0
Serum lysozyme level[a] (μg/ml)	43.4	43.1
Urine lysozyme level[b] (μg/ml)	8.6	Not detected
Immunophenotype	CD7, 13, 33, 34, HLA-DR	CD7, 13, 33, 34, HLA-DR
Karyotype	47,XY,+21[2]/46,XY[38]	47,XX,+21[2]/46,XX[18]
Trisomy 21-positive cells on FISH analysis	26/1000 cells	41/1000 cells
CR after 1st course of chemotherapy	Yes	Yes
Outcome	CR, 4y after diagnosis	CR, 3y after diagnosis

WBC, white blood cell; FAB, French–American–British; FISH, fluorescence in situ hybridization; CR, complete remission.

[a] Reference range, 5.0–10.2 μg/mL.

[b] Undetectable in normal subjects.

with AML-M4 based on the FAB classification because of their peripheral blood monocytosis ($\geq 5 \times 10^9$/L) and elevated serum and/or urine lysozyme levels (>3 × normal values), although blasts in the bone marrow were negative for non-specific esterase and were morphologically classified as AML-M2 [9]. Some reports have suggested that AML with trisomy 21 as a single abnormality is associated with AML-M2, -M4 and -M5 [2,3], but monocyte counts and lysozyme levels were not discussed. Our findings indicate that evaluation of monocyte counts and serum/urine lysozyme levels are important for correct FAB classification of AML with this karyotypic abnormality.

Second, trisomy 21 was observed in only 2 out of the 40 or 20 bone marrow cells examined in Case 1 and 2, respectively, which were much lower percentages than the percentages of morphologically detected blast cells (96% and 61%, respectively). These low fractions of trisomy 21-positive cells were confirmed by interphase FISH analysis (2.6% and 4.1% in Case 1 and 2, respectively). We think that a constitutional mosaic trisomy 21 (mosaic Down syndrome) is not likely, because trisomy 21 was never found on serial cytogenetic analyses performed after remission in either patient (a total of 100 bone marrow cells were analyzed in each patient). These results clearly show that most of the blast cells had normal karyotypes and the blasts that had acquired trisomy 21 were minor clones in both patients.

Third, both patients achieved complete remission after the first course of chemotherapy and have remained in continuous complete remission for 3–4 years after diagnosis. Although several reports have indicated that this chromosomal abnormality is associated with poor prognosis [3,4], the prognostic implications have not been fully evaluated, because there are so few patients with this anomaly. To confirm that pediatric CD7-positive AML with this karyotypic abnormality has a favorable outcome, more patients need to be studied.

In conclusion, we report the first 2 pediatric cases of CD7-positive AML with trisomy 21 as a sole acquired chromosomal abnormality. The patients shared some clinical features including AML-M4 subtype, the presence of minor clones with trisomy 21, and favorable outcomes, and they might have had a distinct subtype of AML.

References

[1] Cortes JE, Kantarjian H, O'Brien S, Keating M, Pierce S, Freireich EJ, et al. Clinical and prognostic significance of trisomy 21 in adult patients with acute myelogenous leukemia and myelodysplastic syndromes. Leukemia 1995;9: 115–7.

[2] Gallego M, Solé F, Acín P, Florensa L, Sans-Sabrafèn J, Woessner S. A new case of trisomy 21 in a patient with an acute nonlymphocytic leukemia (M5b). Cancer Genet Cytogenet 1996;95:213–4.

[3] Chang Y, Wang H, Wang H, Chen Z, Jin J. Trisomy 21 in patients with acute leukemia. Am J Hematol 2008;84:193–4.

[4] Wan TSK, Au WY, Chan JCW, Chan LC, Ma SK. Trisomy 21 as the sole acquired karyotypic abnormality in acute myeloid leukemia and myelodysplastic syndrome. Leuk Res 1999;23:1079–83.

[5] Kondo H, Kobayashi A, Iwasaki H. Trisomy 21 as the sole acquired karyotypic abnormality in an adult patient with CD7-positive acute myeloid leukemia. Cancer Genet Cytogenet 2001;127:77–9.

[6] Yamamoto K, Nagata K, Hamaguchi H. A new case of CD7-positive acute myeloblastic leukemia with trisomy 21 as a sole acquired abnormality. Cancer Genet Cytogenet 2002;133:183–4.

[7] Udayakumar AM, Pathare AV, Muralitharan S, Alghzaly AA, Alkindi S, Raeburn JA. Trisomy 21 as a sole acquired abnormality in an adult Omani patient with CD7- and CD9-positive acute myeloid leukemia. Arch Med Res 2007;38:797–802.

[8] Naithani R, Singhal D, Kumar R, Raina V, Saxena R, Anand H. Sole acquired trisomy 21 in a case of CD7 and CD10 positive acute myeloid leukemia. Indian J Cancer 2008;45:132–4.

[9] Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposed revised criteria for the classification of acute myeloid leukaemia. A report of the French–American–British co-operative group. Ann Intern Med 1985;103:626–9.

[10] Tsukimoto I, Tawa A, Horibe K, Tabuchi K, Kigasawa H, Tsuchida M, et al. Risk-stratified therapy and the intensive use of cytarabine improves the outcome in childhood acute myeloid leukemia: the AML99 trial from the Japanese childhood AML cooperative study group. J Clin Oncol 2009;27: 4007–13.

Ko Kudo

Kiminori Terui*

Shinya Sasaki

Takuya Kamio

Tomohiko Sato

Etsuro Ito

Department of Pediatrics, Hirosaki University Graduate School of Medicine, 5 Zaifu-cho, Hirosaki, Aomori, 036-8562, Japan

*Corresponding author. Tel.: +81 172 39 5070; fax: +81 172 39 5071.

E-mail address: teru@cc.hirosaki-u.ac.jp (K. Terui)

21 March 2011

23 March 2011

18 April 2011

Available online xxx

Serum Prohepcidin Concentrations at Birth and 1 Month After Birth in Premature Infants

Junko Kitajima, MD,[1] Shouichi Ohga, MD, PhD,[1,2]* Tadamune Kinjo, MD,[1] Masayuki Ochiai, MD, PhD,[1,2] Yasushi Takahata, MD, PhD,[1] Satoshi Honjo, MD, PhD,[1,3] and Toshiro Hara, MD, PhD[1,2]

Background. Premature newborns are vulnerable to iron imbalance, although the iron homeostasis during the perinatal period remains unclear. To clarify the iron metabolism of premature infants, we measured serum prohepcidin concentrations of preterm infants, and analyzed the association with iron parameters. **Methods.** Seventy-one (61 preterm and 10 term) infants were enrolled for the study, that had no underlying diseases including asphyxia, bleedings, infection, and anomalies. Serum concentrations of prohepcidin at birth and 1 month after birth were determined by enzyme-linked immunosorbent assay. **Results.** Prohepcidin levels at birth but not 1 month postnatal age positively correlated with gestational age (correlation coefficient [CC]:0.334, $P = 0.005$) and birth weight (CC: 0.367, $P = 0.002$). The levels at birth of preterm infants (median: 29.93 ng/ml, range: 4.0–110.6) were lower than those of full-term infants, and increased thereafter. On the other hand, the levels in small-for-gestational age infants were not associated with gestational age or birth weight. Prohepcidin levels at birth correlated positively with red cell counts (CC = 0.487, $P = 0.025$), unsaturated iron binding capacity (CC = 0.755, $P = 0.001$), total protein (CC = 0.624, $P = 0.005$), and serum albumin levels (CC = 0.500, $P = 0.025$), and negatively with serum iron levels (CC = −0.688, $P = 0.003$), but not ferritin levels. Multivariate analyses indicated that prohepcidin levels at birth were lower in infants with pregnancy-induced hypertension ($P = 0.03$) or premature rupture of membrane ($P = 0.01$). **Conclusions.** Prohepcidin production was physiologically low at birth of preterm infants according to the gestational age, and the levels might be susceptible to the *in utero* stress. The postnatal increase might reflect the maturation and/or adaptation of iron homeostasis. Pediatr Blood Cancer 2011;56:267–272. © 2010 Wiley-Liss, Inc.

