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Coincidental Outbreak of Methicillin-Resistant *Staphylococcus aureus* in a Hematopoietic Stem Cell Transplantation Unit

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common nosocomial pathogens among hospital-acquired infections, and immunocompromised patients are highly susceptive to infection. The molecular typing of isolated strains is a common method for tracing an outbreak of MRSA, but experience with this approach is still limited in the hematopoietic stem cell transplantation (HSCT) ward.

Methods: We experienced 6 cases of MRSA infection/colonization in our 26-bed HSCT ward during a 4-week period. This unusual outbreak strongly suggested that the same MRSA strain was involved despite strict isolation and aseptic patient care. Clarification of the transmission pattern was critical, and we applied pulsed-field gel electrophoresis (PFGE) and amplified fragment length polymorphism (AFLP) assays for evaluation.

Results and conclusion: In four of the six cases, the pattern of bands examined by PFGE and AFLP analyses supported the idea that direct person-to-person transmission was very unlikely and the outbreak was coincidental. This experience highlights the clinical value of molecular typing methods for the clinical epidemiological assessment of MRSA outbreak.
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Key words: outbreak; MRSA; stem cell transplantation

INTRODUCTION

The rapid increase in the incidence of hospital-acquired infection by methicillin-resistant *Staphylococcus aureus* (MRSA) is making infection control procedures very critical, particularly for immunocompromised patients [1]. Hospital-acquired infections also serve as a hallmark of the effectiveness and quality of infection control maneuvers [2]. Outbreaks of infection caused by MRSA have time-consuming and expensive consequences, and genetic analysis is useful, since it can be used to determine the route and origin of MRSA infection [3]. Currently available laboratory methods for determining DNA fragment sizes or sequences in MRSA isolates include Southern blotting [4], ribotyping [5], polymerase chain reaction (PCR) [6], and pulsed-field gel electrophoresis (PFGE) [4,7]. PFGE has become the most common tool for the rapid discrimination of MRSA strains due to its convenience, reliability, and cost-effectiveness [8,9]. However, the interpretation of PFGE bands still needs to be standardized [10]. Alternatively, the amplified fragment length

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TABLE I. Clinical Characteristics of 6 Cases*

UNP	Age	Sex	Disease	Day after transplantation	Cause of admission	Admission to hospital	First admission ward	Admission to SCT unit
1	53	M	MDS	109	GVHD	2002/11/13	Ward A	11/15/2002
2	35	M	NHL	219	GVHD	2002/11/20	Ward B	11/27/2002
3	35	M	GCT	43	GVHD	2002/11/29	Ward C	12/5/2002
4	58	M	MDS	13	SCT	2002/10/31	SCT unit	10/31/2002
5	54	F	NHL	-22	SCT	2002/11/5	SCT unit	11/5/2002
6	63	M	CML	210	GVHD	2002/11/5	SCT unit	11/5/2002

* Cases 4 to 6 were admitted before case 1 showed severe intestinal symptoms induced by gut GVHD, complicated with continuous gastrointestinal bleeding. In 4 of these 6 cases, hospitalization was due to GVHD after transplantation (3 acute and 1 chronic), and 3 of these patients, including case 1, received corticosteroid therapy for the treatment of GVHD.

Note: MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; GCT, germ cell tumor; CML, chronic myelogeneous lymphoma; GVHD, graft versus host disease; PSL, prednisolone; SCT, stem cell transplantation.

polymorphism (AFLP) method is based on the selective amplification by PCR of a subset of restriction fragments from a digest of the whole bacterial genome [11,12]. AFLP has advantages over PFGE since it has more power for discriminating between different strains more quickly with higher specificity in the recognition of digestive fragments of whole bacterial genome [13].

The goal of these laboratory tests is to provide firm evidence that isolates, which are epidemiologically related during an outbreak of the infection, are also genetically related and thus represent the same strain. To enhance the reliability of such molecular laboratory results, the combined use of various genotyping methods appears to be effective [14,15]. An outbreak has been defined as infectious disease derived from the same pathogen, while an outbreak that originates from strains that are indistinguishable from each other by typing methods but for which no direct linkage can be demonstrated is called an "endemic outbreak" [16]. It has been suggested that in disease outbreak due to endemic strains, the common origin may be temporally distant from those in outbreak strains. From the perspective of infection control, this difference is critical, since different procedures are needed to prevent the spread of disease.

We experienced an outbreak of MRSA in our hematopoietic stem cell transplantation (SCT) ward that was initially suspected to be derived from a single origin. To address this serious problem, we tracked down the route of infection and obtained results that highlighted the clinical value of molecular typing using these methods.

PATIENTS AND METHODS

Patients

The routine infection-monitoring procedure in the SCT ward includes surveillance cultures and identification for specific pathogens in the nasal swab, pharyngeal swab or sputum, urine, or stool, which are collected from patients who are suspected to have infection or colonization of the target pathogen including MRSA at the time of admission. In a 4-week period, we experienced six cases (UPN 1 to 6) of MRSA infection or colonization in the SCT ward, while the preceding incidence of MRSA detection in the SCT ward had been only one or two cases per month (mean 0.8/month, range 0–2/month, SD 0.61). Therefore, this was epidemiologically defined as an MRSA outbreak. The patient characteristics are summarized in Table I. We reviewed the medical records of the patients to collect the clinical information required to track down the transmission route. We documented the time course of MRSA identification in relation to patient characteristics, risks of nosocomial infection, and room assignment.

Samples

Isolates were grown from culturing sputum, urine, stool, pus, and blood, and a few were grown from culturing miscellaneous sites such as pharynx and nasal cavity. We examined the first sample isolated in each patient by molecular typing, PFGE, and AFLP analysis.

DNA Isolation and PFGE

Targeted bacterial strains were cultured at 37°C in Luria-Bertani broth. The cell component was lysed by proteinase K to extract DNA. Genomic DNA was digested with *SmaI* and resolved with the CHEF-DRII system (Bio-Rad Laboratories) as described by the manufacturer (traditional typing strategies) [17]. As a control strain, we used MRSA isolated from two groups: (1) two strains isolated from past patients in the same ward, which has no temporal relationship to our present cases (cases 7 and 8) and (2) five strains isolated from a different hospital

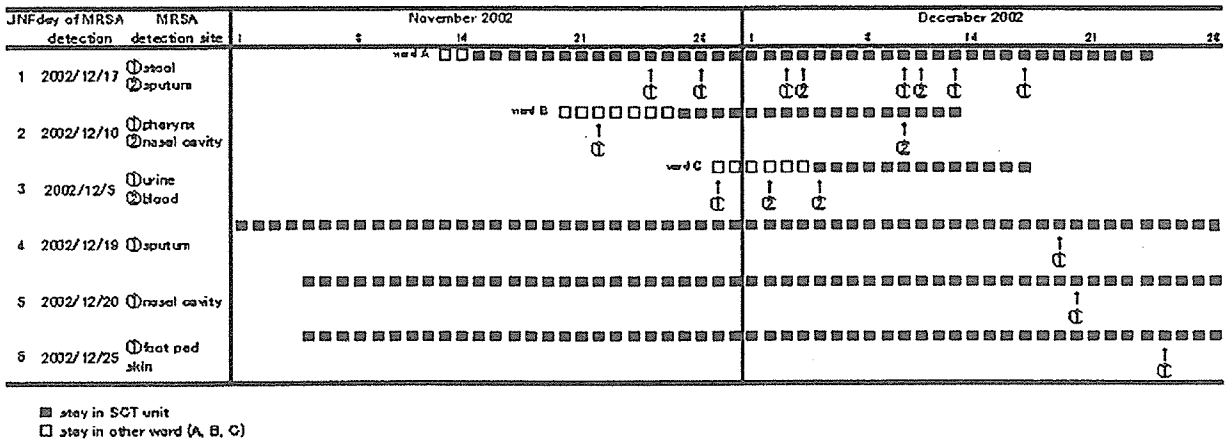

Fig. 1. Time course of MRSA detection in the SCT unit. MRSA was first isolated in the stool of case 1 on 25 November 2002. MRSA had been identified prior to admission to the SCT ward in cases 2 and 3. In contrast, in the other three cases (cases 4, 5, and 6), MRSA was detected after admission to the SCT ward.

(University of Tokyo Hospital, 1150 beds), which was not epidemiologically associated with our hospital (cases 9 to 13). PFGE banding was compared with that in case 1, who was thought to be the origin of this outbreak episode. The criteria described by Tenover et al. [16] were used for the molecular epidemiological interpretation of PFGE banding as follows:

(i) indistinguishable: outbreak was derived from the same isolate;
(ii) closely related: different isolates, closely related to the outbreak pattern;
(iii) possibly related: different isolates, possibly related to the outbreak pattern;
(iv) unrelated: different isolates, unrelated to the outbreak pattern.

One genetic event detected by PFGE was considered meaningful enough as different isolates.

AFLP

Bacterial DNA was prepared with a QIAamp DNA Mini kit (Qiagen) according to the manufacturer's recommendations. DNA was then manipulated with an AFLP Microbial Fingerprinting kit (Applied Biosystems) according to the manufacturer's instructions based on a previous study [11]. Briefly, DNA was digested with *Eco*RI and *Mse*I and then ligated to the corresponding adapters. This was followed by preselective amplification and selective amplification, where *Eco*RI-A (FAM), *Eco*RI-C (NED), *Eco*RI-G (JOE), and *Mse*I-C primers were used. The AFLP reactions were evaluated by analyzing data from samples loaded and run on an ABI 310 Genetic Analyzer with GeneScan software. A dendrogram was constructed from a pairwise distance matrix with the Clustal W version 1.8 software package.

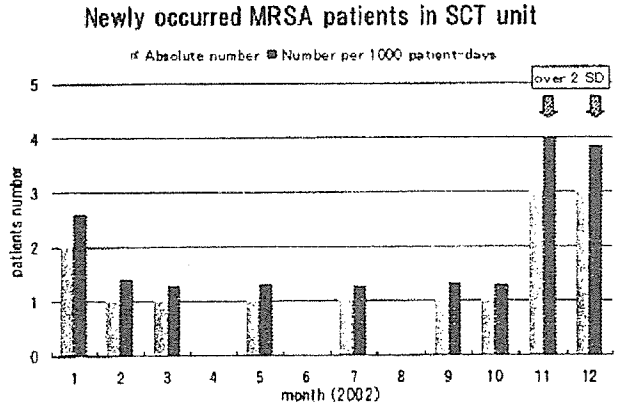

Fig. 2. The incidences of newly detected MRSA cases in SCT unit in 2002. Each bar indicates a number of patients clinically identified as MRSA in 2002.

