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ORIGINAL ARTICLE

Whole brain radiation alone produces favourable outcomes for AIDS-related primary central nervous system lymphoma in the HAART era

Hirokazu Nagai[1], Takashi Odawara[2], Atsushi Ajisawa[3], Shotaro Hagiwara[4], Tomoyuki Watanabe[5], Tomoko Uehira[6], Hideki Uchiumi[7], Mihoko Yotsumoto[8], Toshikazu Miyakawa[9], Akira Watanabe[10], Toshiyuki Kambe[11], Mitsuru Konishi[12], Seiji Saito[13], Soichiro Takahama[14], Masao Tateyama[15], Seiji Okada[16]

[1]Department of Hematology, National Hospital Organization Nagoya Medical Center, Nagoya; [2]Department of Infectious Diseases and Applied Immunology, The Institute of Medical Science, The University of Tokyo, Tokyo; [3]Division of Infectious Disease, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo; [4]Division of Hematology, International Medical Center of Japan, Tokyo; [5]Faculty of Psychological and Physical Science, Aichi Gakuin University, Nisshin; [6]Department of Infectious Diseases, National Hospital Organization Osaka National Hospital, Osaka; [7]Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, Gunma; [8]Department of Laboratory Medicine, Tokyo Medical University, Tokyo; [9]Department of Hematology, Faculty of Medical and Pharmaceutical Sciences Kumamoto University, Kumamoto; [10]Division of Control and Treatment of Infectious Diseases, Chiba University Hospital, Chiba; [11]Department of Respiratory Medicine, Asahi General Hospital, Asahi; [12]Center for Infectious Diseases, Nara Medical University, Nara; [13]Division of Blood Transfusion Services, Hiroshima University Hospital, Hiroshima; [14]Division of Immunology and Infectious Diseases, Clinical Research Institute, National Hospital Organization Kyushu Medical Center, Fukuoka; [15]First Department of Internal Medicine, Faculty of Medicine, University of the Ryukyus, Okinawa; [16]Center for AIDS Research, Kumamoto University, Kumamoto, Japan

Abstract

Primary central nervous system lymphoma (PCNSL) related to acquired immunodeficiency syndrome (AIDS) is a lethal disorder, but the recent application of highly active antiretroviral therapy (HAART) has significantly improved prognosis. This retrospective cohort study of AIDS-related PCNSL examined the actual clinical outcomes and prognostic variables affecting overall survival (OS) in the HAART era. Twenty-three newly diagnosed AIDS-related PCNSL at 12 regional centre hospitals for HIV/AIDS in Japan between 2002 and 2008 were consecutively enrolled. The estimated 3-yr OS rate of the entire cohort was 64% (95%CI, 41.0–80.3%). Whole brain radiation therapy (WBRT) had an independent positive impact on survival (WBRT ≥30 Gy vs. others, $P = 0.02$). Nine of 10 patients with a good performance status (PS) (0–2) remained alive with complete response, whereas 10 (77%) of 13 of those with a poor PS (3–4) died mostly after a short period. The estimated 3-yr OS rate of the groups with a good and poor PS was 100% and 38% (95%CI, 14–63%), respectively ($P = 0.01$). Leukoencephalopathy (grade ≥ 2) developed in 21% of those that survived more than 12 months after radiation. The patients receiving a curative intent radiation dose (≥30 Gy) of WBRT achieved prolonged survival while maintaining a good quality of life in the HAART era, especially among patients with a favourable PS.

Key words acquired immunodeficiency syndrome; primary central nervous system lymphoma; highly active antiretroviral therapy; whole brain radiation; leukoencephalopathy

Correspondence Hirokazu Nagai, Clinical Research Center, National Hospital Organization Nagoya Medical Center, 4-1-1, Sannomaru, Naka-ku, Nagaoya 460-0001, Japan. Tel: 81 52 951 1111; Fax: 81 52 951 9075; e-mail: nagaih@nnh.hosp.go.jp

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Primary central nervous system lymphoma (PCNSL) is one of several acquired immunodeficiency syndrome (AIDS)-defining illnesses (ADI), and it is the second most frequent cerebral mass lesion after toxoplasmosis among those infected with the human immunodeficiency virus (HIV) (1). This type of lymph oma typically arises at the severely immunocompromised late stage of HIV infection, and CD4+ cell counts at diagnosis are

$<20/\mu L$ in most patients (2, 3). The pathological diagnosis is usually diffuse large B cell lymphoma (4, 5). Although Epstein–Barr virus (EBV) is generally absent from PCSNL in immunocompetent patients, about 80–100% of AIDS-related PCNSL is associated with EBV in lymphoma lesions (6). Pathogenetic roles of EBV infection in AIDS-related PCNSL have been suggested. The incidence of PCSNL has significantly decreased since highly active antiretroviral therapy (HAART) was introduced (7), as have all other types of EBV-positive AIDS-related lymphomas (8). Before the introduction of HAART, the prognosis of AIDS-related PCNSL was dismal and median survival was typically <3 months (9–13). After HAART became available, the clinical outcome of AIDS-related PCNSL radically improved (14–19). However, a standard management procedure for these patients remains to be established. We performed a nationwide retrospective survey to elucidate the actual clinical outcome and to identify the significant prognostic variables of AIDS-related PCNSL in the HAART era, in addition to determining the quality of life of long-term survivors of whole brain radiation.

