Serum Tumor Marker CA 125 Is an Early and Sensitive Indicator of Veno-Occlusive Disease in Children Undergoing Bone Marrow Transplantation

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ABSTRACT
Venous-occlusive disease (VOD) is a potentially lethal complication of patients undergoing bone marrow transplantation (BMT). The diagnosis of VOD is currently based on clinical signs and unspecific laboratory findings. CA 125 is an oncofetal antigen used as a tumor marker in various malignancies, especially in those originating from the female reproductive tract or gastrointestinal organs, whereas serum CA 125 levels are not increased in hematological malignancies. Several pathophysiological alterations occurring in VOD may lead to elevations in serum CA 125 levels. Therefore, we explored the behavior of this marker as a diagnostic tool in VOD. Twenty-nine pediatric transplant patients were studied. Eight patients (28%) developed clinical VOD, and a significant increase in serum CA 125 was noted in all of them. During the 7 days preceding the diagnosis of VOD, an increase of at least 57% in serum CA 125 from the pre-BMT value was observed in 6 (86%) of 7 of the evaluable patients with VOD. In contrast, a similar increase was noted only in 6 of the 21 non-VOD patients during the post-BMT period of 30 days. Accordingly, the sensitivity and specificity of serum CA 125 for predicting or detecting VOD were 86% and 71%, respectively. The serum levels of CA 125 were not affected by the presence of Graft-versus-Host Disease (GvHD) or a septic infection. In conclusion, serum CA 125 is of value as an early marker of VOD in children undergoing BMT.

INTRODUCTION
VOD is a potentially life-threatening complication of BMT in children and adults with a mortality of 10–50% (1–3). It typically presents with abdominal pain, ascites, weight gain, and liver dysfunction. Whereas increase in serum bilirubin is characteristic of VOD, such an elevation is also often noted in GvHD; overall, the traditional clinical parameters lack the sensitivity and specificity required for the timely diagnosis of VOD (reviewed in Ref. 1), and additional early indicators would, thus, be desirable for differential diagnosis of VOD. Recently, encouraging results by monitoring serum levels of procollagen type III (4, 5), protein C (6), or plasminogen activator inhibitor-1 (7) have been published. In the present study, we have focused on serum oncofetal antigen CA 125 in search for new markers for VOD in children undergoing BMT.

The monoclonal antibody recognizing CA 125 antigen was originally raised against an ovarian cystadenocarcinoma cell line (8, 9). The exact antigenic structure defined by these antibodies has remained unknown thus far. CA 125 has characteristics of an oncofetal antigen as revealed by its expression in certain fetal tissues (10–12) and in association with various malignancies, especially those originating from the female reproductive tract or gastrointestinal organs (reviewed in Refs. 13–15). Pediatric patients with germ cell tumor or abdominal Burkitt’s lymphoma can also present with elevated serum CA 125 levels at diagnosis (16, 17).

Clinically, serum CA 125 measurements have been used mainly in the diagnosis and follow-up of ovarian and gastrointestinal cancers (reviewed in Ref. 13). Benign conditions such as endometriosis (18) and pelvic inflammations (19) can, however, also increase serum CA 125 levels. Other nonmalignant conditions associated with elevated serum CA 125 levels include acute pancreatitis, peritonitis, renal failure, and various gynecological and liver diseases (15, 20–23). Low levels of CA 125 can also be detected in the serum of healthy individuals (17).

Besides malignancies, CA 125 is synthesized in some normal tissues, including fetal intrahepatic bile ducts and adult endometrium, pleura, and peritoneum (reviewed in Ref. 24). Given the two potential anatomical sources for CA 125 synthesis—namely intrahepatic bile ducts and peritoneum, both affected by the pathogenetic mechanisms during VOD—we hypothesized that CA 125 might be increased in this disease. The second prerequisite for the potential value of this marker as an indicator of VOD is that the clinical conditions that are indications for pediatric BMT would not per se be associated with...
increased levels of CA 125. Because there was no indication that this would be the case, we tested whether CA 125 might be of value in the diagnosis of VOD. In the present study, we demonstrate that VOD is associated with significantly increased serum CA 125. Whereas the mechanisms of the increase in CA 125 are probably multiple, the rise in serum CA 125 during the early course of VOD makes it a feasible marker of this diagnostically challenging condition.

