Remission of lymphoma after withdrawal of methotrexate in rheumatoid arthritis: Relationship with type of latent Epstein-Barr virus infection

Takuya Miyazaki,[1]* Katsumichi Fujimaki,[1] Yukari Shirasugi,[2] Fumiaki Yoshiba,[2] Manabu Ohsaka,[3] Koji Miyazaki,[3] Etsuko Yamazaki,[4] Rika Sakai,[4] Jun-ichi Tamaru,[5] Kenji Kishi,[2] Heiwa Kanamori,[1] Masaaki Higashihara,[3] Tomomitsu Hotta,[2] and Yoshiaki Ishigatsubo[1]

[1] Department of Internal Medicine and Clinical Immunology, Yokohama City University Graduate School of Medicine, Kanagawa, Japan
[2] Department of Hematology, Tokai University School of Medicine, Kanagawa, Japan
[3] Department of Hematology, Kitasato University School of Medicine, Kanagawa, Japan
[4] Department of Hematology and Immunology, Fujisawa City Hospital, Kanagawa, Japan
[5] Department of Pathology, Saitama Medical Center, Saitama, Japan

Rheumatoid arthritis (RA) is associated with an increased risk of developing lymphoma. Although the pathogenesis is still unclear, the increased risk appears to be related to the high inflammatory activity of RA, immunosuppressive agents, or Epstein-Barr virus (EBV) infection. We investigated the relationship between EBV latent infection and methotrexate (MTX)-associated lymphoma in RA patients. Nine patients were diagnosed with non-Hodgkin's lymphoma (NHL) during MTX treatment for RA in a multicenter study. The pathologic findings were consistent with diffuse large B-cell lymphoma in 8 patients and peripheral T-cell lymphoma, unspecified in 1. EBV infection was detected in 3 patients by in situ hybridization. Among all 9 patients who were initially treated by MTX withdrawal alone, 2 obtained spontaneous complete response (CR), 1 had partial response, 2 had stable disease (SD), and 4 had progressive disease. Both patients who had a CR and 1 who had SD were positive for EBV. Further examination of the latent EBV infection patterns revealed that 2 patients who obtained a CR had latency Type III, and the other with SD had latency Type II. These results demonstrate that immunodeficiency caused by MTX treatment is associated with the development of EBV-related NHL in RA patients. In patients who were treated by MTX for RA and developed NHL, remission can be observed following MTX withdrawal especially in NHL with latency Type III EBV infection. The analysis of EBV infection, including the latency types, is useful to decide the optimum therapeutic strategy. Am. J. Hematol. 82:1106–1109, 2007. © 2007 Wiley-Liss, Inc.

Introduction

It is well known that rheumatoid arthritis (RA) is associated with an increased risk of developing lymphoma [1–3]. According to recent epidemiologic studies, the relative risk of developing lymphoma is estimated to be two-times higher in patients with RA or rheumatologic diseases, and the increased risk is greater for Hodgkin lymphoma (HL) than for non-Hodgkin's lymphoma (NHL) [1–5]. Although the pathogenesis of RA-associated lymphoma is still unclear, it is speculated that the increased risk is related to the high inflammatory activity of rheumatologic disease, immunosuppressive agents, or Epstein-Barr virus (EBV) infection [1–16].

Methotrexate (MTX) is currently the most widely used disease-modifying anti-rheumatic drug. Lymphoma has been reported to be a complication of long-term, low-dose MTX treatment. In 1993, Kamel et al. [6] reported that two patients taking MTX for rheumatologic disease who developed lymphoma had a complete regression after withdrawal of MTX alone. Other studies also showed that some MTX-associated lymphomas in RA patients regressed after the withdrawal of MTX alone and do not require treatment with chemotherapy and/or radiation [5,7,11,17–19]. These observations suggest that immunosuppression by MTX may affect development of lymphoma in RA patients, although it is not clear that MTX treatment causes a higher risk of lymphoma than other anti-rheumatoid agents.

Furthermore, EBV infection is often detected in RA-related lymphoma [5,7,9,19–22]. It has been suspected that latent EBV infection may participate in development of lymphoma in RA patients, like EBV-associated B-cell lymphoproliferative disorders occurring in immunosuppressed patients [23,24]. EBV-positive malignancies are associated with the virus's latent cycle, and three different types of EBV latency have been characterized [2,25–27]. Latency Type III EBV infection is usually expressed in lymphoproliferative disorders arising in an immunocompromised host with impaired T-cell immunosurveillance. However, the relationship between the MTX-associated lymphoma in RA patients and the type of EBV latent infection is not yet established. In this study, we investigated the clinical characteristics of lymphomas developing in RA patients receiving MTX and evaluated the association between the pattern of latent EBV infection and the response to MTX withdrawal.

Contract grant sponsor: Yokohama Foundation for the Advancement of Medical Science.

*Correspondence to: Takuya Miyazaki, MD, Department of Internal Medicine and Clinical Immunology, Yokohama City University Graduate School of Medicine, 3–9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan.
E-mail: miyazaki_takuya@ybb.ne.jp

Received for publication 18 January 2007; Revised 15 April 2007; Accepted 25 April 2007

Am. J. Hematol. 82:1106–1109, 2007.

Published online 25 July 2007 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.21003

TABLE I. Characteristics of the 9 RA Patients With NHL Taking MTX

Patient no.	Age/sex	Pathology	Primary location	Clinical stage	Duration of RA (m)	Duration of MTX (m)	Total MTX dose (mg)	Other RA treatment	CRP (mg/dl)	RF (IU/ml)
1	68/F	DLBCL	Nodal	II	88	88	1511	P, Bu	1.2	20
2	70/F	DLBCL	Nodal	I	231	99	2250	P	1.09	9
3	69/F	DLBCL	Extranodal	II	144	34	1020	P, Bu, Sa	0.92	757
4	53/F	DLBCL	Nodal	II	19	9	256	P	3.88	14
5	55/M	DLBCL	Nodal	IV	40	38	984	P	0.18	114
6	66/F	DLBCL	Nodal	III	161	77	420	P	0.6	117
7	69/M	DLBCL	Extranodal	IV	25	25	600	Bu	0.1	2.5
8	68/F	DLBCL	Nodal	III	139	114	1127	P, Mi, G	5.83	24
9	77/M	PTCL-u	Nodal	III	24	22	368	–	2.7	4.2

DLBCL, diffuse large B-cell lymphoma; PTCL-u, peripheral T-cell lymphoma, unspecified; P, prednisolone; Bu, bucillamine; Sa, salazosulfapyridine; Mi, mizoribine; G, gold; CRP, C-reactive protein; RF, rheumatoid factor; m, month.

Results

Patient characteristics

The clinical characteristics of the lymphomas that developed in the RA patients taking MTX are summarized in Table I. At the time of lymphoma diagnosis, the median age was 68 years (range 53–77). According to the WHO classification, 8 patients had diffuse large B-cell lymphoma (DLBCL) and 1 had peripheral T-cell lymphoma, unspecified. Monoclonal proliferation of lymphoma cells were confirmed in all patients. Their clinical stages were distributed as follows: 1 patient had Stage I, 3 had Stage II, 3 had Stage III, and 2 had Stage IV disease. At diagnosis, 2 patients (22%) had extranodal lesions, including 1 gastrointestinal, 1 tonsillar. The median duration from the start of MTX treatment to the diagnosis of NHL was 38 months (range 9–114), and the median total dose of MTX at the diagnosis of NHL was 984 mg (range 256–2,250). Eight patients received other anti-rheumatoid agents in combination with MTX. In particular, prednisolone was used for 7 patients. Rheumatoid factor was positive in 6 patients and C-reactive protein level was elevated in 7 patients at the time of diagnosis for NHL. The duration of RA ranged from 19 to 231 months. Three patients were positive for EBV infection detected by in situ hybridization.

Clinical outcome and EBV infection

The response to therapy is summarized in Table II. All patients were initially managed by withdrawal of MTX. Among the 3 patients with EBV infection, 2 patients with DLBCL obtained a complete response (CR) of their NHL. The time from the MTX withdrawal to CR was 6 and 2 months, respectively. These patients are still alive in CR without additional chemotherapy for 109 and 18 months, respectively (Table II). One patient with stable disease (SD) was positive for EBER1. She obtained a CR following chemotherapy and is alive in CR at 26 months of follow-up. In an analysis of the latent EBV infection pattern, 2 patients with DLBCL who had a CR were categorized as having latency Type III, and 1 with DLBCL in the tonsil who had a SD was defined as having latency Type II (Table II).

On the other hand, among the 6 patients without EBV infection who were managed by MTX withdrawal, 1 patient obtained partial response, 1 had SD, and 4 experienced progressive disease. These patients subsequently received chemotherapy with or without radiotherapy.

Discussion

We analyzed the characteristics of 9 patients with RA who developed NHL while receiving MTX and examined the efficacy of MTX withdrawal and its association with the type of EBV latent infection. To our knowledge, this is the first report of RA patients who developed NHL and had the Type III latency EBV infection seen in the immunocompromised host.

Lymphoma often develops in RA patients, but its pathogenesis has not yet been fully elucidated. Previous studies have suggested that high and long-standing inflammatory activity in RA, by itself, contributes to the development of lymphoma [1,4,8]. Recently, Baecklund et al. [28] identified 378 cases with RA developed lymphoma in Sweden. They found that RA patients with high cumulative disease activity, rather than its treatment, had a dramatically increased risk of developing lymphoma. Although our analysis contained patients who had RA of long duration and/or high RA disease activity, e.g., patients 2, 3, and 8, there was no obvious association with RA disease activity and lymphoma development. Our study also showed lymphoma development even in patients with a short duration of RA and/or low RA disease activity. In this study, DLBCL accounted for 89% of the cases. The high proportion of DLBCL is in accordance with previous reports on the development of lymphoma in RA patients [2,8,9,20]. In a prospective study by Mariette et al. [5], HL was found at a higher rate in RA patients taking MTX compared with the rate in a French population. In contrast, no patient developed HL in our study. This may be related to the fact that the frequency of HL in Japan is less than 10% of all lymphomas [29,30].

In this study, it is noteworthy that both patients who obtained a CR of their NHL after the withdrawal of MTX were positive for EBV. In previous studies, EBV infection has been detected in 12–44% of the lymphomas in RA patients [5,7,9,19,21,28]. These studies suggest that EBV infection may play a role in the development of these lymphomas. However, it is not evident whether EBV itself increases the risk of lymphoma development. In this study, EBV was detected in 3 patients (22%), 2 of whom had a CR of their NHL after the withdrawal of MTX. Mariette et al. [5] reported that 2 of the 3 patients who achieved a CR were positive for EBV. Salloum et al. [7] also reported that 5 of the 6 who had a CR were positive for EBV. Taken together, our results strengthen the concept that EBV-positive lymphoma in RA has a strong tendency to undergo a spontaneous remission after the withdrawal of MTX, although it is also true that there are some EBV-negative lymphomas which regress after MTX withdrawal [5,7,31,32].

Recently, it has been reported that EBV-associated tumors, such as lymphoma, nasopharyngeal carcinoma, and gastric carcinoma, express a variety of EBV latent genes [25–27]. By investigating EBV latent infection patterns, the immunodeficiency state of the lymphoma can be shown more clearly. Therefore, we analyzed the latency of

TABLE II. Response to MTX Withdrawal and Latency of EBV Infection

Patient no.	Effect of MTX withdrawal	Time to CR after MTX withdrawal (m)	Next treatment	Outcome	Follow-up time (m)	EBER1	LMP1	EBNA2	Latency type
1	CR	6	–	Alive in CR	109	+	+	+	III
2	CR	2	–	Alive in CR	18	+	+	+	III
3	SD	–	R-CHOP	Alive in CR	26	+	+	–	II
4	PD	–	R-CHOP	Alive in PR	9	–	NT	NT	NA
5	PD	–	VAD, CHOP-like, IF-RT, auto-PBSCT	Alive in CR	58	–	NT	NT	NA
6	PD	–	CHOP	Alive in CR	43	–	NT	NT	NA
7	PR	–	CHOP, IF-RT	Alive in CR	37	–	NT	NT	NA
8	SD	–	CyclOBEAP	Alive in CRu	3	–	NT	NT	NA
9	PD	–	CHOP	Death with infection	5	–	NT	NT	NA

CR, complete response; SD, stable disease; PD, progressive disease; PR, partial response; CRu, complete response unconfirmed; R, Rituximab; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisolone; VAD, vincristine, doxorubicin, and dexamethazone; IF-RT, involved field-radiation therapy; PBSCT, peripheral blood stem cell transplantation; CyclOBEAP, CHOP-like+etoposide and bleomycin; EBER1, EBV-encoded RNA transcript 1; EBNA2, Epstein-Barr nuclear antigen 2; LMP1, latent membrane protein 1; NT, not tested; NA, not available; m, month.

EBV-positive lymphomas in 3 patients. One patient was categorized as having latency Type II and the other 2 patients as latency Type III. Latency Type III indicates an immunodeficiency state and is associated with lymphoproliferative disorders arising in immunocompromised patients or post-transplant patients. It is believed that the EBV-associated tumors develop following the proliferation of latently EBV-infected B cells, which causes the suppression of the immunosurveillance system by cytotoxic T lymphocytes [3,25,26]. This phenomenon of latent EBV infection and immunosuppressive condition in RA patients developing lymphoma is very similar to post-transplant lymphoproliferative disorder. In our 2 patients having latency Type III, the immune surveillance system essentially might be abolished by the activity of MTX, allowing the EBV-infected B cells to grow aberrantly. It is also assumed that the withdrawal of MTX allowed the immune system to recover, resulting in complete regression of the lymphomas, although we did not examine the reconstitution of EBV-specific immunity in this study. Thus, our results strongly suggest that the immunodeficiency state itself is associated with the development of NHL, and the immunosuppression caused by MTX has an effect on the development and regression of NHL, especially in the latency Type III group of patients. In agreement with this hypothesis, a recent study has shown that MTX may induce EBV replication while producing immunosuppression [33].