Key words: hepcidin; iron metabolism; perinatal period; preterm infants

INTRODUCTION

Anemia of prematurity (AOP) is a multifactorial disease occurring in premature newborns in the context of hemorrhage, hemolysis, shortened survival of red blood cells, insufficient transfer of maternal iron, and iatrogenic blood loss. The primary cause of AOP is inadequate production of erythropoietin (EPO) in the setting of anemia and decreased tissue availability of oxygen [1]. Despite the general use of recombinant human (rh) EPO in preterm infants, recent systematic reviews and meta-analyses of all published data indicated a minor influence of rhEPO on the transfusion need [2,3]. Anemia is occasionally prolonged in very low birth weight (LBW) infants over 16 weeks after birth, because of the increased iron needs on the rapid growth [4]. Neonates receiving both iron and rhEPO show greater reticulocytosis than those receiving rhEPO alone. Minimizing phlebotomy losses and early iron supplementation may prevent the prolonged AOP, while optimal iron therapy remains elusive. There are various factors involving the iron status in neonates, including placental transfer, amount of blood transfusions, bouts of infection, and timing and dose of iron supplementation. Based on the limited information of iron homeostasis in developing fetus and neonate [5], premature infants are more vulnerable to the iron imbalance [6]. Both iron deficiency and excess during the perinatal period have detrimental effects not only on the disease course of LBW infants, but also on the neurodevelopment that may persist into adulthood [7]. There are no sensitive predictors for the iron balance of premature newborns.

Hepcidin, originally identified as a urinary antimicrobial peptide, is a regulator of iron homeostasis [8]. It is synthesized in the liver as an 84-amino acid (aa) prepropeptide, that is processed into a 60-aa prohepcidin and finally into the mature and active form of 25-aa hepcidin. The target for hepcidin is the iron exporter ferroportin 1 that is expressed on duodenal enterocytes, macrophages, and hepatocytes. By the binding and degradation of ferroportin 1, hepcidin inhibits intestinal absorption of iron, recycling by macrophages, and mobilization from hepatic stores [9]. Hepcidin synthesis is increased by iron load or inflammation,

and is decreased by anemia or hypoxia. Elevated hepcidin levels during infections explain the mechanism of anemia of inflammation showing low serum iron and high ferritin levels [8]. Hepcidin metabolism during the early neonatal life is poorly understood. Animal models suggested the inadequate response to hypoxia, iron levels, and interleukin-6 according to the iron need [10]. However, no characteristic changes of hepcidin production have been documented in LBW infants [11–16].

In the present study, we primarily determined serum prohepcidin concentrations in a small series of term and pre-term infants having

[1]Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [2]Comprehensive Maternity and Perinatal Care Center, Kyushu University Hospital, Fukuoka, Japan; [3]Department of Pediatrics, Fukuoka National Hospital, Fukuoka, Japan

*Correspondence to: Shouichi Ohga, Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan.
E-mail: ohgas@pediatr.med.kyushu-u.ac.jp

no underlying diseases at birth and 1 month after birth, with hypothesis-generating explorations between prohepcidin concentrations and other clinical and laboratory variables. Hepcidin production and iron metabolism of premature infants were discussed.

METHODS

Subjects

Of 631 infants admitted to Kyushu University Hospital between October 2005 and April 2009, 71 infants (61 preterm [<37 weeks of gestational age] and 10 full-term) who fulfilled the criteria were enrolled. Inclusion criteria were the absence of asphyxia, infection, bleeding and/or severe anemia requiring blood transfusion within a month after birth. Exclusion criteria were chromosomal aberrations, major anomalies, inherited diseases, gastroenterological and hematological diseases. Two infants suffered from the mild form of bronchopulmonary dysplasia. No infants developed retinopathy of prematurity. Requisite minimum blood was drawn in this study. Association studies between prohepcidin and iron parameters were employed for 21 subjects (13 preterm and 8 full-term infants) who received no iron therapy during the first month of life. Informed consents were obtained from all parents. The Ethics Committee of Kyushu University approved the study.

Measurement of Serum Concentrations of Prohepcidin

Arterial or venous peripheral blood was collected from infants at birth and 4 weeks postnatal age. Serum was separated from blood samples and stored at $-30°C$ until the analysis. Prohepcidin concentrations were determined by an ELISA kit, EIA-466 (DRG Instruments, Marburg, Germany) with high reproducibility and sensitivity, which had been developed with the specific N-terminal hepcidin antibody EG (2)-HepN. The detectable range was from 3.95 to 1,000 ng/ml.

Statistical Analysis

The primarily descriptive study was performed for hypothesis-generating explorations, using STATA Statistical Software 10.0 version (Stata Corporation, College Station, TX). Chi-square test or Fisher's exact test was used to evaluate the distribution of variables. Mann–Whitney U-test was used to compare the group medians. Correlations among continuous variables were examined by Spearman's rank-sum test. Multiple linear regression analysis was performed with adjustment for selected variables. Log-transformed values of prohepcidin were calculated because of the skewed distributions. A P-value <0.05 was considered statistically significant. No study-wide correction of α was used for multiple hypothesis testing.

RESULTS

Demographic and Clinical Profile of Subjects

Clinical characteristics of 71 infants (male/female = 34:37) are shown in Table I. Small-for-gestational age (SGA) infants were 23 (38%) in preterm and 2 (20%) in full-term groups, respectively. Mean gestational age and birth weight of preterm infants were 32 weeks and 1,486 g, respectively. All infants had >8 of Apgar scores at 5 min. No chorioamnionitis was determined. Preterm infants showed higher frequency of respiratory failures than full-term infants ($P = 0.019$). There was no difference between preterm and full-term infants with respect to blood pH at birth and maternal variables including premature rapture of membrane (PROM), pregnancy-induced hypertension (PIH), hemoglobin concentration, and smoking.

Prohepcidin Levels According to the Gestational Age or Birth Weight

Prohepcidin levels of 71 subjects at birth but not 1 month after birth positively correlated with gestational age (correlation coefficient [CC] = 0.334, $P = 0.005$, Fig. 1, left upper) and birth

TABLE I. Clinical Profile of Pre-Term and Full-Term Infants

	Pre-term		Full-term	
Number	61		10	
Gender (male/female)	29:32		5:5	
Small-for-gestational age	23	38%	2	20%
Birth weight[a] (g)	1,486	810–2,498	2,840	1,392–3,350
Gestational age[a] (weeks)	32	26–36	38	37–41
Apgar score[a]				
1 min	8	1–9	8	8–9
5 min	9	9–10	9	9–10
pH[a]	7.299	7.083–7.551	7.377	7.083–7.551
Respiratory failures[b]	31	51%	1	10%
Maternal factors				
PROM	16	26%	0	0%
PIH	13	21%	0	0%
Hemoglobin (g/dl)[a]	10.6	6.4–15.4	10.2	8.0–12.0
Smoking	9	15%	1	10%