PATIENTS AND METHODS

Patients

Twenty-nine patients who underwent 32 BMT procedures, either at the Children’s Hospital, University of Helsinki, Finland (19 patients) or at the Department of Pediatrics, University of Göteborg, Sweden (10 patients), were included in this study. In both institutions, the patients were recruited in a consecutive manner. However, in Helsinki, 7 patients were studied in 1997–1998 and 12 patients in 1986–1990. Selected clinical data of the patients are given in Table 1. Three of the children had two transplantation procedures; the second transplantations were performed 2, 4, and 7 months after the first procedure, respectively. The BMT was autologous (auto-BMT) in 14 cases and allogeneic (allo-BMT) in 18 cases. Allogeneic donors were considered matched if identical at HLA loci A, B, C, and DRB1.

The conditioning regimen for allogeneic BMT included: (a) busulfan (16 mg/kg) and cyclophosphamide (120–200 mg/kg); or (b) TBI (10–12 Gy) followed by cyclophosphamide (120 mg/kg); or (c) melphalan (210 mg/m²; 6 cases) or ara-C (36 g/m²; 2 cases) followed by fractionated TBI (10–12 Gy). The children with an autologous transplant were conditioned with melphalan (210 mg/m²) with or without TBI; some children with neuroblastoma also received cisplatin (90 mg/m²), etoposide (300 mg/m²), or thiotepa (1.125 mg/m²). In haploidentical transplants performed at the University of Göteborg, the graft was in vitro T-cell-depleted with Campath-1 M anti-loci.

\[ \text{Table 1} \quad \text{Clinical data on the patients included in the study (studied for serum CA 125)}^a \]

| No. of marrow transplants (no. of patients) | 32 (29) |
| Female/male ratio | 14/15 |
| Mean age, yr (range) | 6.2 (0.1–17.2) |
| Diagnosis (no. of patients) | |
| Acute lymphoblastic leukemia | 9 |
| Acute myelogenous leukemia | 3 |
| Lymphoma | 2 |
| Neuroblastoma | 4 |
| Retinoblastoma | 1 |
| Small round cell tumor | 2 |
| Other solid tumor | 3 |
| Osteopetrosis | 3 |
| Kostman’s syndrome | 1 |
| Combined immunodeficiency | 1 |
| BMTs (no. of transplants) | |
| Allogeneic (HLA matched/HLA mismatched) | 3/15 |
| Autologous | 14 |

*a Twenty-two of the patients were the same as in a study previously reporting serum procollagen type III as a marker of VOD (6).

Increased levels of CA 125 are probably multiple, the rise in serum CA 125 during the early course of VOD makes it a feasible marker of this diagnostically challenging condition.

Definitions

VOD. The criteria of McDonald were used for VOD (2). VOD was defined as the presence of at least two of the following features before day 30 after BMT: (a) jaundice (bilirubin >20 mmol/L); (b) hepatosplenomegaly; (c) right upper quadrant pain; (d) fluid accumulation (ascites or unexplained weight gain of 5% or more); and (e) other causes of liver disease not identified.

GvHD. Acute GvHD was diagnosed and graded following the guidelines published by the International Bone Marrow Transplantation Registry (25).

Infection. In case no infectious agent was isolated, the patient was judged to have a septic infection requiring i.v. antibiotics when fever was >38.5°C and C-reactive protein (5) was >75 mg/L.

Measurement of Serum CA 125

Serum concentration of CA 125 was measured by a specific RIA (Abbott, Wiesbahn, Germany), with a sensitivity of 15 units/ml. The normal upper 95th percentiles for CA 125 in children less than 0.5, >0.5–1.5, and >1.5 years of age are 45, 25 and 22 units/ml, respectively (16, 17).

Statistical Methods

The two-tailed Student t test was used to compare the results between groups. Spearman rank correlation analysis was used to calculate the correlation coefficients between different parameters. The specificity and sensitivity of various cutoff levels for serum CA 125 levels to detect VOD patients were calculated as described (26).

RESULTS

Serum CA 125 in Children with or without VOD.

