

適応拡大にかかわる医師の責務

抗がん剤併用療法に関する検討委員会の活動により、世界的に標準的に使用されている薬剤の適応拡大に関して短期間に大きな進歩があった。しかしながら、このシステムは治験を行わずに効能・効果、用法・用量の承認を行ったものであり、新規に適応拡大された薬剤に関しては、新薬と同様の重点的な安全対策が必要である。厚生労働省医薬食品局安全対策課と日本癌学会、日本癌治療学会、日本臨床腫瘍学会は共同して、全国がんセンター協議会、地域がん診療拠点病院、特定機能病院等において、新規に適応拡大された薬剤を用いた患者の全数把握と副作用調査を義務づけることとなった。臨床試験結果をベースとした公表論文のデータにおいては、必ずしも十分な安全性情報をカバーするだけの症例数は含んでいないため、理論的にこのような方策は必須であると考えられる。

小児がんに対する治療開発は、高い抗がん剤感受性に基づいた「最大限の効果・利益」を目指し、重篤な有害事象の発生をある程度許容した状況で

行われてきた。骨髄抑制やそれに基づく感染症の発生などに対応して、G-CSF製剤や抗生物質などの支持療法に習熟した医師が行うべきであることはもちろん、長期生存を期待できる腫瘍であるがゆえに、ドキシソルビシンによる心毒性、エトポシドによる二次性悪性腫瘍、イホスファミドによる腎毒性など、晩期障害の管理にも十分に配慮する必要がある。この点で、小児がんの専門家が担う責任はますます重くなっており、現状をより安全で効果的な治療開発に臨むための第一段階と認識し、診療と研究を推進すべきことを再度強調したい。

適応外使用や未承認薬の問題は、政府や製薬企業のみの問題ではなく、医師側の継続的な努力によってこそ解決されるものであると自覚し、Evidence-based medicineと臨床試験・治験を含めた科学的・倫理的な医療の遂行によって、真に患者のためになる治療の開発と医学の発展に寄与していくべきである。

【謝辞】

稿を終えるにあたり、小児がん分野の適応外使用問題の解決にご協力いただいた牧本班作業部会適応拡大検討小委員会の皆様、国立成育医療センターの中村秀文先生、土田尚先生に深謝いたします。

文 献

- 1) 「医の倫理の国際綱領」日本医師会ホームページ (<http://www.med.or.jp/wma/ethics.html>)
- 2) 「処方と医師の裁量」兵庫県整形外科医会ホームページ (<http://hcoa.jp/medical/ho/hashimoto.html>)
- 3) 大西鐘壽：小児医療とオフラベル問題—小児科医が何をしなければならないか。小児内科, 34 : 402-411, 2002
- 4) Bleyer WA : The U.S. pediatric cancer clinical trials programmes: international implications and the way forward. Eur J Cancer, 33 : 1439-1447, 1997
- 5) 健康政策局研究開発振興課長・医薬安全局審査管

- 理課長：適応外使用に係る医療用医薬品の取扱いについて、平成11年2月1日研第4号・医薬審第104号
- 6) 藤原康弘：2000年に承認された新薬と外国臨床データの利用について—適応外使用とブリッジング試験を巡って。医薬品研究, 32(10) : 639-651, 2001
 - 7) 「適応外使用通知に基づく承認について」厚生労働省ホームページ (<http://www.mhlw.go.jp/shingi/2005/01/dl/s0124-9h2.pdf>)
 - 8) 「抗がん剤併用療法に関する報告書について」厚生労働省ホームページ (<http://www.mhlw.go.jp/shingi/2004/05/s0521-5.html>)

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

Osamu Imataki,¹ Atsushi Makimoto,^{1*} Shingo Kato,² Takahiro Bannai,³ Naomi Numa,⁴ Yoko Nukui,⁵ Yuji Morisawa,⁶ Toshihiko Ishida,⁷ Masahiro Kami,¹ Takahiro Fukuda,¹ Shin-ichiro Mori,¹ Ryuji Tanosaki,¹ and Yoichi Takae¹

¹ Hematopoietic Stem Cell Transplantation Unit, National Cancer Center Hospital, Tokyo, Japan

² Department of Microbiology and Immunology Division, Keio University School of Medicine, Tokyo, Japan

³ Laboratory of Microbiology, National Cancer Center Hospital, Tokyo, Japan

⁴ Nursing Division, National Cancer Center Hospital, Tokyo, Japan

⁵ Department of Infectious Diseases, University of Tokyo Hospital, Tokyo, Japan

⁶ Department of Infection Control and Prevention, Jichi Medical School, Tochigi, Japan

⁷ First Department of Internal Medicine, Kagawa Medical University Hospital, Kagawa, Japan

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common nosocomial pathogens among hospital-acquired infections, and immunocompromised patients are highly susceptible 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. *Am. J. Hematol.* 81:664-669, 2006 © 2006 Wiley-Liss, Inc.