PIH, pregnancy induced hypertension; PROM, premature rupture of membrane. [a]Each value represents the median and ranges; [b]Respiratory failures included respiratory distress syndrome, transient tachypnea of the newborn, but no severe cardiopulmonary diseases. Only one infant resulted in mild bronchopulmonary dysplasia. No infants had chorioamnionitis, infection, intracranial hemorrhage, necrotizing enterocolitis, retinopathy of prematurity, and congenital diseases.
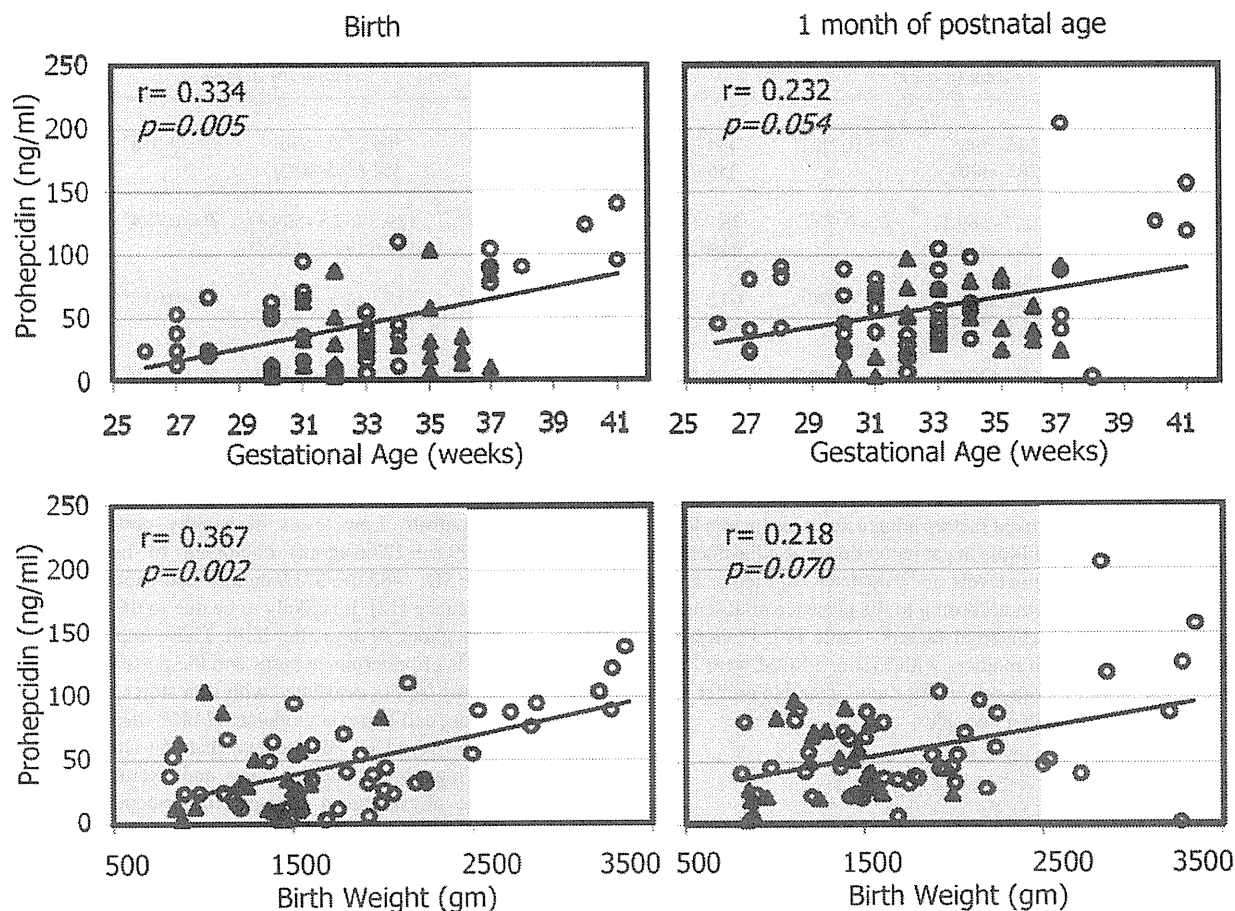
Fig. 1.   Correlations of prohepcidin concentrations with gestational age (**upper columns**) or birth weight (**lower columns**) at birth (**left columns**) and 1 month of postnatal age (**right columns**). Open circles (○) represent appropriate-for-gestational age (AGA) infants, and closed triangles (▲) represent small-for-gestational age (SGA) infants. **Upper left**: Prohepcidin levels at birth correlated with gestational ages of all infants, (correlation coefficient [r] = 0.334, $P = 0.005$, y = 4.924x −119.4), AGA infants, but not SGA infants. **Lower left**: Prohepcidin levels at birth correlated with birth weight of all infants, (r = 0.367, $P = 0.002$, y = 0.0314x −8.2585), AGA infants, but not SGA infants. One month after birth, there was no significant correlation between prohepcidin levels and gestational age or birth weight in any infants. Shaded areas means <37 weeks of gestational age or <2,500 g of birth weight.

weight (CC = 0.367, $P = 0.002$, Fig. 1, left lower). When 46 appropriate-for-gestational age (AGA) infants (○) and 25 SGA infants (▲) were separately analyzed, the correlation was significant only for AGA infants. Prohepcidin levels of 61 preterm infants at birth (median 29.9, range 4.0–110.6) were lower than those at 1 month postnatal age (median 45.8, range 4.0–104.6) ($P < 0.0001$). The levels of 10 full-term infants at birth and 1 month after birth did not differ. The levels of LBW infants but not ≥2,500 g weighing infants at birth were lower than those at 1 month of age ($P < 0.0001$).

Red Cell Counts and Iron Parameters in Premature Infants

Red cell counts and iron parameters are shown in Table II. Red cell counts at birth of preterm infants were lower than those of full-term infants ($P = 0.043$). Serum ferritin levels at birth of preterm infants were lower than those of full-term infants ($P = 0.011$). During the neonatal period, both preterm and full-term infants

became anemic ($P = 0.005$, $P = 0.018$, respectively), while the increment in prohepcidin levels was significant in pre-term ($P = 0.011$) but not full-term infants. Transferrin saturation of preterm infants did not differ between at birth and 1 month after birth.

Association of Prohepcidin With Clinical Variables at Birth or 1 month of Age

The association between prohepcidin and variables including complete blood counts and iron parameters was then examined at birth and 1 month after birth. Prohepcidin levels at birth correlated positively with red cell counts, unsaturated iron binding capacity (UIBC), total protein and albumin concentrations, and negatively with serum iron levels and transferrin saturation, but not with ferritin levels (Table III). No significance was found in each association at 1 month postnatal age. No biochemical data assessing hepatorenal function correlated with prohepcidin levels.

TABLE II. Red Cell Counts and Iron Parameters in Premature Infants at Birth and 1 Month of Age

	Total (n = 21)		Pre-term (n = 13)		Full-term (n = 8)		P-Value[a]
Red cell counts (10^4/μl)							
At birth	467 (336–596)	$P = 0.0002^b$	434 (336–578)	$P = 0.005^b$	509 (393–596)	$P = 0.018^b$	0.043
1 month	335 (265–480)		356 (267–430)		354 (265–480)		0.843
Serum ferritin (ng/ml)							
At birth	107.1 (18.7–569.1)	$P = 0.970^b$	76.7 (18.7–223.2)	$P = 0.347^b$	189.5 (67.5–569.1)	$P = 0.484^b$	0.011
1 month	121.2 (58.1–412.5)		95.4 (58.1–230.3)		152.0 (90.5–412.5)		0.054
Transferrin saturation (%)							
At birth	31.3 (8.3–88.9)	$P = 0.970^b$	64.3 (15.8–88.9)	$P = 0.071^b$	15.1 (8.3–27.5)	$P = 0.012^b$	0.0007
1 month	38.4 (17.1–79.5)		33.2 (17.1–79.5)		44.2 (32.1–57.8)		0.1457
Prohepcidin (ng/dl)							
At birth	49.8 (4.0–139.9)	$P = 0.149^b$	24.2 (4.0–53.4)	$P = 0.011^b$	92.6 (77.3–139.9)	$P = 0.779^b$	0.0001
1 month	45.8 (4.0–204.9)		34.2 (7.1–81.1)		104.1 (4.0–204.7)		0.025

Median (range). [a]P-value: pre-term versus full-term, [b]P-value: at birth versus 1 month after birth.

