Serum Procollagen Type III Is an Early and Sensitive Marker for Veno-Occlusive Disease of the Liver in Children Undergoing Bone Marrow Transplantation

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Veno-occlusive disease of the liver (VOD) is a life-threatening complication occurring in patients undergoing bone marrow transplantation (BMT). Although better patient care has reduced the mortality associated with VOD, it is the third leading cause of transplantation death with a mortality rate of 30% to 50%. Clinical findings include icterus, ascites, and severe abdominal pain with tenderness over the upper right quadrant. While an increase in serum bilirubin is characteristic in patients with VOD, such an elevation can also be seen in graft-versus-host disease (GVHD) and other liver diseases common in BMT patients. Thus, it would be desirable to identify early biochemical markers for VOD. Fibrous alterations in the hepatic venules and small lobular veins occur during development of VOD; these changes are accompanied by the deposition of types I and III collagen in the liver tissue. Since the N-terminal propeptide of type III procollagen (PIIINP) is a sensitive marker of liver and lung fibrosis, we undertook a study to evaluate the usefulness of measurements of serum PIIINP in children with VOD. Seven of the 28 children who underwent BMT, both allogenic and autologous, developed VOD. All seven had an increase of more than 100 ng/mL in the serum PIIINP level, whereas only one of the remaining 21 children not affected by VOD had an increment of PIIINP more than 100 ng/mL (P < .0001). The levels of serum PIIINP were higher in the VOD group during the follow-up period up to 91 days after BMT. The elevation of PIIINP also occurred at a stage of the disease usually preceding any other laboratory or clinical signs of VOD. Serum concentration of PIIINP thus seems to be of value as an early marker for VOD in children undergoing BMT.

MATERIALS AND METHODS

Patients and BMT Procedure

Twenty-nine consecutive patients who underwent 31 BMT procedures either at the Children’s Hospital, University of Helsinki, Finland (13 patients), or at the Department of Pediatrics, University of Göteborg, Sweden (16 patients) were included in the study. The study was approved by the respective ethical committees.

The diagnoses and selected clinical parameters of the children in the study are given in Table 1. Two of the children had two BMTs performed at 2 and 6 months after the first BMT, respectively. The transplantation was autologous (autoBMT) in 18 cases and alllogenic (alloBMT) in 13 cases. For the alloBMTs, two children had an HLA-identical sibling, seven had a haploidentical parent, and one an unrelated HLA-identical volunteer as the donor.

The conditioning regimen for BMT in leukemia, lymphoma, and immunodeficiency patients included busulfan (16 mg/kg) and cyclophosphamide (120 to 200 mg/kg) or total body irradiation (TBI) 10 to 12 GY followed by cyclophosphamide (120 to 200 mg/kg). The children with neuroblastoma were conditioned with melphalan (210 mg/m²) with or without TBI. Some children with neuroblastoma also received cisplatin (90 mg/m²) and etoposide (300 mg/m²) in their conditioning regimen. The children with other solid tumors were given either melphalan or thiopeta (1.125 mg/m²). In the case of haploidentical donors, the marrow was in vitro T-cell depleted with Campath-IM antibodies (CDw52, a generous gift from Drs Waldman and Hale, Department of Pathology, Cambridge University, Cambridge, UK) before infusion into the patient.

The patients were cared for in double door rooms with filtered pressurized air. Supportive care was given with trimethoprim-sulphamethoxazole, aciclovir, an azole derivate, and intravenous immunoglobulin. For alloBMT GVHD prophylaxis was given with low dose methotrexate on days +1, +3, +6, and +11 as well as cyclosporin A from day −1, except when T-cell-depleted marrow was used.

Definitions

VOD. The criteria of McDonalds were used for VOD. VOD was accordingly defined as the presence of at least two of the follow-
ing features before day 30 from BMT: jaundice (bilirubin >20 mmol/L), hepatosplenomegaly, right upper quadrant pain, fluid accumulation (ascites or unexplained weight gain of 5% or more), and other causes of liver disease not identified.

