Case presentation: Nodular sclerosis classical Hodgkin lymphoma bearing Epstein-Barr virus that developed in an older man

Tsutomu Wada1,2, Futoshi Iioka*, Yoshimasa Kamoda1, Yoshitomo Maesako1, Takashi Akasaka1, Gen Honjo3, Takashi Misaki4, Fumiyo Maekawa5, Kayo Takeoka5, Hitoshi Ohno1

1Department of Hematology, Tenri Hospital; 2Department of General Internal Medicine, Tenri Hospital; 3Department of Diagnostic Pathology, Tenri Hospital; 4Radioisotope Center, Tenri Hospital; 5Laboratory of Molecular Genetics, Tenri Institute of Medical Research

Received 2013/4/22; accepted 2013/8/7; released online 2013/12/25

INTRODUCTION

Hodgkin lymphoma (HL) accounts for 4.4 to 8.2% of all lymphomas in Japan.1,2 It is divided into classical HL (CHL) and nodular lymphocyte-predominant HL subgroups, and the former subgroup is further divided into...

A 63-year-old man was admitted to our institution due to loss of 6 kilograms in body weight during the past 2 months. There was no surface lymphadenopathy, and the spleen was palpated 3 finger-breadths below the left costal margin. The hemoglobin level was 11.1 g/dL, white blood cell count 7,100/µL, including 13.0% lymphocytes, and platelet count 121×10³/µL. Serum albumin was 3.4 g/dL, C-reactive protein 18.0 mg/dL, erythrocyte sedimentation rate 78 mm/hr, and soluble interleukin-2 receptor 7,107 U/mL. Computed tomography of the body demonstrated an enlarged spleen containing multiple nodules and marked para-aortic lymphadenopathy, as well as supraclavicular lymph node swelling, and 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET) revealed strong uptake within the enlarged lymph nodes and spleen. A para-aortic lymph node biopsy showed the nodular sclerosis (NS) subtype of classical Hodgkin lymphoma (CHL), and infection with Epstein-Barr virus (EBV) was determined based on the positivity of EBV-encoded small RNAs and polymerase chain reaction amplification of the virus fragment. The patient was diagnosed with CSIII-2B disease of NSCHL and treated with ABVD combination chemotherapy. An ¹⁸F-FDG PET scan performed after 2 cycles of ABVD showed resolution of ¹⁸F-FDG-avid lymphadenopathy and splenomegaly. The patient finally achieved a complete response after the completion of 6 cycles of ABVD. The pathogenesis of the present case of EBV-positive NSCHL involving an older man may not be identical to that of EBV-negative NSCHL that predominantly develops in younger patients. Despite the presence of risk factors before treatment, an early complete metabolic response determined by ¹⁸F-FDG PET suggests long-term progression-free survival.

Keywords: nodular sclerosis classical Hodgkin lymphoma, Epstein-Barr virus, age distribution, ¹⁸F-FDG PET, early complete response

*Correspondence to: Futoshi Iioka, MD
Department of Hematology, Tenri Hospital
200 Mishima, Tenri, Nara 632-8552, Japan
e-mail: iioka@tenriyorozu.jp
nodular sclerosis (NS), mixed cellularity (MC), lymphocyte-rich, and lymphocyte-depleted (LD) subtypes. In any subgroup or subtype, the disease is characterized by a rare neoplastic population of Reed-Sternberg/Hodgkin cells that are surrounded by non-neoplastic cellular infiltrates. On the other hand, Epstein-Barr virus (EBV) is consistently associated with a proportion of cases of CHL, and the virus has been believed to play a causative role in the development of CHL.

With current chemotherapy and/or radiotherapy, approximately 75 to 80% of patients with HL are curable. However, treatment-related toxicities are a cause of late mortality. Thus, many clinical studies have aimed to achieve effective identification of low-risk patients curable with conventional treatment or even less intensive treatment and high-risk patients who require more aggressive treatments to achieve long-term disease control.

Here, we present an older man with advanced-stage NSCHL bearing EBV. We evaluated the response to treatment by 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET) after 2 cycles of chemotherapy, and found that the patient showed an early complete metabolic response.

**CASE REPORT**

A 63-year-old, previously well man was admitted due to body weight loss of 6 kilograms during the past 2 months. He saw his physician 3 weeks before admission, when a blood test revealed a high level of C-reactive protein (CRP); he was then referred to our institution for further investigation. He often noticed night sweats, but there was no fever of >38.0°C.