Definitions [18]

"Methicillin-resistant" is defined according to NCCLS MIC criteria by dilution susceptibility tests. An "outbreak" of MRSA is defined as an increase in the rate of MRSA cases or a clustering of new cases in a specific place during a given period. In this report, we defined an unusual increase in MRSA cases as a multiply repeated isolation of MRSA from a physically independent ward (transplantation unit) with an incidence $\geq$ 2 SD over the baseline. The SCT unit is geographically separate from other wards and has an independent space that is managed to maintain sterilization. Patients from whom MRSA was isolated and who had any concomitant symptoms in the MRSA-detected part were referred to as "MRSA

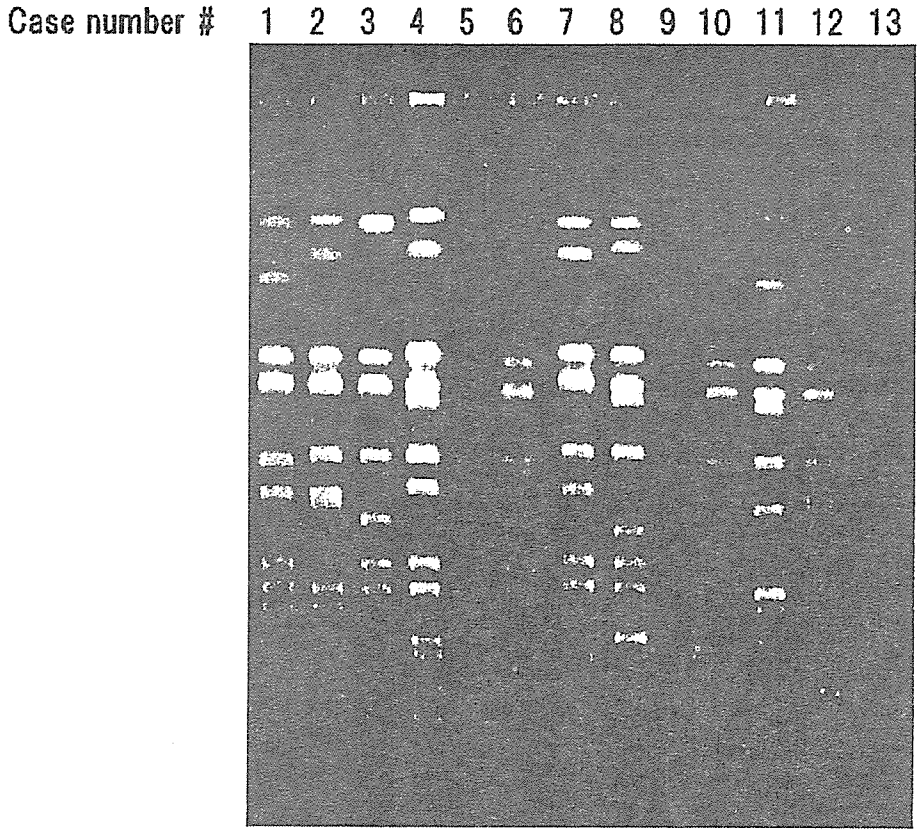

Fig. 3. PFGE analysis of isolated MRSA in the SCT unit. The PFGE pattern showed that there were no detectable differences in bands between cases 3 and 6, but more than two bands were identified in the other four cases (cases 1, 2, 4 and 5). Additionally, two strains that had been previously isolated in the SCT ward (cases 7 and 8) were distinguishable, and the five epidemiologically different isolates (cases 9 to 13) from the University of Tokyo Hospital (1,150 beds) were also distinguishable, with differences in more than two bands.

infection," while those without symptoms were considered "MRSA colonization."

RESULTS

Clinical Course of MRSA Outbreak

The clinical characteristics of six patients in whom MRSA was isolated are presented in Table I. The first patient (case 1) was admitted to the SCT ward because of severe intestinal symptoms induced by gut GVHD, chronic diarrhea, and continuous gastrointestinal bleeding, which occurred at 107 days after SCT. The patient received corticosteroid and intravenous antimicrobial therapy. For 4 weeks prior to his admission, there had been no case of MRSA infection or colonization in the ward. At 13 days after admission, the first isolation of MRSA in his stool was recorded (Table I). Subsequently, five other patients newly developed MRSA events over the next 4 weeks (Figure 1), while the incidence of MRSA detection of SCT ward had remained at one or two cases per month (mean 0.8 /month, range 0–2 /month, SD 0.61, Figure 2). Among these five cases, three (cases 4, 5, and 6) had been admitted to the SCT ward directly from the outpatient clinic without a past history of MRSA infection. The other two cases (cases 2 and 3) were transferred from other wards after the admission of case 1, and MRSA was isolated prior to transfer to the SCT ward (Figure 1). Since, in these two cases, MRSA was identified again in different site in SCT with different drug-sensitivity profile (data not shown) from a previous strain, we included these two cases in the analysis. There were no other patients who were previously identified with MRSA infection or colonization.

Tracing Procedure

The transmission, if any, appeared to take a random pattern, as illustrated in Figure 1. To better evaluate whether the transmission pattern was direct or indirect, we identified the layout of the patients' bed assignments. This revealed that there were nei-

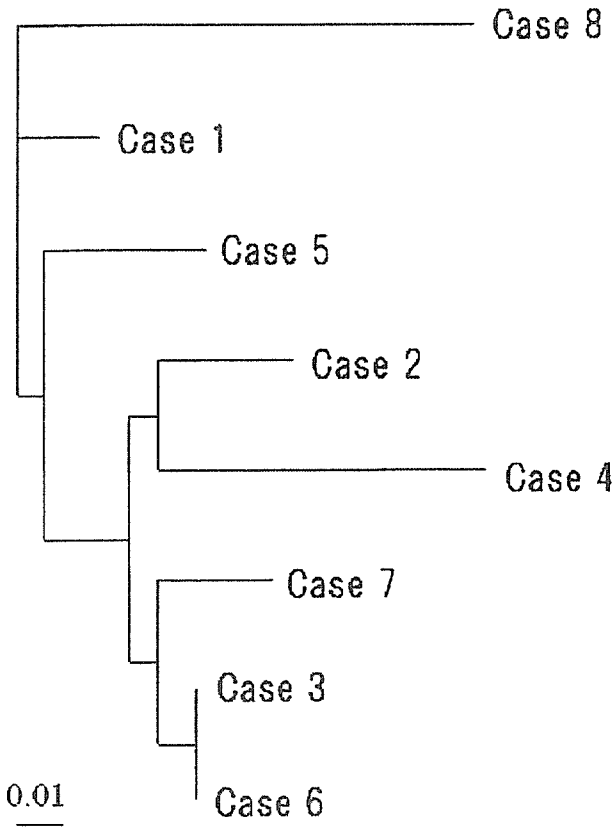

Fig. 4. AFLP pattern of isolated MRSA. AFLP analysis was performed for the same six isolates (case 1 to 6) described in Figure 4. Gene polymorphism showed same result of similarity as PFGE pattern had already indicated, i.e., cases 3 and 6 had the same polymorphism pattern and the others were different strains. The mutual relation of gene polymorphism is presented in the dendrogram and relatedness is indicated by the length of line. The scale bar drawn in the lower part indicated 1.0 % relatedness.

ther overlaps nor coexistence with preceding patients, except that cases 1 and 3 used the same room on different days without an overlap.

PFGE and AFLP Assays of MRSA Isolates

PFGE analysis of the six MRSA strains isolated (Figure 3) showed that two strains (cases 3 and 6) were indistinguishable and therefore considered to be derived from the same isolate, while the remaining four cases (cases 1, 2, 4, and 5) were considered to have different strains. Seven epidemiologically different isolates, i.e., two strains isolated from another ward at different times (cases 7 and 8) and five strains isolated from another hospital (cases 9 to 13) were used as in-hospital and extra-hospital controls, respectively. The results were confirmed by AFLP analyses as a dendrogram shown in Figure 4.

DISCUSSION

The spread of MRSA in highly protected care units, including ICU [19] and neonatal ICU [20], is a well-known major complication in compromised patients. Although few reports have been published on the outbreak of MRSA in a SCT unit, a continuous rise in the incidence of hospital-acquired MRSA infection [21] should influence the incidence of MRSA infection in SCT recipients [22]. Collin et al. reported that the incidence of multidrug resistant *S. aureus* was 15% in isolates from BMT patients with blood stream infection in 1991–1997 [23]. Prolonged neutropenia has been found to be a risk factor for the development of infectious complications in SCT recipients [24]. Since the outbreak of MRSA among immunocompromised patients can greatly affect their mortality, appropriate methods for infection control are strongly warranted. The Consensus Panel's guidelines for preventing the spread of MRSA recommend contact precautions and the isolation of infected or colonized patients in a single room or cohort, i.e., grouping them geographically with designated staff [18]. Also, since MRSA colonization precedes infection because of inpatient circumstances and rather strong treatments [25,26], a local control is very important for controlling MRSA outbreak in selected circumstances such as SCT ward in which many immunocompromised patients are taken care of.

In this report, we described an MRSA outbreak in the SCT ward during a limited period of 4 weeks. Initially, we suspected that all MRSA infections were caused by a single source, such as highly contaminated stool. However, unexpectedly, no direct contact was identified among patients and staff who were involved in their care. The transmission of MRSA mostly occurs through direct person-to-person contact, and transmission from the environment is extremely rare in places where strict precautions are taken and careful decontamination procedures are used. Hence, we undertook a molecular epidemiological analysis to critically examine the suspected break in our procedure. We found that four of the six isolates were genetically different, and our Infection Control Team concluded that horizontal transmission was unlikely. Nevertheless, the interest raised with this event resulted in further enforcement of essential precautions against droplets and contact,and the elimination of new MRSA cases for subsequent months.

Although our observation was well anticipated, in that molecular typing techniques are effective in the diagnosis and tracking of MRSA, the results are still unique, since they highlight the value of these methods over clinical judgment in a critical care situation

with highly immunocompromised patients. Since the molecular typing properties of MRSA are very similar in Japan, especially in the local areas [27], we focused on the genetic event detected by molecular typing and diagnosed those differences as different strain from outbreak. Thus, this report should be helpful for evaluating whether the routine application of these measures should be critically considered in the assessment of outbreak.