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

*Correspondence to: Atsushi Makimoto, Division of Pediatrics, National Cancer Center Hospital, 1-1 Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan
E-mail: amakimoto@ncc.go.jp

Received for publication 12 September 2005; Accepted 24 February 2006

Published online 22 June 2006 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.20668

© 2006 Wiley-Liss, Inc.

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 myelogenous 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 identifi-

cation 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 (UNP 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 *Sma*I 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

Case number # 1 2 3 4 5 6 7 8 9 10 11 12 13

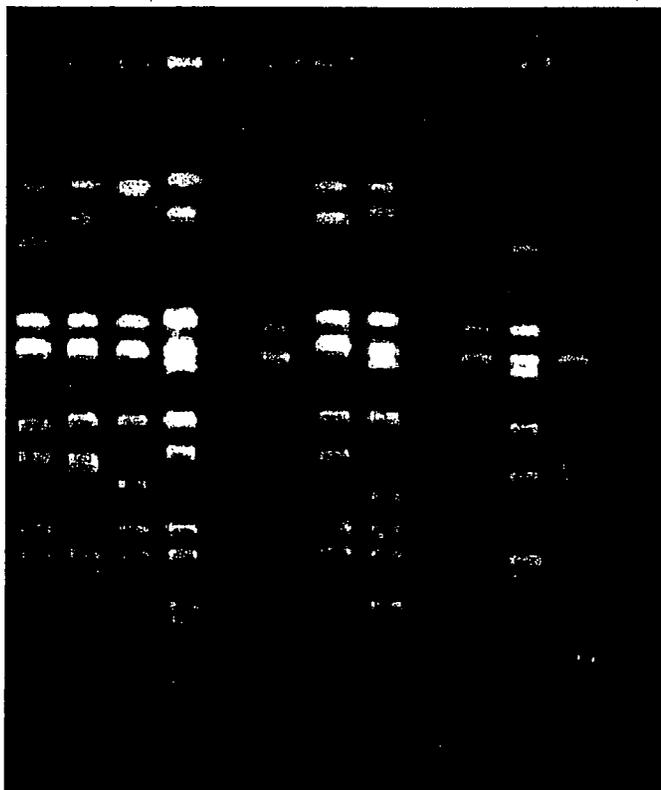


Fig. 3. PFGE analysis of isolated MRSA in the SCT unit. The PFGE pattern showed that there were no detectable differences in bands between cases 3 and 6, but more than two bands were identified in the other four cases (cases 1, 2, 4 and 5). Additionally, two strains that had been previously isolated 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-

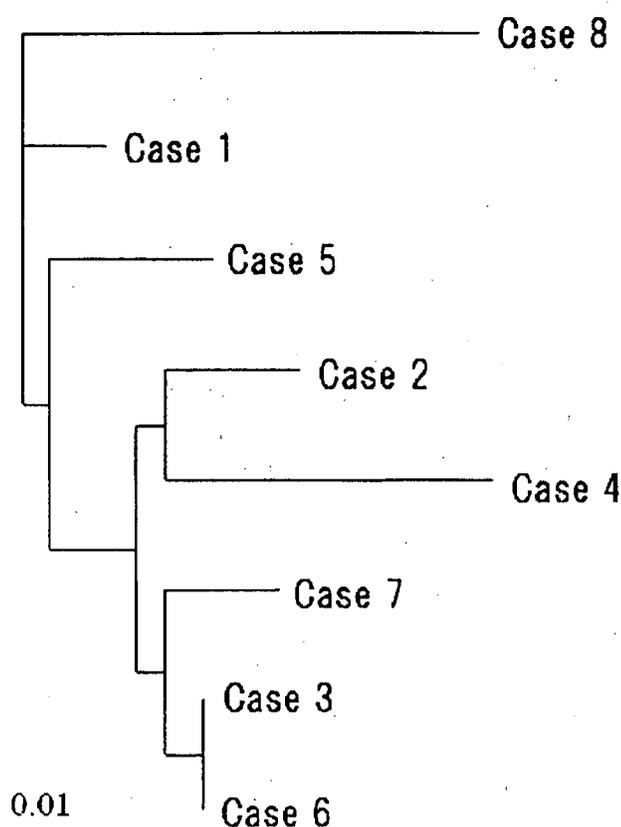


Fig. 4. AFLP pattern of isolated MRSA. AFLP analysis was performed for the same six isolates (case 1 to 6) described in Figure 4. Gene polymorphism showed same result of similarity as PFGE pattern had already indicated, i.e., cases 3 and 6 had the same polymorphism pattern and the others were different 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.