On examination, no surface lymphadenopathy was palpable. The spleen was palpated 3 finger-breadths below the left costal margin. The body temperature was 36.2°C, blood pressure 105/60 mmHg, pulse 52 beats per minute, and oxygen saturation 98% when breathing ambient air. The level of hemoglobin was 11.1 g/dL, white blood cell count 7,100/µL, including 13.0% lymphocytes, and platelet count 121×10³/µL. Blood chemistry values were as follows: total serum protein 6.9 g/dL, albumin 3.4 g/dL, lactate dehydrogenase 180 IU/L, aspartate aminotransferase 21 IU/L, alanine aminotransferase 15 IU/L, and alkaline phosphatase 363 IU/mL. The kidney function and electrolytes were normal. Serum iron was 16 µg/dL, copper 258 µg/dL (normal range, 68 to 128 µg/dL), ferritin 526 ng/mL, CRP 18.0 mg/dL, and erythrocyte sedimentation rate 78 mm/hr. Soluble interleukin-2 receptor was 7,107 U/mL (normal range, 145 to 519 U/mL).

Computed tomography (CT) of the body with contrast material demonstrated an enlarged spleen containing multiple nodules as well as marked para-aortic lymphadenopathy extending from the level of the diaphragm to the bifurcation of the aorta (Figure 1A, left). There were unequivocally swollen lymph nodes in the left supraclavicular fossa. 18F-FDG PET combined with CT revealed marked uptake within the enlarged lymph nodes and spleen (Figure 1A, middle and right).

A para-aortic lymph node was biopsied by a retroperitoneal endoscopic approach. Histopathological examination revealed that the biopsy consisted of nodules separated by fibrous tissues (Figure 2A). The nodules contained a variety of cells including small lymphocytes and immunoblastoid and occasional multinucleated giant cells (Figure 2B). Immunohistochemistry revealed that the giant cells were negative for CD20 (Figure 2C) and ALK (not shown), while they stained positive for CD79a and CD30 (Figures 2D and E), and in situ hybridization for EBV-encoded RNAs (EBERs) was positive in their nuclei (Figure 2F). The majority of background cells were small CD3⁺ lymphocytes. There were so-called “mummified” cells with condensed cytoplasm and pyknotic redish nuclei (Figure 2G), and the cells were positive for CD30 and CD15 (Figures 2H and I). Polymerase chain reaction amplification of DNA extracted by the biopsy detected clonal rearrangement of the immunoglobulin heavy chain gene (IgH) (Figure 3A), and confirmed the presence of the EBV genome (Figure 3B). The bone marrow biopsy was negative.

The histopathological features were consistent with the
Figure 1. CT and ¹⁸F-FDG-PET images. (A) Pretreatment images. Left, coronal section of contrast material-enhanced CT; middle, coronal section of ¹⁸F-FDG PET; and right, axial sections of ¹⁸F-FDG PET combined with CT. The maximum standardized uptake values were 19.1 and 14.7 at the para-aortic lymph node and spleen, respectively. (B) Coronal section of interim ¹⁸F-FDG PET after two cycles of ABVD, and (C) after the completion of 6 cycles of ABVD, showing complete metabolic remission.

Figure 2. Histopathology of the biopsy specimen. (A) H&E staining (loupe image). The capsule is obliterated and the surrounding adipose tissue has been infiltrated. (B to F) B, H&E staining (original magnification, x40 objective); C, anti-CD20 immunostaining (x40 objective); D, anti-CD79a immunostaining (x40 objective); E, anti-CD30 immunostaining (x40 objective); and F, in situ hybridization for EBERs (x40 objective). (G to I) Pictures focusing on mummified cells. G, H&E staining (original magnification, x40 objective); H, anti-CD30 immunostaining (x40 objective); and I, anti-CD15 immunostaining (x40 objective). A mummified cell is indicated by an arrow in G. There were no lacunar cells.
NS subtype of CHL. The disease was stratified into clinical stage III-2 with B-symptoms according to the Cotswolds modification of the Ann Arbor staging system, and the International Prognostic Score (IPS) for advanced disease was 3 (i.e., serum albumin level <4.0 g/dL, male sex, and age ≥45).\textsuperscript{6,7} The patient was treated with ABVD (doxorubicin, 25 mg/m\textsuperscript{2}; bleomycin, 10 mg/m\textsuperscript{2}; vinblastine, 6 mg/m\textsuperscript{2}; and dacarbazine, 375 mg/m\textsuperscript{2} as infusion) chemotherapy, resulting in the prompt resolution of constitutional symptoms and splenomegaly (Figure 4). The