Latency Type II EBV infection is generally observed in HL and nasopharyngeal lymphoma occurring in the immunocompetent host [2,20,27]. We confirmed that the patient with DLBCL in the tonsil had the latency Type II EBV infection in this study. The patient showed a SD after the withdrawal of MTX. It was considered that the infection of EBV influenced the pathogenesis of lymphoma development in the case, but MTX treatment for RA did not affect on the tumor progression. Therefore, there was no effect of MTX withdrawal in the patient.

Materials and Methods

Patients

We investigated the cases of NHL that developed in RA patients taking MTX in four hospitals in Kanagawa, Japan. A retrospective evaluation was conducted for the 9 patients who developed NHL from June 1995 to December 2004. The date of last observation was July 31, 2005. All lymphomas were classified according to the WHO classification [34]. An international workshop to standardize the response criteria for NHL was used to assess the effectiveness of treatment [35]. All patients fulfilled the 1987 American College of Rheumatology criteria for RA [36].

Immunohistochemical staining

Immunohistochemical analyses of formalin-fixed, paraffin-embedded sections of neoplastic tissues were performed during the diagnostic work-up, using a panel of monoclonal antibodies to Epstein-Barr nuclear antigen 2 (EBNA2) (PE2, DakoCytomation, Glostrup, Denmark) and latent membrane protein 1 (LMP1) (CS.1-4, DakoCytomation). The heat antigen retrieval method was employed with different retrieval buffers depending on the antibody used, according to manufacturer's instructions. For CD20, CD45, and CD45RO, the heat antigen retrieval method was not used. To detect, a peroxidase-labelled streptavidin–biotin complex system (BioGenex, San Ramon, CA) was employed. Immunostaining was carried out with an automated immunostainer (i 6,000, BioGenex). Tissue sections were tested for the presence of EBV by in situ hybridization for EBV-encoded RNA transcript 1 (EBER1) using the REMBRANT detection kit (Pan Path, Amsterdam, The Netherlands). The definition of EBV infection latency employed in this study is as follows: latency Type I, characterized by the expression of the EBER1 message alone; latency Type II, characterized by the expression of EBER1 and LMP1; and latency Type III, characterized by the expression of EBER1, LMP1, and EBNA2.

Conclusion

Our results suggest that immunodeficiency caused by MTX treatment is associated with the development of EBV-related NHL in RA patients. In patients who were treated by MTX for RA and developed NHL, remission can be observed following MTX withdrawal especially in NHL with latency Type III EBV infection. Therefore, the analysis of EBV infection, including the latency types, is useful to decide the optimum therapeutic strategy in this setting. Further studies on a larger scale are required to clarify the role of EBV infection and MTX treatment in RA patients who develop lymphoma.

Acknowledgments

Authors thank Drs. Kengo Takeuchi, Shigeki Motomura, Shin Fujisawa, Hiroyuki Fujita, Naoto Tomita, Hideyuki Koharazawa, Takayoshi Tachibana, Eri Tanaka, Shigeki Watanabe, and all members of the Hematology Group of Yokohama City University and the Meeting of Young Hematologists in Kanagawa.

References

1. Wolfe F, Michaud K. Lymphoma in rheumatoid arthritis: The effect of methotrexate and anti-tumor necrosis factor therapy in 18,572 patients. Arthritis Rheum 2004;50:1740–1751.
2. Callan MFC. Epstein-Barr virus, arthritis, and the development of lymphoma in arthritis patients. Curr Opin Rheumatol 2004;16:399–405.

3. Balandraud N, Roudier J, Roudier C, Pharm D. What are the links between Epstein-Barr virus, lymphoma, and tumor necrosis antagonism in rheumatoid arthritis? Semin Arthritis Rheum 2005;34:31–33.
4. Baecklund E, Askling J, Rosenquist R, Ekbom A, Klareskog L. Rheumatoid arthritis and malignant lymphomas. Curr Opin Rheumatol 2004;16:254–261.
5. Mariette X, Cazals-Hatem D, Warszawki J, Liote F, Balandraud N, Sibilia J, the investigators of the Club Rheumatismes et Inflammation. Lymphoma in rheumatoid arthritis patients treated with methotrexate: A 3-year prospective study in France. Blood 2002;99:3909–3915.
6. Kamel OW, van de Rijn M, Weiss LM, et al. Reversible lymphomas associated with Epstein-Barr virus occurring during methotrexate therapy for rheumatoid arthritis and dermatomyositis. N Engl J Med 1993;328:1317–1321.
7. Salloum E, Cooper DJ, Howe G, et al. Spontaneous regression of lymphoproliferative disorders in patients treated with methotrexate for rheumatoid arthritis and other rheumatic disease. J Clin Oncol 1996;14:1943–1949.
8. Baeklund E, Sundström C, Ekbom A, et al. Lymphoma subtypes in patients with rheumatoid arthritis: Increased proportion of diffuse large B cell lymphoma. Arthritis Rheum 2003;48:1543–1550.
9. Dawson TM, Starkebaum G, Wood BL, Willkens RF, Gown AM. Epstein-Barr virus, methotrexate, and lymphoma in patients with rheumatoid arthritis and primary Sjögren's syndrome: Case series. J Rheumatol 2001;28:47–53.
10. Ferraccioli GF, Casatta L, Bartoli E, et al. Epstein-Barr virus associated Hodgkin's lymphoma in a rheumatoid arthritis patient treated with methotrexate and cyclosporin A. Arthritis Rheum 1995;38:867–868.
11. Lioté F, Pertuiset É, Cochand-Priollet B, et al. Methotrexate related B lymphoproliferative disease in a patients with rheumatoid arthritis. Role of Epstein-Barr virus infection. J Rheumatol 1995;22:1174–1178.
12. Kamel OW, van de Rijn M, Hanasono MM, Warnke RA. Immunosuppression-associated lymphoproliferative disorders in rheumatoid patients. Leuk Lymphoma 1995;16:363–368.
13. Sibilia J, Liote F, Mariette X. Lymphoproliferative disorders in rheumatoid arthritis patients on low-dose methotrexate. Rev Rheum, Eng Ed 1998;65:267–273.
14. Chevrel G, Berger F, Miossec P, et al. Hodgkin's disease and B cell lymphoproliferation in rheumatoid arthritis patients treated with methotrexate. Arthritis Rheum 1999;42:1773–1776.
15. Kamel OW, Holly EA, van de Rijn M, Lele C, Sah A. A population based, case control study of non-Hodgkin's lymphoma in patients with rheumatoid arthritis. J Rheumatol 1999;26:1676–1680.
16. Ellman MH, Hurwitz H, Thomas C, Kozloff M. Lymphoma developing in a patient with rheumatoid arthritis taking low dose weekly methotrexate. J Rheumatol 1991;18:1741–1743.
17. Baird RD, van Zyl-Smit RN, Dilke T, Scott SE, Rassam SMB. Spontaneous remission of low-grade B-cell non-Hodgkin's lymphoma following withdrawal of methotrexate in a patient with rheumatoid arthritis: case report and review of the literature. Br J Haematol 2002;118:567–568.
18. Bachman TR, Sawitzke AD, Perkins SL, Ward JH, Cannon GW. Methotrexate-associated lymphoma in patients with rheumatoid arthritis. Arthritis Rheum 1996;39:325–329.
19. Usman AR, Yunus MB. Non-Hodgkin's lymphoma in patients with rheumatoid arthritis treated with low dose methotrexate. J Rheumatol 1996;23:1095–1097.
20. Hoshida Y, Tomita Y, Zhiming D, et al. Lymphoproliferative disorders in autoimmune disease in Japan: Analysis of clinicopathological features and Epstein-Barr virus infection. Int J Cancer 2004;108:443–449.
21. van de Rijn M, Cleary ML, Variakojis D, Warnke RA, Chang PP, Kamel OW. Epstein-Barr virus clonality in lymphomas occurring in patients with rheumatoid arthritis. Arthritis Rheum 1996;39:638–642.
22. Kamel OW, van de Rijn, Le Brun DP, Weiss LM, Warnke RA, Dorfman RF. Lymphoid neoplasms in patients with rheumatoid arthritis and dermatomyositis: Frequency of Epstein-Barr virus and other features associated with immunosuppression. Hum Pathol 1994;25:638–643.
23. Loren AW, Porter DL, Stadtmauer EA, Tsai DE. Post-transplant lymphoproliferative disorder: A review. Bone Marrow Transplant 2003;31:145–155.
24. Abed N, Casper JT, Camitta BM, et al. Post transplant lymphoproliferative disorder: Evaluation of histogenesis of B-lymphocytes in pediatric EBV-related post-transplant lymphoproliferative disorders. Bone Marrow Transplant 2004; 33:321–327.
25. Heslop HE. Biology and treatment of Epstein-Barr virus-associated non-Hodgkin lymphomas. Hematology 2005;1:260–266.
26. Macsween KF, Crawford DH. Epstein-Barr virus—Recent advances. Lancet Infect Dis 2003;3:131–140.
27. Rickinson AB, Kieff E. Epstein-Barr Virus. Field's Virology. Philaderphia. Lippincott Williams & Wilkins; 2001. pp 2575–2627.
28. Baecklund E, Iliadou A, Askling J, et al. Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis. Arthritis Rheum 2006;54:692–701.
29. Lymphoma Study Group of Japanese Pathologists. The WHO classification of malignant lymphomas in Japan. Incidence of recently recognized entities. Pathol Int 2000;50:696–702.
30. Glaser SL, Hsu JL. Hodgkin's disease in Asians: Incidence patterns and risk factors in population-based data. Leuk Res 2002;26:261–269.
31. Ogata M, Kikuchi H, Ono K, et al. Spontaneous remission of Epstein-Barr virus-negative non-Hodgkin's lymphoma after withdrawal of cyclosporine in a patient with refractory anemia. Int J Hematol 2004;79:161–164.
32. Svensson AM, Jacobson ER, Ospina D, Tindle BH. Reversible Epstein-Barr virus-negative lymphadenopathy and bone marrow involved by Hodgkin lymphoma in a rheumatoid arthritis patient undergoing long-term treatment with low dose methotrexate: A case report and review of the literature. Int J Hematol 2006;83:47–50.
33. Feng W, Cohen JI, Fischer S, et al. Reactivation of latent Epstein-Barr virus by methotrexate: A potential contributor to methotrexate-associated lymphomas. J Natl Cancer Inst 2004;96:1691–1702.
34. Jaffe ES, Harris NL, Stein H, Vardiman JW, editors. Pathology and Genetics, Tumours of Hematopoietic and Lymphoid Tissues. World Health Organization Classification of Tumours. Lyon: IARC Press; 2001.
35. Cheson BD, Horning SJ, Coiffier B, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. J Clin Oncol 1999;17:1244–1253.
36. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–324.

^{18}F-FDG PET for Posttherapy Assessment of Hodgkin's Disease and Aggressive Non-Hodgkin's Lymphoma: A Systematic Review

Teruhiko Terasawa[1], Takashi Nihashi[2], Tomomitsu Hotta[1], and Hirokazu Nagai[1]

[1]*Clinical Research Center for Blood Diseases, National Hospital Organization Nagoya Medical Center, Nagoya, Japan; and*
[2]*Department of Radiology, National Center for Geriatrics and Gerontology, Obu City, Japan*

Although studies have shown that ^{18}F-FDG PET, when used to assess the response of malignant lymphoma after treatment, has a strong ability to predict relapse, its diagnostic accuracy in clinical practice remains unclear. The aim of this study was to systematically review the diagnostic accuracy of ^{18}F-FDG PET in detecting residual disease at the completion of first-line therapy of Hodgkin's disease (HD) and aggressive non-Hodgkin's lymphoma (NHL). **Methods:** We searched relevant articles from 1966 to July 2006 using MEDLINE, EMBASE, SCOPUS, Biological Abstracts, bibliographies, review articles, and textbooks without language restriction. One assessor (for non–English-language studies) or 2 assessors (for English-language studies) independently reviewed each article to abstract relevant study characteristics and results. Relevant individual patient data or subgroup data were provided by the investigators if they were unavailable from the publications. We estimated summary receiver operating characteristic curves and confidence regions for summary sensitivity and specificity. **Results:** Nineteen studies consisting of 474 HD and 254 aggressive NHL patients were included. These studies had heterogeneity and suboptimal methodologic quality and reporting. Reported ranges for the sensitivity and specificity of ^{18}F-FDG PET in predicting disease relapse were 0.50–1.00 and 0.67–1.00, respectively, for HD and 0.33–0.77 and 0.82–1.00, respectively, for NHL. These estimates were similar when conventional imaging tests showed a residual mass. For HD studies, the summary receiver operating characteristic curves were similar irrespective of whether a residual mass was detected by conventional tests. Factors explaining the variability of diagnostic estimates were not identified. **Conclusion:** Although currently available evidence is still limited, ^{18}F-FDG PET seems to have good diagnostic accuracy for assessing residual HD at the completion of first-line treatment. Clinical data on this use of ^{18}F-FDG PET for aggressive NHL are more limited. Prospective studies with a more rigorous research design, conduct, and reporting would more reliably reveal the clinical diagnostic accuracy of this imaging modality.