Patients and methods

This retrospective cohort study examined the clinical outcomes of patients diagnosed with AIDS-related PCNSL (in the HAART era) who visited the 12 regional hospitals for HIV/AIDS in Japan during the period January 2002–December 2008. HAART was defined as two kinds of nucleoside reverse transcriptase inhibitor combined with protease inhibitor or non-nucleoside reverse transcriptase inhibitor. HAART was introduced in 1997 in Japan. This study received approval from the responsible ethics committee.

Patients

The patients included in this study were newly diagnosed with AIDS-related PCNSL during the study period. The pathological diagnosis of each institution was accepted. Those with disseminated lymphoma lesions other than CNS were excluded, whereas those diagnosed with possible AIDS-related PCNSL according to some clinical-based modalities were included. All patients who satisfied the above-mentioned criteria were serially enrolled. Data from all patients registered in this study were statistically analysed.

Clinical characteristics of the patients

Data regarding age, Eastern Cooperative Oncology Group (ECOG) performance status (PS) at diagnosis, number of CD4+ cells at diagnosis, HIV viral load at diagnosis, prior AIDS, concurrent opportunistic diseases, presence of severe neurological symptoms at diagnosis and prior HAART were analysed. Diagnostic modalities and the primary therapy of all enrolled patients were also determined and analysed.

A complete response (CR) to treatment was defined as the disappearance of all clinical evidence of disease at the completion of first induction therapy. The presence of residual disease but with ≥50% decrease in the sum of the products of the greatest diameter was defined as a partial response (PR). Intra-ocular lesions were not assessed in any of the patients. Overall survival (OS) was defined as the interval from diagnosis to death from any cause. Grades of leukoencephalopathy were evaluated based on each institutional decision according to CTCAE v3.0 (20).

Statistical analysis

The primary endpoint of this study was the identification of factors that significantly impacted OS. Both multivariate and univariate Cox regression analyses were performed to assess the effects of treatment and the various baseline prognostic factors on OS. All P values are two-tailed. OS was assessed using the Kaplan–Meier method. Groups divided by clinical variables were compared using the log-rank test. Data were statistically analysed using STATA 10.0 (STATACORP LP, College Station, TX, USA).

Results

Patients' background

Table 1 shows the characteristics of the 23 registered patients with AIDS-related PCNSLs. The median age was 41 (21–60), and male gender accounted for 96% of the patients. Eleven patients developed PCNSL as ADI, and 12 patients were diagnosed with AIDS before the development of PCNSL. Radiological imaging examinations were carried out in all 23 patients. Eleven were diagnosed with PCNSL based on both imaging features and the presence of EBV DNA in cerebrospinal fluid by PCR without a brain biopsy, while three were diagnosed by radiological MRI and SPECT imaging, and the favourable response of brain tumour by radiation therapy. One patient was diagnosed at autopsy. PCR tests of EBV genome in cerebrospinal fluid were performed in 20 patients, and 16 patients out of them showed positivity (80%, 16/20). Seven (30%) were treated with HAART at diagnosis; and finally, HAART was administered to 91% of the patients. Concurrent opportunistic diseases were identified in 15 (65%). Twelve patients had other ADIs before the diagnosis of PCSNL. The median count

Table 1 Characteristics of patients with AIDS-related PCNSL (n = 23)

Gender	*n* (%)
Male	22 (96%)
Female	1 (4%)
Age(years)	
Median	41
Range	21–60
AIDS diagnosed before PCNSL, n (%)	12 (52 %)
Pneumocystis jiroveci pneumonia	6
Cytomegalovirus infection	2
Candidiasis	3
Cryptosporidiosis	1
HAART therapy before PCNSL, *n* (%)	7 (30%)
Opportunistic diseases at diagnosis of PCNSL, *n* (%)	15 (65%)
CD4+ cell count at PCNSL diagnosis (cells/mL)	
Median	22
Range	1–657
HIV viral load at diagnosis of PCSNL (copy/mL)	
Median	77000
Range	0–1.23 × 10^7
PS at diagnosis of PCNSL, n (%)	
0	2 (7%)
1	4 (17%)
2	4 (17%)
3	5 (22%)
4	8 (35%)
Ataxia and/or cognitive disturbance (grade ≥ 3) at PCNSL diagnosis, *n* (%)	12 (52%)
Diagnostic modality, n (%)	
Biopsy	8 (35%)
Imaging only	3 (13%)
Autopsy	1 (4%)
Positive for EBV genome in CSF by PCR	11 (48%)
PCR test of EBV genome in CSF	(n = 20)
Positive	16/20
Negative	4/20
HAART after diagnosis of PCNSL, *n* (%)	21 (91%)

HAART, highly active antiretroviral therapy; PCNSL, Primary central nervous system lymphoma.
Neurological symptoms graded according to CTCAE v3.0.

of CD4+ cells at PCNSL diagnosis was 22/μL (1–657), and 13 (57%) of 23 patients had a poor PS at diagnosis (3–4). Twelve patients (52%) had severe neurological symptoms defined as ataxia or cognitive disturbance grade ≥ 3 according to CTCAE v3.0 at the time of PCNSL diagnosis.