American Journal of Hematology DOI 10.1002-ajh

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

- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- Shopsin B, Kreiswirth BN. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus*. *Emerg Infect Dis* 2001;7:323-326.
- Vos P, Hogers R, Blecker M, Reijans M, van de Lee T, et al. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res* 1995;23:4407-4414.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- Peacock JE, Jr., Marsik EJ, Wenzel RP. Methicillin-resistant *Staphylococcus aureus*: introduction and spread within a hospital. *Ann Intern Med* 1980;93:526-532.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.

CASE REPORT

Osamu Imataki · Atsushi Makimoto · Rie Kojima
Michiyo Sakiyama · Ako Hosono · Yoichi Takaue

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

Received: August 8, 2005 / Accepted: December 15, 2005

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)

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-fourth 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).

O. Imataki · A. Makimoto (✉) · R. Kojima · M. Sakiyama · A. Hosono · Y. Takaue
Hematopoietic Stem Cell Transplantation Unit and Pediatric Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
Tel. +81-3-3542-2511; Fax +81-3-3542-3815
e-mail: amakimot@ncc.go.jp

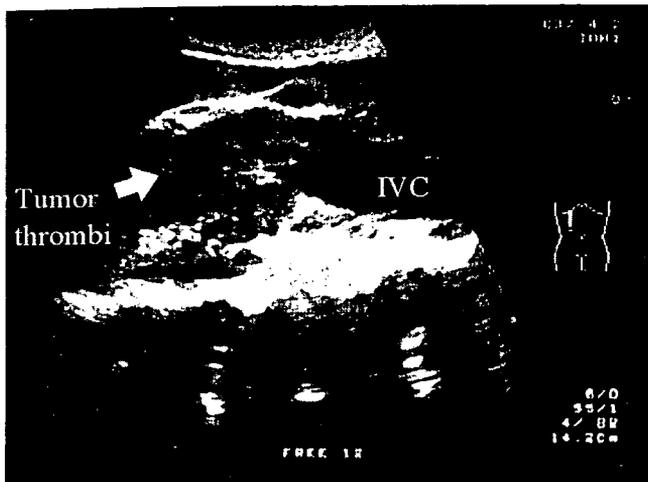


Fig 1. Abdominal echography at diagnosis showing a large right-side adrenal tumor with intravascular extension and massive liver metastasis. IVC, inferior vena cava

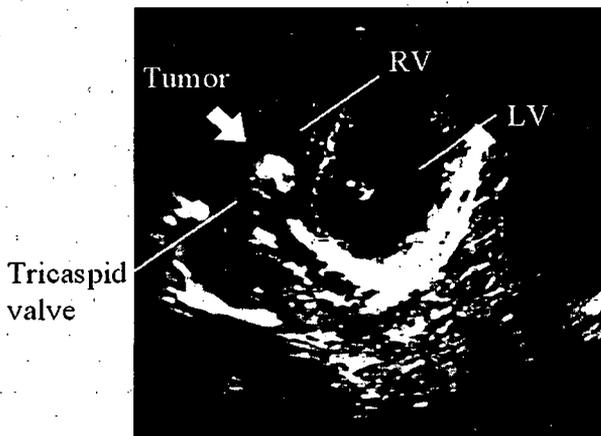


Fig 2. Cardiac echography showing a polypoid lesion in the right ventricle through the tricuspid valve during the diastolic phase. RV, right ventricular; LV, left ventricular

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/m², day 1), ifosfamide (IFM, 1200 mg/m², days 2–6), and cisplatin (CDDP, 20 mg/m², 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/m² for 6 days), busulfan (4 mg/kg/day for 2 days) and antithymocyte globulin (25 mg/m² 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/m² on day 1, and 7 mg/m² 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 disproportionately located in the lesser curvature, which ap-

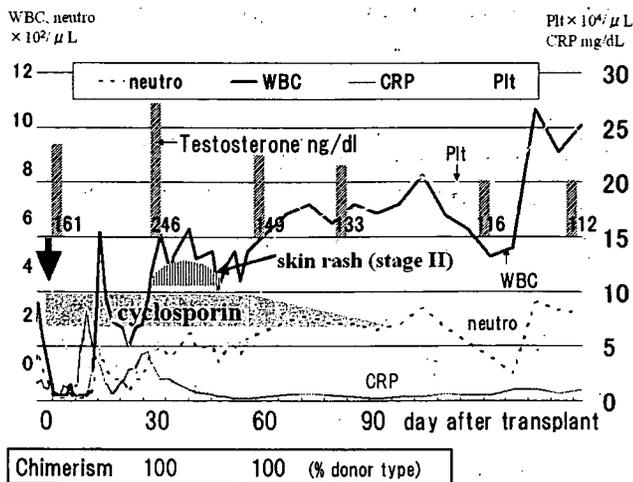


Fig 3. Clinical course of transplantation. The serum level of testosterone (normal range 10–60 ng/dl) as a tumor marker continued to decrease after the occurrence of acute graft-versus-host disease. White blood cell count (*WBC*) and platelets (*plt*) slowly increased after hematopoietic engraftment, 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 in peritonitis 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 histology⁴ 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 consoli-

ation 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

- Copeland PM (1983) The incidentally discovered adrenal mass. *Ann Intern Med* 98:940–945
- 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
- Venkatesh S, Hickey RC, Sellin RV, et al. (1989) Adrenal cortical carcinoma. *Cancer* 64:765–769
- 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
- Cagle PT, Hough AJ, Pysker TJ, et al. (1986) Comparison of adrenal cortical tumors in children and adults. *Cancer* 57:2235–2237
- 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
- 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
- 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
- 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
- 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
- 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