**Graft-versus-host disease.** Acute GvHD was evaluated using the International Bone Marrow Transplantation Registry recommendations.

**Infection.** In case no infectious agent was isolated, the patient was judged to have a septic infection requiring intravenous antibiotics when fever was >38.5°C and C-reactive protein (CRP) >75 mg/L.

**Measurement of Serum PIIINP**

Serum was collected 1 to 2 weeks before BMT and weekly after the procedure. Altogether 188 serum samples, 2 to 15 samples per patient (median, 4 samples), were obtained and stored at −70°C until analysis. The last sample was obtained between day 7 and 91 (median day 35) from BMT. Three patients, one with osteopetrosis, one with Hodgkin’s disease, and one with combined immunodeficiency had no preBMT serum collected. The PIIINP values from these children were excluded from the statistical analyses.

A specific radioimmunoassay was used for measuring serum PIIINP levels (Hoechst AG, Germany). The measurements were run in duplicate and the intra-assay coefficient of variation was 4.8%. As PIIINP concentrations are age dependent with high postnatal values declining from birth to age 2 years before another increase at puberty, the increment of serum PIIINP was used besides the absolute serum concentration. When presenting the data, an increment of more than 100 ng/mL was used as a cut-off point.

**Statistical Methods**

χ² Test and the analysis of variance were used to compare results between the groups; the Spearman rank analysis was used for the correlation analyses. The predictive value, specificity, and sensitivity of various cut-off levels of the serum PIIINP increment to detect VOD patients within the BMT group were calculated as described.

**RESULTS**

**Serum PIIINP in Children With or Without VOD**

Of the 28 BMT patients whose serum samples were available both before and after the transplantation, 7 children developed VOD. The mean age at sampling was not different between VOD and nonVOD patients (mean age and range for samples in VOD group, 3.1 and 0.1 to 12 years and in nonVOD group, 5.7 and 0.4 to 14 years, respectively), thus the absolute serum concentrations could be compared between these groups (Fig 1). Serum PIIINP was higher during the whole posttransplantation period in children with VOD than in those without VOD with a statistically significant difference at day 0, through days 14 to 35, and at day 49 after BMT.

Table 2 gives the results of important laboratory tests and central clinical findings and signs on the seven VOD patients, and the baseline and maximal serum PIIINP concentrations. The youngest patients showed a clear increase in their PIIINP levels during BMT procedure, in contrast to the declining levels in healthy children from birth to age 2 years. An increase in PIIINP was observed on average 9 days (range, 0 to 23 days) earlier than the diagnosis of VOD was made (Fig 2).

Two of the three patients lacking preBMT samples developed VOD. After BMT, both of them had high postBMT PIIINP values, which were in the same range as other VOD patients (maximal value, 238 and 482 ng/mL, respectively). Moreover, this was the second transplantation for the latter patient, and the serum PIIINP after first transplantation, not complicated with VOD, had been significantly lower (260 ng/mL).

All 7 children with VOD had an increase of serum PIIINP more than 100 ng/mL during the postBMT period (Tables 2 and 3). Among the remaining 21 children without VOD, only 1 had an increment of PIIINP more than 100 ng/mL (P = .0001 between VOD and nonVOD group). The sensitivity and predictive value for the cut-off levels are given in Table 3.