Treatment and initial response

Twenty-one patients were treated by radiotherapy alone, and only one received combined modality treatment (high-dose methotrexate and cytoxan followed by whole brain radiation (WBRT)). One patient received only best supportive care (BSC). Thirteen patients received a curative intent radiation dose (≥30 Gy) of WBRT. The overall response rate to all of these strategies including BSC was 65%, while the response rate to a curative intent WBRT was 77% (Table 2).

Table 2 Initial treatment modality and early clinical response

Treatment modality	*n* (%)	CR/PR, *n* (%)
Whole brain radiation ± local boost (≥30 Gy)	13 (57%)	10 (77%)
Whole brain radiation (<30 Gy)	5 (22%)	1 (20%)
Local brain radiation	3 (13%)	3 (100%)
Combined modality therapy	1 (4%)	1 (100%)
Best supportive care	1 (4%)	0 (0%)
Total	23	15 (65%)

Combined modality therapy: high-dose methotrexate and high-dose cytoxan followed by WBRT.

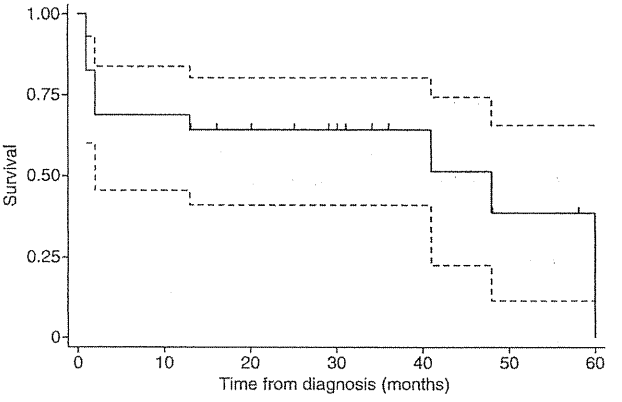

Figure 1 Overall survival curves (All patients with Primary central nervous system lymphoma). Kaplan–Meier estimate with 95% CI (dashed line). Marks indicated censored observation. The total number of censored cases was 12.

Clinical variables affecting OS

The estimated 3-yr OS rate of all patients was 64% (95% CI, 41.0–80.3%) with a 20-month median follow-up (Fig. 1).

Significant clinical variables that affected OS were distinguished using univariate and multivariate analyses. Univariate analysis showed that better PS (ECOG) at diagnosis (0–2 vs. 3–4) and receiving curative intent radiation dose (≥30 Gy) of WBRT (WBRT ≥ 30 Gy vs. others) were significant positive survival predictors (P = 0.01 and <0.01, respectively), and younger age (<40 yr vs. ≥40) also tended to affect positively on OS but did not reach statistical significance (P = 0.12) (Table 3). Multivariate analysis of these three variables revealed that receipt of WBRT (≥30Gy) had an independent positive impact on OS (P = 0.02) (Table 4). Favourable PS (ECOG) was second strong predictor to

Table 3 Factors affecting OS (univariate analysis)

Clinical variables	No. of patients	Median survival (Month)(95% CI)	*P* value*
Age (yr)			
<40	9	41 (1.7–80.3)	0.12
≥40	14	2 (2–34.1)	
PS (ECOG)			
0–2	10	48 (N/A)	0.01
3–4	13	2 (0–12.6)	
CD4 (cells/mL)			
<50	18	60 (N/A)	0.77
≥50	5	41 (0–97.7)	
Prior AIDS			
(−)	11	48 (0–114.1)	0.69
(+)	12	41(0–93.2)	
Prior HAART			
(−)	16	41 (0.6–81.4)	0.52
(+)	7	48(12.2–83.9)	
HIV viral load (copy/mL)			
$\leq 1 \times 10^5$	13	13 (N/A)	0.07
$>1 \times 10^5$	10	60 (N/A)	
Severe neurological symptoms at PCNSL onset			
(−)	12	NR	0.12
(+)	11	41 (12.3–69.7)	
Opportunistic disease			
(−)	8	48 (33.8–62.2)	0.34
(+)	15	NR	
Therapy			
WBRT (≥30 Gy)	13	60 (N/A)	<0.01
Other	10	2 (0.7–3.3)	
Response rate			
SD/PD	8	2 (N/A)	0.14
CR/PR	15	48(31.7–64.3)	

*Log-rank test.

CR, complete response; ECOG, Eastern Cooperative Oncology Group; HAART, highly active antiretroviral therapy; N/A, not applicable; NR, not reached; OS, overall survial; PCNSL, Primary central nervous system lymphoma; PR, partial response; WBRT, whole brain radiation therapy.