Key Words: ^{18}F-FDG PET; lymphoma; response assessment; residual disease

J Nucl Med 2008; 49:13–21
DOI: 10.2967/jnumed.107.039867

Persistence of a mass after completion of first-line therapy is a common problem in assessing the response of both Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL) (*1,2*). Based on the likelihood of residual disease, clinicians need to proceed to further therapeutic interventions such as applying radiotherapy to the residual mass or salvage chemotherapy with or without high-dose chemotherapy followed by stem cell transplantation. Therefore, more accurate diagnostic tests to distinguish a posttherapy disease mass from fibrosis or scar tissue are needed to reduce the likelihood of unnecessary and potentially toxic therapy (*3*).

^{18}F-FDG PET is a promising functional imaging test for patients with malignant lymphoma and other malignancies and has gained wide use during the last decade (*4*). Its routine use has been recommended to assess the posttherapy response of HD, especially if CT reveals a residual mass (*3*). Others have also recommended its use for the same purpose on the basis of its high negative predictive value for HD and high positive predictive value for NHL (*5*). A recent survey of physicians caring for lymphoma patients found that intended management plans were often changed on the basis of ^{18}F-FDG PET results (*6*).

Recently, a systematic review explored the diagnostic accuracy of ^{18}F-FDG PET for this purpose and assessed the quality of the included studies (*7*). Similar to studies of diagnostic tests in other medical fields, this review revealed several methodologic problems affecting both the internal and the external validity of the published studies. The authors, however, estimated summary diagnostic accuracy without considering the effect of major methodologic variability on diagnostic tests underlying the original studies. For example, some of the studies included a mix of NHL histologies consisting of indolent, aggressive, and highly

Received Jan. 15, 2007; revision accepted Aug. 18, 2007.
For correspondence or reprints contact: Teruhiko Terasawa, MD, Institute for Clinical Research and Health Policy Studies, Tufts-New England Medical Center, 750 Washington St., Tufts-NEMC #63, Boston, MA 02111.
E-mail: tterasawa@tufts-nemc.org

aggressive lymphomas, and others included a mix of patients consisting of those who received first-line therapy and those who received salvage therapy. The diagnostic accuracy estimates reported from these studies have limited external validity and cannot be directly applied to specific clinical scenarios; each distinct histologic subtype has its unique clinical profiles, such as treatment strategies, responses, and prognoses. Also, the review did not consider the different ways that the primary studies presented results (e.g., individual patient vs. each lymphoma lesion, all involved sites vs. only bulky disease, or single vs. multiple inclusions of a patient).

This study was an updated systematic review of the diagnostic accuracy of ^{18}F-FDG PET in assessing the response of HD and aggressive NHL after first-line therapy, with special emphasis on the methodologic issues discussed above.

MATERIALS AND METHODS

Study Identification

We searched MEDLINE and EMBASE from January 1966 through July 2006 without language restriction. The detailed search strategy can be found in Supplemental Table 1 (supplemental materials are available online only at http://jnm.snmjournals.org). This search was augmented by SCOPUS and Biological Abstracts. We also manually reviewed the reference lists of eligible studies, review articles, and textbooks.

Study Selection

Two of us reviewed the pertinent studies to determine eligibility. We included prospective or retrospective studies evaluating posttherapy response assessment by ^{18}F-FDG PET exclusively for patients with HD or aggressive NHL followed by clinical follow-up, with or without pathologic confirmation as a reference standard. We included studies that evaluated at least 10 patients. We considered aggressive NHL to be mantle cell lymphoma; follicular center lymphoma, grade III; diffuse large B-cell lymphoma (DLBCL); primary mediastinal large B-cell lymphoma; peripheral T-cell lymphoma, unspecified; angioimmunoblastic T-cell lymphoma; angiocentric lymphoma; and anaplastic large cell lymphoma, T- and null-cell type by the Revised European–American Classification of Lymphoid Neoplasms (REAL)/World Health Organization classification, or corresponding subcategories for the International Working Formulation classification, the Kiel classification, or the Rappaport classification (*8*). We included studies of only those patients who completed first-line chemotherapy, radiotherapy, or combined-modality therapy and underwent conventional imaging tests such as CT, ultrasonography, or MRI for posttreatment restaging just before or after undergoing PET. We focused our analysis on studies that reported individual patients as the unit of analysis irrespective of the number of relapses or of the sites of relapse or residual disease, because this is the most appropriate perspective for clinical decision making. We excluded abstracts, editorials, comments, letters, review articles, and case reports. We excluded studies enrolling patients with HIV-associated or post-transplant lymphoproliferative disorders.

Many studies did not meet all the rigorous inclusion criteria but did partially include a relevant patient population. For these studies, we contacted the authors by mail or email to ask for individual patient data or subgroup data relevant to our inclusion criteria. When there was no response within 3 wk, another correspondence was sent. When there was no response to the third communication attempt, we considered the request rejected.

Data Abstraction

Two independent, board-certified hematologists abstracted relevant data for English-language articles. For non–English-language articles, data were extracted by a single reviewer working with a physician native speaker of the relevant language. We used an abstraction form consisting of items recommended in the Standards for Reporting of Diagnostic Accuracy (*9*). One nuclear medicine specialist evaluated the technical specifications and quality of the PET procedure by using recommended guidelines (*10*). The reviewers knew in which journals the studies had been published. Based on the enrollment of participants before PET, we categorized studies into 2 groups: "posttherapy evaluation," or studies that included patients irrespective of restaging results on conventional imaging tests, and "residual mass evaluation," or studies that evaluated only patients for whom visible residual mass lesions were shown on conventional imaging tests. In the posttherapy evaluation, we also abstracted the data on the subgroup of patients who had a residual mass shown on imaging. If the relevant data were unavailable from the published literature, we contacted the authors of the paper to request the subgroup data. Inconsistencies between reviewers were either clarified by the paper authors or resolved by consensus.

Assessment of Study Quality and Applicability

To evaluate the quality and applicability of the studies included in this review, we used an established quality rating system for diagnostic studies (*11*) and a recently proposed quality evaluation tool (*12*). In the established system, we examined 6 aspects of study quality: quality and application of the reference standard, independence of test interpretation, description of patient characteristics, cohort assembly, and sample size. We then rated each study as "a" (the highest quality), "b," "c," or "d" (the lowest quality) according to the predefined score. The recently proposed quality evaluation tool, which was designed exclusively for studies of diagnostic accuracy, comprehensively explores both methodologic quality and reporting. The tool consists of 14 items addressing patient spectrum, reference standard, disease progression bias, partial and differential verification bias, test review bias, clinical review bias, incorporation bias, test execution of both index test and reference standard, study withdrawals, and indeterminate test results.

Data Synthesis and Statistical Analysis

For each study, we constructed a 2 × 2 contingency table consisting of true-positive, false-positive, false-negative, and true-negative, where all patients were categorized as being PET-positive or -negative and as being positive or negative for disease according to the results determined through ^{18}F-FDG PET and the reference standard, respectively. We defined as disease-positive a patient who had biopsy-confirmed residual disease or whose disease had relapsed during clinical follow-up. We did not independently combine the sensitivity and specificity of the included studies because this approach does not take into account the interdependence of these 2 test parameters. We instead estimated summary receiver operating characteristic (ROC) curves and elliptic 95% confidence regions of summary sensitivity and specificity by the hierarchical summary ROC method (*13*). This model is a more sophisticated approach than the conventional linear regression model to compute summary diagnostic measures taking account of variations both within a study and between studies. We fitted the model by using maximum-

likelihood estimation implemented in the NLMIXED procedure of SAS/STAT (version 8; SAS Institute) (*14*). Then, we depicted the summery ROC curves and confidence regions for summary sensitivity and specificity by using Stata (version 8.2.; Stata Corp.) (*15*). Also, we estimated the area under the curve and the Q* statistic, the point on the curve where sensitivity equals specificity, as global measures for the summary ROC curves. We explored heterogeneity between studies by visual assessment of ROC plots for the following predetermined items: study design (prospective vs. retrospective), rates of patients with residual mass as found on conventional imaging tests, relapse rates, follow-up period, timing of PET scan after completion of therapy, and publication year. We also performed post hoc subgroup analyses for the items used for the technical specifications of PET and quality assessment.

RESULTS

Study Characteristics

We included 19 studies: 9 studies (*16–24*) that met all eligibility criteria and 10 studies (*25–34*) with relevant published data or with unpublished data obtained by contacting the authors of the paper (Table 1). The detailed process by which articles were selected can be found in the Supplemental Appendix and in Supplemental Figure 1. Because one group of researchers reported a "residual therapy evaluation" for HD and aggressive NHL and a "posttherapy evaluation" exclusively for aggressive NHL—and included in both groups the data on some participants (*23,29*)—we separately extracted the relevant data. Another group of researchers published an updated "posttherapy evaluation" for HD, with some participants overlapping from the previous report (*27,31*). We contacted the principal investigator and obtained updated combined results. For 5 studies that reported both HD and aggressive NHL (*25–28,30*), the contacted investigators provided unpublished subgroup data for each category. As a result, we finally had for further evaluation 15 studies consisting of 474 patients with HD and 8 studies consisting of 254 patients with aggressive NHL (Table 2).

Most studies were retrospective (Table 1). Four studies exclusively evaluated patients for whom a posttherapy visual residual mass was found on conventional imaging tests (*25,29,32,34*). Generally, patients underwent PET 1–3 mo after completing therapy, but some underwent PET at less than 1 mo. Most studies included both adult and adolescent patients, but 3 studies also included pediatric patients (*16,21,24*).

For HD studies, nodular sclerosis was the leading histologic subcategory and either ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) or MOPP (nitrogen mustard, vincristine, procarbazine, prednisone) plus ABVD or ABV (doxorubicin, bleomycin, vincristine) with or without radiotherapy was the most widely reported first-line treatment (Table 2). In posttherapy evaluations, 35%–72% of patients were found to have a residual mass on conventional imaging (Supplemental Table 2). Relapse rates were similar for both posttherapy evaluations and residual mass evaluations, ranging from 4% to 55% and 0% to 50%, respectively.

For aggressive NHL studies, DLBCL was the leading histologic subcategory, and the most widely adopted first-line therapy was CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) or comparable doxorubicin-containing regimens with or without radiotherapy (Table 2). In posttherapy evaluations, 26%–56% of patients were found to have a residual mass on conventional imaging (Supplemental Table 2). Relapse rates were 33%–60% for posttherapy evaluations and 33%–67% for residual mass evaluations.

Concerning imaging techniques and technologies, although their reporting was limited, the included studies generally followed the guidelines of the Society of Nuclear Medicine for performing ^{18}F-FDG PET (Supplemental Table 3). Only a single study used PET/CT (*30*). Most studies adopted qualitative diagnostic criteria: foci of elevated ^{18}F-FDG uptake unexplained by physiologic uptake. Two studies (*32,34*) also adopted quantitative diagnostic criteria: standardized uptake values (the ratio of ^{18}F-FDG uptake in tumor sites to that in normal sites). Only 2 studies clearly reported that all the included participants underwent pretherapy PET (*17,23*). Generally, experienced nuclear medicine physicians interpreted the results.

Sensitivity, Specificity, and Summary ROC Curves

HD studies reported widely ranging sensitivities and specificities for ^{18}F-FDG PET. For posttherapy evaluations, sensitivity ranged from 0.50 to 1.00 and specificity ranged from 0.67 to 1.00 (Supplemental Table 2; Fig. 1). For residual mass evaluations, reported estimates had a similarly wide range: 0.43–1.00 for sensitivity and 0.67–1.00 for specificity. The summary ROC curves and confidence regions for summary sensitivity and specificity for posttherapy evaluations and residual mass evaluations were similar (Fig. 2): The area under the curve for the summary ROC curve was 0.94 for posttherapy evaluations and 0.93 for residual mass evaluations, and the Q* statistic was 0.88 for posttherapy evaluations and 0.86 for residual mass evaluations.

Studies of aggressive NHL also reported a widely ranging sensitivity but a narrower high specificity. For posttherapy evaluations, sensitivity ranged from 0.33 to 0.77 and specificity ranged from 0.82 to 1.00 (Supplemental Table 2; Fig. 1). For residual mass evaluations, sensitivity ranged from 0.33 to 0.87 and specificity ranged from 0.75 to 1.00. We did not perform the metaanalysis because there were too few data points to reliably estimate the summary ROC curves and confidence regions for summary sensitivity and specificity.

Investigating Heterogeneity

We did not identify any clinical or ^{18}F-FDG PET test characteristics, or any items that assessed the quality and applicability of each study, to explain the heterogeneity of sensitivity and specificity (data not shown).