No other differences, such as incidence or severity of GvHD or infection, were found between children with or without increased PIIINP. The proportion of alloBMT was
Table 2. Clinical and Laboratory Data of VOD Patients

<table>
<thead>
<tr>
<th>F/M Patient</th>
<th>Age (y)</th>
<th>BMT Type</th>
<th>GvHD (d)</th>
<th>Infection</th>
<th>Asc (d)</th>
<th>Oed (d)</th>
<th>Weight Gain (d)</th>
<th>Hep. meg.</th>
<th>Outcome</th>
<th>Bilirubin (ng/mL)</th>
<th>PredBMT PIIINP (ng/mL)</th>
<th>Max&gt; PIIINP (ng/mL) (d)</th>
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<tbody>
<tr>
<td>F</td>
<td>2.6</td>
<td>Allo</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Died</td>
<td>175</td>
<td>18</td>
<td>147</td>
</tr>
<tr>
<td>F</td>
<td>0.3</td>
<td>Allo</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Died</td>
<td>32</td>
<td>130</td>
<td>230</td>
</tr>
<tr>
<td>O-petr CMV</td>
<td>0.1</td>
<td>Allo</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Recov</td>
<td>77</td>
<td>178</td>
<td>360</td>
</tr>
<tr>
<td>O-petr</td>
<td>0.5</td>
<td>Allo</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Died</td>
<td>490</td>
<td>134</td>
<td>612</td>
</tr>
<tr>
<td>SCID</td>
<td>4.6</td>
<td>Auto</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Recov</td>
<td>30</td>
<td>22</td>
<td>124</td>
</tr>
<tr>
<td>AML</td>
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<td>Allo</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Died</td>
<td>18</td>
<td>32</td>
<td>299</td>
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<tr>
<td>ALL</td>
<td>12.2</td>
<td>Auto</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Died</td>
<td>95</td>
<td>29</td>
<td>152</td>
</tr>
<tr>
<td>Gloma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

* Infection: +, sepsis; additional microbes causing systemic infection shown in respective cases.
† Unexplained weight gain more than 5% during BMT procedure recorded.
‡ Outcome at 3 months from the transplant; Recov, recovered.
§ Day of maximal value. All time points refer to the day of BMT (day 0).

higher in the group with increased PIIINP, but this difference was not statistically significant.

Serum PIIINP in Liver Dysfunction Not Caused by VOD and in GvHD

Among the patients who did not develop VOD, there were 7 who had at least one serum bilirubin value at or above 20 mmol/L (1 with serum bilirubin >34 mmol/L), and 6 additional cases had at least one liver enzyme value (aspartyl aminotransferase, alanine aminotransferase, or gamma-glutamyltransferase) above the normal range. The maximal increment of PIIINP in these 13 patients ranged from 3 to 78 ng/mL (mean 37 ng/mL) in 12, and 1 had an increment of 129 ng/mL. He was the only 1 in the nonVOD group with a change of more than 100 ng/mL. Grade I GvHD occurred in 4 children and the increment of serum PIIINP in these children ranged from 3 to 26 ng/mL (mean, 15 ng/mL). The only patient with severe GvHD showed only a maximal increase of 79 ng/mL in serum PIIINP concentration during the BMT procedure.

A significant positive correlation in the whole study group and VOD group was observed between serum PIIINP concentration and serum bilirubin (N = 102, P = .0008), aspartyl aminotransferase (N = 45, P = .01), and total serum protein (N = 46, P = .02), but not with other laboratory parameters. Also in the VOD group, a significant positive correlation was observed between serum PIIINP and serum bilirubin (N = 33, P = .005) and total serum protein (N = 20, P = .05), whereas there were no such correlations between these parameters in the nonVOD group.

Table 3. Patient Characteristics According to Serum PIIINP Levels

<table>
<thead>
<tr>
<th>Maximal Increment of PIIINP</th>
<th>&gt;100 ng/mL</th>
<th>&lt;100 ng/mL</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of transplantations</td>
<td>8</td>
<td>20</td>
<td>0.0001</td>
</tr>
<tr>
<td>VOD</td>
<td>7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sepsis†</td>
<td>3</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>Generalized BCG</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>GvHD grade 2-4</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>AlloBMT</td>
<td>6</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>AutoBMT</td>
<td>4</td>
<td>14</td>
<td>NS</td>
</tr>
</tbody>
</table>

* x² test.
† For detecting VOD, the sensitivity and predictive value for 100 ng/mL increment cut-off level are 100% and 88%, respectively; the respective values for 75 ng/mL increment cut-off level are 100% and 75%.
‡ CRP >75 mg/L plus a positive blood culture or CRP >75 mg/L and fever >38.5°C.
PROCOLLAGEN TYPE I11 AS A MARKER FOR VOD

DISCUSSION

This investigation was designed to study serum PIINP concentrations in pediatric patients undergoing BMT, focusing on serum PIINP levels in relation to the development of VOD.