Eight of the 29 children studied developed VOD (Table 2). The diagnostic criteria were fulfilled 18 days, on average, after BMT (range, 6–30 days). The children with VOD (mean age, 2.8; range, 0.1–12.2 years) were significantly younger than the children without VOD (mean age, 7.4; range, 0.3–17.2 years; P < 0.05). No significant correlation between the age and serum pre-BMT CA 125 values was noted, and there was a trend toward higher serum CA 125 in older children. The mean serum levels of CA 125 were very similar before and during the first post-BMT week in children both with and without VOD but then rapidly increased in the VOD group (Fig. 1). A significant increase in serum CA 125 was noted in every patient developing bodies (CDw52, a generous gift from Drs. Waldman and Hale, Department of Pathology, Oxford University, Oxford, United Kingdom) before infusion to the patient.

The patients were cared for in double-door rooms with normal or filtered pressurized air. Supportive care was given with cotrimoxazole/trimethoprim/sulfa, aciclovir, and i.v. immunoglobulin in some cases. For allogeneic BMT, 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.

The study was approved by the ethical committees of both institutions.
VOD, and, in 6 of 8 VOD patients, the increase either preceded or coincided with the diagnosis of VOD (Fig. 2A). One patient was diagnosed with VOD on post-BMT day 6, and no CA 125 sample was drawn between BMT and VOD. In this patient, the first post-VOD CA 125 level was significantly increased (by 2.1-fold) when compared with the pre-BMT value. Because of the lack of a representative sample, this patient was excluded from the sensitivity and specificity calculations. During the 7-day period preceding the diagnosis of VOD (the day of diagnosis included), an increase of 57% or more in serum CA 125 from the pre-BMT value was observed in 6 (86%) of 7 of the evaluable VOD patients. In contrast, such an increase in the 21 non-VOD patients was noted in only 6 patients before the day-30 post-BMT (the limit for VOD by definition). Thus, with this cutoff level, the sensitivity and specificity of serum CA 125 for predicting or detecting VOD were 86 and 71%, respectively. When an absolute increase of at least 17 units/ml from the pre-BMT value in CA 125 was used as a cutoff, the sensitivity remained at 86%, but the specificity increased to 76%.

**Serum CA 125 in Children with GvHD or Severe Infections.** Four patients developed a grade II-to-IV GvHD, on average, 24 days after BMT (range, 12–48 days). Elevated levels of serum CA 125 were observed in several samples from these patients (Fig. 2B). However, serum CA 125 level did not detect or predict GvHD. During the week preceding the diagnosis of GvHD, serum CA 125 was increased by 0–42% when compared with the pre-BMT value. One of the GvHD patients, who developed acute GvHD on day 48 post-BMT, was diagnosed as having VOD on day 14. The organ-specific sequence of GvHD did not affect the serum CA 125 pattern; the serum CA 125 values obtained during the consecutive 1-week periods (the indicated day ± 3 days) were pooled for the analysis. For each patient, the serum CA 125 value that was obtained closest to the indicated time point was used.

The table below presents the serum CA 125 concentration at the beginning of cytoreductive therapy. For the other time points, serum CA 125 levels of serum CA 125 were observed in several samples from these patients. Day –7 presents the serum CA 125 concentration at the beginning of cytoreductive therapy. For the other time points, serum CA 125 values obtained during the consecutive 1-week periods (the indicated day ± 3 days) were pooled for the analysis. For each patient, the serum CA 125 value that was obtained closest to the indicated time point was used.

<table>
<thead>
<tr>
<th>F/M Diagnosis</th>
<th>Age (yr)</th>
<th>BMT type</th>
<th>GvHD (day)</th>
<th>VOD (day)</th>
<th>Infection</th>
<th>Ascites</th>
<th>Edema</th>
<th>Weight gain</th>
<th>Hep. meg.</th>
<th>Outcome</th>
<th>Maximum bilirubin</th>
<th>CA 125, units/ml</th>
<th>pre-BMT/maximum</th>
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<tr>
<td>F Kostman</td>
<td>2.6</td>
<td>Allo</td>
<td>23</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Died</td>
<td>175</td>
<td>30/320</td>
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<td></td>
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<tr>
<td>F Osteopetrosis</td>
<td>0.3</td>
<td>Allo</td>
<td>19</td>
<td>+ (CMV)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Died</td>
<td>32</td>
<td>15/35</td>
<td></td>
<td></td>
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<tr>
<td>M Osteopetrosis</td>
<td>0.1</td>
<td>Allo</td>
<td>14</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Died</td>
<td>77</td>
<td>15/150</td>
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<tr>
<td>M SCID</td>
<td>0.5</td>
<td>Allo</td>
<td>13</td>
<td>+(BCG)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Died</td>
<td>490</td>
<td>23/200</td>
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<tr>
<td>F AML</td>
<td>4.6</td>
<td>Auto</td>
<td>20</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Recov</td>
<td>30</td>
<td>15/21</td>
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<tr>
<td>M ALL</td>
<td>1.1</td>
<td>Allo</td>
<td>30</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Died</td>
<td>18</td>
<td>15/220</td>
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<tr>
<td>M Gioma</td>
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<td>Auto</td>
<td>6</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>Died</td>
<td>95</td>
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<td>48</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>Recov</td>
<td>52</td>
<td>15/47</td>
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</table>