Quality Assessment of Published Studies

Overall, the quality and reporting of the included studies were limited (Supplemental Table 4), suggesting that they are subject to bias and variation limiting the internal and

TABLE 1
Studies Included in the Systematic Review

Study	Clinical indication	Study design	Time from therapy completion to PET (mo)		Follow-up (mo)		No. of patients	No. of female patients	Age (y)		Exclusion of patients with PIF or PD during treatment
			Median	Range	Median	Range			Median	Range	
HD and NHL											
de Wit et al. (*25*)	Residual mass	Retrospective	2	<10	13	1–26	34	NR	43	21–73	NR
Foo et al. (*26*)	Posttherapy	Retrospective	NR	NR	NR or 13*	3–65	24†	33%‡	33‡	20–67	NR
Jerusalem et al. (*27*)	Posttherapy	Prospective	NR	1–3	23 or 21*	NR	54	27 (50%)	47	15–80	Yes
Mikosch et al. (*28*)	Posttherapy§‖	Retrospective	NR	>1	NR	>12	93	NR	NR	NR	NR
Mikhaeel et al. (*29*)	Residual mass	Retrospective	NR	<1	38	16–68	32	31 (31%)	NR	NR	NR
Schaefer et al. (*30*)	Posttherapy¶	Retrospective	3	NR	9.3#	NR	41†	38%‡	39.6#	NR	NR
HD											
Filmont et al. (*16*)	Posttherapy	Retrospective	5.2	1–87	14	6–39	32	22 (53%)	30	6–65	NR
Friedberg et al. (*17*)	Posttherapy	Prospective	NR	<1	24	10–32	32†	36%‡	30‡	18–60	NR
Guay et al. (*18*)	Posttherapy	Retrospective	2	0–55	15	1–60	48	25 (50%)	38	17–78	NR
Jerusalem et al. (*31*)	Posttherapy§	Prospective	1	NR	NR§	NR	36	23 (64%)‡	28	13–71	Yes
Keresztes et al. (*32*)	Residual mass‖	Retrospective	3#	2–5	50 or 62**	5–98	31†	42%‡	31 or 37**	13–70	NR
Mocikova et al. (*33*)	Posttherapy‖	Retrospective	NR	NR	25	NR	94	44 (47%)‡	30	28–85	NR
Rigacci et al. (*19*)	Posttherapy	Prospective	4	2–8	45	NR	28	14 (50%)	30.6	16–73	NR
Spaepen et al. (*20*)	Posttherapy	Retrospective	NR	1–3	30	12–48	60	33 (45%)	35	11–75	NR
Weihrauch et al. (*34*)	Residual mass§	Prospective	NR	<4	28	16–68	28	8 (26%)	36	18–76	NR
Wickmann et al. (*21*)	Posttherapy	Retrospective	NR	0–7	32	1–56	48	36	NR	<18	NR
NHL											
Juweid et al. (*22*)	Posttherapy	Retrospective	1	0–5	35	25–60	54	NR	58	21–79	No
Mikhaeel et al. (*23*)	Posttherapy	Retrospective	NR	<2	30	7–56	45†	37%‡	NR	NR	Yes
Spaepen et al. (*24*)	Posttherapy	Retrospective	NR	1–3	21	11–50	93	34 (37%)	47	2–77	NR

*Patients without or with residual mass, respectively.
†Only subset of participants who were evaluated with PET after completion of therapy.
‡Data abstracted from total participants, not limited to patients who were evaluated with PET at end of therapy.
§Some PET scans were taken at the end of salvage therapy.
‖Some participants underwent PET multiple times.
¶Each lymphoma lesion was evaluated as the unit of analysis.
#Mean.
**Patients with or without bulky mass before therapy, respectively.
NR = not reported; PD = progressive disease; PIF = primary induction failure.

TABLE 2
Patient Characteristics for Studies Included in the Systematic Review

Study	No. of patients*	Histologic class	Pretherapy stage†	Type of therapy
HD				
de Wit et al. (*25*)	5	NA	NA	Chemotherapy ± radiotherapy
Filmont et al. (*16*)	32	22 NS, 7 MC, 2 LP, 1 LD	NA	Chemotherapy ± radiotherapy ± surgery or autologous transplantation
Foo et al. (*26*)	9	7 NS, 1 MC, 1 unclassifiable	8 II, 1 III	Chemotherapy ± radiotherapy
Friedberg et al. (*17*)	32	20 NS, 6 MC, 3 LP, 7 unclassifiable	3 I, 23 II, 6 III, 4 IV	ABVD or MOPP + ABVD ± radiotherapy
Guay et al. (*18*)	48	NA	2 I, 23 II, 15 III, 8 IV	MOPP or ABVD ± radiotherapy
Jerusalem et al. (*27,31*)	31	27 NS, 3 MC, 1 LD	NA	Chemotherapy ± radiotherapy
Keresztes et al. (*32*)	26	NA	NA	C-MOPP + ABVD or C-MOPP + ABV + CEP ± radiotherapy
Mikosch et al. (*28*)	31	NA	NA	Chemotherapy ± radiotherapy
Mikhaeel et al. (*29*)	15	NA	8 I + II, 7 III + IV	ABVD ± radiotherapy
Mocikova et al. (*33*)	71	NA	NA	Chemotherapy ± radiotherapy
Rigacci et al. (*19*)	28	22 NS, 5 MC, 1 LD	21 II, 5 III, 2 IV	ABVD ± radiotherapy
Schaefer et al. (*30*)	18	17 NS, 1 MC	NA	NA
Spaepen et al. (*24*)	60	45 NS, 9 MC, 3 LP, 3 unclassifiable	25 II, 19 III, 16 IV	Stanford V or MOPP + ABV ± radiotherapy
Weihrauch et al. (*34*)	20	NA	1 I, 9 II, 7 III, 2 IV	ABVD, C-MOPP + ABVD, or BEACOPP ± radiotherapy‡
Wickmann et al. (*21*)	48	NA	NA	GPOH-HD 95§
Aggressive NHL				
de Wit et al. (*25*)	6	Aggressive by REAL/WHO criteria	NA	Chemotherapy ± radiotherapy
Foo et al. (*26*)	12	11 diffuse large, 1 PTCL	3 I, 5 II, 2 III, 2 IV	Chemotherapy ± radiotherapy
Jerusalem et al. (*31*)	35	27 DLBCL, 8 FL grade 3	NA	Chemotherapy ± radiotherapy
Juweid et al. (*22*)	54	47 diffuse large cell, 4 ALCL, 2 diffuse mixed, 1 angioimmunoblastic T-cell	19 I + II, 35 III + IV	22 CHOP, 29 R-CHOP, 2 ProMACE-CytaBOM, 1 CNOP
Mikosch et al. (*28*)	24	Aggressive by REAL/WHO criteria	NA	Chemotherapy ± radiotherapy
Mikhaeel et al. (*23*)	45	"Aggressive" (diffuse mixed, diffuse large cell, large cell immunoblastic)	30 I + II, 19 III + IV‖	Radiotherapy only or CHOP ± radiotherapy
Schaefer et al. (*30*)	5	5 DLBCL	NA	NA
Spaepen et al. (*24*)	73	50 DLBCL, 8 ALCL, 7 ALCL HD-like, 5 MCL, 3 PTCL	NA	Doxorubicin-containing regimen or UKCCSG protocol (pediatric cases)

*Who met inclusion criteria of this systematic review.
†According to Ann Arbor staging system.
‡Eight relapsed cases (excluded) received salvage therapy with or without high-dose therapy followed by stem-cell transplantation.
§Treatment strategy for HD in children and adolescents (*35*).
‖Data abstracted from total participants, not limited to patients who were evaluated with PET at end of therapy.

ALCL = anaplastic large cell lymphoma; BCL = B-cell lymphoma; BEACOPP = bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone; CEP = CCNU, etoposide, prednimustine; C-MOPP = cyclophosphamide, vincristine, procarbazine, prednisone; CNOP = cyclophosphamide, mitoxantrone, vincristine, prednisone; FL = follicular lymphoma; LD = lymphocyte depleted; LP = lymphocyte predominance; MC = mixed cellularity; MCL = mantle cell lymphoma; NA = not available; NS = nodular sclerosis; ProMACE-CytaBOM = prednisone, cyclophosphamide, doxorubicin, etoposide, cytarabine, bleomycin, vincristine, methotrexate; PTCL = peripheral T-cell lymphoma; R-CHOP = rituximab plus CHOP; Stanford V = doxorubicin, vinblastine, mechlorethamine, vincristine, bleomycin, etoposide, prednisone; UKSCCG = United Kingdom children cancer study group; WHO = World Health Organization.

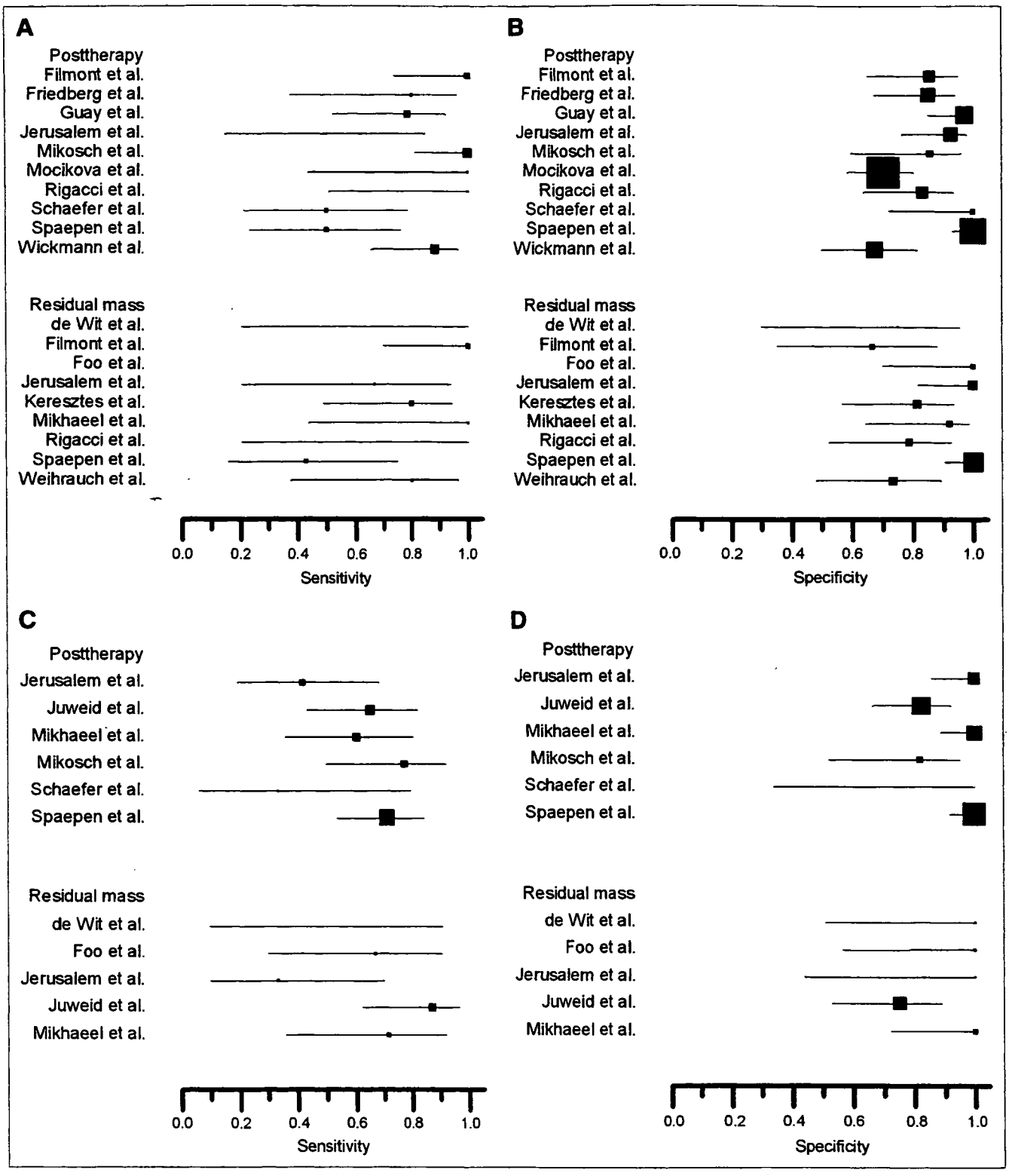

FIGURE 1. Sensitivity and specificity for HD (A and B) and aggressive NHL (C and D). Size of square plotting symbol is proportional to sample size (for sensitivity, number of patients who relapsed; for specificity, number of patients in remission) for each study. Horizontal lines are 95% confidence intervals. One study (Foo et al.) is omitted for sensitivity for HD because there were no cases of relapse.

external validities, respectively, of the test results. The detailed results of the quality assessment can be found in the Supplemental Appendix.

DISCUSSION

This systematic review showed that the reported sensitivities and specificities of ^{18}F-FDG PET to assess residual

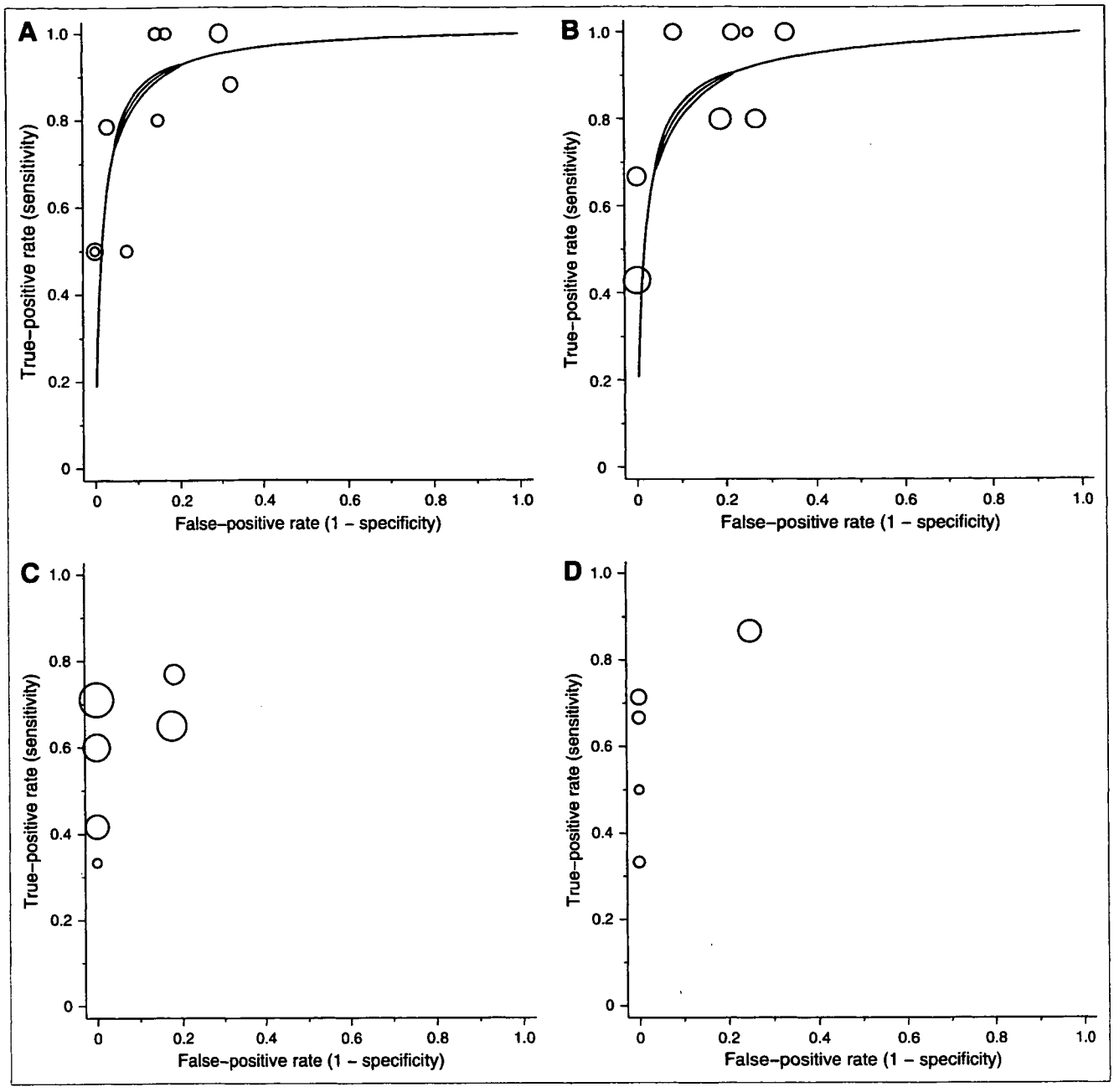

FIGURE 2. ROC plotting and summary ROC curve of ^{18}F-FDG PET for HD and aggressive NHL. Individual study estimates of sensitivity and 1 – specificity are shown for posttherapy evaluation and residual mass evaluation of HD (A and B) and aggressive NHL (C and D). Size of each circle is proportional to sample size for each study (all study participants). Solid crescent boundary represents 95% confidence region for summary sensitivity and specificity.

