We report here a 16-year-old male with primitive neuroectodermal tumor (PNET)-associated probable microangiopathy with multiple bone metastases. Laboratory findings excluded the possibility of amegakaryocytic or immune thrombocytopenia and disseminated intravascular coagulation. He was first treated with plasma-exchange (PE), followed by platelet transfusions, steroid pulse therapy and combined chemotherapy. PE and steroid pulse therapy reduced his plasma CRP level. Combined chemotherapy drastically increased his platelet count until it had almost normalized without further transfusion. The plasma level of von Willebrand factor-cleaving protease (ADAMTS13) activity measured before PE was not severely deficient (48% of normal) and an unusually large von Willebrand factor multimer (UL-VWFM) was detected. We consider that this therapeutic strategy has the following benefits: (1) reduction of plasma levels of factors that are harmful to both platelet activation and endothelial cell injury; and (2) the safe transfusion of platelet concentrate in thrombotic microangiopathy. This strategy should be confirmed in further cases.

Key words: PNET – microangiopathy – chemotherapy – ADAMTS13 – UL-VWFM

INTRODUCTION
Malignancy-associated thrombotic microangiopathy (TMA), characterized by thrombocytopenia and microangiopathic hemolytic anemia, is a rare but life-threatening complication of sarcoma and its treatment remains controversial. Recent studies, however, have indicated that such patients usually have normal plasma von Willebrand factor-cleaving protease (ADAMTS13) activity (1,2), and that platelet transfusions are generally contra-indicated in these patients because transfusions have been associated with disease exacerbation (3,4). We report here a case of PNET-associated probable TMA that was successfully treated by platelet transfusion after extensive plasmapheresis (PE) followed by chemotherapy.

CASE REPORT
The patient was a 16-year-old male (body weight, 60 kg) who had complained of a high fever and fatigue beginning in June 2002. He was admitted to a nearby hospital on 14 June and received penicillin injections for three days. Suspicion of meningitis, sepsis, viral infection and immunologic diseases was excluded by negative results of leucocytosis in the cerebrospinal fluid, culture of blood and cerebrospinal fluid and antibodies against certain viruses and nucleus. A lytic area in the right eighth rib was then noted on radiography. Bone scintigraphy showed multiple hot lesions on 18 June. Bone marrow examination performed on 19 June was normal without invasion of malignant cells. Pathological examination using biopsy specimens together with the demonstration of EWS-FLI1 translocation confirmed a diagnosis of a PNET (Fig. 1). On 25 June, he developed slight bilirubinemia (1.2 mg/dl) and thrombocytopenia (94×10^9/l), which then rapidly progressed together with hemolytic signs consisting of rouleaux formation and poikilocytosis of erythrocytes in the peripheral blood, and microscopic hematuria. Normoblasts and immature myeloid cells in the peripheral blood were also found as leucoerythroblastic features. Because of this complex clinical picture, he was transferred to our hospital on 8 July. On admission, he had anemia (Hb 105 g/l) (normal range: 135–176), thrombocytopenia (26×10^9/l), and high serum levels of CRP
(251 mg/l), LDH (1787 U/l) (normal range: 106–211 U/l), GOT (49 U/l) (normal range: 12–32 U/L), and ALP (2,100 U/l) (normal range: 106–211 U/l). Other laboratory findings were as follows: BUN 6.78 mg/dl (normal range: 2.85–7.12 mg/dl), creatinine 53.04 mg/dl (normal range: 26.52–79.56 mg/dl), and total bilirubin 18.8 mg/dl (normal range: 5.1–18.8 mg/dl). His blood type was O-Rho (D) positive and both direct and indirect Coombs tests were negative. Antiplatelet antibody determined by mixed passive hemaglutination assay was negative. He had never previously undergone chemotherapy or blood transfusion. Coagulation screening tests including the levels of antithrombin (86%) and fibrinogen (5.91 g/l) were within normal ranges; however, his serum FDP level had increased slightly to 43.7 mg/ml. Plasma ADAMTS13 activity determined by the multimer assay was not immediately available. Based on these clinical and laboratory findings, we suspected that the patient had PNET-associated TMA rather than immune thrombocytopenia or disseminated intravascular coagulation (DIC). Because of his extremely poor general condition, surgical and/or chemotherapeutic approaches were thought to be inadvisable.