REFERENCES

1. Crossley K, Loesch D, Landesman B, Mead K, Chern M, et al. An outbreak of infections caused by strains of *Staphylococcus aureus* resistant to methicillin and aminoglycosides. I. Clinical studies. J Infect Dis 1979;139:273–279.
2. Scheckler WE, Brimhall D, Buck AS, Farr BM, Friedman C, et al. Requirements for infrastructure and essential activities of infection control and epidemiology in hospitals: a consensus panel report. Society for Healthcare Epidemiology of America. Infect Control Hosp Epidemiol 1998;19:114–124.
3. Strausbaugh LJ, Jacobson C, Sewell DL, Potter S, Ward TT. Methicillin-resistant *Staphylococcus aureus* in extended-care facilities: experiences in a Veterans' Affairs nursing home and a review of the literature. Infect Control Hosp Epidemiol 1991;12:36–45.
4. Prevost G, Jaulhac B, Piemont Y. DNA fingerprinting by pulsed-field gel electrophoresis is more effective than ribotyping in distinguishing among methicillin-resistant *Staphylococcus aureus* isolates. J Clin Microbiol 1992;30:967–973.
5. Blumberg HM, Rimland D, Kiehlbauch JA, Terry PM, Wachsmuth IK. Epidemiologic typing of *Staphylococcus aureus* by DNA restriction fragment length polymorphisms of rRNA genes: elucidation of the clonal nature of a group of bacteriophage-nontypeable, ciprofloxacin-resistant, methicillin-susceptible *S. aureus* isolates. J Clin Microbiol 1992;30:362–369.
6. van Belkum A, Bax R, Peerbooms P, Goessens WH, van Leeuwen N, et al. Comparison of phage typing and DNA fingerprinting by polymerase chain reaction for discrimination of methicillin-resistant *Staphylococcus aureus* strains. J Clin Microbiol 1993;31:798–803.
7. Ichiyama S, Ohta M, Shimokata K, Kato N, Takeuchi J. Genomic DNA fingerprinting by pulsed-field gel electrophoresis as an epidemiological marker for study of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 1991;29:2690–2695.
8. Bannerman TL, Hancock GA, Tenover FC, Miller JM. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. J Clin Microbiol 1995;33:551–555.
9. Tenover FC, Arbeit RD, Goering RV. How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infections: a review for healthcare epidemiologists. Molecular Typing Working Group of the Society for Healthcare Epidemiology of America. Infect Control Hosp Epidemiol 1997;18:426–439.
10. Shopsin B, Kreiswirth BN. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis 2001;7:323–326.
11. Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, et al. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 1995;23:4407–4414.
12. van den Braak N, Simons G, Gorkink R, Reijans M, Eadie K, et al. A new high-throughput AFLP approach for identification of new genetic polymorphism in the genome of the clonal microorganism *Mycobacterium tuberculosis*. J Microbiol Methods 2004;56:49–62.
13. Grady R, Blanc D, Hauser P, Stanley J. Genotyping of European isolates of methicillin-resistant *Staphylococcus aureus* by fluorescent amplified-fragment length polymorphism analysis (FAFLP) and pulsed-field gel electrophoresis (PFGE) typing. J Med Microbiol 2001;50:588–593.
14. Fang FC, McClelland M, Guiney DG, Jackson MM, Hartstein AI, et al. Value of molecular epidemiologic analysis in a nosocomial methicillin-resistant *Staphylococcus aureus* outbreak. J Am Med Assoc 1993;270:1323–1328.
15. Yoshida T, Kondo N, Hanifah YA, Hiramatsu K. Combined use of ribotyping, PFGE typing and IS431 typing in the discrimination of nosocomial strains of methicillin-resistant *Staphylococcus aureus*. Microbiol Immunol 1997;41:687–695.
16. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995;33:2233–2239.
17. Tenover FC, Arbeit R, Archer G, Biddle J, Byrne S, et al. Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus*. J Clin Microbiol 1994;32:407–415.
18. Wenzel RP, Reagan DR, Bertino JS, Jr., Baron EJ, Arias K. Methicillin-resistant *Staphylococcus aureus* outbreak: a consensus panel's definition and management guidelines. Am J Infect Control 1998;26:102–110.
19. Mulligan ME, Murray-Leisure KA, Ribner BS, Standiford HC, John JF, et al. Methicillin-resistant *Staphylococcus aureus*: a consensus review of the microbiology, pathogenesis, and epidemiology with implications for prevention and management. Am J Med 1993;94:313–328.
20. Regev-Yochay G, Rubinstein E, Barzilai A, Carmeli Y, Kuint J, et al. Methicillin-resistant *Staphylococcus aureus* in neonatal intensive care unit. Emerg Infect Dis 2005;11:453–456.
21. Peacock JE, Jr., Marsik FJ, Wenzel RP. Methicillin-resistant *Staphylococcus aureus*: introduction and spread within a hospital. Ann Intern Med 1980;93:526–532.
22. Kato N, Tanaka J, Mori A, Tutumi Y, Yonezumi M, et al. The risk of persistent carriage of methicillin-resistant *Staphylococcus aureus* in hematopoietic stem cell transplantation. Ann Hematol 2003;82:310–312.
23. Collin BA, Leather HL, Wingard JR, Ramphal R. Evolution, incidence, and susceptibility of bacterial bloodstream isolates from 519 bone marrow transplant patients. Clin Infect Dis 2001;33:947–953.
24. Ninin E, Milpied N, Moreau P, Andre-Richet B, Morineau N, et al. Longitudinal study of bacterial, viral, and fungal infections in adult recipients of bone marrow transplants. Clin Infect Dis 2001;33:41–47.
25. Pujol M, Pena C, Pallares R, Ariza J, Ayats J, et al. Nosocomial *Staphylococcus aureus* bacteremia among nasal carriers of methicillin-resistant and methicillin-susceptible strains. Am J Med 1996;100:509–516.
26. Davis KA, Stewart JJ, Crouch HK, Florez CE, Hospenthal DR. Methicillin-resistant *Staphylococcus aureus* (MRSA) nares colonization at hospital admission and its effect on subsequent MRSA infection. Clin Infect Dis 2004;39:776–782.
27. Kikuchi K, Takahashi N, Piao C, Totsuka K, Nishida H, et al. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* strains causing neonatal toxic shock syndrome-like exanthematous disease in neonatal and perinatal wards. J Clin Microbiol 2003;41:3001–3006.

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CASE REPORT

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Intensive multimodality therapy including paclitaxel and reduced-intensity allogeneic hematopoietic stem cell transplantation in the treatment of adrenal cancer with multiple metastases

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Abstract Adrenocortical carcinoma is a rare malignancy in adolescents and young adults. The prognosis of unresectable/metastatic adrenocortical carcinoma remains very poor because the rarity of the tumor has made it difficult to establish treatment guidelines, and diagnosis and the resultant treatment can be greatly delayed. We treated a 24-year-old woman who was diagnosed with adrenocortical carcinoma of the right adrenal gland which extended to the inferior vena cava. Although she underwent surgical resection of the extensive tumor as the primary treatment, the disease recurred in the lung and liver as multiple metastases shortly after surgery. She received intensive multimodality therapy, including chemotherapy with paclitaxel, ifosfamide, and cisplatin (TIP regimen), embolization of the feeding arteries, and proton irradiation for the liver mass. Finally, she underwent reduced-intensity allogeneic hematopoietic stem cell transplantation from an HLA 1-locus-mismatched sibling donor. A prolonged survival of 39 months after the onset of the disease was achieved. Although this experience is limited, we suggest that TIP chemotherapy was effective for adrenocortical carcinoma, and a graft-versus-tumor effect after reduced-intensity stem cell transplantation may have contributed to the prolonged survival.

Key words Allogeneic stem cell transplantation (allo-SCT) · Reduced-intensity stem cell transplantation (RIST) · Adrenocortical carcinoma (ACC) · Graft-versus-tumor (GVT) reaction · Graft-versus-host disease (GVHD)

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Introduction

Adrenocortical carcinoma (ACC) is a rare neoplasm with an incidence of approximately 0.5–2.0 per million per year.[1] The sex ratio is 2.5, with greater female involvement. Patients typically present with endocrine symptoms caused by the excessive production of hormones by the tumor, and abdominal symptoms including fullness, tenderness, nausea, and vomiting. It has been reported that tumor reduction contributes to long-term survival, and whenever possible radical surgery is recommended for all patients, including those with recurrent disease. More than three-forth of these patients have a functioning tumor independent of their clinical manifestations, and this suggests that early resection of the tumor could lead to a better chance of survival. However, in those without these early clinical manifestations, complete surgical resection becomes difficult or impossible since the diagnosis is delayed. The reported median survival time was 14.5 months, with a significantly lower survival in those aged 40 years or more, or those who had distant metastasis at the time of diagnosis.[2] Although it has been reported that chemotherapy with mitotane (o,p′-DDD, 1,1-dichlorodiphenyl-dichloroethane) could contribute to longer survival in an adjuvant setting,[2,3] the optimal therapeutic approach for those with systemic disease has not yet been established. Hence, an accumulation of experience is urgently required to identify an effective multidisciplinary strategy.

We describe here a patient with advanced ACC who was treated very intensively with radical resection of the primary tumor, followed by a combination of chemotherapy consisting of paclitaxel, ifosfamide, and cisplatin (TIP) for lung metastases, a combination of arterial embolization and proton irradiation therapy for liver metastases, and allogeneic stem cell transplantation (SCT) with a reduced-intensity regimen (RIST).

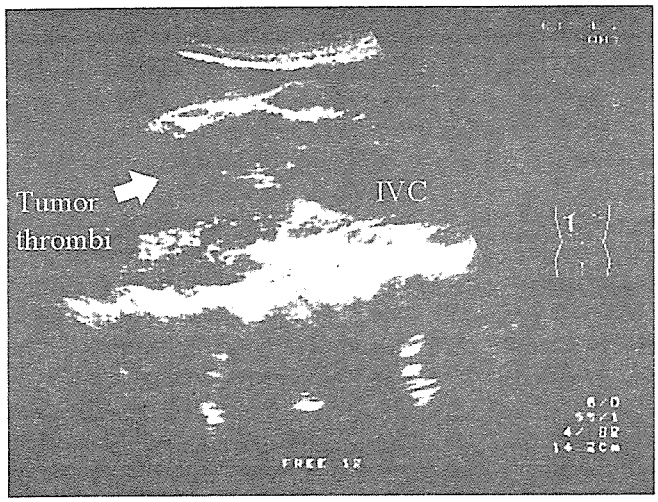

Fig 1. Abdominal echography at diagnosis showing a large right-side adrenal tumor with intravascular extension and massive liver metastasis. *IVC*, inferior vena cava

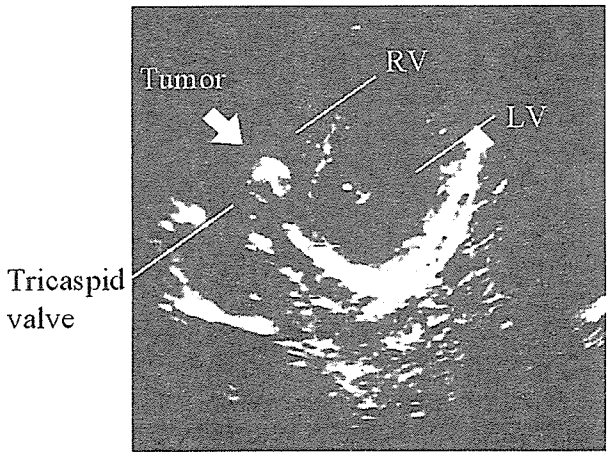

Fig 2. Cardiac echography showing a polypoid lesion in the right ventricle through the tricuspid valve during the diastolic phase. *RV*, right ventricular; *LV*, left ventricular

Case report

A 24-year-old woman developed general fatigue and amenorrhea in January 2000, and a giant tumor in the right adrenal gland was disclosed in October 2000 by evaluation, including ultrasound echography and computed tomography (CT). The liver mass extended into the inferior vena cava (IVC) and caused thrombi and mass lesion in the right atrium (Fig. 1). Cardiac echography revealed direct invasion of the cardiac tricuspid valve by the tumor that originated from the abdomen (Fig. 2). A systemic survey using CT scanning disclosed no other distant metastatic lesion. The patient underwent complete surgical resection of the tumor under support with an extracorporeal circulation device. A pathological diagnosis of adrenocortical carcinoma was established, and the production of androgen was confirmed by an elevated serum level of androgen.