*Hep.meg., hepatosplenomegaly; Allo, allogenic; Auto, autologous; CMV, cytomegalovirus; BCG, Bacillus Calmette-Guérin; SCID, severe combined immunodeficiency; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; Recov, recovered.

**Figure 1** Serum CA 125 levels in children undergoing BMT. Serum CA 125 concentration (units/ml; mean ± SE) after BMT in patients who developed VOD (n = 8, upper curve) and in patients who had no VOD (n = 21, lower curve). *P < 0.05 between the VOD and non-VOD groups. Day –7 presents the serum CA 125 concentration at the beginning of cytoreductive therapy. For the other time points, serum CA 125 values obtained during the consecutive 1-week periods (the indicated day ± 3 days) were pooled for the analysis. For each patient, the serum CA 125 value that was obtained closest to the indicated time point was used.

**Figure 2** The upper curve shows the serum CA 125 pattern in the two children with only gastrointestinal involvement. The lower curve presents the serum CA 125 pattern in the two children with only respiratory involvement. The organ-specific sequence of VOD did not affect the serum CA 125 pattern; the serum CA 125 values obtained during the consecutive 1-week periods (the indicated day ± 3 days) were pooled for the analysis. For each patient, the serum CA 125 value that was obtained closest to the indicated time point was used.

**Table 2** Clinical and laboratory data of VOD patients

- The evaluable VOD patients. In contrast, such an increase in the 125 from the pre-BMT value was observed in 6 (86%) of 7 of the day-30 post-BMT (the limit for VOD by definition). Thus, with this cutoff level, the sensitivity and specificity of serum CA 125 for predicting or detecting VOD were 86 and 71%, respectively. When an absolute increase of at least 17 units/ml from the pre-BMT value in CA 125 was used as a cutoff, the sensitivity remained at 86%, but the specificity increased to 76%.

**Serum CA 125 in Children with GvHD or Severe Infections.** Four patients developed a grade II-to-IV GvHD, on average, 24 days after BMT (range, 12–48 days). Elevated levels of serum CA 125 were observed in several samples from these patients (Fig. 2B). However, serum CA 125 level did not detect or predict GvHD. During the week preceding the diagnosis of GvHD, serum CA 125 was increased by 0–42% when compared with the pre-BMT value. One of the GvHD patients, who developed acute GvHD on day 48 post-BMT, was diagnosed as having VOD on day 14. The organ-specific sequence of GvHD did not affect the serum CA 125 pattern; the serum CA 125 pattern in the two children with only gastrointestinal involvement did not differ from that in the remaining two GvHD children with combined liver and gastrointestinal involvement (data not shown).

Acute sepsis did not affect the serum CA 125 concentration in the children studied (Fig. 2C). However, a moderate positive correlation was found between serum CA 125 and C-reactive protein (P < 0.001; r = 0.33; n = 136).