Thus, we prepared a protocol consisting an initial plasma exchange (PE) followed by transfusion of a single-donor platelet concentrate (PC) supplied by the Japan Red Cross Blood Center. This regimen was repeated for five consecutive days, together with steroid pulse therapy (methylprednisolone 1 g/d for 3 days). Using this approach, PC was transfused without any appreciable adverse reactions. The expected rise in platelet count was identified 1 h after each infusion. It then decreased to the pre-infusion level (23–33 × 10^9/l) over the next few days. Bone marrow examination on the fourth hospital day (11 July) demonstrated a normal nuclear cell count (137 × 10^9/l), of which malignant cells accounted for 29.5%. Meanwhile, the megakaryocyte count was normal or had increased slightly (200 × 10^9/l), supporting the concept of enhanced consumption of newly-produced platelets. After sequential PE, a marked decrease in the CRP level was observed, and the general condition of the patient appeared to improve. However, the LDH level remained elevated and even increased slightly while the anemia worsened, indicating invasive expansion of tumor cells. Thus, on 13 July, we started combined chemotherapy, consisting of vincristine (VCR), adriamycin (ADR), and cyclophosphamide (CPA), that resulted in a dramatic increase in the platelet count with a concomitant decrease in LDH. Furthermore, the anemia ceased to progress, with no red blood cell transfusion required throughout this clinical course. Partial response was confirmed by resection of the right eighth rib after chemotherapy. Total body and local irradiation was performed after the Hi-MEC regimen, resulting in an absence of abnormal accumulation on bone scintigraphy 10 months after diagnosis. However, 13 months after diagnosis, recurrences in the right hip joint and orbit were detected and the patient died of disease. Survival after diagnosis was 23 months.

Before PE, his plasma VWF antigen level was elevated (298%) and an unusually-large VWF multimer (UL-VWF) was present (Fig. 2).
The diagnosis of TMA was based on schistocytosis and evidence of hemolysis. In our case, an elevated level of LDH was evident; however, schistocytosis was not tested in our hospital. Thrombocytopenia occurred as a result of low platelet production and/or increased breakdown. In our case, a normal or slight increase in the production of platelets in the bone marrow was confirmed during hospitalization. Therefore, the increased breakdown of platelets was assumed. Spherocytes in the peripheral blood, which are characteristic in immune and hereditary hemolysis, were not found before transition. Considering these data along with the negative results on direct and indirect Coombs tests, the possibility of immune hemolysis was considered highly improbable and DIC was also excluded by the absence of signs indicating decreased ATIII.

Though a high CRP value persisted, severe infection, including meningitis, sepsis and viral infection were excluded by intensive examination. High fever and leucocytosis were thus considered characteristic symptoms of PNET and not owing to infection. Splenomegaly as a sign of increased breakdown of platelets was not confirmed by CT. There was no history of blood transfusion.

Invasion of malignant cells as confirmed by bone marrow examination occurred between 11 June and 19 July, and thrombocytopenia with hemolytic anemia occurred concomitant with this invasion, though multiple bone metastases had

**DISCUSSION**

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Invasion of malignant cells as confirmed by bone marrow examination occurred between 11 June and 19 July, and thrombocytopenia with hemolytic anemia occurred concomitant with this invasion, though multiple bone metastases had
already been confirmed by bone scintigraphy. Therefore the formation of bone metastases is insufficient to explain thrombocytopenia in this case. We consider that thrombocytopenia was probably as a result of malignant tumor-associated TMA.

Detection of UL-VWF, released from endothelial cells and cleaved by ADAMTS13, and subnormal activity of ADAMTS13, reported as a marker to differentiate between TTP and HUS (3), were also confirmed later. Detection of UL-VWF suggests injury of the endothelial cells or obstruction of cleavage by ADAMTS13. In our case, ADAMTS13 activity was subnormal, which agreed with the findings in the majority of TMA reported (1). Therefore, detection of UL-VWF suggested injury of endothelial cells (2). Invasion of malignant cells, synchronously occurring, may have caused endothelial cell injury. Histological examination may help clarify the mechanism.

Cytokines have recently been reported to mediate UL-VWF release from vascular endothelial cells (5). Furthermore, it was proposed that cytokines that injure vascular endothelial cells may interfere with the efficient supply of ADAMTS13 (5). Thus, cytokines may be another cause of TMA.

Systemic chemotherapy is usually indicated except in cases of chemotherapy-induced TMA. However, low platelet count made the initiation of this therapy inadvisable. Thus, transfusion of platelets was performed after PE to prevent adverse reaction. It has been proposed that PC transfusion is contra-indicated in TMA because uncleaved UL-VWF induces platelet aggregation under high shear stress and exacerbates thrombosis (3). However, after removal of factors including UL-VWF and cytokines from the circulation by PE, PC transfusion was performed safely and quickly resulted in raising the platelet count over the short time. However, the basic conditions, for example, expansion of tumor cells, may gradually lower the platelet count again. Thus, treatment of the tumor itself is necessary. Combined chemotherapy after PE dramatically improved TMA in our case. This also supports the hypothesis that malignant cells were related to injury of the endothelial cells. Prognosis of TMA depends on the chemosensitivity of the tumor itself. Further experience is necessary to confirm this regimen.

The present findings may improve our understanding the reason why malignancy-associated TMA responds poorly to PE therapy alone, as has been commonly accepted.

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Conflict of interest statement

None declared.

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