---

**Figure 3.** Ethidium bromide-stained gene electrophoresis of PCR products. (A) PCR amplification of the CDR sequences of the \(\text{IgH}\) gene. Lane \(M\), molecular weight marker (bp, base pairs); lane 1, positive amplification control; lane 2, placenta DNA as a negative amplification control; and lane 3, DNA from the lymph node biopsy. PCR products were electrophoresed on a polyacrylamide gel. Primers were FR2 (5'-TGG[A/G]TCCG[A/C/G]CAG[G/C]C[T/C]CC[G/A/T/C]GG-3') and JH consensus (5'-ACCTGAGGAAGACGGTGACC-3') sequences.\textsuperscript{20} (B) PCR amplification of the EBV BamW fragment. Lane \(M\), molecular weight marker; lane 1, DNA from EBV+ Burkitt lymphoma Raji cells as a positive amplification control; lane 2, placenta DNA as a negative amplification control; and lane 3, DNA from the lymph node biopsy. The PCR products were electrophoresed on an agarose gel. Primers were PER1 (5'-TTTGTCCCCACGCGCGCATA-3') and PER2 (5'-AGGTGGCCTAGCAACGCGAA-3'), and the size of amplified products was 186 bp.\textsuperscript{21,22} The product indicated by the asterisk is inconsistently amplified in EBV-negative cells.

---

**Figure 4.** Clinical course, showing the duration and severity of symptoms, treatments, and the values of C-reactive protein (CRP), soluble interleukin-2 receptor (sIL-2R), and ferritin. One cycle of ABVD chemotherapy consisting of two doses of each drug was given every 4 to 6 weeks. ABVD: doxorubicin, bleomycin, vinblastine, and dacarbazine.
value of CRP was normalized. An $^{18}$F-FDG PET/CT scan performed after 2 cycles of ABVD showed resolution of $^{18}$F-FDG-avid lymphadenopathy and splenomegaly (Figure 1B). We completed a total of 6 cycles of ABVD, and the patient achieved a complete response; imaging studies including a third $^{18}$F-FDG PET/CT scan confirmed the resolution of the lymphoma lesions (Figures 1C and 4).

**DISCUSSION**

Here, we described a case of advanced-stage NSCHL that developed in an older man. The disease presumably arose in the para-aortic lymph nodes within the abdomen, spread to the spleen, and then upward to the supraclavicular nodes via a functionally contiguous lymphatic channel, accounting for the apparent bypassing of the mediastinum (Figures 1A and B). Although positive EBV status has been reported to be related to a poor prognosis in patients 50 to 74 years old, our patient achieved a complete response with the standard ABVD chemotherapy.

The epidemiology of CHL and rate of EBV positivity vary markedly depending on geographic location, socioeconomic status, histologic subtype, and most importantly age. In industrial countries including Japan, NSCHL, which is the predominant histologic subtype, shows a bimodal age distribution curve; there is one major peak in young adults (approximately 20 years old) and another minor peak at an older age (approximately 65 years old). In economically disadvantaged areas, on the other hand, MCCHL is more frequent in children and older adult patients. With regard to the involvement of EBV, positivity of the virus is most commonly associated with the MC and LD subtypes, while the virus is infrequently detected in the NS subtype; in one report, the rate of EBV positivity in NSCHL was as low as 4% in Tokyo metropolitan area. In contrast, Asano et al., in their large series of Japanese CHL, discriminated the NS grade 2 (NS2) subtype defined by MacLennan et al. in 1989 and showed that the median age of NS2 was 50 years and the rate of EBV positivity was as high as 53%. Although it remains to be determined whether the present case matches the criteria of NS2, the pathogenesis of the present case of EBV-positive NSCHL involving an older man may not be identical to that of EBV-negative NSCHL that predominantly develops in younger patients.