Table 4 Factors affecting OS (multivariate analysis)

Clinical variables	Hazard Ratio (95% CI)	*P* value
Age		
<40	1	0.09
≥40	5.27 (0.76–36.1)	
PS		
0–2	1	0.06
3–4	9.24 (0.86–96.43)	
Therapy		
WBRT (≥30Gy)	1	0.02
Other	8.10 (1.35–48.43)	

OS, overall survial; PS, performance status; WBRT, whole brain radiation therapy.

better OS with highest hazard ratio but was not statistically significant ($P = 0.06$).

Nine of ten patients with a good PS (0–2) remained alive with CR (all received curative intent WBRT), nevertheless 10 (77%) of 13 of those with a poor PS (3–4) died mostly within 2 months (7/10; 70%). The estimated 3-yr OS rate of each group was 100% and 38% (95% CI, 14–63%), respectively ($P = 0.01$, log-rank test) (Fig. 2A).

The 3-yr OS rates for 13 patients who received WBRT (≥30Gy) estimated from Kaplan–Meier survival curves and in the group that received a different type of treatment were 92% (95% CI, 57–99%) and 24% (95% CI, 4–58%), respectively ($P < 0.01$, log-rank test) (Fig. 2B).

Leukoencephalopathy and PS in survivors

Leukoencephalopathy is a late-onset, serious adverse event associated with radiation therapy to the brain

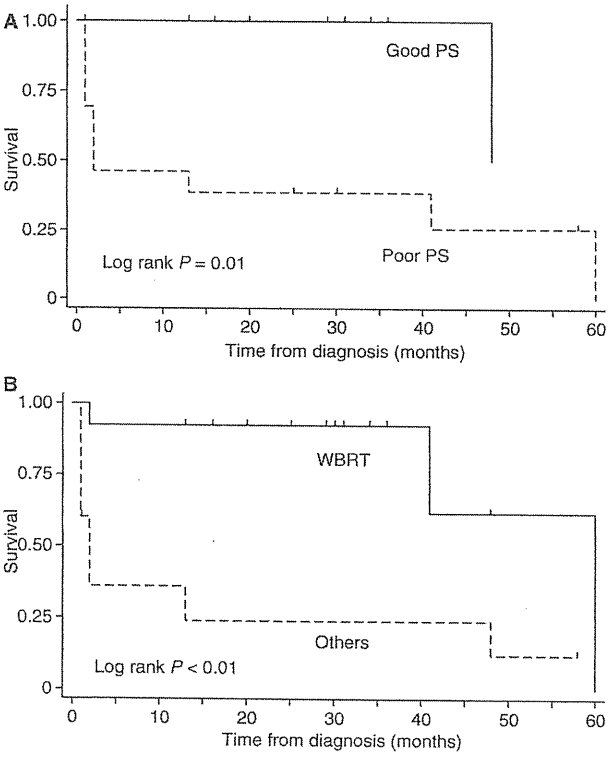

Figure 2 Overall survival curves. (A) Survival according to performance status (PS) at Primary central nervous system lymphoma(PCNSL) diagnosis. Solid line, patients with PS 0–2 (good PS); dashed line, patients with PS 3–4 (poor PS). Marks indicated censored observation. The number of censored cases was nine in good PS group and three in poor PS group. (B) Survival according to initial therapy for PCNSL. Solid line, patients receiving WBRT (≥30 Gy); dashed line, patients receiving other therapy. Marks indicated censored observation. The number of censored cases was 10 in WBRT (≥30 Gy) group and two in others' group.

Table 5 Current status and neurological symptoms of patients who survived ≥12 months

Patient No.	Survival (months)	Neurological symptoms Ataxia/cognitive disturbance	Leukoencephalopathy	PS
1	48	0/2	1	1
2	25	4/3	3	4
3	30	0/0	0	0
4	31	3/0	0	1
5	34	2/1	0	1
6	13	0/0	0	0
7	58	1/0	1	1
8	20	0/1	0	1
9	16	0/0	0	0
10	29	0/0	0	0
11	36	0/0	0	0

PS, performance status.
Neurological symptoms and leukoencephalopathy graded according CTCAE v3.0.

(21–23). We analysed the incidence and grade of radiation-related leukoencephalopathy, which was assessed among the patients who survived for ≥12 months after initial radiation therapy. Leukoencephalopathy was graded according to CTCAE v3.0. Twelve patients survived for ≥12 months after WBRT (≥30 Gy), and two patients survived for ≥12 months after local brain radiation. Among these fourteen patients, five (36%) were diagnosed with leukoencephalopathy by CT or MRI imaging, and three of them had leukoencephalopathy grade ≥_2 (median follow-up, 30 months; range, 13–58 months). No signs of leukoencephalopathy have developed in eight of the 12 survivors who received WBRT (≥30 Gy).

We also analysed the current neurological symptoms and PS of 11 living patients. The PS of all patients except for one with severe neurological symptoms was ≤1 (Table 5).

Discussion

AIDS-related PCNSL was a highly lethal ADI in the pre-HAART era, with survival being generally quoted as <3 months (9–13). Many studies have indicated improved survival of patients with AIDS-related PCNSL after the introduction of HAART (14–19), but standard management for such patients has not been established.