disease for patients with HD who completed first-line chemo- or chemoradiotherapy had large between-study heterogeneity. In addition, the summary ROC curves and confidence regions for sensitivity and specificity showed similar and good overall diagnostic accuracy irrespective of the presence of a visible residual mass on conventional imaging modalities such as CT or MRI. For aggressive NHL, although our review showed a relatively stable high specificity and variable sensitivity, there were too few studies to calculate a reliable overall diagnostic accuracy.

Many potential factors may explain the heterogeneity. For test characteristics, differences in the type of PET scanner, in the timing of PET after the completion of therapy, in positive test criteria, and in the clinical experience of the interpreters are relevant. For patient characteristics, several differences should be considered: in the type of histology, especially for aggressive NHL (e.g., DLBCL vs. other aggressive NHLs); in therapeutic strategy (e.g., chemotherapy vs. combined-modality therapy); in the presence or absence of a visible residual mass on conventional imaging tests; and in relapse

risk groups, such as the international prognostic score for advanced-stage HD (*36*) or the international prognostic index for aggressive NHL (*8*). For study characteristics, differences such as method of patient selection (e.g., prospective vs. retrospective), type and application of a reference standard, and patient follow-up have been reported to affect the variability of sensitivity and specificity (*9,37*). Because the available data were limited, we could not identify specific factors that explain the heterogeneity, which should be further addressed in future studies. Although the reported positive PET criteria appeared almost identical in all HD studies, the large crescent shape of the confidence regions implies a negative correlation between sensitivity and specificity across the included studies, suggesting that a variation in threshold may partially explain the between-study heterogeneity.

Our study had several important limitations. Because we selected only those studies for which pertinent data were available, several important investigations (some of which were included in the previous metaanalysis) may have been excluded. Also, because most data were derived from retrospective studies with poor-quality design and reporting, our conclusions are subject to the bias and variations in the original studies (*37*). In addition, our review included only a single study in which patients with DLBCL received rituximab in addition to CHOP (*22*). Because the combination of rituximab with CHOP is a current standard therapy (*38*), our results may be less applicable to clinical practice. Further, we included only a single study (*30*) that used a relatively new and promising additional technique—PET/CT—that may overcome the current technical limitations of PET (*39*).

In the recently revised consensus recommendations, ^{18}F-FDG PET has become an important component of posttherapy response assessments in clinical trials for HD and DLBCL (*40*). Although the currently available data have limitations, our systematic review would probably support the clinical relevance of the response criteria for high-risk HD; incomplete response defined by positive PET findings would have an excellent ability to predict relapse irrespective of whether a residual tumor mass is found on conventional imaging. For favorable-risk HD, patients labeled as incomplete responders after first-line therapy should still have a moderate possibility of long-term remission. Thus, clinical investigators adopting the recommendations into therapeutic efficacy trials would need to decide how to manage patients in this category before implementing the trials. For DLBCL and other aggressive NHLs, our results based on the limited clinical evidence would not suffice to support the criteria, and further research is necessary to validate them.

Our review shows that currently available data do not suffice to answer the phase 3 question of diagnostic accuracy studies: What is the diagnostic accuracy of ^{18}F-FDG PET for posttherapy response assessment of malignant lymphoma? (*41*). Reliable clinical evidence is especially limited for aggressive NHL. Further investigation should include prospective diagnostic accuracy studies (phase 3) of PET or PET/CT that adopt a more rigorous research methodology. We propose that, ideally, diagnostic accuracy studies should accompany prospective clinical trials to answer efficacy questions. Data on additional therapy, such as involved-field radiotherapy or high-dose chemotherapy with autotransplantation, for posttherapy PET-positive patients are limited. Before the routine clinical implementation of a treatment strategy based on posttherapy PET findings, randomized studies should assess the impact on patients' clinical outcomes, if appropriate (phase 4) (*41*). Also, determination of the cost-effectiveness of treatment strategies adopting PET is necessary to allow a better understanding of the role of posttherapy PET.

CONCLUSION

The currently available data show that ^{18}F-FDG PET has good overall accuracy in detecting residual disease in patients with HD who have completed first-line therapy. The current literature has methodologic weaknesses that may overestimate accuracy. Because data from original studies are limited, our review could not find robust evidence to answer the question of whether clinicians should routinely use PET to assess the posttherapeutic response, suggesting that they should be cautious about making clinical decisions based solely on a PET result.

ACKNOWLEDGMENTS

We thank Maike de Wit, MD, Jerusalem Guy, MD, Malik Juweid, MD, Katalin Keresztes, MD, Peter Mikosch, MD, Heidy Mocikov, MD, Robert Pichler, MD, Niklaus Schaefer, MD, and Martin Weihrauch, MD, for providing the data on their original work; Yinghua Li, MD, and Leo Kawaguchi, MD, for assistance in translating Chinese- and German-language publications; Jonathan Deeks, PhD, Kengo Ito, MD, and Kazuo Kubota, MD, for helpful comments; Petra Macaskill, PhD, for providing statistical programs; and Bruce Cheson, MD, and Joseph Lau, MD, for reviewing the manuscript. Part of this work was supported by Grant-in-Aid 15-2 for Cancer Research from the Ministry of Health, Labor, and Welfare of Japan, by the Japanese Society of Nuclear Medicine, and by Nihon Medi-Physics Corporation Limited.

REFERENCES

1. Radford JA, Cowan RA, Flanagan M, et al. The significance of residual mediastinal abnormality on the chest radiograph following treatment for Hodgkin's disease. *J Clin Oncol.* 1988;6:940–946.
2. Cheson BD, Horning SJ, Coiffier B, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. *J Clin Oncol.* 1999;17:1244–1253.
3. Meyer RM, Ambinder RF, Stroobants S. Hodgkin's lymphoma: evolving concepts with implications for practice. *Hematology Am Soc Hematol Educ Program.* 2004:184–202.
4. Gambhir SS, Czernin J, Schwimmer J, Silverman DH, Coleman RE, Phelps ME. A tabulated summary of the FDG PET literature. *J Nucl Med.* 2001;42(suppl):1S–93S.
5. Burton C, Ell P, Linch D. The role of PET imaging in lymphoma. *Br J Haematol.* 2004;126:772–784.

6. Hillner BE, Tunuguntla R, Fratkin M. Clinical decisions associated with positron emission tomography in a prospective cohort of patients with suspected or known cancer at one United States center. *J Clin Oncol.* 2004;22:4147–4156.
7. Zijlstra JM, Lindauer-van der Werf G, Hoekstra OS, Hooft L, Riphagen II, Huijgens PC. ^{18}F-Fluoro-deoxyglucose positron emission tomography for post-treatment evaluation of malignant lymphoma: a systematic review. *Haematologica.* 2006;91:522–529.
8. A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. *N Engl J Med.* 1993;329: 987–994.
9. Bossuyt PM, Reitsma JB, Bruns DE, et al. Standards for Reporting of Diagnostic Accuracy. Towards complete and accurate reporting of studies of diagnostic accuracy: The STARD Initiative. *Ann Intern Med.* 2003;138:40–44.
10. Schelbert RS, Hoh CK, Royal HD, et al. Society of Nuclear Medicine procedure guideline for tumor imaging using F-18 FDG, version 2.0 [SNM Web site]. 1999. Available at: http://interactive.snm.org/index.cfm?PageID=772&RPID=969. Accessed October 18, 2007.
11. Kent DL, Haynor DR, Larson EB, Deyo RA. Diagnosis of lumbar spinal stenosis in adults: a metaanalysis of the accuracy of CT, MR, and myelography. *AJR.* 1992;158:1135–1144.
12. Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Med Res Methodol.* 2003;3:25.
13. Rutter CM, Gatsonis CA. A hierarchical regression approach to meta-analysis of diagnostic test accuracy evaluations. *Stat Med.* 2001;20:2865–2884.
14. Macaskill P. Empirical Bayes estimates generated in a hierarchical summary ROC analysis agreed closely with those of a full Bayesian analysis. *J Clin Epidemiol.* 2004;57:925–932.
15. Harbord RM, Deeks JJ, Egger M, Whiting P, Sterne JA. A unification of models for meta-analysis of diagnostic accuracy studies. *Biostatistics.* 2007;8:239–251.
16. Filmont JE, Yap CS, Ko F, et al. Conventional imaging and 2-deoxy-2-[^{18}F]fluoro-D-glucose positron emission tomography for predicting the clinical outcome of patients with previously treated Hodgkin's disease. *Mol Imaging Biol.* 2004;6:47–54.
17. Friedberg JW, Fischman A, Neuberg D, et al. FDG-PET is superior to gallium scintigraphy in staging and more sensitive in the follow-up of patients with de novo Hodgkin lymphoma: a blinded comparison. *Leuk Lymphoma.* 2004;45:85–92.
18. Guay C, Lepine M, Verreault J, Benard F. Prognostic value of PET using ^{18}F-FDG in Hodgkin's disease for posttreatment evaluation. *J Nucl Med.* 2003; 44:1225–1231.
19. Rigacci L, Castagnoli A, Dini C, et al. 18FDG-positron emission tomography in post treatment evaluation of residual mass in Hodgkin's lymphoma: long-term results. *Oncol Rep.* 2005;14:1209–1214.
20. Spaepen K, Stroobants S, Dupont P, et al. Can positron emission tomography with [(18)F]-fluorodeoxyglucose after first-line treatment distinguish Hodgkin's disease patients who need additional therapy from others in whom additional therapy would mean avoidable toxicity? *Br J Haematol.* 2001;115:272–278.
21. Wickmann L, Luders H, Dorffel W. 18-FDG-PET-findings in children and adolescents with Hodgkin's disease: retrospective evaluation of the correlation to other imaging procedures in initial staging and to the predictive value of follow up examinations [German]. *Klin Padiatr.* 2003;215:146–150.
22. Juweid ME, Wiseman GA, Vose JM, et al. Response assessment of aggressive non-Hodgkin's lymphoma by integrated International Workshop Criteria and fluorine-18-fluorodeoxyglucose positron emission tomography. *J Clin Oncol.* 2005; 23:4652–4661.
23. Mikhaeel NG, Timothy AR, O'Doherty MJ, Hain S, Maisey MN. 18-FDG-PET as a prognostic indicator in the treatment of aggressive non-Hodgkin's lymphoma: comparison with CT. *Leuk Lymphoma.* 2000;39:543–553.
24. Spaepen K, Stroobants S, Dupont P, et al. Prognostic value of positron emission tomography (PET) with fluorine-18 fluorodeoxyglucose ([^{18}F]FDG) after first-line chemotherapy in non-Hodgkin's lymphoma: is [^{18}F]FDG-PET a valid alternative to conventional diagnostic methods? *J Clin Oncol.* 2001;19:414–419.
25. de Wit M, Bumann D, Beyer W, Herbst K, Clausen M, Hossfeld DK. Whole-body positron emission tomography (PET) for diagnosis of residual mass in patients with lymphoma. *Ann Oncol.* 1997;8(suppl):57–60.
26. Foo SS, Mitchell PL, Berlangieri SU, Smith CL, Scott AM. Positron emission tomography scanning in the assessment of patients with lymphoma. *Intern Med J.* 2004;34:388–397.
27. Jerusalem G, Beguin Y, Fassotte MF, et al. Whole-body positron emission tomography using ^{18}F-fluorodeoxyglucose for posttreatment evaluation in Hodgkin's disease and non-Hodgkin's lymphoma has higher diagnostic and prognostic value than classical computed tomography scan imaging. *Blood.* 1999;94: 429–433.
28. Mikosch P, Gallowitsch HJ, Zinke-Cerwenka W, et al. Accuracy of whole-body ^{18}F-FDP-PET for restaging malignant lymphoma. *Acta Med Austriaca.* 2003; 30:41–47.
29. Mikhaeel NG, Timothy AR, Hain SF, O'Doherty MJ. 18-FDG-PET for the assessment of residual masses on CT following treatment of lymphomas. *Ann Oncol.* 2000;11(suppl):147–150.
30. Schaefer NG, Hany TF, Taverna C, et al. Non-Hodgkin lymphoma and Hodgkin disease: coregistered FDG PET and CT at staging and restaging—do we need contrast-enhanced CT? *Radiology.* 2004;232:823–829.
31. Jerusalem G, Beguin Y, Fassotte MF, et al. Early detection of relapse by whole-body positron emission tomography in the follow-up of patients with Hodgkin's disease. *Ann Oncol.* 2003;14:123–130.
32. Keresztes K, Lengyel Z, Devenyi K, Vadasz G, Miltenyi Z, Illes A. Mediastinal bulky tumour in Hodgkin's disease and prognostic value of positron emission tomography in the evaluation of post-treatment residual masses. *Acta Haematol.* 2004;112:194–199.
33. Mocikova H, Markova J, Belohlavek O, et al. *Cas Lek Cesk.* 2004;143:476–479.
34. Weihrauch MR, Re D, Scheidhauer K, et al. Thoracic positron emission tomography using ^{18}F-fluorodeoxyglucose for the evaluation of residual mediastinal Hodgkin disease. *Blood.* 2001;98:2930–2934.
35. Dorffel W, Luders H, Ruhl U, et al. Preliminary results of the multicenter trial GPOH-HD 95 for the treatment of Hodgkin's disease in children and adolescents: analysis and outlook. *Klin Padiatr.* 2003;215:139–145.
36. Hasenclever D, Diehl V. A prognostic score for advanced Hodgkin's disease. International Prognostic Factors Project on Advanced Hodgkin's Disease. *N Engl J Med.* 1998;339:1506–1514.
37. Whiting P, Rutjes AW, Reitsma JB, Glas AS, Bossuyt PM, Kleijnen J. Sources of variation and bias in studies of diagnostic accuracy: a systematic review. *Ann Intern Med.* 2004;140:189–202.
38. Cheson BD. What is new in lymphoma? *CA Cancer J Clin.* 2004;54:260–272.
39. Barrington SF, O'Doherty MJ. Limitations of PET for imaging lymphoma. *Eur J Nucl Med Mol Imaging.* 2003;30(suppl):S117–S127.
40. Cheson BD, Pfistner B, Juweid ME, et al. The International Harmonization Project on Lymphoma: revised response criteria for malignant lymphoma. *J Clin Oncol.* 2007;25:579–586.
41. Sackett DL, Haynes RB. The architecture of diagnostic research. *BMJ.* 2002; 324:539–541.