Four months after surgery, the patient had tumor recurrence with multiple liver and lung metastases detected by a follow-up CT examination. Because the lesions grew rapidly, in April 2001 she began to receive chemotherapy which consisted of paclitaxel (TXL, 175 mg/m^2, day 1), ifosphamide (IFM, 1200 mg/m^2, days 2–6), and cisplatin (CDDP, 20 mg/m^2, days 2–6), i.e., the "TIP regimen," every 3–4 weeks. After 3 courses of TIP chemotherapy, lung lesions showed complete remission (CR), while liver metastases remained stable. For liver lesions, the patient underwent a transarterial embolism (TAE) procedure using a mixture of Lipiodol and falmorubicin following transarterial infusion (TAI) of cisplatin (CDDP) and mitomycin C (MMC). A total of 3 TAE/TAI procedures were performed up to August 2001, when all therapies were suspended owing to the development of severe myelosuppression. In November 2001, when the liver mass started to increase in size, 60 Gy proton-beam irradiation was administered concurrently with each course of TIP chemotherapy and TAE.

In April 2002, the patient decided to participate in a phase I trial of RIST for refractory solid tumors, which was approved by our institutional review board (IRB), in the expectation of a powerful graft-versus-tumor (GVT) effect. Prior to the patient's registration on the trial, the medical team held a thorough discussion with the patient and her family regarding possible treatment options such as mitotane. Because of the lack of evidence of curability with the mitotane therapy, the patient did not decide to receive mitotane. She therefore chose the option to receive RIST from an HLA 1-locus-mismatched brother under sufficient informed consent (recipient's HLA typing: A33, A24, B60, B52, DR12, and DR2; donor's HLA typing: A33, A11, B60, B52, DR12, and DR2). The RIST regimen consisted of fludarabine (30 mg/m^2 for 6 days), busulfan (4 mg/kg/day for 2 days) and antithymocyte globulin (25 mg/m^2 for 2 days). At the time of RIST, her disease was CR in the lung, while the liver lesion was not evaluable because of the earlier intensive local treatments. A combination of cyclosporine (3 mg/kg) and methotrexate (10 mg/m^2 on day 1, and 7 mg/m^2 on days 3 and 6) was administered for graft-versus-host disease (GVHD) prophylaxis. The early course after RIST was uneventful except for transient neutropenic fever for 4 days, which was successfully treated with antimicrobial therapy. Hematopoietic engraftment was observed on day 13. On day 33 after RIST, the patient developed grade 1, stage II, skin GVHD, which resolved spontaneously in 3 weeks. The dose of cyclosporine was gradually tapered until it was discontinued on day 80, while the serum androgen level decreased remarkably between day 60 and day 150. The relationship between the occurrence of GVHD and tumor response is illustrated in Fig. 3. Although a CT scan examination on day 90 confirmed PD in the lung lesions, the tumors appeared to be slowly progressive. Thereafter, the patient maintained a relatively high performance status with indolent tumors until April 2003, when she suddenly experienced serious hematemesis. A upper gastrointestinal endoscopy revealed extensive mucosal damage disproportionally located in the lesser curvature, which ap-

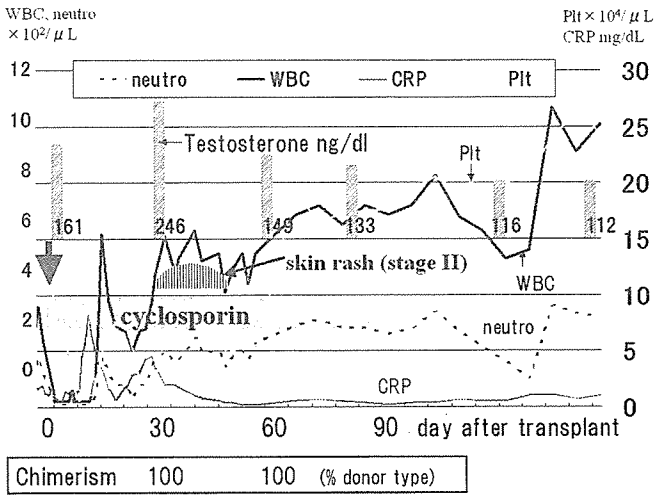

Fig 3. Clinical course of transplantation. The serum level of testosterone (normal range 10–60ng/dl) as a tumor marker continued to decrease after the ocurrence of acute graft-versus-host disease. White blood cell count (*WBC*) and platelets (*plt*) slowly increased after hematopoietic engraftment, which was observed at day 13. Chimerism achieved 100% donor type at 30 days after SCT and continued to keep 100% donor type. *CRP*, C-reactive protein

pears secondary to proton irradiation. The patient experienced a silent perforation of the gastric wall resulting panperitonitis the next day, which led to her death 12 months after transplantation. Necropsy was not approved.

Discussion

The prognosis of localized ACC primarily depends on the histology[4] and tumor size,[5] and there have been reports on the value of radical tumor resection and the efficacy of mitotane therapy.[2] In contrast, no effective therapy has been reported for patients with distant metastases or unresectable tumor, and the reported median survival period is 8 months.[6] Here, we report the case of a patient who had an extended tumor and survived 39 months after the onset of the disease, which was much longer than the reported median value. In addition, this patient maintained a high performance status and qualified daily life for 9 months after progression until the day before her death, although the tumor recurred shortly after RIST.

We believe that three factors may have contributed to this patient's ability to achieve a durable asymptomatic period. First, the TIP regimen, which has been shown to be effective for germ cell tumors and other types of endocrine tumor as a salvage chemotherapy,[7] might also be effective for ACC. Considering the lack of adequate therapeutic options, we feel that even this limited single-patient experience could still be of value for future evaluations. Second, this patient underwent an experimental RIST procedure from a partially HLA-mismatched sibling as a final consolidation therapy, as part of clinical trials in patients with various types of solid tumor. Clinical studies that have been reported to date provide proof-of-principle that allogeneic T cells can induce clinically relevant GVT effects in solid tumors, including renal cell carcinoma (RCC)[8] and others.[9,10] It is widely accepted that the GVT effect is closely associated with GVHD. Hence, it is possible that the GVT effect may be enhanced by selecting a mismatched graft. Although this patient had no measurable lesions at the time of RIST, the decrease in the serum level of androgen between day 60 and day 150 when GVHD emerged and the indolent course of tumor progression after RIST might be considered evidence of a GVT effect. Third, this patient received intensive procedures for local tumor control, including extensive primary tumor resection, TAE/TAI, and proton-beam irradiation, which may have made the tumor more sensitive to a GVT effect, as previously suggested.[11]

In summary, successful allogeneic SCT requires a highly integrated program of donor selection, preparative regimen, and management of GVHD. Nevertheless, it is possible that the combination of TIP chemotherapy, intensive local tumor controls, and a GVT effect may have contributed to the prolonged survival of this patient.

References

1. Copeland PM (1983) The incidentally discovered adrenal mass. Ann Intern Med 98:940–945
2. Luton JP, Cerdas S, Billaud L, et al. (1990) Clinical features of adrenocortical carcinoma, prognostic factors, and the effect of mitotane therapy. N Engl J Med 322:1195–1201
3. Venkatesh S, Hickey RC, Sellin RV, et al. (1989) Adrenal cortical carcinoma. Cancer 64:765–769
4. Lack EE, Mulvihill JJ, Travis WD, et al. (1992) Adrenal cortical neoplasms in the pediatric and adolescent age group. Clinicopathologic study of 30 cases with emphasis on epidemiological and prognostic factors. Pathol Annu 27(Pt 1):1–53
5. Cagle PT, Hough AJ, Pysher TJ, et al. (1986) Comparison of adrenal cortical tumors in children and adults. Cancer 57:2235–2237
6. Icard P, Chapuis Y, Andreassian B, et al. (1992) Adrenocortical carcinoma in surgically treated patients: a retrospective study on 156 cases by the French Association of Endocrine Surgery. Surgery 112:972–979; discussion 979–980
7. Motzer RJ, Sheinfeld J, Mazumdar M, et al. (2000) Paclitaxel, ifosfamide, and cisplatin second-line therapy for patients with relapsed testicular germ cell cancer. J Clin Oncol 18:2413–2418
8. Childs R, Chernoff A, Contentin N, et al. (2000) Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. N Engl J Med 343:750–758
9. Pedrazzoli P, Da Prada GA, Giorgiani G, et al. (2002) Allogeneic blood stem cell transplantation after a reduced-intensity, preparative regimen: a pilot study in patients with refractory malignancies. Cancer 94:2409–2415
10. Koscielniak E, Gross-Wieltsch U, Treuner J, et al. (2005) Graft-versus-Ewing sarcoma effect and long-term remission induced by haploidentical stem-cell transplantation in a patient with relapse of metastatic disease. J Clin Oncol 23:242–244
11. Kami M, Makimoto A, Heike Y, et al. (2004) Reduced-intensity hematopoietic stem cell transplantation (RIST) for solid malignancies. Jpn J Clin Oncol 34:707–716

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Nutritional Support for Patients Suffering From Intestinal Graft-versus-Host Disease After Allogeneic Hematopoietic Stem Cell Transplantation

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Background: Patients who exhibit gastrointestinal (GI) involvement due to graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (SCT) are often recommended to withhold oral intake (NPO) to avoid further damage to the GI mucosa. However, it is possible that continuing oral intake could be beneficial in many patients compared to total parenteral nutrition (TPN).

Objective: The primary objective of this prospective study was to evaluate whether programmed step-ladder oral dieting (enteral nutrition; EN) is feasible and beneficial for these patients.

Methods: A total of 18 patients who exhibited GI-acute GVHD (stage I to III gut GVHD) after SCT received an EN dieting program, and changes in clinical and laboratory parameters were compared to those in a control cohort of 17 patients who were placed on NPO with TPN. Patients with GVHD were included prospectively and those with intestinal bleeding/obstruction, severe pancreatitis, and cytomegalovirus enterocolitis were excluded.

Results: None of the patients in the EN group experienced significant adverse events, including exacerbation of GI symptoms. Although there was no statistically significant difference in the volume or frequency of diarrhea or the time to complete dietary recovery, parameters including body weight and serum levels of total protein and albumin tended to improve faster in the EN group.