In patients developing VOD after BMT, a marked elevation of PIINP was always observed. The maximal increment of PIINP level, more than 100 ng/mL, occurred in all 7 BMT patients with concurrent VOD and only in 1 of the other 21 BMTs without VOD. We also observed very high postBMT levels of PIINP in 2 patients, in whom no immediate preBMT values were available, supporting also the value of serum PIINP measurements in this context. Even the youngest patients with VOD showed a clear elevation in their PIINP when developing the disease, while the levels in healthy children decrease during the first 2 years of life.11 All the other VOD patients were prepubertal, and the increase in serum PIINP thus cannot be attributed to the known elevation occurring at the time of growth acceleration.11 Furthermore, serum bilirubin, a key laboratory parameter for VOD, also correlated positively with the serum PIINP levels. Importantly, the PIINP levels were generally elevated earlier than any traditional clinical or laboratory signs of VOD were noted. In addition, 3 of our VOD patients did not have markedly elevated serum bilirubin levels (>34 mmol/L) and still showed a clear elevation of serum PIINP. A marked increase in serum PIINP levels after BMT thus seems to be an early, sensitive, and reliable indicator of VOD in children, although earlier histochemical studies have shown that the accumulation of type III collagen at the tissular level becomes clear only in the cases where liver samples were studied 50 days or more after BMT.13

Although most patients with a maximal increase in serum PIINP concentration of more than 100 ng/mL and VOD had a concurrent proven or suspected infection, this is not likely to affect the alterations in the PIINP levels, since the infection rate was similar in the patients without VOD. Earlier studies have also shown that hepatitis in children does not affect the serum PIINP levels, whereas elevations have been noted in adults with liver infection10 and fibrosis, especially with alcohol liver disease.4 Consistent with these findings, the children having one or several abnormal liver tests not caused by VOD did not have a significant alteration in their serum PIINP concentrations in the present study. Furthermore, liver fibrosis in children with polycystic liver-kidney disease does not significantly increase serum PIINP levels (Heikinheimo et al, unpublished results).

There was no association with mild GvHD and PIINP levels in our patients. Only one patient without VOD had a severe GvHD with liver involvement, and his PIINP level was not significantly elevated during the follow-up. More such patients, however, have to be evaluated to rule out the possible effect of severe GvHD on serum PIINP levels. Elevated serum levels of PIINP have earlier been described in adults with myelofibrosis and related conditions.14.15 These patients have been reported to have increasing PIINP serum levels with advancing bone marrow fibrosis. Our patients had no such conditions. Various other conditions with bone marrow involvement were included in our study, but the preBMT PIINP concentrations were within age related reference values in all patients. It is notable that the elevations of serum PIINP concentrations in children with VOD were remarkably higher than those described in adults with myelofibrosis, in which median increments were in the order of 15 to 30 ng/mL.

We conclude that serum PIINP concentration reliably indicates the development of VOD in children undergoing BMT. In addition to the sensitivity of this marker, an increase of serum PIINP usually precedes any other sign of this life-threatening condition.

NOTE ADDED IN PROOF

While this manuscript was under evaluation, Eltumi et al17 reported serum PIINP concentrations in children undergoing BMT; the results are essentially the same as in our study in regard to the finding of elevated serum PIINP usually preceding the clinical diagnosis of this complication. A preliminary report on PIINP levels in four adult patients has also appeared.18 In that study, high baseline levels of PIINP in VOD patients make interpretation of the results difficult.

ACKNOWLEDGMENT

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REFERENCES


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