Our retrospective cohort study of AIDS-related PCNSL in the HAART era showed favourable survival especially in patients with a good PS who underwent WBRT at the dosage of ≥30 Gy designed for curative intent. Univariate analysis showed that significant clinical factors for a favourable OS were a good PS (ECOG 0–2) at diagnosis and the receipt of WBRT (≥30 Gy). Multivariate analysis selected the receipt of WBRT (≥30 Gy) as the statistically significant clinical factor for a favourable OS. Even in the HAART era, low CD4+ cell counts was reported to be a significant poor prognostic factors for AIDS-related systemic non-Hodgkin lymphoma (24). Our data could not show that CD4+ cell count had the prognostic effect in AIDS-related PCNSL in the HAART era. Systemic non-Hodgkin lymphomas were usually treated with systemic chemotherapy, which could impair host immune status, and one of the major causes of death was severe infection during treatment. Thus CD4+ cell count in AIDS-related systemic lymphoma would be more important than in PCNSL treated with brain radiation, which might have minimal damages to host immunity, in the context of control of infectious complications.

Some reports during the HAART era have indicated improved survivals of patients with PCNSL after treatment with curative intent WBRT. However, in each study, all patients with PCNSL were not reported to be actually treated with this modality in the HAART era; the largest study comprised 25 patients (16), but only 10 of the patients described in that study underwent both WBRT (≥30 Gy) and therapy with two or three anti-retroviral agents. All of our 23 patients were diagnosed in the HAART era, 12 were treated with both HAART and the curative intent WBRT, and we followed up the survivors for longer (median: 18 months) than any other studies (14–16). The 3-yr OSs of the entire cohort, the group with a favourable PS, and the group that underwent WBRT were 64%, 100% and 92%, respectively. These data showed that the survival of patients with AIDS-related PCNSL could be favourable if treated with curative WBRT under a relatively good general PS during the HAART era. The reported 3-yr OS of patients with non-AIDS-related PCNSL is 29% when treated only with brain radiation (25) and 50–70% when treated with high-dose MTX-based chemotherapy plus brain radiation (26, 27). Our survival findings were comparable with those of immunocompetent patients and might be superior if PS is favourable at diagnosis.

One major difference between AIDS-related and immunocompetent PCNSL is considered the consistent association with EBV. The presence of EBV in the setting of prolonged immunosuppression might cause B cell activation that result in the development of PCNSL. Anti EBV therapy or HAART with/without ganciclovir and interleukin two have been applied to treat AIDS-related PCNSL, with some good responses (28–30). In the context of these concepts, the role of chemotherapy in AIDS-related PCNSL remains obscure. The adequacy of such therapeutic modalities, as WBRT, a high-dose MTX-based regimen, and com-

bined therapy should be further analysed in prospective clinical trials.

Our long-term follow-up allowed an analysis of the incidence of leukoencephalopathy, general status and neurological symptoms after therapy was completed. The adverse effects of brain radiation comprise an acute type that can occur even during radiation, an early-delayed type that occurs 2–4 months later, and a late type that manifests about 9–12 months later. Leukoencephalopathy is a late-onset complication that requires long-term follow-up. Our patients were followed up for 13–56 months, which should have allowed most leukoencephalopathy to be recognised. The incidence was 36% (5/14), and severe events (grade $\geq$ 2) developed in three patients. The PS of all eleven survivors except for one with grade 3 leukoencephalopathy was $\leq$1. Two patients showed cognitive disturbance of grade $\geq$ 2, and three showed ataxia of grade $\geq$ 2. PCNSL itself, even in the remission status, might account for some neurological symptoms. Longer observation might be required to determine the final outcome of late-onset radiation-damage to the brain.

Our findings suggested that patients with AIDS-related PCNSL achieved durable remission after curative intent WBRT, especially those with a good PS during the HAART era. These findings indicate that early diagnosis of this disease before symptoms can affect general status could result in prolonged survival with a favourable outcome. Thus, surveillance of a high-risk population for HIV infection and close follow-up of patients infected with HIV should improve the outcomes of AIDS-related PCNSL.

Acknowledgements

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Impact of Peripheral Lymphocyte Count on the Sensitivity of 2 IFN-γ Release Assays, QFT-G and ELISPOT, in Patients with Pulmonary Tuberculosis

Kosaku Komiya[1,2], Haruyuki Ariga[1], Hideaki Nagai[1], Shinji Teramoto[1], Atsuyuki Kurashima[1], Syunsuke Shoji[1] and Yutsuki Nakajima[1]

Abstract

Objective This study evaluated the effect of peripheral lymphocyte count on 2 interferon-gamma release assays [QuantiFERON TB-Gold (QFT-G) and enzyme-linked immunospot (ELISPOT)] and their sensitivity in patients with pulmonary tuberculosis, including HIV-negative immunocompromised patients.

Patients and Methods Two hundred thirty patients with microbiologically confirmed active pulmonary tuberculosis were subjected to the tests. Lymphocyte counts were analyzed simultaneously.