To assess the independent factors influencing prohepcidin levels at birth, we employed multiple linear regression with adjustment for red cell counts and albumin levels, and calculated adjusted means of prohepcidin levels at birth according to the presence or absence of respiratory failure and maternal factors (Table IV). Prohepcidin levels of newborns from mothers with PIH or PROM were lower than of those born to mothers without each factor. No prohepcidin levels differed among other variables.

DISCUSSION

The notable finding of this study was that preterm infants had lower concentration of serum prohepcidin at birth than full-term newborns. This finding did not hold for SGA infants when examined separately. The prohepcidin levels of preterm infants increased during the first month of life. There are six reports of prohepcidin levels in neonates. Tiker et al. [11] found no difference in the prohepcidin levels between preterm and term infants assessed by the high sensitivity EIA system. The discrepancy may be explained by the later blood sampling (during 7 days after birth) and distinct grouping of subjects. Balogh et al. [14] revealed that prohepcidin levels increased within a few days after birth in half of healthy term infants, indicating the active synthesis for perinatal adaptation. Recently, Yapakçi et al. [12] reported that prohepcidin levels were higher in either preterm or term newborns with sepsis than

seen in each controls. Low levels of healthy preterm infants (mean ± SD: 279.8 ± 227.6 ng/ml) compared to healthy term infants (mean ± SD: 482.0 ± 371.9 ng/ml) did not reach the statistical significance [12]. It is likely to be due to the wide range of sampling throughout 30 days after birth. These implied the low prohepcidin levels of preterm newborns and the postnatal increase after birth. Various factors associated with iron stores, erythropoiesis, inflammation, and hypoxia modulate HAMP (hepcidin gene) expression in the liver [17]. We believe that the strict protocol employed with regard to sampling dates and exclusion criteria ensured that the physiological changes in prohepcidin in the premature infants were accurately determined.

Maternal and placental factors including smoking or infections lead to tissue hypoxia and hypercytokinemia in the fetus. Interleukin-6, serum iron, and soluble hemojuvelin stimulate HAMP expression via the proper receptor on hepatocytes [18], while hypoxia modulates the expression via unknown receptor [19]. The multivariate analysis indicated that PIH and PROM independently affected the prohepcidin levels at birth. PIH-associated intrauterine hypoxia might reduce the hepcidin production in the fetus. The lack of a link between prohepcidin levels and gestational age or body weight in SGA infants might involve other placental effects on the hepcidin system. In the study infants, there was no association between PROM and infections including chorioamnionitis. There is little information about the regulatory mechanism of

TABLE III. Association of Prohepcidin with Clinical Variables at Birth or 1 Month of Age

	Birth		1 month of age	
	CC[a]	P-Value	CC[a]	P-Value
Red cell counts (/μl)	0.487	0.025	0.043	0.858
Hemoglobin (g/dl)	0.301	0.171	0.041	0.859
Serum iron (μg/dl)	−0.688	0.003	−0.036	0.872
UIBC (μg/dl)	0.755	0.001	0.044	0.844
Transferrin saturation (%)	−0.797	<0.001	0.132	0.568
Ferritin (ng/ml)	0.297	0.184	0.317	0.168
Total protein (g/dl)	0.624	0.005	0.274	0.220
Albumin (g/dl)	0.500	0.025	0.188	0.401

CC, correlation coefficient; UIBC, unsaturated iron binding capacity. [a]CC was calculated for the evaluation by Spearman's rank sum test. All data were used in 21 subjects having no missing data including iron parameters.

TABLE IV. Association Variables Influencing on the Prohepcidin Levels at Birth

Variables	n	Adjusted means[a]	95% Confidence interval		P-Value
			Lower	Upper	
Red cell counts					
336	5	14.2	4.5	45.4	Reference
393	5	25.0	6.7	93.3	0.49
467	5	58.6	18.0	191.5	0.10
514	6	36.7	12.0	112.2	0.25
Serum albumin					
2.7	5	45.4	14.5	142.4	Reference
3.1	3	16.7	3.2	88.6	0.30
3.2	5	13.9	4.4	44.1	0.14
3.5	8	46.0	16.4	128.6	0.99
Respiratory failure					
No	14	48.2	20.9	111.2	0.14
Yes	7	11.5	2.8	46.6	
PIH					
No	18	38.7	23.0	65.2	0.03
Yes	3	6.3	1.5	26.2	
PROM					
No	18	38.9	23.9	63.2	0.01
Yes	3	6.1	1.7	21.4	

PIH, pregnancy induced hypertension; PROM, premature rupture of membrane. [a]Multiple linear regression was performed with adjustment for selected variables of red cell counts and serum albumin levels at birth. Prohepcidin and albumin values were calculated after logarithmic transformation (n = 21).

feto-maternal iron balance such as the biased iron transfer from mother to fetus [20]. Further study should be directed towards the placental factors modulating hepcidin production during the perinatal period.

There were significant associations between prohepcidin levels and total protein, albumin or UIBC levels (Table III). Physiological basis of low prohepcidin production in preterm newborns might denote an immaturity of liver-dependent iron homeostasis and protein synthesis. In rat models [21], liver-dependent iron regulatory mechanism via divalent metal transporter 1 (DMT1) was established by day 20 after birth, and the intestinal expression of DMT1 and ferroportin 1 (FPN1) increased by day 40 after birth. Insufficient hepcidin leads to the excess of free iron, and the oxidant stress could be a risk factor for lung disease and retinopathy of prematurity [22]. In this setting, oral iron therapy for preterm infants should be carefully started within a month of life [23,24].

The limitation of our study was the lack of assessment for total body iron content and direct hepcidin levels. Liver biopsy or magnetic resonance imaging for all LBW newborns was impractical. Quantification of *HAMP* gene also needs liver tissues. Active 25-aa hepcidin has become measurable by mass spectrometry [25,26] or immunoassays [27,28], although neither was established at the start of this study. Multivariable analyses for small selected samples make no tenable inferences to the whole population of preterm infants. Our preliminary results need further evaluation in prospective studies.

ACKNOWLEDGMENT

REFERENCES

1. Bishara N, Ohls RK. Current controversies in the management of the anemia of prematurity. Semin Perinatol 2008;33:29–34.
2. Mainie P. Is there a role for erythropoietin in neonatal medicine? Early Hum Dev 2008;84:525–532.
3. Bell EF. When to transfuse preterm babies. Arch Dis Child Fetal Neonatal Ed 2008;93:F469–F473.
4. Rao R, Georgieff MK. Iron in fetal and neonatal nutrition. Semin Fetal Neonatal Med 2007;12:54–63.
5. Collard KJ. Iron homeostasis in the neonate. Pediatrics 2009; 123:1208–1216.
6. Rao R, Georgieff MK. Iron therapy for preterm infants. Clin Perinatol 2009;36:27–42.
7. Fredriksson A, Schröder N, Eriksson P, et al. Maze learning and motor activity deficits in adult mice induced by iron exposure during a critical postnatal period. Brain Res Dev Brain Res 2000; 119:65–74.
8. Bergamaschi G, Villani L. Serum hepcidin: A novel diagnostic tool in disorders of iron metabolism. Haematologica 2009;94:1631–1633.
9. Ganz T, Nemeth E. Iron imports. IV. Hepcidin and regulation of body iron metabolism. Am J Physiol Gastrointest Liver Physiol 2006;290:G199–G203.
10. Nemeth E, Rivera S, Gabayan V, et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest 2004;113:1271–1276.
11. Tiker F, Celik B, Tarcan A, et al. Serum pro-hepcidinlevels and relationships with iron parameters in healthy preterm and term newborns. Pediatr Hematol Oncol 2006;23:293–297.