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

Osamu Imataki,¹ Shigetoshi Nakatani,² Terumi Hasegawa,² Miki Kondo,³ Kiyoko Ichihashi,³ Mitsuko Araki,³ Toshihiko Ishida,⁴ Sung-Won Kim,¹ Shin-ichiro Mori,¹ Takahiro Fukuda,¹ Kensei Tobinai,¹ Ryuji Tanosaki,¹ Atsushi Makimoto,¹ and Yoichi Takaue^{1*}

¹ Division of Hematopoietic Stem Cell Transplantation and Hematology, National Cancer Center Hospital, Tokyo, Japan

² Clinical Nutrition Care and Management, National Cancer Center Hospital, Tokyo, Japan

³ Transplantation Nursing Unit, National Cancer Center Hospital, Tokyo, Japan

⁴ First Department of Internal Medicine, Kagawa Medical University Hospital, Kagawa, Japan

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, pa-

tients 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

Grant sponsor: Grant-in-Aid for Scientific Research from the Ministry of Health, Labor and Welfare, with no conflict of interest.

*Correspondence to: Yoichi Takaue, Department of Medical Oncology, National Cancer Center Hospital, 1-1 Tsukiji 5-Chome, Chuo-ku, Tokyo 104-0045, Japan. E-mail: ytakaue@ncc.go.jp

Received for publication 30 August 2005; Accepted 21 March 2006

Published online 24 July 2006 in Wiley InterScience (www.interscience.wiley.com).

DOI: 10.1002/ajh.20700

© 2006 Wiley-Liss, Inc.

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, consommé, juice, miso soup | Calories 300-350 kcal Protein 5-7 g Fat 15-2 g Dietary fiber 15 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,

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 | | |
| I | 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 myelogenous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous 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.

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.

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

American Journal of Hematology DOI 10.1002/ajh

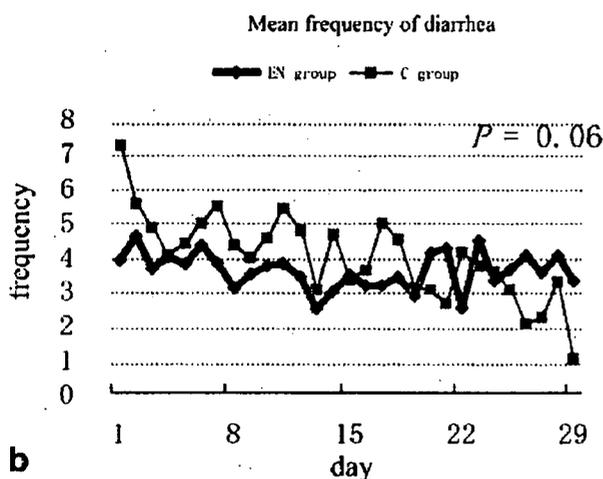
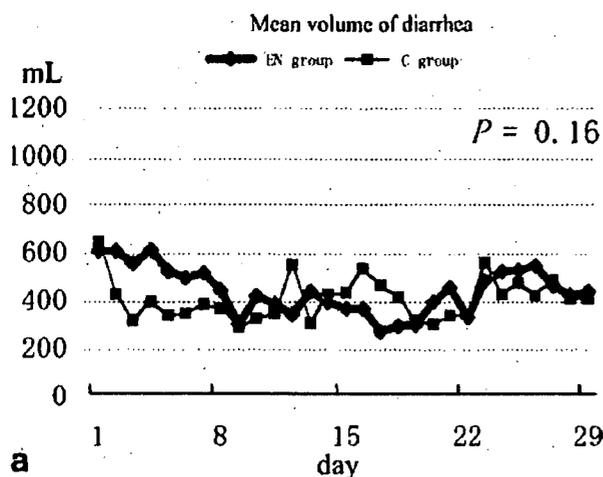


Fig. 1. Changes in mean volume and frequency of diarrhea. No difference was observed between the C and EN groups in the time-course of diarrhea as evaluated by volume ($P = 0.16$) (a) and frequency ($P = 0.06$) (b).