original article

Annals of Oncology 18: 122–128, 2007
doi:10.1093/annonc/mdl349
Published online 16 October 2006

Clinicopathological features of pyothorax-associated lymphoma; a retrospective survey involving 98 patients

H. Narimatsu[1], Y. Ota[2], M. Kami[3]*, K. Takeuchi[4], R. Suzuki[5], K. Matsuo[6], T. Matsumura[3], K. Yuji[3], Y. Kishi[7], T. Hamaki[8], U. Sawada[9], S. Miyata[10], T. Sasaki[11], K. Tobinai[12], M. Kawabata[13], Y. Atsuta[14], Y. Tanaka[15], R. Ueda[16] & S. Nakamura[17]

[1]*Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya;* [2]*Department of Pathology, Toranomon Hospital, Tokyo;* [3]*Division of Exploratory Research, the Institute of Medical Science, the University of Tokyo, Tokyo;* [4]*Department of Pathology, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Tokyo;* [5]*Division of Molecular Medicine, Aichi Cancer Center, Nagoya;* [6]*Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya;* [7]*Division of Genetic Therapeutics, Center for Molecular Medicine, Jichi Medical School, Tochigi;* [8]*Department of Transfusion Medicine, Metropolitan Fuchu Hospital, Tokyo;* [9]*First Department of Internal Medicine, Nihon University School of Medicine, Tokyo;* [10]*Department of Radiology, Toyama Prefectural Central Hospital, Toyama;* [11]*Department of Chemotherapy, Tokyo Metropolitan Komagome Hospital, Tokyo;* [12]*Hematology Division, National Cancer Center Hospital, Tokyo;* [13]*Division of Respiratory Diseases, Toranomon Hospital, Tokyo;* [14]*Division of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya;* [15]*Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo;* [16]*Department of Internal Medicine and Molecular Science, Nagoya City University School of Medicine, Nagoya;* [17]*Department of Clinical Pathology, Nagoya University Graduate School of Medicine, Nagoya, Japan*

Received 17 June 2006; revised 14 August 2006; accepted 23 August 2006

To investigate clinicopathological features of pyothorax-associated lymphoma (PAL), we examined medical records of 98 patients (88 males and 10 females) with PAL at a median age of 70 years (range 51–86). Seventy-nine patients had a history of artificial pneumothorax. Median interval between diagnosis and artificial pneumothorax was 43 years (range 19–64). At diagnosis, performance status (PS) was 0–1 (n = 56) and 2–4 (n = 42). Clinical stages were I (n = 42), II (n = 26), III (n = 8) and IV (n = 22). Pathological diagnosis comprised diffuse large-B-cell (n = 78) and peripheral T-cell lymphoma (n = 1). Seventeen were treated supportively. The other 81 received aggressive treatments; chemotherapy (n = 52), radiotherapy (n = 7), surgery (n = 4) and combination (n = 18). Five-year overall survival (OS) was 0.35 (95% confidence interval, 24% to 45%). Causes of deaths were PAL (n = 39), respiratory failure (n = 13) and others (n = 12). Multivariate analysis identified prognostic factors for OS; lactate dehydrogenase levels [hazard ratio (HR) = 2.36; P = 0.013], sex (female versus male) (HR = 0.15; P = 0.01), PS (2–4 versus 0–1) (HR = 2.20; P = 0.02), clinical stages (III/IV versus I/II) (HR = 1.95; P = 0.037) and chemotherapy (HR = 0.31; P = 0.01). Most patients with PAL are elderly and have comorbidities, while some of them achieve durable remission with appropriate treatments. These findings prompt us to establish an optimal treatment strategy on the basis of risk stratification of individual patients.
Key words: artificial pneumothorax, diffuse large B-cell lymphoma, Epstein–Barr virus, malignant lymphoma, tuberculosis

introduction

Pyothorax-associated lymphoma (PAL) is a lymphoproliferative disorder developing in the pleural cavity after a long-standing history of pyothorax. While the pathogenesis, clinical features and optimal treatment have not been clarified, PAL represents an entity distinct from other malignant lymphomas [1]. PAL usually develops in patients who have undergone artificial pneumothorax for the treatment of pulmonary tuberculosis, and the interval between the operation and development of PAL has been reported to be 22–55 years [1–7]. Most studies on PAL are reported from Japan, but occasionally from other Asian [8] and Western countries [9] in the recent literature. Artificial pneumothorax, as a form of surgical treatment of lung tuberculosis, had been more widely carried out in Japan than in the Western countries, especially in the 1930s to 1950s [10]. Artificial pneumothorax is the most significant risk factor for development of PAL [10]. Approximately 2% of patients with chronic pyothorax develop PAL [7]. In the majority of these patients, the lymphoma cells are classified as large atypical B cells, and they express latent gene products of Epstein–Barr virus (EBV) [11, 12].

Optimal management of PAL is unclear. In Japan, ~70% of patients with PAL receive chemotherapy and/or radiotherapy [1]. Recent studies showed that PAL is responsive to chemotherapy [6] and radiotherapy, but the overall prognosis is poor, with a 5-year survival of 21.6% [6]. Some case series

Correspondence to: Dr M. Kami, Division of Exploratory Research, the Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan. Tel: +81-3-6409-2068; Fax: +81-3-6409-2069; E-mail: kami-tky@umin.ac.jp

indicated that surgical interventions including open-window thoracostomy and pleuropneumonectomy are beneficial for the treatment of PAL [1]; however, their studies are too small to make a definite conclusion. Since most patients with PAL are elderly and/or have comorbidities, invasive approaches are not feasible options in these patients. Treatment strategy of PAL must be established on the basis of the risk stratification of individual patients. We reviewed 98 cases of patients with PAL to investigate its clinicopathological features, treatment outcomes and prognostic factors.

patients and methods

patient selection and clinical records

We collected data from a total of 139 cases of PAL from 88 collaborating institutions. Clinical, pathological, immunophenotypic and karyotypic data were obtained from patients' records, autopsy request forms and pathological reports in 98 patients. Four cases were reported previously in the nationwide retrospective analysis in Japan [2, 6]. All the patients were diagnosed from November 1980 to May 2001. Performance status (PS) was evaluated by using Eastern Cooperative Oncology Group (ECOG) PS [13]. International Prognostic Index was evaluated as previously described [14].

Histological diagnosis was done on the basis of institutional diagnosis. Discrepancies in nomenclature among centers were resolved according to the synonyms in the World Health Organisation classification [15].

diagnostic criteria of PAL

PAL was defined as lymphoma developing in the pyothorax with or without involvement of regional lymph nodes or other visceral tissues [6]. Diagnosis of PAL was made by pathological examination of biopsy specimens obtained from the lesion in the pleural cavity or autopsy. Diagnosis of PAL was not established in patients without lesions in the pleural cavity or those without a history of pyothorax. Clinical stages were evaluated according to the proposed criteria [16].

end points and statistical analysis

The end points of this study are to (i) describe clinicopathological features of PAL, (ii) clarify its prognostic factors and (iii) investigate its optimal treatment. The probability of survival was calculated as a function of time with the Kaplan–Meier method. The estimated survival was calculated as of 31 July 2001. A log-rank test was applied to assess the impact by the factor of interest when appropriate. Uni- and multivariate Cox proportional hazard models were applied to assess the impact of potential prognostic factors. Multivariate model was built with forward/backward stepwise method using threshold P value for removal and adding in the model as 0.20 and 0.10, respectively. We applied P value <0.05 as statistical significance. All the analyses were conducted by STATA version 9.2 (STATA Corp., College Station, TX).

results

patients' characteristics

Patients' characteristics at the diagnosis of PAL are shown in Table 1. There were 88 males and 10 females, and their median age was 70 years (range 51–86). Seventy-nine patients (81%) had a history of artificial pneumothorax, and median interval between diagnosis of PAL and artificial pneumothorax was 43 years (range 19–64). At the diagnosis of PAL, PS was 0–1 (n = 56) and 2–4 (n = 42), and 49 patients (50%) had B symptoms. Clinical stages were I (n = 42), II (n = 26), III (n = 8) and IV (n = 22).

Table 1. Patient characteristics at the diagnosis of PAL

Variables	
Patient background	
Number of patients	98
Median age (range), years	70 (51–86)
Sex (male/female)	88/10
Artificial pneumothorax[a]	79
Median age at the onset of pyothorax (range), years	30 (14–50)
Side of pyothorax (right/left/unknown)	40/57/1
Interval between pyothorax and development of PAL, median (range), years	43 (19–64)
Clinical stage (I/II/III/IV)	42[b]/26/8/22
B symptoms (present/absent)	49/49
LDH, median (range), IU/l	523 (109–3100)
IPI (low risk/low-intermediate risk/high-intermediate risk/high risk)	5/21/33/39
ECOG PS (0/1/2/3/4)	21/35/20/15/7
Immunohistochemical characteristics of lymphoma cell	
CD3 (positive/negative)	0/43
CD4 (positive/negative)	0/6
CD5 (positive/negative)	1/6
CD8 (positive/negative)	0/6
CD10 (positive/negative)	1/6
CD20 (positive/negative)	41/2
CD30 (positive/negative)	3/8
CD33 (positive/negative)	0/8
CD34 (positive/negative)	0/7
CD43 (positive/negative)	4/5
CD56 (positive/negative)	0/6
CD79a (positive/negative)	14/1

[a]Information was not available in one patient.
[b]All are classified as stage IE.
ECOG: Eastern Cooperative Oncology Group; IPI: International Prognostic Index; LDH: lactate dehydrogenase; PAL: pyothorax-associated lymphoma; PS: performance status.

pathological examination

In situ hybridization using a probe for EBV-encoded RNA-1 demonstrated the presence of the EBV genome in the nucleus of tumor cells in 28 of 29 (88%) patients. Immunohistochemical studies revealed that 12 of 14 (86%) and 11 of 17 (65%) patients were positive for Epstein–Barr virus nuclear antigen (EBNA)-2 and latent membrane protein (LMP)-1, respectively. Immunohistochemical analysis for human herpes virus (HHV)-8 detection in lymphoma cells was not conducted.

Information on pathological diagnosis was available in 79 patients. The diagnosis included diffuse large B-cell lymphoma (n = 78) and peripheral T-cell lymphoma (n = 1). Immunohistochemical characteristics of lymphoma cells are shown in Table 1. Information on cytogenetic analysis was obtained in seven patients. Two patients had a normal karyotype. One patient had the abnormality of dicentric

doi:10.1093/annonc/mdl349

(15;18)(p11:p11). The remaining four patients had a complex karyotype, including 56–64, XX, −X, add(1)(q11), add(4)(q31) (n=1), add(3)(p11), −18, +mar (n=1) and hyperdiploid with abnormality of 3p, 8q, 15p (n=1). The information was not available in one patient.

treatment and outcomes

Seventeen patients (17%) were treated supportively, and the other 81 patients (83%) received aggressive treatments (Table 2). Response rates to chemotherapy, radiotherapy and chemoradiotherapy were 56%, 71% and 83%, respectively. Since assessable lesions were completely removed by surgery, response was not assessable in the remaining 10 patients who received it. Clinical characteristics and outcomes according to clinical stages are shown in Table 2.