Conclusion: The EN diet is safely applicable to patients suffering from GI involvement by GVHD. Am. J. Hematol. 81:747–752, 2006. © 2006 Wiley-Liss, Inc.

Key words: graft-versus-host disease (GVHD); enteral nutrition; immunonutrition

INTRODUCTION

Graft-versus-host disease (GVHD) is a major complication of allogeneic hematopoietic stem cell transplantation (SCT) that influences the ultimate prognosis of patients [1]. Gut involvement due to GVHD particularly impairs the host nutritional status and QOL due to long-lasting diarrhea and anorexia. Hence, effective supportive care of patients suffering from GVHD should include attention to intense nutritional support and bone mineral retention, since many receive concomitant steroid therapy. Additionally, normal intestinal architecture and functions are required to prevent biliary stasis, retarded bowel movement, bacterial translocation, and resultant systemic infection [2,3]. With the development of gut GVHD, patients are often recommended to withhold oral intake (NPO, "bowel rest") to avoid further damage to the gastrointestinal (GI) mucosa. However, this raises a serious concern since NPO care can induce atrophic deficit of the GI mucosa and resultant dysfunction

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TABLE I. Grade of Programmed EN Dieting

Step	Staple food (form of rice)	Side dishes (approved foods and cuisines)	Nutritive value
0	Liquid	Juice (without grain, without oranges), electrolytic supplement solution	500–2000 ml
1	Liquid	Water gruel, starch gruel, clear soup, consomme, juice, miso soup	Calories 300–350 kcal Protein 5–7 g Fat 1.5–2 g Dietary fiber 1.5 g
2	Mush	Potato, vegetables, canned fruits, vegetable juices, noodles, tofu, whitefish	Calories 600–650 kcal Protein 20–25 g Fat 5–8 g Dietary fiber 1.5–8 g
3	Rice gruel	Eggs, breads, banana, apple	Calories 900–1000 kcal Protein 30–35 g Fat 10–13 g Dietary fiber 8–9 g
4	Boiled rice	Blue-skinned fish, oil (~3 g/day)	Calories 1200–1300 kcal Protein 40–45 g Fat 15–20 g Dietary fiber 9–10 g
5	Boiled rice	Chicken (low fat), yogurt, oil (~8 g/day)	Calories 1500–1600 kcal Protein 60–65 g Fat 30–35 g Dietary fiber 12–13 g

Note: A patient-oriented stepped-up dieting program was gradually applied over six steps that varied with regard to the solidity, intensity, and acceptability by the patient

of the GI system. Moreover, it has recently been reported that enteral nutrition (EN) was more effective than parenteral nutrition for the nutritional support of patients with an injured intestine due to trauma or an invasive operation [4,5]. Taken together, these findings suggest that the current patient management procedure that includes the interruption of oral feeding to enforce "bowel rest" in SCT patients suffering from GVHD should be critically reevaluated. Furthermore, EN, if tolerable, may be a preferred route for maintaining digestive and absorptive function as intact as possible.

In those suffering from GI involvement of GVHD, such evaluation becomes more complex since diarrhea is very often multifactorial and includes secretory dysfunction, osmotic factors, and rapid passage. Hence, the establishment of a standard care procedure remains very difficult. To address these concerns, we conducted a controlled cohort study to evaluate the benefit of different nutritional support measures for patients suffering from acute gut GVHD after SCT. Our clinical hypothesis was that a programmed and controlled scheduled oral nutritional support with EN is beneficial for patients who have mild to moderately progressing acute symptoms of gut GVHD.

PATIENTS AND METHODS

Patients

Seventy patients who were treated at the National Cancer Center Hospital from January 2001 to December 2003 and who developed GI symptoms by GVHD were involved in this prospective study. Forty among those eligible patients met the following inclusion criteria: (i) pathologically diagnosed GVHD with biopsied specimens, (ii) presented symptoms within 100 days after SCT, and (iii) clinically diagnosed as stage I to III gut GVHD and grade II to III acute GVHD according to the clinical grading criteria [6,7]. Patients who had intestinal tract bleeding, intestinal obstruction, or severe pancreatitis were excluded from this analysis, since these pathophysiologies are considered contraindications for EN. Additionally, patients with pathologically diagnosed cytomegalovirus enterocolitis were also excluded, and thus a total of 35 patients were left for this study.

Methods

In the study periods, two different nutritional intervention procedures were applied; patients who developed gut GVHD before July 2002 ($n = 17$) were treated with NPO and total parenteral nutrition (TPN) (C group), while the remaining patients who developed gut GVHD after July 2002 ($n = 18$) were treated by programmed GVHD dieting intervention (EN group). The patients were consecutively registered to our database at National Cancer Center Hospital, and this prospective study was approved by the IRB. The programmed EN dieting consisted of six steps with regard to solidity, intensity, and acceptability for intestinal digestion, as shown in Table I. Each food and nutrient was made more solid and dense

in a step-up manner, after the confirmation of stable symptoms that lasted for a minimum of 3 days. Each step of programmed EN dieting was suitably stepped down when intolerance or exacerbation of gut GVHD symptoms developed. Patients were made NPO with the appearance of significant abdominal symptoms (nausea, vomiting, and abdominal pain). Patients in the EN group only received oral intake without enteral tube feeding. On the other hand, the patients in group C were adequately allowed to eat according to their symptoms with TPN.

We evaluated "time to complete dietary recovery," which was defined as the duration from the start of nutritional management (stopping oral intake or start of programmed EN dieting) to the restoration of a normal diet with the recovery of nutritional parameters. Nutritional parameters evaluated in this study included (1) clinical symptoms, including volume and frequency of diarrhea, and body weight and (2) laboratory data, including total serum protein and albumin. Body mass index (BMI) was calculated as BMI = {height (m)}2/body wt (kg).

Statistical Analysis

Our clinical hypothesis was that a programmed and controlled schedule of nutritional support with oral intake (EN dieting) could be effective in the support of patients suffering from acute gut GVHD with mild to moderately progressing symptoms. We evaluated "the time to complete dietary recovery," which was defined as the duration from the start of nutritional management (stopping oral intake or start of EN dieting) to the recovery to normal diet, various enteral symptoms, and nutritional parameters. The time to complete dietary recovery is shown with a time–event cumulative curve, and the log-rank test was used to compare groups C and EN. Nutritional parameters are given as the mean of each group by time course, and the data in groups C and EN were compared by an analysis of variance (ANOVA). A P value of less than 0.05 was considered significant.

RESULTS

Patients' Characteristics

The patients' clinical backgrounds are summarized in Table II, which shows that there are no essential differences between groups C and EN. Older patients tended to receive a reduced-intensity regimen more often than a conventional regimen.

Safety of Programmed EN Dieting

Throughout the study, no severe adverse events associated with nutritional intervention were observed, indicating that our procedure with gradual stepped-up or -down dieting was safe. No severe infectious episodes were observed in each group. EN dieting had to be terminated early in 2 of 18 cases due to prolonged GI symptoms and exacerbation of an underlying malignant disorder. There were 4 censored cases in group C, mainly due to recurrence of the basic malignant disorder.

TABLE II. Patients' Characteristics

	EN group (N = 18)	C group (N = 17)
Age median (range)	53 (22–64)	53 (23–69)
Sex male:female	12:6	14:3
Disease		
AML	6	8
MDS	3	2
ALL	4	2
CML	3	1
NHL	1	2
ATL	1	1
Solid tumors	0	1
Transplantation source		
BM	1	3
PBSC	17	14
Transplantation regimen		
Conventional	5	7
Reduced intensity	13	10
Donor HLA typing		
Full match	14	14
1 locus mismatch	4	0
2 loci mismatch	0	3
GVHD prophylaxis		
CSP alone	10	8
CSP + MTX	6	4
CSP + ATG	2	2
Others	0	3
Gut GVHD stage		
1	5	9
2	7	3
3	6	5
GVHD grade		
II	6	8
III	12	9
Onset day of gut GVHD (mean of day)	74	68

Note: Patients who underwent SCT and developed gut GVHD were enrolled in this study. Patients who developed gut GVHD before July 2002 (n = 17) were treated with no oral intake (C group), while the EN group (n = 18) was treated by programmed GVHD dieting. AML, acute myelogeneous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CML, chronic myelogeneous leukemia; NHL, non-Hodgkin lymphoma; ATL, adult T-cell leukemia; BM, bonemarrow; PBSC, peripheral blood stem cell; CSP, cyclosporine; MTX, methotrexate; ATG, anti-thymocyte globulin.

Efficacy of Programmed EN Dieting

Although there was a wide variation in each patient in diarrhea volume and frequency of diarrhea, we adapted ANOVA to evaluate whether there is a statistically significant difference between the two groups

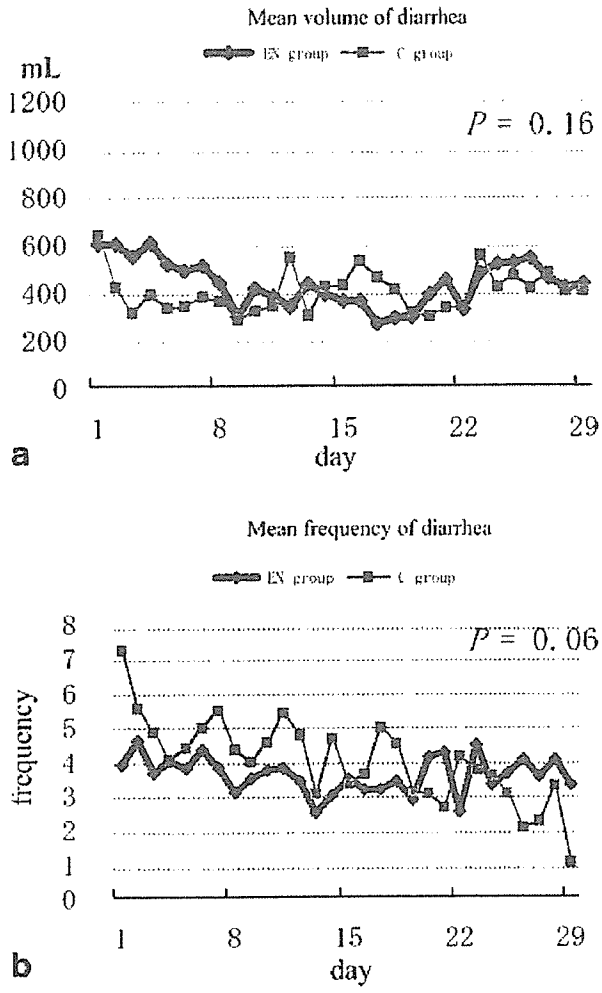

Fig. 1. Changes in mean volume and frequency of diarrhea. No difference was observed between the C and EN groups in the time-course of diarrhea as evaluated by volume ($P = 0.16$) (a) and frequency ($P = 0.06$) (b).