($P = 0.16$ 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

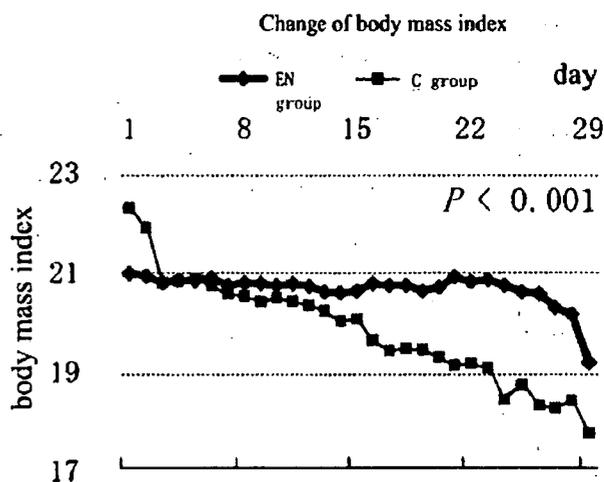


Fig. 2. Changes in BMI. The mean changes in BMI, with the first evaluation as a control, were compared between the two groups. A slower decrease in body weight tended to be observed in the EN group, while patients retained their BMI significantly better in the EN group than in the C group ($P < 0.001$).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

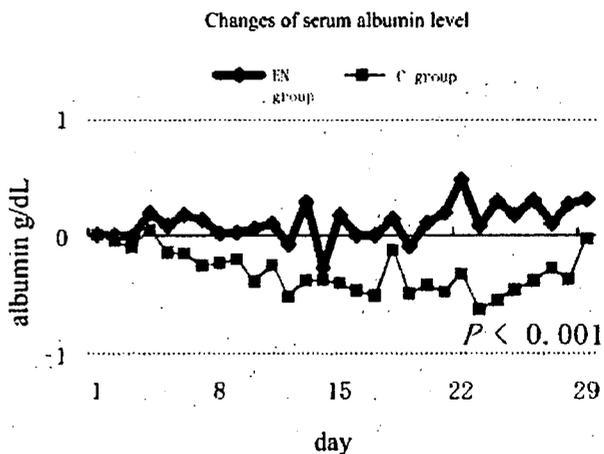


Fig. 3. Changes in albumin as nutritional parameter. One of the nutritional parameters, albumin (Alb), was evaluated between the C and EN groups. In the EN group, patients maintained significantly more stable levels of Alb ($P < 0.001$).

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

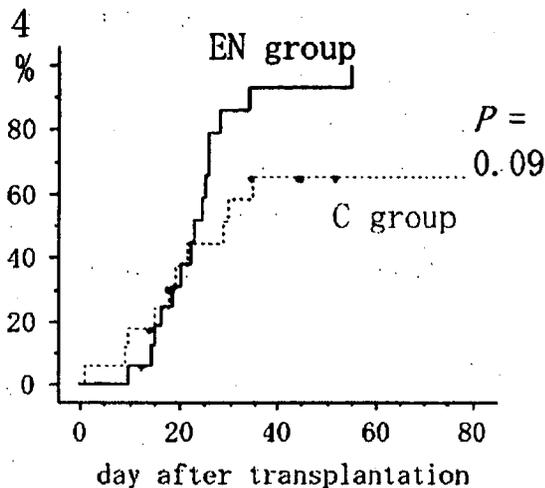


Fig. 4. Time to complete dietary recovery. The number of days required for return to a normal diet was 38 days in group C, while it was 31 days in group EN, with no statistically significant difference ($P = 0.09$).

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, ω -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;17:55A-65A.

Case Report

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

Minoru Hamazaki,¹ Hajime Okita,² Jun-ichi Hata,³ Shin-ichi Shimizu,⁴ Hiroshi Kobayashi,⁴ Katsuhiko Aoki⁵ and Taemi Nara⁶

Departments of ¹Pathology, ⁵Radiology and ⁶Oncology, Shizuoka Children's Hospital, Shizuoka, ²Department of Developmental Biology, National Research Institute for Child Health and Development, ³National Center for Child Health and Development, Tokyo and ⁴Department of Pathology, Seirei Hamamatsu General Hospital, Hamamatsu, Japan

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,

Correspondence: Minoru Hamazaki, MD, Department of Pathology, Shizuoka Children's Hospital, 860 Urushiyama, Aoi-ku, Shizuoka 420-8660, Japan. Email: mhamasa@sch.pref.shizuoka.jp

Received 13 March 2006. Accepted for publication 1 May 2006.
© 2006 Japanese Society of Pathology