12. Yapakçi E, Tarcan A, Celik B, et al. Serum pro-hepcidin levels in term and preterm newborns with sepsis. Pediatr Int 2009;51:289–292.

13. Yapakçi E, Ecevit A, Gökmen Z, et al. Erythrocyte transfusions and serum prohepcidin levels in premature newborns with anemia of prematurity. J Pediatr Hematol Oncol 2009;31:840–842.

14. Balogh A, Szabó M, Kelen D, et al. Prohepcidin levels during human perinatal adaptation. Pediatr Hematol Oncol 2007;24:361–368.

15. Amarilyo G, Mimouni FB, Oren A, et al. Prohepcidin concentrations and erythroid progenitors in cord blood of appropriate versus small for gestational age neonates. J Perinatol 2010;30:396–398.

16. Kulaksiz H, Gehrke SG, Janetzko A, et al. Pro-hepcidin: Expression and cell specific localisation in the liver and its regulation in hereditary haemochromatosis, chronic renal insufficiency, and renal anaemia. Gut 2004;53:735–743.

17. Darshan D, Anderson GJ. Liver-gut axis in the regulation of iron homeostasis. World J Gastroenterol 2007;13:4737–4745.

18. Truksa J, Lee P, Beutler E. Two BMP responsive elements, STAT, and bZIP/HNF4/COUP motifs of the hepcidin promoter are critical for BMP, SMAD1, and HJV responsiveness. Blood 2009;113:688–695.

19. Choi SO, Cho YS, Kim HL, et al. ROS mediate the hypoxic repression of the hepcidin gene by inhibiting C/EBPalpha and STAT-3. Biochem Biophys Res Commun 2007;356:312–317.

20. Rao R, Georgieff MK. Perinatal aspects of iron metabolism. Acta Paediatr Suppl 2002;91:124–129.

21. Leong WI, Bowlus CL, Tallkvist J, et al. Iron supplementation during infancy—Effects on expression of iron transporters, iron absorption, and iron utilization in rat pups. Am J Clin Nutr 2003;78:1203–1211.

22. Hirano K, Morinobu T, Kim H, et al. Blood transfusion increases radical promoting non-transferrin bound iron in preterm infants. Arch Dis Child Fetal Neonatal Ed 2001;84:F188–F193.

23. Franz AR, Mihatsch WA, Sander S, et al. Prospective randomized trial of early versus late enteral iron supplementation in infants with a birth weight of less than 1301 grams. Pediatrics 2000;106:700–706.

24. Braekke K, Bechensteen AG, Halvorsen BL, et al. Oxidative stress markers and antioxidant status after oral iron supplementation to very low birth weight infants. J Pediatr 2007;151:23–28.

25. Kearney SL, Nemeth E, Neufeld EJ, et al. Urinary hepcidin in congenital chronic anemias. Pediatr Blood Cancer 2007;48:57–63.

26. Castagna A, Campostrini N, Zaninotto F, et al. Hepcidin assay in serum by SELDI-TOF-MS and other approaches. J Proteomics 2010;73:527–536.

27. Ganz T, Olbina G, Girelli D, et al. Immunoassay for human serum hepcidin. Blood 2008;112:4292–4297.

28. Grebenchtchikov N, Geurts-Moespot AJ, Kroot JJ, et al. High-sensitive radioimmunoassay for human serum hepcidin. Br J Haematol 2009;146:317–325.

CASE REPORT

High-dose chemotherapy followed by autologous and allogeneic peripheral blood stem cell transplantation for recurrent disseminated trilateral retinoblastoma

Toshihisa Tsuruta · Yasuo Aihara · Hitoshi Kanno · Chikako Kiyotani ·
Katsuya Maebayashi · Masako Sakauchi · Makiko Osawa · Hisaichi Fujii ·
Osami Kubo · Yoshikazu Okada

Abstract

Introduction Trilateral retinoblastoma (TRb) is an intracranial neurogenic tumor associated with unilateral or bilateral retinoblastoma and has very poor prognosis. Patients typically die from leptomeningeal tumor dissemination.

Case report A 3-year-old girl who had been diagnosed with TRb had a disseminated relapse after a tumorectomy, cerebrospinal irradiation, and conventional chemotherapy. The disseminated tumor disappeared after the first autologous peripheral blood stem cell transplantation (PBSCT) with high-dose melphalan and thiotepa. During the second complete remission, a second autologous PBSCT with high-dose busulfan and melphalan was performed. Seven months after the first PBSCT, the second relapse occurred, and we subsequently performed an allogeneic PBSCT with myeloablative chemotherapy consisting of melphalan, thiotepa, and cyclophosphamide. The patient showed clinical improvement after the allogeneic PBSCT.

Conclusion Although high-dose chemotherapies have a curative effect for some patients with TRb, the prognoses of disseminated tumors are still poor. Further examination of the high-dose chemotherapy is necessary for the time, the conditioning drugs, and the hematopoietic stem cell sources.

Keywords Trilateral retinoblastoma · Recurrence · High-dose chemotherapy · Allogeneic · Stem cell transplantation

Contribution The first author, Toshihisa Tsuruta, and the second author, Yasuo Aihara, contributed equally to this work.

T. Tsuruta (⊠)
Department of Clinical Examination,
National Hospital Organization Kumamoto Medical Center,
1-5, Ninomaru, Kumamoto-shi,
Kumamoto 860–0008, Japan
e-mail: ttsuruta@kumamoto2.hosp.go.jp

Y. Aihara · O. Kubo · Y. Okada
Department of Neurosurgery,
Tokyo Women's Medical University,
Tokyo, Japan

H. Kanno · H. Fujii
Department of Transfusion and Cell Processing,
Tokyo Women's Medical University,
Tokyo, Japan

C. Kiyotani
Department of Pediatric Oncology,
National Center of Child Health and Development,
Tokyo, Japan

K. Maebayashi
Department of Radiology, Tokyo Women's Medical University,
Tokyo, Japan

M. Sakauchi · M. Osawa
Department of Pediatrics, Tokyo Women's Medical University,
Tokyo, Japan

🖄 Springer

Introduction

Trilateral retinoblastoma (TRb) is a disease in which unilateral or bilateral retinoblastoma is associated with an intracranial primitive neuroectodermal tumor (PNET) in the pineal (pineoblastoma) or suprasellar region [14]. This disease was first recognized in 1977 [13] and officially termed TRb in 1980 [4]. TRb is found in approximately 3% of all retinoblastoma children but is more prevalent in patients with unilateral familial retinoblastoma [6]. The prognosis of TRb patients is very poor, and the mean time from diagnosis to death is 6.6–17 months [3, 6, 12, 20]. Patients typically die from leptomeningeal tumor dissemination, despite the lack of progression in the intracranial tumor [20]. Recently, high-dose chemotherapy is thought to be potentially curative for patients with TRb especially without metastasis [5, 7]. We performed high-dose chemotherapy followed by autologous and allogeneic peripheral blood stem cell transplantation (PBSCT) in a patient with recurrent intracranial and leptomeningeal disseminated TRb and obtained dramatic clinical results.