Results Overall sensitivity was 74% (159/215; 95% CI, 68-80%) for QFT-G and 92% (198/215; 89-96%) for ELISPOT ($p<0.0001$). In patients with peripheral lymphocyte counts of $\geq 1000/\mu L$, sensitivity was high for both QFT-G (88%, 111/126; 82-94%) and ELISPOT (97%, 122/126; 94-100%). However, the sensitivity decreased significantly with decreasing peripheral lymphocyte count for both QFT-G (test for trend $p<0.0001$) and ELISPOT (test for trend $p=0.007$). When lymphocyte counts were $<500/\mu L$, the sensitivity was 81% (25/31; 66-96%) for ELISPOT, but only 39% (12/31; 21-57%) for QFT-G.

Conclusion Both QFT-G and ELISPOT are sensitive methods for detecting active pulmonary tuberculosis, but their sensitivity partly depends on peripheral lymphocyte counts. At low lymphocyte count conditions, ELISPOT is superior to QFT-G for detecting tuberculosis, irrespective of age, gender, and nutrition.

Key words: IFN-γ release assay, lymphocyte count, pulmonary tuberculosis

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Introduction

Until recently, the detection of latent *Mycobacterium tuberculosis* infection (LTBI) was only based on tuberculin skin testing (TST). With the advent of highly specific interferon-gamma (IFN-γ) release assays (IGRAs), however, alternatives are now available. False-positive results with TST occur because of antigenic cross-reactivity of the purified protein derivative with non-*M. tuberculosis* infections, including those caused by Bacille Calmette-Guerin (BCG) vaccination. In contrast, IGRAs are not affected by BCG vaccination and most non-*M. tuberculosis* infections, and are at least as sensitive as TST for detecting active tuberculosis (1, 2). Therefore, the Centre for Disease Control and Prevention (CDC) guideline recommends that IGRAs are more useful than TST for detection of LTBI (3, 4).

Although the overall sensitivity of IGRAs such as QuantiFERON TB Gold (QFT-G) and enzyme-linked immunospot (ELISPOT) were reported to be very high, these were evaluated only in studies that excluded distinctly immunocompromised patients (5-20). However, immunocompromised hosts, including elderly and patients receiving immunosuppressive agents, have a greater risk of tuberculosis than usual hosts. Some studies on immunocompromised hosts have reported that IGRAs are more useful than TST, but the sensitivity of IGRAs was diminished in those patients (1, 6-9). It has also been reported that CD4 lympho-

[1]Department of Pulmonary Medicine, National Hospital Organization Tokyo National Hospital, Tokyo and [2]Department of Internal Medicine 2, Oita University, Oita
Received for publication March 15, 2010; Accepted for publication June 1, 2010
Correspondence to Dr. Kosaku Komiya, komiyakh1@yahoo.co.jp

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cyte counts affect the positive and intermediate responses of QFT-G (8, 10-15).

Therefore, this study was conducted to quantify the effect of peripheral blood lymphocyte count on QFT-G and ELISPOT and to evaluate their sensitivity in patients with pulmonary tuberculosis, including HIV-negative immunocompromised patients.

Methods

Setting, patient recruitment, and eligibility

In total, 602 patients who were clinically suspected of pulmonary tuberculosis infection were tested with QFT-G and ELISPOT simultaneously, between January 2008 and June 2009 at the Department of Pulmonary Medicine, Tokyo National Hospital, National Health Organization (Tokyo, Japan). Patients provided written and informed consent. Two hundred and fifteen HIV-negative patients with *M. tuberculosis* infection confirmed by positive culture and/or PCR for *M. tuberculosis* from sputum or bronchoalveolar lavage were recruited prospectively. Patients were tested before or within 14 days of initiation of chemotherapy. Information on immunosuppressive therapy, malignancies, and bedridden status was collected from each patient at the time of enrollment. Laboratory findings [peripheral blood cell count, lymphocyte count, serum albumin (Alb), C-reactive protein (CRP), and haemoglobin A1c (HbA1c)], sputum smear status, and radiological findings were obtained at the same time.

QFT-G and ELISPOT were performed by the following procedures. Furthermore, the sensitivity of each assay was calculated and the results were analysed according to peripheral lymphocyte counts. We compared the mean peripheral lymphocyte counts between groups with positive, negative, and indeterminate results of the 2 IGRAs.

Furthermore, univariate and multivariate analyses were performed with respect to the significant contributing factors for the results of the sensitivity of these assays in detecting pulmonary tuberculosis.

This study was approved by the ethics committee of Tokyo National Hospital, National Health Organization (Tokyo, Japan).

QFT-G test

The QFT-G test was performed according to the recommendations of the manufacturer (Cellestis, Ltd., Carnegie, Victoria, Australia), and the test results were evaluated according to the guidelines of CDC (21).