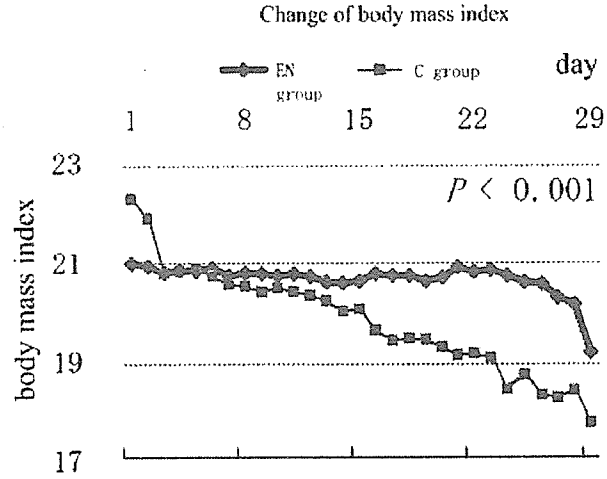

Fig. 2. Changes in BMI. The mean changes in BMI, with the first evaluation as a control, were compared between the two groups. A slower decrease in body weight tended to be observed in the EN group, while patients retained their BMI significantly better in the EN group than in the C group ($P < 0.001$). BMI was calculated as BMI = {height (m)}^{2}/body wt (kg).

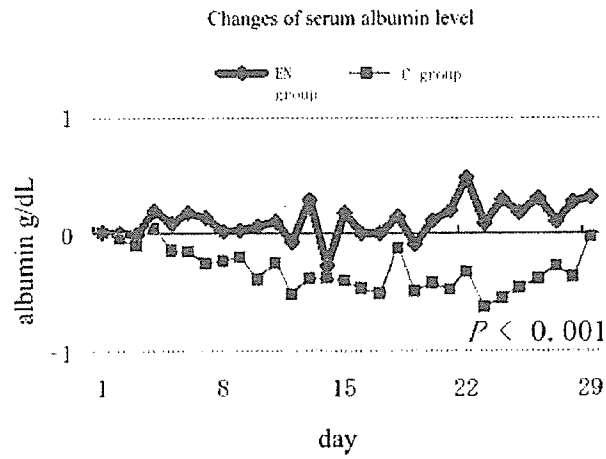

Fig. 3. Changes in albumin as nutritional parameter. One of the nutritional parameters, albumin (Alb), was evaluated between the C and EN groups. In the EN group, patients maintained significantly more stable levels of Alb ($P < 0.001$).

($P = 0.16$ and 0.06, respectively. Figure 1a and b). The mean body weight values in each group were compared by considering the absolute changes after adjusting by the value at the initial evaluation. In comparing the two groups, the decrease in body weight after the start of nutritional management was more obvious in group C than in group EN but this difference was not statistically significant ($P = 0.09$), since there was a wide interpatient variation. On the other hand, the change in BMI was significantly different between the two groups (Figure 2, $P < 0.001$).

Nutritional status was also estimated by laboratory parameters, including serum levels of total protein and albumin (Alb), which were determined as absolute changes by adjusting by the value at the first evaluation at the starting point of nutritional management, and a significantly slower decrease was noted in the EN group ($P < 0.001$) (Figure 3). These nutritional parameters remained higher in group EN than in group C. During the study period, no patient actually met with stopping rules mentioned above and consequently, the total number of days for NPO was not evaluated. The time to complete dietary recovery was compared between the two groups. While 38 days were required for the

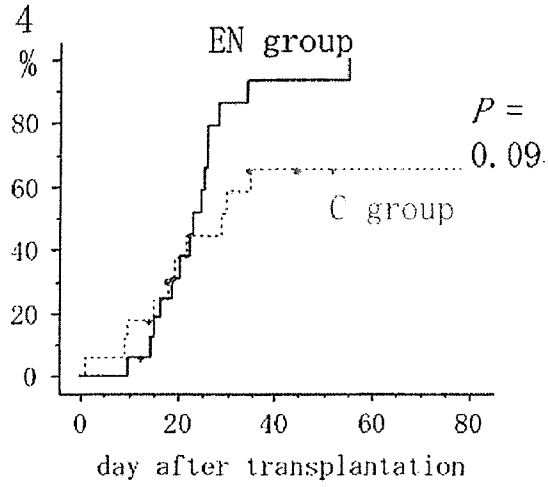

Fig. 4. Time to complete dietary recovery. The number of days required for return to a normal diet was 38 days in group C, while it was 31 days in group EN, with no statistically significant difference ($P = 0.09$).

recovery to a normal diet in group C. 31 days were required in group EN (Figure 4).

DISCUSSION

Since Weisdorf et al. reported that central venous parenteral nutritional support improved long-term survival in patients who underwent bone marrow transplantation (BMT) [8], intravenous TPN has been widely used in SCT. However, it has not yet been confirmed which procedure, enteral or parenteral nutrition, can provide more effective and safer nutritional support. In this study, we considered that the patients in the EN group may have preserved nutritional parameters better than the other group and ate sooner, although no differences were found in the time to complete dietary recovery. A clinical study group at Johns Hopkins University randomized BMT patients into two groups to receive different types of nutritional support, TPN or EN, and they did not observe any differences in nutritional parameters between the two groups [9]. In their study, patients who received TPN were allowed to eat anything they liked, while those with EN had few chances to receive TPN treatment. Moreover, those who had been receiving TPN were allowed to take oral intake and thus were not on strict NPO. Additionally, in our study, the two groups of patients were evaluated in different study periods, and there was a significant difference in the modality of the supportive measures. These points make a direct and strict comparison between the TPN and EN groups very difficult and unreliable. These biases, which are inherent to studies in this field, also existed in our study, which might explain why we failed to detect significant differences in clinical benefits.

We used to routinely advise patients to stop oral intake with the development of gut GVHD. Thereafter, they were encouraged to drink or eat gradually, since it has been suggested that inadequate nutritional support further deteriorates gut GVHD symptoms. To establish clearly defined subjective guidelines, we conducted this interventional cohort study. We found that both controlled and uncontrolled EN can be administered safely. Since the time to complete dietary recovery was almost comparable in the two groups, the results suggest that any EN program is acceptable and does not harm or degrade the QOL of patients suffering from GVHD. If this is confirmed, a restricted diet would not be necessary for those with moderately symptomatic gut GVHD. Nevertheless, the evaluation of nutritional parameters in this study suggested that controlled EN did a better job of maintaining body weight and serum nutritional status, compared to the results in the NPO group. The random administration of food intake may be inadequate compared to scheduled dieting, which attempts a gradual build-up of intestinal mucosa by the comprehensive supply of nutrients including glucose, protein, fat, fiber, etc. This may have a secondary advantage of keeping the mucosal barrier intact and preventing bacterial translocation through the GI tract.

Nevertheless, since the cause of diarrhea is multifactorial, it is inherently difficult to assess the effectiveness of and standardize nutritional intervention procedures. In the literature, four pathologies have been reported to be contraindications for EN since they cause undesirable bowel movement, i.e., presence of gastrointestinal bleeding, intestinal obstruction, severe pancreatitis, and intestinal perforation. The pathophysiology of diarrhea associated with gut GVHD includes osmotic and secretory diarrhea. Hypertonic EN is considered to further deteriorate symptoms of diarrhea. Hence, it is reasonable to suggest that dietary foods in EN adequately maintain an isotonic status as well as nutritional status to improve immunologic function. An intact GI system is vital for maintaining normal immune functions, and a novel concept of nutrition support, "immunonutrition," has been introduced, which focuses on the maintenance of the comprehensive biological protection system against external pathogens to maintain normal immune function [10]. Clinical benefits of immunonutrition, including improvement of nutritional parameters, decreased risk of infection, and shorter duration of hospitalization, have been reported in patients in the perioperation period and in those who required care in the ICU [11,12]. However, currently a precise evaluation

of the efficacy of each component of immunonutritional agents is difficult [13], and controversy still exists regarding the value of immunonutrition after SCT. This study did not evaluate this proposed immunonutrition, and to accomplish this in SCT practice, prospective monitoring of immune parameters would be required.

The serum level of albumin can be significantly affected by many variables including diarrhea associated with GVHD and, hence, would not be a very good marker for the evaluation of protein status in the HSCT population. However, in our experience, serum albumin decreased after SCT to suggest the possibility of the use in the estimation of patient's nutrition status at least for a short period of follow-up, when referring to the general description in the guideline by American Society for Parenteral and Enteral Nutrition, i.e., "low serum levels indicate which hospitalized patients are at increased risk of morbidity and mortality" [14].

In conclusion, the current study is hampered by preexisting biases including a small number of studied patients, a cohort analysis in different periods, and a lack of adequate measures for data evaluation. Nevertheless, it appears that patients supported by programmed EN experienced no exacerbation of gut GVHD symptoms, with a suggested benefit of enhanced maintenance of nutrition status. Further study is warranted to prospectively evaluate the value of various nutrients including arginine, o-3 fatty acid, and nucleic acid [13] and various clinical outcomes including the cost, complications, and QOL in an attempt to improve the nutritional and immune status of transplanted patients.

REFERENCES

1. Antin JH. Clinical practice. Long-term care after hematopoietic-cell transplantation in adults. N Engl J Med 2002;347:36–42.
2. Welsh FK, Farmery SM, MacLennan K, et al. Gut barrier function in malnourished patients. Gut 1998;42:396–401.
3. Weisdorf SS, Schwarzenborg SJ. Hematopoietic cell transplantation. 2nd ed. Nutritional support of hematopoietic stem cell recipients. Oxford: Blackwell; 1999. p 723.
4. Moore FA, Moore EE, Jones TN, et al. TEN versus TPN following major abdominal trauma—reduced septic morbidity. J Trauma 1989;29:916–922; discussion 922–923.
5. Archer SB, Burnett RJ, Fischer JE. Current uses and abuses of total parenteral nutrition. Adv Surg 1996;26:165–189.
6. Thomas ED, Storb R, Clift RA, et al. Bone-marrow transplantation (second of two parts). N Engl J Med 1975;292:895–902.
7. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant 1995;15:825–828.
8. Weisdorf SA, Lysne J, Wind D, et al. Positive effect of prophylactic total parenteral nutrition on long-term outcome of bone marrow transplantation. Transplantation 1987;43:833–838.
9. Szeluga DJ, Stuart RK, Brookmeyer R, et al. Nutritional support of bone marrow transplant recipients: a prospective, randomized clinical trial comparing total parenteral nutrition to an enteral feeding program. Cancer Res 1987;47:3309–3316.
10. Suchner U, Kuhn KS, Furst P. The scientific basis of immunonutrition. Proc Nutr Soc 2000;59:553–563.
11. Lipman TO. Grains or veins: is enteral nutrition really better than parenteral nutrition? A look at the evidence. J Parenter Enter Nutr 1998;22:167–168.
12. Heyland DK, Novak F, Drover JW, et al. Should immunonutrition become routine in critically ill patients? A systematic review of the evidence. J Am Med Assoc 2001;286:944–953.
13. Gianotti L, Braga M, Fortis C, et al. A prospective, randomized clinical trial on perioperative feeding with an arginine-, omega-3 fatty acid-, and RNA-enriched enteral diet: effect on host response and nutritional status. J Parenter Enter Nutr 1999;23:314–320.
14. Guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients: A.S.P.E.N. Board of directors. J Parenter Enter Nutr 1993;17:5SA–6SA.