Clonal IgH rearrangement and the expression of CD79a suggest that the differential diagnosis should include EBV-positive diffuse large B-cell lymphoma (DLBCL) of the elderly. Clonal IgH rearrangement and the expression of CD79a suggest that the differential diagnosis should include EBV-positive diffuse large B-cell lymphoma (DLBCL) of the elderly. In the present case, although the expression of OCT2 and BOB.1 was not studied, the presence of “mummified” cells stained positive by both CD30 and CD15 antibodies supports the diagnosis of CHL.

It has been established that IPS can effectively predict the treatment outcome in advanced-stage HL patients before treatment. On the other hand, the initial tumor response to induction treatment is highly prognostic. $^{18}$F-FDG PET is currently considered the standard modality for not only primary staging, but also evaluation of the treatment response in patients with HL. Several prospective studies of patients with advanced-stage HL scanned with $^{18}$F-FDG PET after 2 cycles of ABVD (PET-2) showed long-term progression/failure-free survival of $\geq$95% in PET-2-negative patients compared with 0 to 12.8% in PET-2-positive patients. The value of a negative early “interim” $^{18}$F-FDG PET result for the prediction of treatment success is independent of a low or high IPS risk. Our patient, with IPS score 3, had a predicted 5-year freedom from progression before treatment of 60%. In contrast, the early complete metabolic response determined by PET-2 suggests that the patient has a strong likelihood of achieving long-term progression-free survival with 6 cycles of ABVD. Nevertheless, at present, it is premature to introduce early evaluation of
the treatment response by $^{18}$F-FDG PET into routine clinical practice to determine whether a patient will be cured with conventional treatment or require switching to more aggressive treatment, including high-dose chemotherapy with autologous hematopoietic stem cell rescue.

REFERENCES

中高年男性に発症したEpstein-Barrウイルス陽性結節硬化型古典的ホジキンリンパ腫の1例

和田 努1,2, 飯岡 大1, 鴨田吉正1, 前迫善智1, 赤坂尚司1, 本庄 原3, 御前 隆4, 前川ふみよ5, 竹岡加陽5, 大野仁嗣1

1天理よろづ相談所病院 血液内科
2天理よろづ相談所病院 総合診療教育部
3天理よろづ相談所病院 病理診断部
4天理よろづ相談所病院 RIセンター
5天理よろづ相談所 医学研究所 遺伝子解析室

症例: 63歳男性。体重減少（2か月間に6kg）のため入院。身体診察では、表在リンパ節は触知しなかったが、脾臓を左季肋下3横指触知した。

検査結果: ヘモグロビン11.1g/dL、白血球数7,100/µL（リンパ球13.0％）、血小板数121×10³/µL、アルブミン3.4g/dL、C反応性蛋白18.0mg/dL、赤沈1時間値78mm、可溶性IL-2レセプター7,107U/mLであった。

CTでは、傍大動脈・腹腔動脈周囲の著しいリンパ節腫大と、脾腫及び脾臓内多発腫瘤を認め、左鎖骨下リンパ節腫大も認められた。

PET検査では、これらの腫大したリンパ節と脾臓にFDGの強い集積を認めた。後腹膜鏡下に傍大動脈リンパ節を生検したところ、リンパ節は線維性の隔壁で囲まれた複数の結節からなり、多彩な炎症細胞を背景に大型細胞が認められた。

免疫染色では、大型細胞はCD30+、CD15+、CD20-、CD79a+、ALK-で、ISH法によるEpstein-Barr virus (EBV)-encoded small RNAs陽性であった。

PCR法で、免疫グロブリン重鎖遺伝子の再構成を認め、EBVゲノムのフラグメントが増幅された。

経過: EBV陽性結節硬化型古典的ホジキンリンパ腫、臨床病期Ⅲ-2Bと診断した。ABVD療法2サイクル終了後にPET検査（interim PET）を実施したところ、FDGの異常集積は完全に消滅していた。ABVD療法を合計6サイクル実施し、完全覚解に至った。

考察: 本例のような中高年発症のEBV陽性結節硬化型古典的ホジキンリンパ腫の病理発生は、若年者に好発するEBV陰性結節硬化型古典的ホジキンリンパ腫のそれと異なるともかもしれない。本例は治療前に3つの危険因子（低アルブミン血症、男性、高齢発症）を伴っていたが、interim PET評価によって早期完全覚解を認めたことから、良好な治療予後が予測される。

キーワード: 結節硬化型古典的ホジキンリンパ腫、Epstein-Barrウイルス、年齢分布、¹⁸F-FDG PET、早期完全覚解