ELISPOT assays

Peripheral blood mononuclear cells were separated from the heparinized blood sample by density centrifugation using BD Vacutainer® Cell Preparation Tubes (Becton, Dickinson and Company, Franklin Lakes, NJ). We seeded pre-coated IFN-γ ELISPOT plates (BD) with 2.5×10^5 cells per well in AIM-V medium (GIBCO), and then incubated with ESAT-6 (5 g/mL) and CFP-10 (5 g/mL) peptides at 37℃ in 5% carbon dioxide for 16 h. A negative control (no mitogen or antigen) and a positive control (phytohemagglutinin, 5 g/mL) were included. After incubation, the wells were washed and developed with a conjugate against the antibody used and an enzyme substrate. Spot-forming units were counted using a KS ELISPOT system. The results of ELISPOT were also interpreted according to the following criteria: The test result is positive if 1) the negative control has 0-5 spots and (ESAT-6 or CFP-10 spot count) (negative control spot count) 6, and/or 2) if the negative control has 6-10 spots and (ESAT-6 or CFP-10 spot count) 2× (negative control spot count). The test result is negative if the above criteria are not met and the positive control is valid. If the positive control is indeterminate (<20 spots) and both antigens are negative, the test result is regarded as indeterminate.

Statistical analysis

Data were entered using PASW statistics 17.0 for Windows and analyzed. Analyses were 2-sided, confidence intervals (CI) were 95%, and results were considered significant when $p<0.05$. Continuous variables were tested for normality using the Shapiro-Wilk test and compared using Student's t-test distribution. In other cases, the Mann-Whitney test and Kruskal-Wallis test were used. The chi-square test was applied for comparing categorical variables unless 1 of the categories had less than 20 observations, in which case, the Fisher's exact test was applied. A chi-square test and a linear trend test were conducted for testing trends. Odds ratio analysis for risk factors was performed by both univariate and multivariate analyses.

Results

Patient characteristics and IGRA results

Two hundred and thirty patients were examined in the study. Demographic and clinical characteristics of the study patients are summarized in Table 1. Two hundred and thirteen were Japanese in origin, and the others were either Chinese or Filipino. A total of 215 were HIV negative. The primary diseases of the 18 patients who were undergoing immunosuppressive treatment were rheumatoid arthritis (7 patients), idiopathic interstitial pneumonia (4), microscopic polyangitis (2), sarcoidosis (1), autoimmune hepatitis (1), aplastic anaemia (1), Crohn disease (1), and Still disease (1).

Overall sensitivity is shown Table 2. Figure 1 and 2 show the distribution of results of ELISPOT according to the QFT-G result and vice versa, respectively. Among the 3 subgroups of 160 (74%) positive, 49 (23%) negative, and 6 (3%) indeterminate QFT-G result subjects, the median peripheral lymphocyte count was highest in the positive group [median: 1,247; interquartile range (IQR): 860, 1,680], intermediate in the negative group (median: 732; IQR: 475, 1,120), and lowest in the indeterminate group (median: 164; IQR: 141, 630) ($p<0.0001$). On the other hand, among the 2

Inter Med 49: 1849-1855, 2010 DOI: 10.2169/internalmedicine.49.3659

Table 1. Summery Demographic and Clinical Characteristics of Subjects

Category	Subcategory	n/N(%) or median(IQR)
Male sex		156/215(73)
Age		67(50,79)
Nationality	Japanese	213(99)
Smear status	Positive	182(84)
Photographic findings	Cavitation	105(49)
Immunosuppressive therapy		18/215(12)
Bedridden		38/215(18)*
Malignancies		10/215(4.7)
Laboratory findings	WBC count cells/μL	6400(5000,8125)
	Lymphocyte count cells/μL	1132(635,1556)
	Serum albumin g/dL	3.3(2.5,3.9)
	CRP mg/dL	4.27(0.99,8.81)
	HbA1c %	5.9(5.4,6.3)

IQR:Inter-quartile range
* Among bedridden group, two people overlapped with malignancies group, and other two people overlapped with immunosuppressive therapy group.

Table 2. QFT-G and ELISPOT Results for All Study Participants

	QFT-G	ELISPOT	p-value*
Positive, n(%)	160(74)	199(93)	p<0.0001
Negative, n(%)	49(23)	16(7.4)	p<0.0001
Indeterminate, n(%)	6(2.8)	0(0)	ND

*p-value for differentce between QFT-G and ELISPOT analysis.

subgroups of 199 (93%) positive and 16 (7%) negative ELISPOT result patients, the median peripheral lymphocyte count was higher in the positive group (median: 1,172; IQR: 704, 1,587) than in the negative group (median: 616; IQR, 387, 1,178) (p=0.012).

Patient characteristics in various subgroups were as follows. Of 6 patients with indeterminate QFT-G results, 2 had malignancies, 1 of which was a terminal status of gastric cancer. Among the 16 with negative results for both QFT-G and ELISPOT, 5 (31%) were receiving immunosuppressive therapy, 6 (38%) were bedridden (1 of whom was also receiving immunosuppressive therapy), and 1 had a malignancy. Among the 33 patients with negative results for QFT-G and positive results for ELISPOT, 2 (6%) were receiving immunosuppressive therapy, 9 (27%) were bedridden, and 2 (6%) had malignancies. All patients with positive QFT-G results were also ELISPOT-positive. Of the 160 patients with positive results on both assays, 11 (7%) were receiving immunosuppressive therapy, 22 (14%) were bedridden (1 of whom was also receiving immunosuppressive therapy), and 1 had a malignancy (and was also bedridden).