BRIEF REPORT
Ewing Sarcoma/Primitive Neuroectodermal Tumor of the Kidney in a Child

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A 6-year-old female was admitted with abdominal pain and a mass in the right abdomen. Her lactose dehydrogenase level was 1,200 IU/L, and neuron specific enolase was 120 ng/ml. Computed tomography scan confirmed a large right renal mass with necrosis. A right radical nephrectomy was performed. The tumor was completely encapsulated. Based on small round cell histology, strong MIC-2 (CD99) positive tumor cells, and EWS-FLI-1 fusion transcript, Ewing sarcoma/primitive neuroectodermal tumor of the kidney was diagnosed. Induction and follow-up with seven cycles of chemotherapy were given after surgery. She has had no evidence of recurrence 90 months from diagnosis. Pediatr Blood Cancer

Key words: electron microscopy; Ewing sarcoma/primitive neuroectodermal tumor; EWS-FLI-1; immunohistochemistry; kidney

INTRODUCTION

Ewing sarcoma/primitive neuroectodermal tumor (ES/PNET) of the kidney is a rare and highly malignant neoplasm. It affects young adults, and only a few pediatric cases (younger than 15 years) have been reported [1–9]. ES/PNET arising in the kidney act aggressively and show poor response to therapy [1]. ES/PNET of the kidney needs to be differentiated from other small round cell tumors of the kidney, because each type of tumor is treated differently. The diagnosis of this neoplasm is currently based on a combination of light microscopy, immunohistochemistry, electron microscopy, chromosomal analyses, and specific chimeric transcripts. Our patient, who was diagnosed by histochemistry and molecular biology analysis of the resected kidney and treated with chemotherapy, has remained alive more than 90 months after diagnosis.

CASE

A 6-year-old female was admitted to our hospital with abdominal pain and an abdominal mass. On physical examination, a large and firm mass was evident in the right abdomen. Laboratory evaluation showed a lactate dehydrogenase level of 1,200 IU/L (normal 218–411 IU/L), a neuron specific enolase level of 120 ng/ml (normal <10 ng/ml), and ferritin level of 160 ng/ml (normal 15–89 ng/ml). Urine catecholamine levels were within normal limits. Abdominal computed tomography (CT) scan confirmed a large right renal mass with areas of necrosis and bleeding. There was no obvious lymphadenopathy and no intra-abdominal metastasis. Bone scintigraphy and CT scan of the thorax did not detect metastasis.

A right radical nephrectomy was performed. The tumor involved a large portion of the lower part of the kidney. The tumor was completely encapsulated and was $5.0 \times 4.5 \times 4.5$ cm. Lymph nodes were negative for malignancy. Histologic examination revealed a small round cell tumor with massive necrosis, but no rosette formations. Periodic acid-Schiff (PAS) staining revealed diastase sensitive material in the tumor cell cytoplasm. Immunohistochemistry revealed that tumor cells were strongly positive for MIC-2 (CD99) as well as vimentin. The tumor cells were negative for chromogranin A, neurofilament, and synaptophysin. Electron microscopic examination showed a high nuclear-cytoplasm ratio and aggregated glycogen granules in the cytoplasm (Fig. 1A). A higher magnification of tumor cells showed neurosecretory-type granules, microtubules, and desmosome-like structures (Fig. 1B). The expression of EWS-FLI-1 fusion transcript was demonstrated by molecular biology (Fig. 2). A single 330 base pair cDNA product was detected by ethidium bromide staining, corresponding to the EWS-FLI-1 as previously reported by Sorensen et al. [10]. Direct DNA sequencing confirmed the presence of a fusion of EWS exon 7 to the FLI-1 exon 6. Unfortunately chromosomal findings failed because proliferation of the tumor cells was poor. According to results on small round cell histology and immunohistochemical profiles, electron microscopic findings, and EWS-FLI-1 fusion transcript, the tumor was diagnosed as an ES/PNET of the kidney. Therapy was initiated with 1.5 gm/m^2 vincristine on days 1, 8, 15, 22, 29, and 36; 500 mg/m^2 cyclophosphamide on days 2, 9, 30, and 37; and 0.45 mg/m^2 dactinomycin on days 16–20 for induction and then a total of seven cycles of 4-drug chemotherapy, consisting of 1.5 gm/m^2 vincristine on days 1, 15, 22, 29, 36, and 43; 0.45 mg/m^2 dactinomycin on days

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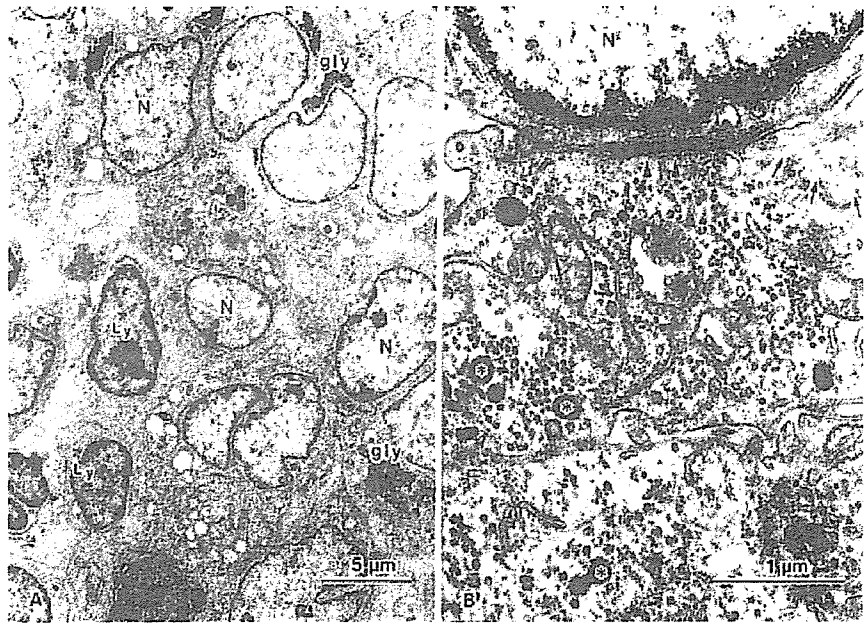

Fig. 1. Ultrastructural findings in the tumor cells. **A**: Tumor cells are oval and small (about 8–10 μm in a diameter). Nuclear-cytoplasm ratio is high. Nucleus has a few heterochromatin. Aggregated glycogen granules (gly) are observed in the cytoplasm. Ly, lymphocytes; N, nuclei. **B**: Neurosecretory granules (asterisks), microtubules (arrows), and desmosome-like structures (arrowheads) are observed in the tumor cells under higher magnification.

1–5; 500 mg/m^2 cyclophosphamide on days 16, 23, 30, 37, and 44; and 60 mg/m^2 doxorubicin on day 44 after surgery. She had no serious adverse effects during chemotherapy. She had no evidence of recurrence after 90 months from diagnosis and no late effects have been noted.

DISCUSSION

Though the existence of renal PNET was reported in 1975 in a review of pediatric PNETs [11], only a small number of cases have been reported. Recently, Parham et al. [12] from National Wilms Tumor Study Group Pathology Center reported that 79 of 146 cases of primary malignant neuroepithelial tumors of the kidney in adults and children were considered to be ES/PNET. Follow-up information, however, was only provided for 14 of 146 cases, and it is unclear which, if any, of those were actually ES/PNET [8]. Pediatric cases (younger than 15 years old) of ES/PNET of the kidney are extremely rare, and only ten cases have been reported previously [1–9]. Clinical characteristics, pathologic features, treatments, and outcomes of those cases are summarized in Table I.

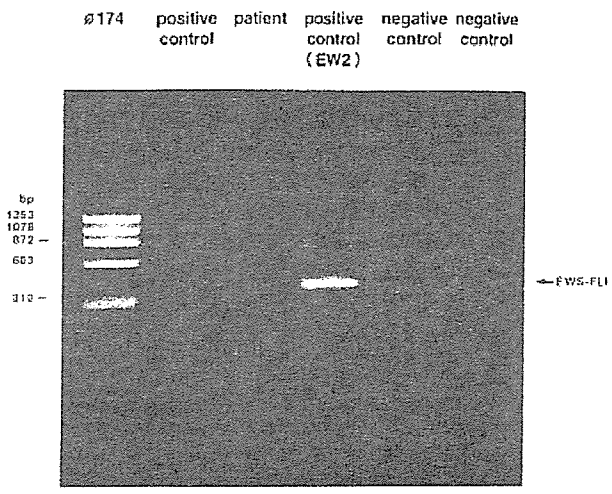

Fig. 2. A single 330 base pair transcript is detected in the patient sample following reserve transcriptase polymerase chain reactor (RT-PCR) performed on RNA extract from tumor tissue.

Several approaches can be used to arrive at a diagnosis of ES/PNET. The first approach is light microscopic examination of tumor tissue including immunohistochemistry. These tumors consist of primitive-appearing round cells with high nucleus to cytoplasmic ratios. The immunohistochemical features of ES/PNET are positive for CD99 (MIC2); however, expression of CD99 is by no means specific for ES/PNET among round cell tumors [13]. Although FLI-1 is a variable histochemical marker for ES/PNET, it is also positive in lymphoblastic lymphoma [14]. In contrast, WT-1 is a positive marker of Wilms tumor and desmoplastic round cell tumors, whereas it is a negative marker for ES/PNET, neuroblastoma and rhabdomyosarcoma. The second approach is electron microscopic examination of tumor tissue. Electron microscopic features include a specific high nuclear-cytoplasm ratio and aggregated glycogen granules in the cytoplasm. Neural differentiation appears on some cells with polar processes, which may contain microtubules or

TABLE I. Clinical and Pathological Features of ES/PNET of the Kidney in Pediatric Cases