Case report

A 3-year-old girl was examined in a nearby hospital after she experienced vomiting and facial bruising after playing in the bathroom and exhibited drowsiness for 3 days. Previously, her eyeball had been enucleated due to right unilateral retinoblastoma, and she had been given an artificial eye 2 weeks after birth. Computed tomography (Fig. 1a) and magnetic resonance imaging (MRI) (Fig. 1b) of her brain revealed an intraventricular tumor in the third ventricle and obstructive hydrocephalus. A tumorectomy was performed using a transtentorial approach, and a histological examination of the tumor showed pineoblastoma or PNET (Fig. 1c, d). Based on these results, she was diagnosed with trilateral retinoblastoma. One month after the diagnosis, the patient started 24 Gy whole brain irradiation and 26 Gy local radiation to the posterior fossa, and the tumor almost completely disappeared.

The patient was administered weekly vincristine (VCR, 1.5 mg/m^2) treatment in combination with radiation therapy. After the irradiation was performed, five courses

Fig. 1 Initial intracranial tumor images and pathological findings. a Axial view of a contrast-enhanced computed tomography scan. b Sagittal view of gadolinium-enhanced T1-weighted magnetic resonance imaging. c Hematoxylin and eosin staining of the tumor showed Flexner–Wintersteiner rosettes (original magnification ×200). d Immunostaining of MIB-1. Over 50% of the cells were MIB-1 positive (original magnification ×200)
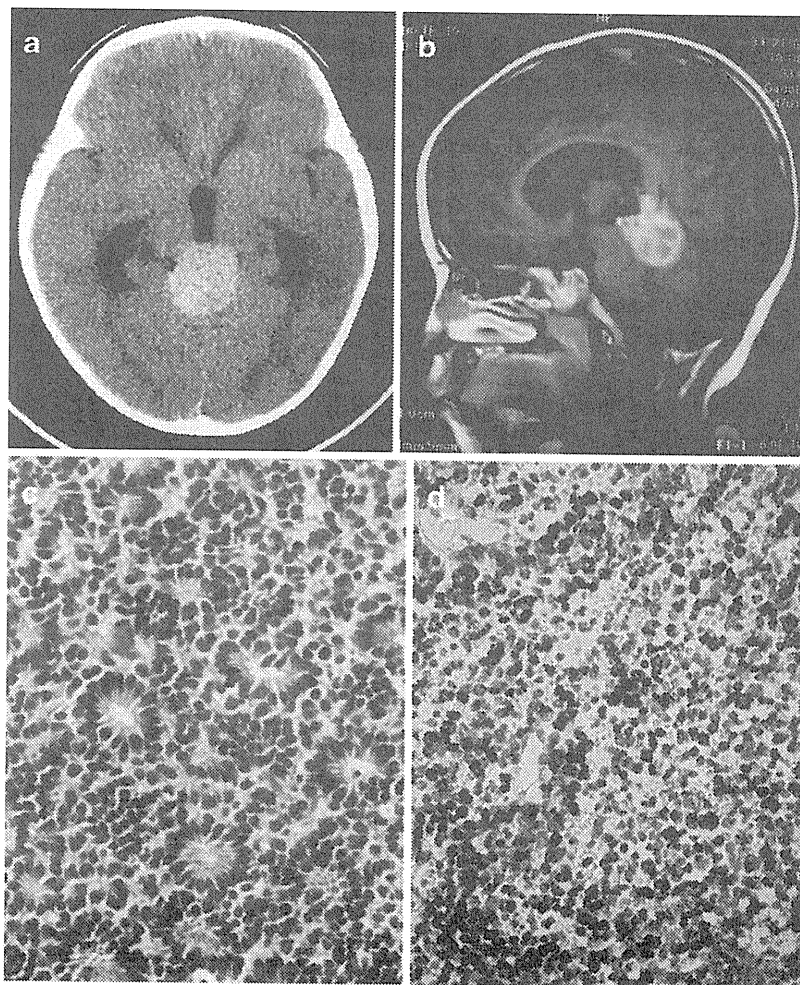
of monthly ICE + VCR therapy consisting of VCR (1.5 mg/m^2/day×1 day), carboplatin (280 mg/m^2/day×2 days), ifosfamide (1,800 mg/m^2/day×5 days), and etoposide (100 mg/m^2/day×5 days) were administered. She suffered from the chicken pox after the third course and severe candidemia after the fourth course of ICE + VCR therapy. After administering systemic antifungal therapy with intravenous fluconazole and micafungin, a fifth course of ICE + VCR therapy without carboplatin was administered after a 1.5-month delay. More than 1.5 months after the last chemotherapy treatment, she began complaining of unsteadiness and within 2 days, her consciousness level decreased, and she became less responsive with a Glasgow coma score (GCS) of 9 to 10 [24]. After performing head and spine MRI and examining gadolinium-enhanced radiograms (Fig. 2), it was determined that her disseminated tumor had recurred. In several days, her consciousness level decreased to 4 (GCS), and it did not improve with symptomatic therapy to lower the intracranial pressure. Thirteen days after she presented the initial symptoms, her breathing became irregular. We administered high-dose chemotherapy consisting of thiotepa (TESPA, 170 mg/m^2×4, days −5, −4) and melphalan (L-PAM, 60 mg/m^2×2, days −5, −4) followed by autologous PBSCT with artificial respiration assistance. After the high-dose chemotherapy was complete, her consciousness level gradually improved, and she could converse with her mother within 1 week. After 1 month, gadolinium-enhanced MRI revealed that the disseminated tumor had disappeared (Fig. 3).

We performed a second round of high-dose chemotherapy with oral busulfan (4 mg/m^2/day×4, days −8~−5) and L-PAM (90 mg/m^2×2, days −4, −3) with autologous PBSCT to make the remission last. After the second

PBSCT was performed, she was discharged from our hospital. We performed maintenance therapy with intrathecally administered TESPA (10 mg) and cytosine arabinoside (25 mg) every 2 weeks.

Three months after the second PBSCT, MRI of the head and spine indicated that the tumor had reappeared. However, we could not give the patient autologous stem cell support because her bone marrow had not completely recovered from the second PBSCT. Fortunately, one of her two elder sisters had an identical HLA haplotype, and we performed an allogeneic PBSCT using her HLA-matched sister 10 months after the second autologous PBSCT. The conditioning regimen for the allogeneic PBSCT was high-dose L-PAM (60 mg/m^2×2, days −12, −11) with TESPA (170 mg/m^2×4, days −12, −11, −5, −4) and cyclophosphamide (50 mg/kg×2, days −3, −2). The patient was prophylactically treated for graft-versus-host disease (GVHD) with a short course of methotrexate (15 mg/m^2, days +1, and 10 mg/m^2, days +3, +6, +11) and cyclosporin A (3 mg/kg, from day −1). The tumors regressed, and no GVHD was observed after the high-dose chemotherapy and allogeneic PBSCT (Fig. 4). Before the allogeneic PBSCT, she was on artificial respiratory respiration, but the mechanical intubation was removed after the PBSCT, and she was able to play cards with her mother. Unfortunately, she died due to tumor regrowth 2 months after the allogeneic PBSCT.