Table 2. Clinical stage and outcome

Variables	Stage I	Stage II	Stage III–IV
Number of patients	42	26	30
ECOG PS (0/1/2/3/4)	14/18/8/1/1	4/12/4/5/1	3/5/8/9/5
Treatment of PAL			
Aggressive therapy[a]			
Chemotherapy	15[b]	18[c]	19[d]
Irradiation	4	2	1
Surgery	4	0	0
Chemotherapy and irradiation	10	2	0
Chemotherapy and surgery	4	0	1
Chemotherapy, irradiation and surgery	1	0	0
Response rate of aggressive therapy	76%	76%	32%
Palliative therapy	4	4	9
Alive/dead at last follow-up	18/24	10/16	6/24
Median survival (months)	51.8	12.2	7.5
Cause of death			
PAL	15	11	13
Respiratory failure	7[e]	2[f]	4[g]
Sepsis	0	0	3
Hemorrhage	0	0	2
Renal failure	1	0	0
Multiple organ failure	0	1	1
Heart failure	0	1	0
Others[h]	1	1	1
Death with PAL in remission	3	1	0
Death within 1 year after onset of PAL	9	12	19

[a]No patient received rituximab.
[b]Those patients who received CHOP or its related regimen.
[c]Those patients who included CHOP or its related regimen (n = 17) and ProMACE-CytaBOM (n = 1).
[d]Those patients who included CHOP or its related regimen (n = 18) and MACOP-B (n = 1).
[e]Caused by pneumonia (n = 2), pulmonary tuberculosis (n = 1), pyothorax (n = 1) and bronchopleural fistula (n = 1). The information was not available in remaining two patients.
[f]Caused by pulmonary emphysema (n = 1) and pneumonia (n = 1).
[g]Caused by pneumonia (n = 3) and pyothorax (n = 1).
[h]Included suicide (n = 1) and unknown (n = 2).
CHOP, combination chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisone; ECOG, Eastern Cooperative Oncology Group; PAL, pyothorax-associated lymphoma; PS, performance status. ProMACE-CytaBOM, the combination chemotherapy with cyclophosphamide, doxorubicin, etoposide, prednisone, cytarabine, bleomycin, vincristine, methotrexate and leucovorin.

Sixty-four patients died during their clinical courses. Their causes of deaths included PAL (n = 39), respiratory failure (n = 13) and others (n = 12) (Table 2). Four patients died without recurrence of PAL. Autopsy was conducted in 22 patients. Either ante-mortem or post-mortem examination revealed multiorgan involvement of PAL in 69 patients (70%). These organs included lymph nodes (n = 32), liver (n = 12), bone (n = 9), bone marrow (n = 8), contralateral lung (n = 13), gastrointestinal tract (n = 9), central nervous system (n = 8), spleen (n = 6), diaphragm (n = 4), pancreas (n = 5), skin (n = 8), kidney (n = 5), heart (n = 3), bladder (n = 2), prostate (n = 2) and testis (n = 2).

Median follow-up of the surviving patients was 33 months (range 1–241 months). The 5-year overall survival (OS) rate was 0.35 [95% confidence interval (CI) 0.24–0.45] (Figure 1). The 5-year OS rate was 0.47 (95% CI 0.30–0.63), 0.35 (95% CI 0.16–0.54) and 0.15 (95% CI 0.04–0.33) in patients with stages I, II and I–IV disease, respectively (Figure 2). Of the 41 patients with stage I disease, four received surgery alone as a primary treatment. Two patients died at 8 and 47 months after the diagnosis of PAL due to progression of underlying disease and respiratory failure, respectively. The remaining two patients are alive 32 and 62 months after the diagnosis of PAL.

prognostic factors of PAL

Prognostic factors of PAL are shown in Table 3. Multivariate analysis identified several prognostic factors for OS; lactate dehydrogenase (LDH) levels [hazard ratio (HR) = 2.36;

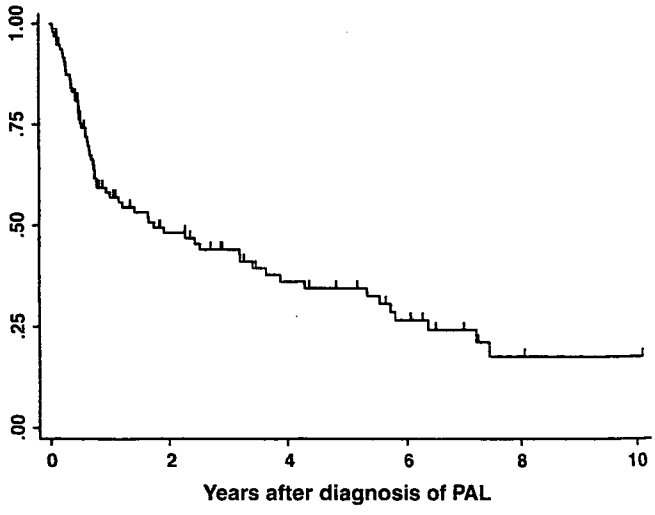

Figure 1. Overall survival (OS) of patients with pyothorax-associated lymphoma. The 5-year OS rate was 0.35 (95% confidence interval 0.24–0.45). PAL indicates pyothorax-associated lymphoma.

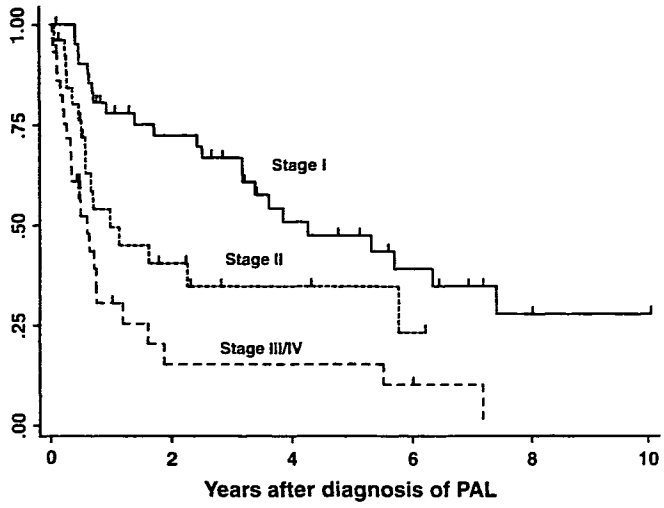

Figure 2. Overall survival (OS) of the patients with stage I, II and III/IV diseases. The 5-year OS rates were 0.47 [95% confidence interval (CI) 0.30–0.63], 0.35 (95% CI 0.16–0.54) and 0.15 (95% CI 0.04–0.33) in patients with stages I, II and I–IV disease, respectively. OS is different among the three groups ($P < 0.0001$). PAL indicates pyothorax-associated lymphoma.

Table 3. Prognostic factors of overall survival

	Hazard ratio	95% CI	P value
Univariate factors			
Age	1.00	0.97–1.04	0.94
Sex (female versus male)	0.18	0.04–0.74	0.02
Interval between onset of pyothorax and PAL	1.00	0.99–1.02	0.63
Side of pyothorax (right versus left)	0.77	0.46–1.29	0.32
LDH (elevated versus normal)	1.06	0.86–1.30	0.58
B symptoms	1.63	0.99–2.70	0.06
History of artificial pneumothroax	0.88	0.47–1.66	0.70
Clinical stage (stage III/IV versus I/II)	2.89	1.70–4.89	<0.001
Irradiation	0.86	0.49–1.51	0.60
Chemotherapy	0.67	0.37–1.22	0.19
Operation	0.40	0.14–1.09	0.07
ECOG PS (2–4 versus 0–1)	3.38	1.98–5.77	<0.001
Multivariate factors			
Age	1.01	0.97–1.05	0.72
LDH (elevated versus normal)	2.36	1.20–4.65	0.01
B symptoms	1.65	0.95–2.87	0.08
Clinical stage (stage III/IV versus I/II)	1.95	1.04–3.65	0.04
Sex (female versus male)	0.15	0.03–0.64	0.01
Irradiation	0.49	0.22–1.10	0.09
Chemotherapy	0.31	0.13–0.75	0.01
Operation	0.31	0.09–1.08	0.07
ECOG PS (2–4 versus 0–1)	2.20	1.15–4.20	0.02

CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; PAL, pyothorax-associated lymphoma; PS, performance status; LDH, lactate dehydrogenase.

$P = 0.013$], sex (female versus male) (HR = 0.15; $P = 0.01$), PS (2–4 versus 0–1) (HR = 2.20; $P = 0.02$), clinical stages (III/IV versus I/II) (HR = 1.95; $P = 0.037$) and chemotherapy (HR = 0.31; $P = 0.01$).

discussion

Clinical features of PAL shown in the present study are comparable to previous reports [2, 6, 7, 17]. PAL develops in elderly patients with a history of artificial pneumothorax, and median interval between artificial pneumothorax and the diagnosis of PAL was 43 years (range 19–64). Approximately two thirds of patients had localized diseases, whereas the PS was poor even in patients with early-stage disease. The present nationwide study showed that the ECOG PS was ≥3 in 43% of the patients with PAL, while the patients with good general conditions were reported in a previous small-sized case report from Japan [18].

Underlying chronic pyothorax or respiratory failure, which is probably associated with the poor PS, can be an obstacle to the treatment of PAL. Male predominance has been demonstrated in reports on PAL, and most researchers discussed that it is attributable to the male dominance of tuberculosis [2, 6, 7, 17]. The male : female ratio in the present study and in the previous study from Japan [19] was 9 : 1 and 12.3 : 1, respectively. While these are not comparable, these are remarkably higher than the ratio of 2 : 1 in the Japanese patients with tuberculosis [20]. It is reasonable to assume that genetical and/or environmental factors other than tuberculosis might be involved in the male dominance of PAL and that these might be associated with the pathogenesis of PAL. In consideration of future clinical significance of PAL, it should be noted that 20% of the patients have not received artificial pneumothorax in the present study. Artificial pneumothorax is now rarely conducted in Japan; however, the present study indicates the possibility that PAL can occur in patients with structural lung diseases, genetic abnormalities, and disorders of innate or acquired immunity [21]. PAL remains an important clinical entity, and further investigations are awaited on PAL without a history of artificial pneumothorax. Immunohistochemical analyses for HHV-8 detection in lymphoma cells were required in those patients to exclude the possibility of HHV-8-associated primary effusion lymphoma, which has been reported in elderly patients without immune deficiency [22].

The present study showed that the clinical stage of PAL is an important variable in the therapeutic decision making for PAL. Advanced PAL has a poor prognosis; the 5-year survival rate was 15% in patients with PAL at stage 3–4. Approximately 30% of these patients were treated with supportive measures due to advanced ages and/or comorbidities, while the remaining 70% of the patients received aggressive treatments. These findings indicate that it is difficult to control advanced PAL with the current therapeutic procedures on the basis of combination chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP). Considering that no patients were given either rituximab or ibritumomab in the present study, clinical significance of these molecular agents warrant further investigation.

In contrast, some patients with PAL at an early stage achieved durable remission in the present study. PAL at an early stage can be cured with the current therapeutic measures. Development of PAL is associated with chronic inflammation, and these situations are similar with malignant lymphoma of mucosa-associated lymphoid tissue and that of thyroid gland, in which

doi:10.1093/annonc/mdl349 | 125

Helicobacter pylori-induced gastritis and Hashimoto's thyroiditis contribute to their pathogenesis [23, 24]. Surgery generally plays a limited role in the treatment of malignant lymphoma, while surgical resections of lymphoma lesions are frequently curative in these lymphomas [25–27]. Four patients with PAL at stage 1–2 were treated with surgery alone in the present study, and two achieved durable remission. Surgical resection might be curative for early-stage PAL. Further studies are warranted to investigate its role in the treatment of PAL. On the other hand, one patient died of recurrence of PAL, and distant lesions of PAL can be overlooked using the current diagnostic procedures. Since PAL occasionally involves the extra nodal organs [28], precise evaluation of clinical stage is essential to establish optimal treatments for PAL. Novel diagnostic modalities, such as magnetic resonance imaging and positron emission tomography, may be useful for making an accurate staging of PAL [29].

Immunohistochemical examination of PAL cells in the present study has shown the same expression pattern as reported previously [1, 6, 7, 30–32]. PAL cells expressed some EBV-associated antigens; EBNA-2 and LMP-1 in 12 of 14 and 11 of 17 patients, respectively. These antigens are targets of the cytotoxic T lymphocytes [33], and the expression pattern of EBV-associated antigens in PAL is categorized as latency III, which is same as the pattern of malignant lymphomas developing in immunocompromised patients. These findings raise the possibility of underlying immune defects in PAL; however, a majority of the patients with PAL are immunocompetent. It remains unknown how PAL evades the immune system in immunocompetent patients, but it is to be noted that PAL develops in the lung abscess and that it sometimes stays there without any nodal involvement. In the present study, 66 of the 98 patients with PAL had not presented nodal involvement through their clinical courses. Since the immune system cannot work well in the space of abscess [30], PAL may be able to survive in the lung abscess even in immunocompetent patients. Nodal involvement rarely occurs until progression of PAL, and some genetic events such as loss of tumor suppressor genes may be required for the dissemination of PAL. Clinicopathological studies using molecular [34, 35] and immunological techniques [36] are warranted to clarify the mechanism of immune escape and progression of PAL.