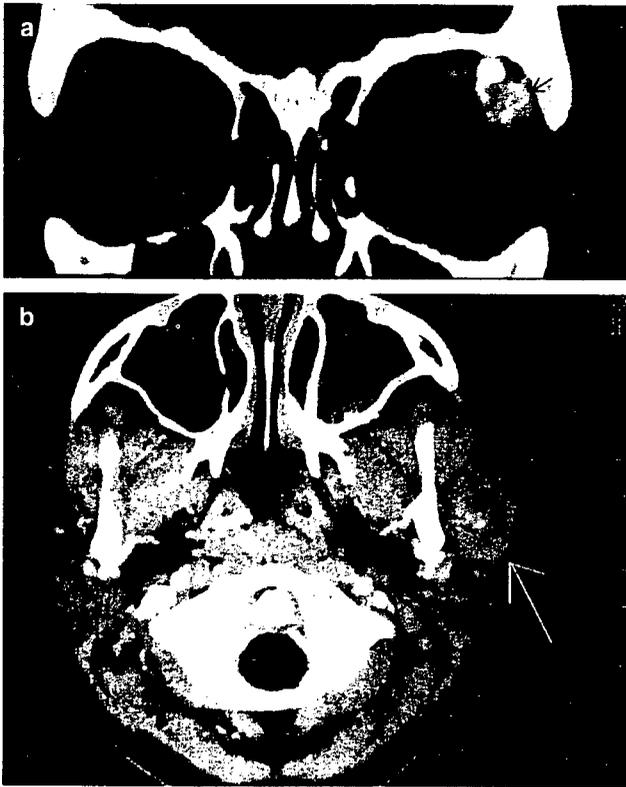


Figure 1 Radiological appearance. (a) The initial tumor mass (arrow) is visible in the left upper lateral aspect of the orbital region as a partially calcified, low-density mass on a CT image. (b) CT of the first recurrence showing an irregular mass (arrow) with a central low-density area near the upper aspect of the left parotid gland.

although the possibility of future recurrence cannot be ruled out.

PATHOLOGICAL FINDINGS

A conventional morphological examination was performed using formalin-fixed paraffin sections stained with HE. Histological examination of the primary tumor suggested an epithelial neoplasm, like a lacrimal gland carcinoma or an adenoid cystic carcinoma, or a rhabdomyosarcoma or metastasis of an abdominal tumor. The first recurrent tumor was characterized by conspicuously atypical small cell proliferation, forming solid cellular nodules or trabecular, basaloid epithelial lesions, and was accompanied by prominent interstitial fibrous intervention (Fig. 2). The tumorous tissue was intermingled with the ducts of the parotid gland, and the tumor itself was located adjacent to the serous acini of the parotid gland. The second recurrent main tumor exhibited diffuse cellular proliferation but did not have a desmoplastic stroma. The nodal metastases in the lower portion of the left neck, however, exhibited a distinct desmoplastic morphology similar to that of the first recurrent

tumor. These histological findings were consistent with a desmoplastic small cell tumor of soft tissue with divergent differentiation, and were almost identical to those of the initial orbital tumor.

Immunohistochemical study

The immunohistochemical investigation was performed using formalin-fixed, paraffin-embedded tissues and the streptavidin–biotin peroxidase indirect method. Primary antibodies were prepared against the following antigens: desmin (clone DE-R-11, diluted 1:50; DakoCytomation, Glostrup, Denmark), cytokeratin (AE1/AE3, diluted 1:50, proteinase K-treated; DakoCytomation), vimentin (Vim3B4, diluted 1:50, microwave-treated; DakoCytomation), epithelial membrane antigen (EMA; E29, diluted 1:50; DakoCytomation), myogenin (F5D, diluted 1:50, microwave-treated; DakoCytomation), WT1 (C-19, diluted 1:50, proteinase K-treated; Santa Cruz Biotechnology, Santa Cruz, CA, USA), CD99 (F5D, diluted 1:50; DakoCytomation), sarcomeric actin (α -Sr-1, diluted 1:40; DakoCytomation), CD45 (T29/33, diluted 1:50; DakoCytomation), glial fibrillary acidic protein (GFAP; 6F2, diluted 1:50, microwave-treated; DakoCytomation), neuron-specific enolase (NSE; BBS/NC/VI-H14, diluted 1:50; DakoCytomation), S-100 protein (rabbit polyclonal, diluted 1:400; DakoCytomation), Ki-67 antigen (MIB-1, diluted 1:50, microwave-treated; DakoCytomation), and P53 (PAb240, diluted 1:50, microwave-treated; DakoCytomation).

The solid areas of the first and second recurrent tumors were positive for vimentin, but the trabeculae were negative. In contrast, the trabecular or epithelioid components were positive for EMA, but the solid cellular area was negative. Distinct positive immunoreactivity for desmin and cytokeratin was expressed in the cytoplasm of the small tumor cells in both the solid cellular and trabecular architectures (Fig. 3). No evidence of myogenin, WT1, CD99, sarcomeric actin, CD45, GFAP, NSE, or S-100 protein positivity was seen. Ki-67 labeling index was 40–60% in the solid area of tumor and 10–20% at the trabeculae. P53 labeling index was <1–5%.

Electron microscopy

The electron microscopy specimens were prepared using conventional 2% glutaraldehyde and 1% osmium tetroxide fixation followed by epoxy embedding. Ultrathin sections stained with uranyl acetate and lead citrate were then observed using a JEM 1010 transmission electron microscope (JEOL, Tokyo, Japan).