Discussion

The patient in this case report was diagnosed with TRb when she was 3 years old. Generally, suprasellar TRb is

Fig. 2 Sagittal (a), coronal (b), axial (c), and sagittal spinal (d) gadolinium-enhanced T1-weighted magnetic resonance imaging after the first relapse. Disseminated tumors were seen in almost all of the leptomeningeal spaces
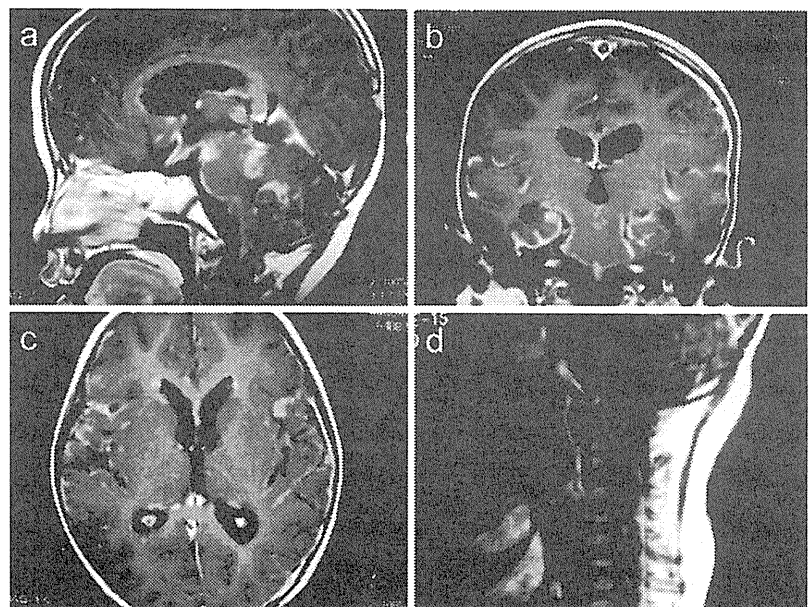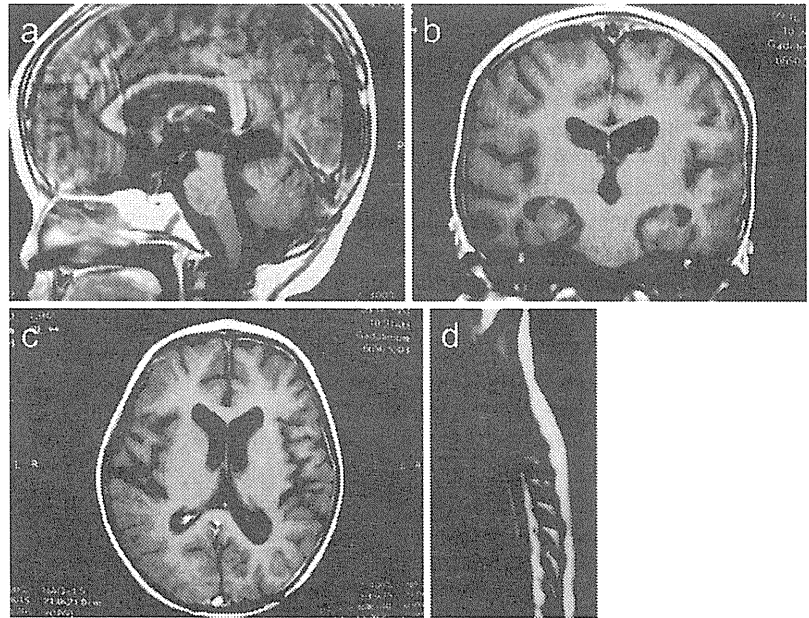
Fig. 3 The disseminated tumors disappeared after the first autologous PBSCT. Sagittal (a), coronal (b), axial (c), and sagittal spinal (d) gadolinium-enhanced T1-weighted magnetic resonance images are shown


diagnosed earlier and may arise earlier than pineal TRb, and screening by neuroimaging can improve the cure rate if the tumor is detected when it is 15 mm or smaller in size [15]. Because this patient had a tumor that was larger than 15 mm and recognized as a pineal region tumor, her prognosis was thought to be very poor.

When the first autologous PBSCT was performed, the patient was ventilated, and she could not tolerate the long-term conditioning regimen. Although we administered only half of the original double conditioning regimen of TESPA and L-PAM reported by Hara et al. [10], the disseminated tumor dramatically disappeared after the PBSCT. L-PAM is considered an effective drug for retinoblastoma and is often administered via the intra-arterial route [1]. TESPA has a good spinal fluid transitional ratio [11]. L-PAM and TESPA are often used as a conditioning regimen for PBSCT in pediatric brain tumor patients [18, 19, 21].

We performed the second PBSCT using a conditioning regimen of BU + L-PAM to obtain a better stage of complete remission. After the second PBSCT, the patient was discharged, and she was able to walk and stay home for over 4 months. However, it is unclear whether this second PBSCT was clinically effective. We used oral BU that also has a good spinal fluid transitional ratio [26] because it is water-insoluble and has anti-tumor effects for various brain tumors, including when it is used as part of the PBSCT conditioning regimen for medulloblastoma and PNET [18, 19, 21]. Recently, a water-soluble microcrystalline formulation of BU (Busulfex™, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) became available, and intrathecal use is now a possibility [9].

Allogeneic hematopoietic stem cell transplantation (allogeneic HSCT), including PBSCT for patients with malignant solid tumors, has two main purposes. One is the effects

Fig. 4 Nonenhanced computed tomography images before (a) and after (b) allogeneic PBSCT
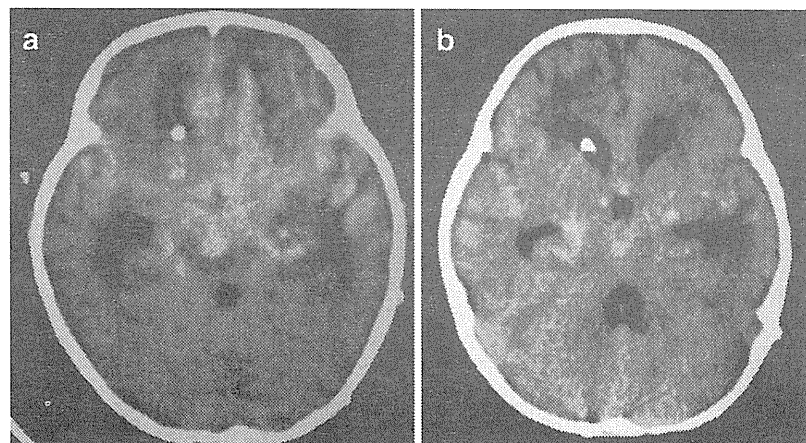
of high-dose chemotherapy, which are similar to autologous HSCT [27]. Another is the immunotherapeutic effects that can be obtained due to the graft-versus-tumor effect [17]. Although we did not observe any immunological effects, the chemotherapy was efficacious with the conditioning regimen of the allogeneic HSCT. Because allogeneic HSCT is thought to have great potential as an immunotherapy for malignant tumors, nonmyeloablative conditioning regimens have recently become available [8, 17]. Immunotherapy for malignant brain tumors has been performed for over 30 years [23], but there are few reports on allogeneic HSCT for brain tumors [16, 22].

Because there is a report that tandem high-dose chemotherapy (etoposide/carboplatin and TESPA/L-PAM) followed by local radiotherapy has a good effect for TRb patients with a better disease stage than our case, an early and aggressive treatment may improve the prognosis of TRb [5]. On the other hand, Dunkel et al. reported that the prognosis of TRb patients with leptomeningeal dissemination is still poor even if high-dose chemotherapy is performed [7].

We previously reported that there is a poor-prognostic type in disseminated medulloblastoma, and some molecular markers, e.g., ERBB2 may be useful for detecting such a type [2]. Because double high-dose chemotherapy followed by autologous HSCT could not increase the prognosis of such poor-prognostic type, further study of the methods of HSCT, including nonmyeloablative allogeneic HSCT, is necessary. On the other hand, many molecular target therapies are available recently. We also reported that there are shared molecular targets for pediatric brain tumor [25]. There may be a special molecular target for poor-prognostic TRb. The studies of molecular markers of TRb may be useful in the development of further therapy.