Identification of prognostic factors of PAL is important to establish an optimal treatment strategy, considering that some patients with PAL at early stage can be cured with adequate treatments and that approximately two thirds of the patients were at stage 1–2 when the diagnosis of PAL was established. The present study has demonstrated that sex is significantly associated with survivals as well as conventional prognostic factors including serum levels of LDH, clinical stage and PS [37]. Interestingly, none of the 10 female patients died of disease progression (data not shown). The prognosis is more favorable in females than in males. These observations will provide an important clue to investigate the pathogenesis of PAL. Sex hormones *per se* might play a role in the development and progression of PAL. Alternatively, some factors associated with sex might be involved in the lymphomagenesis and progression of PAL. Previous epidemiological studies have identified several risk factors of malignant lymphoma, and some of them are associated with sex. These included pregnancy [38], smoking history [39] and alcohol consumption [40]. Further studies are warranted to investigate the association between sex and PAL.

While the present study provided novel information on PAL, it has some limitations to be discussed. Firstly, this is a retrospective study, involving a small number of patients. It might have been influenced by unrecognized bias. Secondly, the present study provided little information on morphological, immunohistochemical, karyotypic and genetic findings of PAL. More detailed information on these findings has to be investigated. The more detailed macroscopic findings, such as size of lesions and the depth of the invasion of pleura, are also needed. Thirdly, clinical features of PAL, which is not associated with pneumothorax, are not clarified, while these kinds of PAL may become a significant problem in the future. Fourthly, information of the type and the degree of comorbidities is limited in the present study although these comorbidities have an impact on response rate and survival in the elderly patients. Lastly, optimal treatments for PAL have not been established. These findings prompt us to conduct further large-scale prospective studies.

appendix

This study was conducted at the following institutions under the auspices of the following investigators in Japan: M. Kami and M. Kawabata (Toranomon Hospital, Tokyo); Y. Tanaka and A. Yamazaki (Tokyo University Hospital, Tokyo); K. Mori (Juntendo University Hospital, Tokyo); M. Mori (Tokyo Metropolitan Geriatric Hospital, Tokyo); S. Sunaga (Hitachi General Hospital, Hitachi); J. Kojima and S. Komastumoto (Dokkyo Medical University School of Medicine, Tochigi and Ashikaga Red Cross Hospital, Ashikaga); S. Miyata (Toyama Prefectural Central Hospital, Toyama); T. Hashizume (National Hospital Organization Kanagawa Hospital, Hatano); M. Fukase (Shonai Hospital, Tsuruoka); S. Okamoto (Keio University School of Medicine, Tokyo); Y. Irie (Saga Prefectural Hospital Koseikan, Saga); T. Miki and Y. Hashimoto (Himeji Brain and Heart Center, Himeji); K. Nakayama (Nihon University School of Medicine, Tokyo); Y. Atsuta and T. Nishida (Nagoya First Red Cross Hospital); A. Hirasawa (Yokohama Rosai Hospital, Yokohama); A. Taguchi (Yokohama City University Hospital, Yokohama); M. Yagita (Kitano Hospital, Osaka); T. Kumagai and Y. Adachi (NTT Kanto Medical Center, Tokyo); K. Tanaka (Kurume University Hospital, Kurume); M. Takagawa (Ishinomaki Red Cross Hospital, Ishinomaki); H. Yanai (Hiroshima City Hospital, Hiroshima); S. Hara (Kurashiki Daiichi Hospital, Kurashiki); S. Taniguchi (Hamanomachi Hospital, Fukuoka); J. Suzumiya (Fukuoka University Hospital, Fukuoka); T. Ishibashi (Shizuoka Hospital, Shizuoka); H. Ishihara (Yamanashi University Hospital, Yamanashi); S. Fukuda (Okayama Medical Center, Okayama); M. Kurosawa (Sapporo Kosei Hospital, Sapporo); A. Wakita (Nagoya City University Hospital, Nagoya); H. Saito (Nagano Red Cross Hospital, Nagano); K. Tsukazaki (Nagasaki University Hospital, Nagasaki); F. Nagamura (Institute of Medical Science, University of Tokyo, Tokyo); K. Suga (Saga Medical University Hospital, Saga); Y. Hasegawa (Tsukuba University Hospital, Tsukuba); H. Mizuno (Chukyo Hospital, Nagoya); A. Oyama

(Aichi Prefectural Hospital, Okazaki); R. Suzuki (Aichi Cancer Center Hospital, Nagoya); T. Kato (Higashi Nagoya Hospital, Nagoya); M. Kajita (Hekinan Municipal Hospital, Hekinan); T. Kataoka (Nagoya Memorial Hospital, Nagoya); Y. Morishita (JA Aichi Showa Hospital, Konan); Y. Hamano (Showa University Hospital, Tokyo); H. Ikeda (Sapporo City General Hospital, Sapporo); T. Miyoshi (Kitakyushu Municipal Medical Center, Kitakyushu); S. Ozaki (Tokushima University Hospital, Tokushima); K. Tobinai, A. Hori and Y. Matsuno (National Cancer Center Hospital, Tokyo); M. Daibata (Kochi Medical School Hospital, Kochi); S. Tamaki (Yamada Red Cross Hospital, Ise); Y. Inoue (Japanese Red Cross Medical Center, Tokyo); T. Ishizaka (University of Fukui Hospital, Fukui); T. Miyakuni (Naha Hospital, Naha); T. Takahashi (Kobe City General Hospital, Kobe); T. Izumi (Jichi Medical School Hospital, Shimotsuke); Y. Kashiwayama (Okayama Red Cross Hospital); T. Tananka (Ota Nishinouchi Hospital, Koriyama); A. Sato (Iwate Prefectural Central Hospital, Morioka); K. Kawakami (Suzuka General Hospital, Suzuka); U. Sawada (Nihon University Itabashi Hospital); T. Hotta (Tokai University Hospital, Isehara); M. Tsuyuguchi (Tokushima Municipal Hospital, Tokushima); Y. Tsunoda (Fujita General Hospital, Kunimi); A. Fujita (Showa General Hospital, Tokyo); T. Katagiri (Tokyo Medical University Hospital, Tokyo); H. Okazaki (Sakai Municipal Hospital, Sakai); T. Yoshino and K. Shinagawa (Okayama University, Okayama) and T. Nakaseko (Chiba University Hospital, Chiba).

acknowledgements

We are grateful to Hisashi Baba (Department of Infectious Diseases, Nagoya University Hospital) and Katsuyuki Aozasa (Department of Pathology, Osaka University Graduate School of Medicine) for their critical reading of the manuscript and for providing us useful discussion. We thank all the staff and resident members of the participating institutions in Japan. A complete list of participating institutions appears in the Appendix. This study was supported by Health and Labour Sciences Research Grants.

references

1. Aozasa K, Takakuwa T, Nakatsuka S. Pyothorax-associated lymphoma: a lymphoma developing in chronic inflammation. Adv Anat Pathol 2005; 12: 324–331.
2. Iuchi K, Aozasa K, Yamamoto S et al. Non-Hodgkin's lymphoma of the pleural cavity developing from long-standing pyothorax. Summary of clinical and pathological findings in thirty-seven cases. Jpn J Clin Oncol 1989; 19: 249–257.
3. Cheung C, Schonell M, Manoharan A. A variant of pyothorax-associated lymphoma. Postgrad Med J 1999; 75: 613–614.
4. Mori N, Yatabe Y, Narita M et al. Pyothorax-associated lymphoma. An unusual case with biphenotypic character of T and B cells. Am J Surg Pathol 1996; 20: 760–766.
5. Nakamura S, Sasajima Y, Koshikawa T et al. Ki-1 (CD30) positive anaplastic large cell lymphoma of T-cell phenotype developing in association with long-standing tuberculous pyothorax: report of a case with detection of Epstein–Barr virus genome in the tumor cells. Hum Pathol 1995; 26: 1382–1385.
6. Nakatsuka S, Yao M, Hoshida Y et al. Pyothorax-associated lymphoma: a review of 106 cases. J Clin Oncol 2002; 20: 4255–4260.
7. Aozasa K. Pyothorax-associated lymphoma. Int J Hematol 1996; 65: 9–16.
8. Kim Y, Lee SW, Choi HY et al. A case of pyothorax-associated lymphoma simulating empyema necessitatis. Clin Imaging 2003; 27: 162–165.
9. Androulaki A, Drakos E, Hatzianastassiou D et al. Pyothorax-associated lymphoma (PAL): a western case with marked angiocentricity and review of the literature. Histopathology 2004; 44: 69–76.
10. Aozasa K, Ohsawa M, Iuchi K et al. Artificial pneumothorax as a risk factor for development of pleural lymphoma. Jpn J Cancer Res 1993; 84: 55–57.
11. Fukayama M, Ibuka T, Hayashi Y et al. Epstein–Barr virus in pyothorax-associated pleural lymphoma. Am J Pathol 1993; 143: 1044–1049.
12. Sasajima Y, Yamabe H, Kobashi Y et al. High expression of the Epstein–Barr virus latent protein EB nuclear antigen-2 on pyothorax-associated lymphomas. Am J Pathol 1993; 143: 1280–1285.
13. Oken MM, Creech RH, Tormey DC et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982; 5: 649–655.
14. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. A predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med 1993; 329: 987–994.
15. Jaffe ES, Harris NL, Stein H, Vardiman JW (eds). Pathology & Genetics. Tumours of Hematopoietic and Lymphoid Tissues. Geneva: World Health Organization 2001.
16. Lister TA, Crowther D, Sutcliffe SB et al. Report of a committee convened to discuss the evaluation and staging of patients with Hodgkin's disease: Cotswolds meeting. J Clin Oncol 1989; 7: 1630–1636.
17. Iuchi K, Ichimiya A, Akashi A et al. Non-Hodgkin's lymphoma of the pleural cavity developing from long-standing pyothorax. Cancer 1987; 60: 1771–1775.
18. Aruga T, Itami J, Nakajima K et al. Treatment for pyothorax-associated lymphoma. Radiother Oncol 2000; 56: 59–63.
19. Nakatsuka S-i, Yao M, Hoshida Y et al. Pyothorax-associated lymphoma: a review of 106 cases. J Clin Oncol 2002; 20: 4255–4260.
20. Ohmori M, Ishikawa N, Yoshiyama T et al. Current epidemiological trend of tuberculosis in Japan. Int J Tuberc Lung Dis 2002; 6: 415–423.
21. Mansharamani NG, Koziel H. Chronic lung sepsis: lung abscess, bronchiectasis, and empyema. Curr Opin Pulm Med 2003; 9: 181–185.
22. Nador RG, Cesarman E, Chadburn A et al. Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi's sarcoma-associated herpes virus. Blood 1996; 88: 645–656.
23. Aozasa K, Inoue A, Tajima K et al. Malignant lymphomas of the thyroid gland. Analysis of 79 patients with emphasis on histologic prognostic factors. Cancer 1986; 58: 100–104.
24. Parsonnet J, Hansen S, Rodriguez L et al. *Helicobacter pylori* infection and gastric lymphoma. N Engl J Med 1994; 330: 1267–1271.
25. Zucca E, Conconi A, Pedrinis E et al. Nongastric marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue. Blood 2003; 101: 2489–2495.
26. Zinzani PL, Magagnoli M, Galieni P et al. Nongastrointestinal low-grade mucosa-associated lymphoid tissue lymphoma: analysis of 75 patients. J Clin Oncol 1999; 17: 1254.
27. Derringer GA, Thompson LD, Frommelt RA et al. Malignant lymphoma of the thyroid gland: a clinicopathologic study of 108 cases. Am J Surg Pathol 2000; 24: 623–639.
28. Hara S, Kami M, Miyakoshi S et al. Central nervous system involvement in pyothorax-associated lymphoma: ring enhancement on CT scan. Ann Hematol 2001; 80: 174–177.
29. Asakura H, Togami T, Mitani M et al. Usefulness of FDG-PET imaging for the radiotherapy treatment planning of pyothorax-associated lymphoma. Ann Nucl Med 2005; 19: 725–728.
30. Fukayama M, Hayashi Y, Ooba T et al. Pyothorax-associated lymphoma: development of Epstein–Barr virus-associated lymphoma within the inflammatory cavity. Pathol Int 1995; 45: 825–831.
31. Kanno H, Aozasa K. Mechanism for the development of pyothorax-associated lymphoma. Pathol Int 1998; 48: 653–664.
32. Yamato H, Ohshima K, Suzumiya J, Kikuchi M. Evidence for local immunosuppression and demonstration of c-myc amplification in pyothorax-associated lymphoma. Histopathology 2001; 39: 163–171.
33. Straus SE, Cohen JI, Tosato G, Meier J. NIH Conference. Epstein–Barr virus infections: biology, pathogenesis, and management. Ann Intern Med 1993; 118: 45–58.

34. Liu A, Takakuwa T, Fujita S et al. Alterations of DNA damage-response genes ATM and ATR in pyothorax-associated lymphoma. Lab Invest 2005; 85: 436–446.
35. Nishiu M, Tomita Y, Nakatsuka S et al. Distinct pattern of gene expression in pyothorax-associated lymphoma (PAL), a lymphoma developing in long-standing inflammation. Cancer Sci 2004; 95: 828–834.
36. Takakuwa T, Ham MF, Luo WJ et al. Loss of expression of Epstein–Barr virus nuclear antigen-2 correlates with a poor prognosis in cases of pyothorax-associated lymphoma. Int J Cancer 2006; 118: 2782–2789.
37. A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. N Engl J Med 1993; 329: 987–994.
38. Olsson H, Olsson ML, Ranstam J. Late age at first full-term pregnancy as a risk factor for women with malignant lymphoma. Neoplasma 1990; 37: 185–190.
39. Herrinton LJ, Friedman GD. Cigarette smoking and risk of non-Hodgkin's lymphoma subtypes. Cancer Epidemiol Biomarkers Prev 1998; 7: 25–28.
40. Chiu BC, Cerhan JR, Gapstur SM et al. Alcohol consumption and non-Hodgkin lymphoma in a cohort of older women. Br J Cancer 1999; 80: 1476–1482.