We also analyzed the correlation of serum CA 125 with multiple clinically relevant laboratory parameters. In agreement with the findings associating serum CA 125 and VOD, a significant positive correlation was observed between CA 125 and serum bilirubin levels (P < 0.0001; r = 0.418; n = 106). This correlation seemed mainly to be due to the VOD, inasmuch as the correlation was stronger in patients with VOD (n = 39; r = 0.567; P < 0.001) and weaker in the patients without VOD (n = 67; r = 0.225; P = 0.07). The correlation between serum CA 125 and bilirubin was also separately analyzed in patients with sepsis but no VOD (n = 12; r = 0.147; P = 0.6) and in patients with GvHD but no VOD (n = 15; r = 0.329; P = 0.2).
Considering the potential mechanisms of CA-125 increases, we were especially interested in its associations with liver and renal functions. There were no significant correlations between serum CA 125 and alanine aminotransferase, aspartyl aminotransferase, or thromboplastin time. Somewhat surprisingly, a moderate negative correlation was observed between alkaline phosphatase and CA 125 ($r = -0.251$; $P < 0.05$; $n = 71$). Serum creatinine correlated positively ($r = 0.292$; $P < 0.05$; $n = 73$), and serum albumin correlated negatively with CA 125 ($r = -0.349$; $P < 0.001$; $n = 117$). There was a moderate positive correlation between serum procollagen type III and CA 125 ($r = 0.185$; $P < 0.05$; $n = 139$).

**DISCUSSION**

In the present study, we have evaluated the value of serum CA 125 determinations in the diagnosis of VOD in children undergoing BMT. Thus far, the studies on the role of CA 125 in children have focused on its use as a diagnostic tool in cancer. These studies have demonstrated that serum CA 125 may be elevated in children with premalignant or malignant liver diseases (27, 28) and in some patients with immature teratomas or germ-cell tumors (17). The sites of origin of CA 125 antigen in these patient groups are not clear, but the fetal type of gene expression in tumors is a possible explanation for elevated serum CA 125 in children with embryonal tumors. Abdominal Burkitt’s lymphoma in children and adults is also often associated with increased serum CA 125 concentration at diagnosis (16, 29).

Our results demonstrate that serum CA 125 measurement can be used as an early and sensitive indicator of VOD. Importantly, the main clinically relevant processes to be considered as differential diagnostic options for VOD did not cause significant rapid changes in serum CA 125 levels. However, as is evident from Fig. 2, A and B, the clinical usefulness of CA 125 measurement may depend on repeated measurements because VOD was associated with rapid increase from the baseline level whereas the maximum concentrations achieved were not invariably high. Therefore, we want to underscore that a follow-up of CA 125 levels is mandatory and is far more informative than single estimations. Although there is currently no effective therapy available for established VOD in the BMT setting, its timely and reliable differentiation from other, and possibly concurrently, emerging processes, such as acute GvHD, remains of paramount clinical importance.

The mechanisms of CA 125 increase in the VOD patients remains unspecified thus far but most likely are multiple. Unspecific rise due to hepatocellular damage is unlikely because no correlation between CA 125 and aminotransferases or thromboplastin time were found. Our original hypothesis included altered CA 125 synthesis in intrahepatic bile ducts. However, the negative correlation between CA 125 and alkaline phosphatase does not support this assumption. Ascites formation is a central criterion for VOD. Given that the peritoneum synthesizes CA 125 antigen (30), we hypothesized that, irrespective of whether ascites formation in VOD involves irritation of peritoneal epithelium or merely reflects passive stasis at the hepatic level, altered homeostasis of peritoneal epithelium might affect CA 125 synthesis. Supporting this hypothesis, we observed a significant negative correlation between the serum albumin and CA 125 levels. Finally, decreased renal clearance may contribute to the increased levels of serum CA 125 because a positive correlation was found between serum CA 125 and creatinine levels.
We and others have earlier shown that serum PIIINP levels are elevated in most patients with VOD when or before the diagnostic criteria are fulfilled (4, 5), and that PIIINP may, thus, serve as a marker for VOD. In the present study, we found a significant correlation between serum CA 125 and serum PIIINP levels, and these two markers—probably reflecting the peritoneal and liver involvement, respectively—could, thus, be used to complement each other as biochemical indicators for VOD. Serum CA 125 was at least as sensitive a marker of VOD as serum PIIINP, and the availability of the CA 125 determinations in most centers gives it an additional advantage over PIIINP measurements. Yet, the final role of each of the putative new parameters, including PIIINP, protein C, plasminogen activator inhibitor-1, and CA 125 in the clinical diagnosis of VOD remains to be established.

Taken together, our results demonstrate that the pathophysiological process in VOD leads to increased serum concentrations of CA 125 early in the disease in the vast majority of patients. These results suggest that the measurement of serum CA 125 is of benefit in diagnosing VOD in children undergoing BMT. With emerging new therapies for VOD, the identification of sensitive and easily measurable markers will probably be of great benefit in the management of these patients.

REFERENCES
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