The tumor cells were characterized by prominent desmosome-type intercellular junctions with tonofilaments (Fig. 4). A small amount of intermediate fibrils without striated muscle



Figure 2 Histological findings. (a) Conspicuous proliferation of small atypical cells with increased mitotic figures is apparent. A salivary gland duct is visible at the right of the figure. (b) The tumor tissue is characterized by trabecular, epithelioid proliferation surrounded by a hyaline basal structure, with fibrous proliferation in the interstitium.

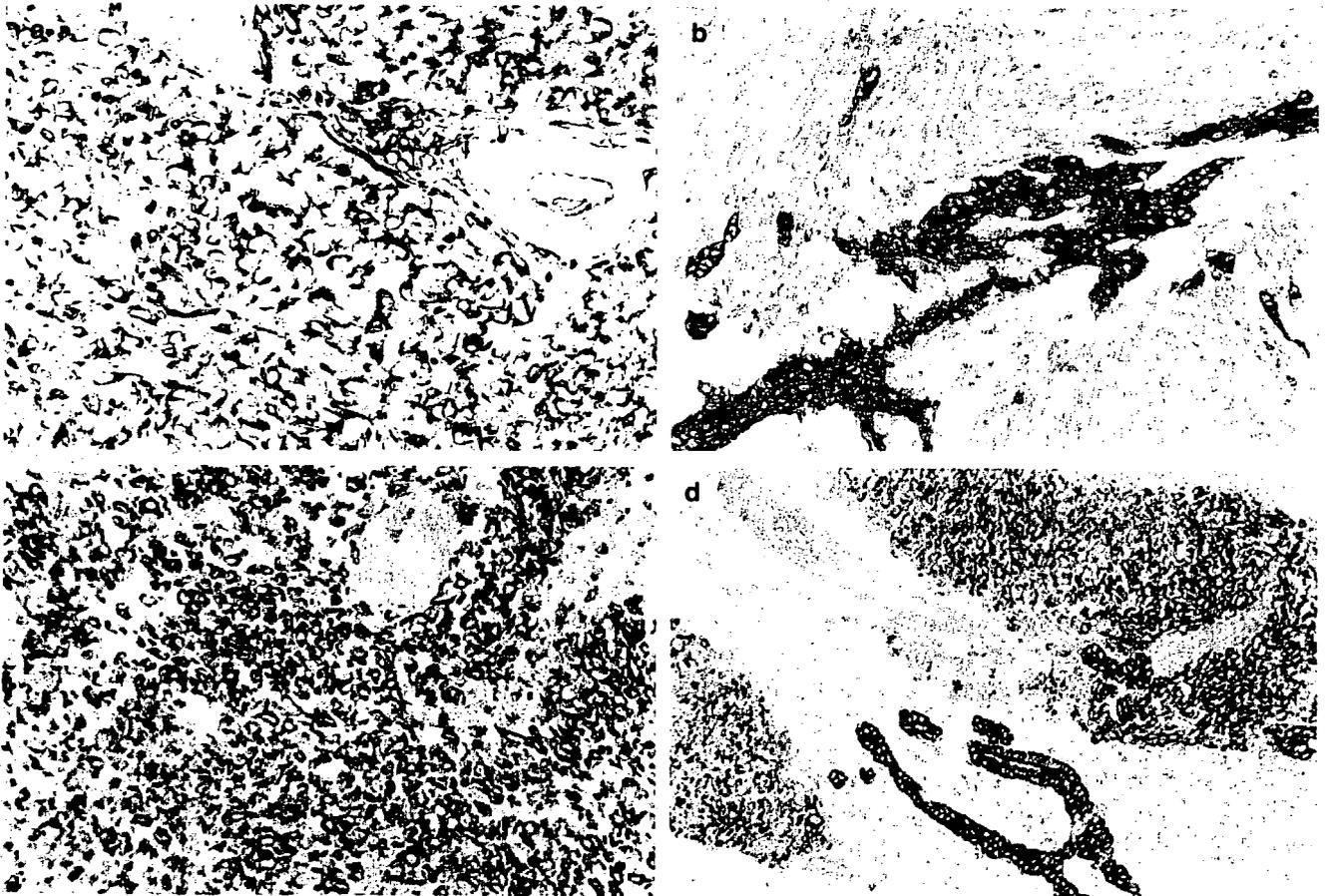


Figure 3 Immunohistochemical findings. (a) Vimentin is reactive in the cytoplasm of the tumor cells. (b) EMA is positive at the membrane of the tumor cells, especially in the trabecular region. Immunostaining was positive for (c) desmin and (d) cytokeratin in both solid and trabecular portions of the tumor.