Conclusion

We reported about a three-year-old girl who had been diagnosed with TRb and had a disseminated relapse after the initial multiplier therapy including conventional chemotherapies. The disseminated tumor disappeared after the first autologous PBSCT. Although we performed a second autologous PBSCT during the second complete remission, the second relapse occurred. We subsequently performed an allogeneic PBSCT, and the patient showed clinical improvement without any transplantation-related side effects. This is the first report of high-dose chemotherapy for leptomeningeal disseminated TRb that can induce complete remission and is also the first report of allogeneic HSCT for TRb.

References

1. Abramson DH, Dunkel IJ, Brodie SE, Kim JW, Gobin YP (2008) A phase I/II study of direct intraarterial (ophthalmic artery) chemotherapy with melphalan for intraocular retinoblastoma initial results. Ophthalmology 115(8):1398–1404, 1404 e1391
2. Aihara Y, Tsuruta T, Kawamata T, Kanno H, Maebayashi K, Sakauchi M, Wada E, Osawa M, Fujii H, Kubo O, Hori T (2010) Double high-dose chemotherapy followed by autologous peripheral blood stem cell transplantation for primary disseminated medulloblastoma: a report of 3 cases. J Pediatr Hematol Oncol 32 (2):e70–74
3. Amoaku WM, Willshaw HE, Parkes SE, Shah KJ, Mann JR (1996) Trilateral retinoblastoma. A report of five patients. Cancer 78(4):858–863
4. Bader JL, Miller RW, Meadows AT, Zimmerman LE, Champion LA, Voute PA (1980) Trilateral retinoblastoma. Lancet 2 (8194):582–583
5. De Ioris MA, Fidani P, Munier FL, Serra A, Ilari I, Popovic MB, Natali G, Secco DE, Cozza R (2010) Successful treatment of trilateral retinoblastoma with conventional and high-dose chemotherapy plus radiotherapy: a case report. J Pediatr Hematol Oncol 32(8):e343–345
6. De Potter P, Shields CL, Shields JA (1994) Clinical variations of trilateral retinoblastoma: a report of 13 cases. J Pediatr Ophthalmol Strabismus 31(1):26–31
7. Dunkel IJ, Jubran RF, Gururangan S, Chantada GL, Finlay JL, Goldman S, Khakoo Y, O'Brien JM, Orjuela M, Rodriguez-Galindo C, Souweidane MM, Abramson DH (2010) Trilateral retinoblastoma: potentially curable with intensive chemotherapy. Pediatr Blood Cancer 54(3):384–387
8. Espinoza-Delgado I, Childs RW (2004) Nonmyeloablative transplantation for solid tumors: a new frontier for allogeneic immunotherapy. Expert Rev Anticancer Ther 4(5):865–875
9. Gururangan S, Petros WP, Poussaint TY, Hancock ML, Phillips PC, Friedman HS, Bomgaars L, Blaney SM, Kun LE, Boyett JM (2006) Phase I trial of intrathecal spartaject busulfan in children with neoplastic meningitis: a Pediatric Brain Tumor Consortium Study (PBTC-004). Clin Cancer Res 12(5):1540–1546
10. Hara J, Osugi Y, Ohta H, Matsuda Y, Nakanishi K, Takai K, Fujisaki H, Tokimasa S, Fukuzawa M, Okada A, Okada S (1998) Double-conditioning regimens consisting of thiotepa, melphalan and busulfan with stem cell rescue for the treatment of pediatric solid tumors. Bone Marrow Transplant 22(1):7–12
11. Heideman RL, Cole DE, Balis F, Sato J, Reaman GH, Packer RJ, Singher LJ, Ettinger LJ, Gillespie A, Sam J et al (1989) Phase I and pharmacokinetic evaluation of thiotepa in the cerebrospinal fluid and plasma of pediatric patients: evidence for dose-dependent plasma clearance of thiotepa. Cancer Res 49(3):736–741
12. Holladay DA, Holladay A, Montebello JF, Redmond KP (1991) Clinical presentation, treatment, and outcome of trilateral retinoblastoma. Cancer 67(3):710–715
13. Jakobiec FA, Tso MO, Zimmerman LE, Danis P (1977) Retinoblastoma and intracranial malignancy. Cancer 39(5):2048–2058
14. Jurkiewicz E, Pakula-Kosciesza I, Rutynowska O, Nowak K (2010) Trilateral retinoblastoma: an institutional experience and review of the literature. Childs Nerv Syst 26(1):129–132
15. Kivela T (1999) Trilateral retinoblastoma: a meta-analysis of hereditary retinoblastoma associated with primary ectopic intracranial retinoblastoma. J Clin Oncol 17(6):1829–1837
16. Lundberg JH, Weissman DE, Beatty PA, Ash RC (1992) Treatment of recurrent metastatic medulloblastoma with intensive

chemotherapy and allogeneic bone marrow transplantation. J Neurooncol 13(2):151–155

17. Lundqvist A, Childs R (2005) Allogeneic hematopoietic cell transplantation as immunotherapy for solid tumors: current status and future directions. J Immunother 28(4):281–288

18. Perez-Martinez A, Lassaletta A, Gonzalez-Vicent M, Sevilla J, Diaz MA, Madero L (2005) High-dose chemotherapy with autologous stem cell rescue for children with high risk and recurrent medulloblastoma and supratentorial primitive neuro-ectodermal tumors. J Neurooncol 71(1):33–38

19. Perez-Martinez A, Quintero V, Vicent MG, Sevilla J, Diaz MA, Madero L (2004) High-dose chemotherapy with autologous stem cell rescue as first line of treatment in young children with medulloblastoma and supratentorial primitive neuroectodermal tumors. J Neurooncol 67(1–2):101–106

20. Provenzale JM, Gururangan S, Klintworth G (2004) Trilateral retinoblastoma: clinical and radiologic progression. AJR Am J Roentgenol 183(2):505–511

21. Ridola V, Grill J, Doz F, Gentet JC, Frappaz D, Raquin MA, Habrand JL, Sainte-Rose C, Valteau-Couanet D, Kalifa C (2007) High-dose chemotherapy with autologous stem cell rescue followed by posterior fossa irradiation for local medulloblastoma

recurrence or progression after conventional chemotherapy. Cancer 110(1):156–163

22. Secondino S, Pedrazzoli P, Schiavetto I, Basilico V, Bramerio E, Massimino M, Gambacorta M, Siena S (2008) Antitumor effect of allogeneic hematopoietic SCT in metastatic medulloblastoma. Bone Marrow Transplant 42(2):131–133

23. Takakura K, Miki Y, Kubo O (1975) Adjuvant immunotherapy for malignant brain tumors in infants and children. Childs Brain 1(2–3):141–147

24. Teasdale G, Jennett B (1974) Assessment of coma and impaired consciousness. A practical scale. Lancet 2(7872):81–84

25. Tsuruta T, Aihara Y, Kanno H, Funase M, Murayama T, Osawa M, Fujii H, Kubo O, Okada Y (2011) Shared molecular targets in pediatric gliomas and ependymomas. Pediatr Blood Cancer. doi:10.1002/pbc.23009

26. Vassal G, Deroussent A, Hartmann O, Challine D, Benhamou E, Valteau-Couanet D, Brugieres L, Kalifa C, Gouyette A, Lemerle J (1990) Dose-dependent neurotoxicity of high-dose busulfan in children: a clinical and pharmacological study. Cancer Res 50 (19):6203–6207

27. Wolff JE, Finlay JL (2004) High-dose chemotherapy in childhood brain tumors. Onkologie 27(3):239–245