Case	Ref.	Age	Gender (yr)	Symptoms	Metastasis	Pathology (immunohistochemistries)	Chimeric transcript	Therapy	Outcome (follow-up [Mo])
1	1	4	F	Abdominal pain, fever	RPLN, liver	CD99(+),NSE(+),S-100(+), Ker(+),Act(−),Vim(−),Chro(−)	NS	IFO, CBP, VP-16 radiation	Died (1)
2	1	14	M	Bone pain, weight loss	Lung, bone, bone marrow	CD99(+),NSE(+),Vim(+),Synapto(+) (±)S-100(−),Ker(−),Act(−),Chro(−)	EWS/FLI-1(−) EWS/ERG(−)	CY, VCR, DOX,IFO,VP-16 auto BMT	Alive (under treatment)
3	2	13	NS	Abdominal pain, hematuria	No	MIC2(+),NSE(+),ker(−),Des(−), Act(−)	EWS/FLI-1(+)	Nephrectomy chemotherapy	NS
4	3	10	M	Abdominal mass	No	MIC2(+),NSE(+),Leu7(+), S-100(−),Ker(−), Des(−),Vim(−),Chro(−)	EWS/FLI-1(+)	Nephrectomy chemotherapy	Alive (6)
5	4	5	F	NS	IVC, right heart	NS	NS		NS
6	5	15	F	Abdominal pain, abdominal distention	No	MIC2(+),Vim(+),NSE(−), S-100(−)	NS	Nephrectomy CY, VCR, DOX,IFO,VP-16	Alive (8)
7	6	9	M	Abdominal pain, abdominal mass, weight loss	No	MIC2(+),NSE(−),Vim(−),Ker(−), LCA(−)	NS	Nephrectomy CY, VCR, DOX,IFO,VP-16	Alive (relapse+) (10)
8	7	9	F	Abdominal distention, abdominal mass	No	CD99(+),LCA(−),Ker(−),Act(−), NFM(−)	EWS/FLI-1(+)	Nephrectomy IFO,VP-16,CY,DOX,VCR auto BMT	Died (5)
9	8	11	M	Gross hematuria, abdominal mass	No	CD99(+)	NS	Nephrectomy VCR, DOX, VP-16, CY, DAC	Alive (64)
10	9	14	F	Abdominal pain, abdominal mass	IVC, right heart, liver	NS	NS	Chemotherapy	Died (24)
11	Present case	6	F	Abdominal pain, abdominal mass	No	MIC2(+),Vim(+)NFM(−),Chrom(−)	EWS/FLI-1(+)	Nephrectomy VCR, DAC, CY, DOX,	Alive (90)

RPLN, retroperitoneal lymphonode; IVS, inferior vena cava; NSE, neuron specific enolase; Ker, keratin; Act, actin; Vim, Vimentin; Chro, chromogrn A; MIC2, B microgloblin; Des, desmin; NFM, neurofibrofilament; Synapto, sinaptophilin; IFO, ifomide; CBD, carboplatinum; CY, cyclophosphamide; VCR, vincristine; DOX, doxrubicine; DAC, actinomycin D; BMT, bone marrow transplantation.

neurosecretory glands [15]. The third approach is chromosomal translocation, such as t(11;22) (q24;q12) which is positive in 88–95% of ES/PNET cases [16]. The final approach involves a molecular biologic examination. In 90–95% of cases of ES/PNET, the chimeric transcript is EWS-FLI-1; the remaining 5–10% are EWS-ERG. Other transcripts, including EWS-ETV1 and EWS-EIAF, have also been reported [16].

In terms of prognosis, the 5-year disease-free survival rate of ES/PNET is 45–55% [17], but the prognosis of ES/PNET of the kidney appears worse [1,18]. In pediatric cases (Table I), 5 of 8 patients were alive when the cases were reported; however, 1 patient (no. 6) was alive with disease, 2 patients (no. 3 and no. 5) were followed-up only for 6 and 8 months, and 1 patient was under treatment (no. 9). The follow-up duration was not described in this case. Only 2 patients (no. 8 and our case) were alive after 5 years. For 2 patients, it was not defined whether they were alive or not (Table I). Jimenez et al. [8] described that 3 of 11 patients were alive for 4–64 months, and 5 patients had local recurrence or distance metastasis then died of their disease, and 3 patients were lost to follow-up. Most of the recent therapeutic protocol for children with ES/PNET consists of vincristine, doxorubicin, cyclophsphamide, ifosphamide, and etoposide. Radiation and surgery have been used; some patients have been treated with myeloablative chemoradiotherapy followed by autologous bone marrow rescue. In spite of a lack of radiation therapy and our not using ifosphamide and etoposide for chemotherapy, our patient has survived for a relatively long period with no recurrence. Possible reasons for this good outcome might include the pathologic features of the tumor, the well-encapsulated nature of the tumor with no involvement beyond the capsule and the accurate diagnosis followed by prompt treatment with chemotherapy. Several approaches including cytogenetical methods are important for early, accurate diagnosis of ES/PNET.

REFERENCES

1. Rodriguez-Galindo C, Marina NM, Fletcher BD, et al. Is primitive neuroectodermal tumor of he kidney a distinct entity? Cancer 1997;79:2243–2250.
2. Quezado M, Benjamin DR, Tsokos M. EWS/FLI-1 fusion transcripts in three peripheral primitive neuroectodermal tumors of the kidney. Hum Pathol 1997;28:767–771.
3. Takeuchi T, Iwasaki H, Ohjma Y, et al. Renal primitive neuroectodermal tumor: A morphologic, cytogenetic, and molecular analysis with the establishment of two cultured cell lines. Diag Mol Pathol 1997;6:309–317.
4. Hasanbegovic E, Terzic R, Sabanovic S, et al. Ewing's soft-tissue sarcoma-case report. Med Arh 1998;52:157–158.
5. Antoneli ABG, Coasta CML, de Camargo B, et al. Primitive neuroectodermal tumor (PNET)/extraosseous Ewing sarcoma of the kidney. Med Ped Oncol 1998;30:303–307.
6. Kuczynski AP, Gugelmin ES, Netto RAS. Primitive neuroectodermal tumor of the kidney in children. J Ped (Rio J) 2001;77:49–51.
7. Vicha A, Stejskalvo E, Sumerauer D, et al. Malignant peripheral primitive neuroectodermal tumor of the kidney. Cancer Genet Cytogenet 2002;139:67–70.
8. Jimenez RE, Folpe AL, Laspham RL, et al. Primitive Ewing's sarcoma/primitive neuroectodermal tumor of the kidney. Am J Surg Pathol 2002;26:320–327.
9. Ng AWH, Lee PSF, Howerd RG. Primitive neuroectodermal kidney tumor. Austral Radiol 2004;48:211–213.
10. Sorensen PHB, Liu XF, Delattre O, et al. Reverse transcriptase PCR amplification of EWS/FLI1 fusion transcripts as a diagnostic test for peripheral primitive neuroectodermal tumors of childhood. Diagn Mol Pathol 1993;2:147–157.
11. Seemayer TA, Thelmo WL, Bolande RP, et al. Peripheral neuroectodermal tumors. Perspect Pediatr Pathol 1975;2:151–172.
12. Parham DM, Roloson GJ, Feely M, et al. Primary malignant neuroepithelial tumors of the kidney. Am J Surg Pathol 2001;25:133–146.
13. Stevenson A, Chatten J, Bertoni F, et al. CD99 (p30/32MIC2) neuroectodermal/Ewing's sarcoma antigen as an immunohistochemical marker. Review of more than 600 tumors and literature experience. Appl Immunohistochemistry 1994;2:231–240.
14. Folpe AL, Hill CE, Parham DM, et al. Immunohistochemical detection of FLI-1 protein expression: A study of 132 round cell tumors with on CD99-positive mimics of Ewing's sarcoma/primitive neuroectodermal tumor. Am J Surg Pathol 2000;24: 1657–1662.
15. Suh CH, Ordonez NG, Hocks J, Mackay B. Ultrastracture of the Ewing's sarcoma family of tumor. Ultrastruct Pathol 2002;26:67–76.
16. Stephenson CF, Bridge JA, Sandberg AA. Cytogenetic and pathologic aspects of Ewing's sarcoma and neuroectodermal tumors. Human Pathol 1992;23:1270–1277.
17. Kushner BH, Hajdu SI, Gulati SC, et al. Extracranial primitive neuroectodermal tumors: The memorial Sloan-Kettering Cancer Center experience. Cancer 1991;67:1825–1829.
18. Benesch M, Urban C. Is primitive neuroectodermal tumor of the kidney a distinct entity? Cancer 1998;82:1414–1415.

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Case Report

Desmoplastic small cell tumor of soft tissue: Molecular variant of *EWS-WT1* chimeric fusion

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A 7-year-old girl was hospitalized because of a tumorous mass in her left periorbital region. The tumor was removed by local excision. The soft-part tumor recurred in the parotid gland region 4 months later, and a second recurrence was noted on the left side of the neck 3 years and 3 months thereafter. The patient had not received chemotherapy or local irradiation. Histological and immunohistochemical examinations of the recurrent masses revealed morphological characteristics of small cell proliferation with desmoplastic stroma that were similar to those of the initial tumor. The cellular components showed immunoreactivity for desmin, cytokeratin, vimentin, and epithelial membrane antigen in part, but the cells were negative for myogenin, CD99, and neuron-specific enolase. These findings suggested a diagnosis of desmoplastic small cell tumor, despite its extra-abdominal location. The histological diagnosis was confirmed by reverse transcriptase polymerase chain reaction, which demonstrated an *EWS-WT1* chimeric fusion gene. An in-frame fusion of *EWS* exon 9 and *WT1* exon 8 was subsequently identified by cloning and sequencing. The chimeric fusion gene might be related to the tissue-specific phenotype of desmoplastic small cell tumors, although further investigation of this speculation is necessary.

Key words: desmoplastic small cell tumor, *EWS-WT1* fusion transcript, extra-abdominal

Desmoplastic small cell tumor (DSCT) occurs usually in the intra-abdominal region of children or adolescents and consists of proliferating small cells with a desmoplastic stroma. DSCT is also characterized by the presence of an *EWS-WT1* chimeric fusion transcript, typically confirmed by reverse transcriptase–polymerase chain reaction (RT-PCR). Tumors with similar histological appearances have been reported in other soft tissues,[1] but such tumors are extremely rare. Here, we describe a patient with a desmoplastic small cell tumor that developed primarily in the orbital soft tissue.

CLINICAL SUMMARY

A 7-year-old girl complained of an insidious swelling in the soft tissue of her left upper eyelid. On hospitalization, a reconstructed CT image revealed a partially calcified, low-density mass in the upper lateral aspect of the left orbital region (Fig. 1). The mass was excised surgically. The patient was not treated with systemic chemotherapy or local irradiation.

Four months after the initial surgery, a local recurrence was detected in the left periorbital region. A CT image revealed an irregular mass, measuring 26 × 20 mm in greatest diameter, with central low-density and calcified foci; the mass was located near the upper aspect of the left parotid gland (Fig. 1). Systemic gallium citrate scintigraphy demonstrated an abnormally localized accumulation at the mass lesion, but no distant metastases were noted. The local tumor was completely re-excised surgically, and the patient was carefully followed thereafter. Chemotherapy or irradiation was not performed.

Three years and 3 months later, at the age of 11 years, the patient was again hospitalized because of a second recurrence in her left lower neck region. CT showed a tumorous lesion located between the left lower margin of the parotid gland and the piriform fossa. The main cervical recurrent tumor and nodal metastases were removed. The tumorous tissue weighed 38.9 g in total. The patient has been followed for 8 months and has shown no signs of local recurrence,

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