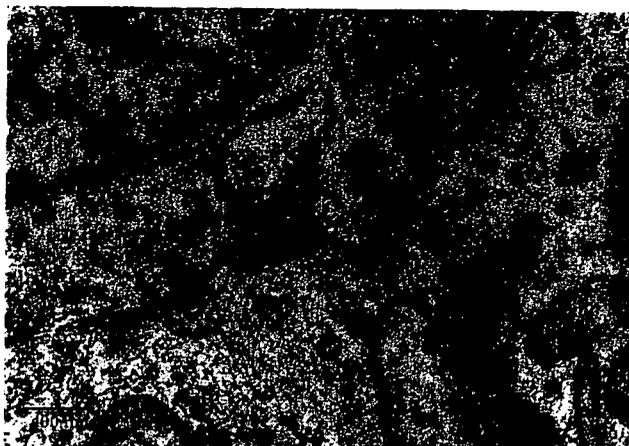


Figure 4 Electron microscopy. The tumor cells exhibit intercellular desmosomes with distinct tonofilaments.

differentiation were seen in the cytoplasm. No specific neurosecretory vesicle was found in the cytoplasm.

Gene analysis

Total RNA was extracted from frozen tumor tissue using an RNeasy Kit (Qiagen, Hilden, Germany) and was reverse transcribed to cDNA using a First-Strand cDNA synthesis Kit (Amersham, Piscataway, NJ, USA). PCR was performed using the following primers: EWS/WT1, ESBP1 (CGAC TAGTTATGATCAGAGCAGT)² and 10BA (TGCTGCCTGG GACACTGAAC); EWS-FLI1, 22.3 (TCCTACAGCCAAGCT CCAAGTC)³ and 11.3 (ACTCCCCGTTGGTCCCCTCC); and EWS/ERG, ESBP1 and EU15 (CATGTACGGGAGGTCT GAGGGGT).⁴ The amplified products were run on a 2% agarose gel. The products were then purified using MicroSpin S-400 Columns (Amersham) and cloned into pGEM-T vectors via the pGEM-T Vector System (Promega, Madison, WI, USA). Sequencing of the subcloned PCR products was performed using a DyEnamic ET Terminator Cycle Sequencing Kit (Amersham) with M13-40 and reverse primers.

The aforementioned histological diagnosis was also confirmed by RT-PCR of snap-frozen tumor tissues taken from the first recurrent tumor and the second recurrent masses, including both the solid cellular portion and the desmoplastic portion (Fig. 5). Each tissue specimen exhibited the same *EWS-WT1* chimeric fusion (data not shown). The chimeric transcript was finally confirmed by cloning and sequencing of the PCR product, resulting in the identification of an in-frame fusion of *EWS* exon 9 and *WT1* exon 8 (Fig. 6). Neither *EWS-FLI1* nor *EWS-ERG* chimeric transcripts associated with the Ewing family of tumors were detected (data not shown).

1 2 3 4



Figure 5 Molecular analysis. Reverse transcriptase–polymerase chain reaction of the first recurrent tumor tissue shows an in-frame fusion of *EWS* and *WT1* in lane 2, which contains a longer band than that of the positive desmoplastic small cell tumor (DSCT) control. A smaller product, corresponding to the fusion of *EWS* exon 7 to *WT1* exon 8, is also visible. Lane 1, 100 bp ladder marker; lane 2, present case; lane 3, positive DSCT control; lane 4, *NCR-NB3* negative control neuroblastoma cell.

EWS exon 9 | WT1 exon 8
 Present case cgaggtggcctcaataagcctggtgtgagaaccataccagtgtgact

Figure 6 Sequence of *EWS-WT1* junction. The fusion point of the longer product is shown. A vertical line indicates the nucleotide position of the junction.

DISCUSSION

Several epithelial neoplasms, many of which are malignant in adults, were initially considered in the differential diagnosis of the slow-growing tumor and repeated recurrences seen in the present case. Several kinds of malignant small cell tumors of soft tissues including rhabdomyosarcoma (especially alveolar type), extraskeletal Ewing sarcoma family of tumors (ESFT),⁵ neuroblastoma metastases, and non-Hodgkin lymphoma (in addition to DSCT) must be ruled out in pediatric patients. The immunohistochemical reactivity of desmin or vimentin was not necessary to confirm a diagnosis of rhabdomyosarcoma because myogenin, which is a myogenic regulatory protein, was not reactive. The absence of CD99, CD45, NSE, S100, and GFAP reactivity enabled ESFT, non-Hodgkin lymphoma, and neuronal tumors to be ruled out. Electron microscopy revealed an epithelioid fine structure with distinct desmosomes, similar to the findings for a previously reported extra-abdominal DSCT.⁶ No evidence of intracytoplasmic neurosecretory vesicles, often seen in neuroblastomas, or aggregates of thick myofilaments with electron-dense zones, indicating rhabdomyosarcoma, was found.

Another possibility in the differential diagnosis of small round cell tumors in children is DSCT. Recently, intra-