Influence of lymphocyte count on performance of QFT-G and ELISPOT analyses

When the peripheral lymphocyte count was ≥1,000/μL, the sensitivity of both tests was high: QFT-G (88%, 114/129; 95% CI, 82-94%) and ELISPOT (97%, 125/129; 95% CI, 94-100%). However, a clear trend of decreasing sensitivity with decreasing peripheral lymphocyte count was evident for both QFT-G (test for trend p<0.0001) and ELISPOT (test for trend p=0.007). This decline was more notable for QFT-G than for ELISPOT. ELISPOT was 81% (25/31; 95% CI, 66-96%) sensitive even when the lymphocyte count was less than 500/μL, whereas QFT-G was less than 70% sensitive when the lymphocyte count was <1,000/μL and less than 39% (12/31; 95% CI, 21-57%) when the lymphocyte count was <500/μL (Fig. 3).

For further analysis of factors affecting sensitivity, QFT-G and ELISPOT results were transformed to binary variables by combining negative and indeterminate results after logistic regression analysis. In order to increase statistical power, continuous variables were redefined as dichotomous variables using the following cut-off values: age 67 years (arbitrary), serum Alb 3.3 g/dL and CRP 4.72 mg/dL (median of study population), lymphocyte count 1,000 cells/μL (definition of lymphocytopenia), and HbA1c 6.5% (recommended by the American Diabetes Association). Odds ratios for factors affecting positive QFT-G results are listed in Table 3. The following factors were associated with a positive QFT-G result: lymphocyte count greater than 1,000 cells/μL (by both univariate and multivariate analyses), serum Alb greater than 3.3 g/dL (by both univariate and multivariate analyses), CRP greater than 4.72 mg/dL (by univariate analysis only), and bedridden status (by univariate analysis only). On the other hand, odds ratios for factors affecting positive ELISPOT results are listed in Table 4. Factors associated with a positive ELISPOT result were a lymphocyte count of greater than 1,000 cells/μL (by both univariate and multivariate analyses), immunosuppressive therapy (by both univariate and multivariate analyses), and serum Alb of greater than 3.3 g/dL (by univariate analysis only).

Inter Med 49: 1849-1855, 2010 DOI: 10.2169/internalmedicine.49.3659

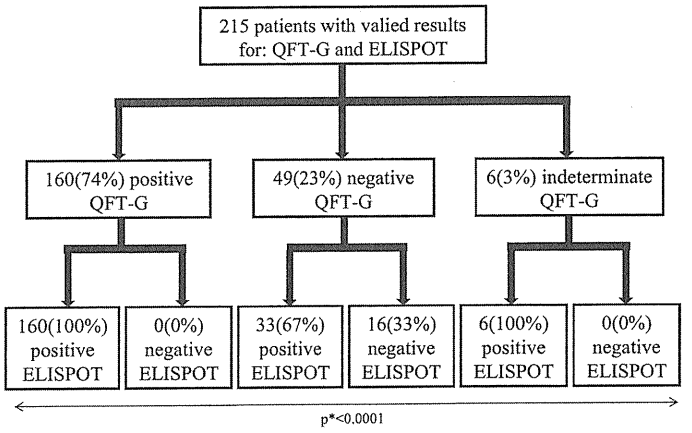

Figure 1. Distribution of ELISPOT results, according to results obtained with QFT-G. *P-values indicate the difference between positive, negative and indeterminate results for QFT-G, for Kruskal-Wallis exact test.

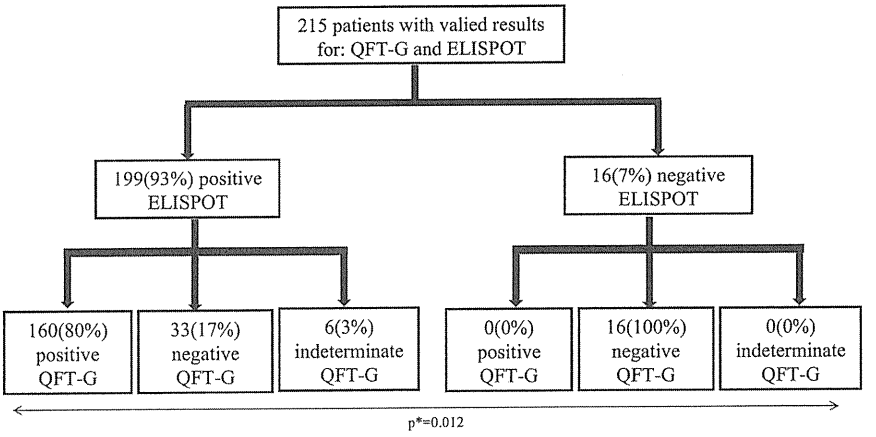

Figure 2. Distribution of QFT-G results, according to results obtained with ELISPOT. *P-values indicate the difference between positive and negative results for ELISPOT, for Mann-Whitney exact test.

Discussion

This is the first study that directs comparison of the sensitivity of 2 IGRA tests, QFT-G and ELISPOT, for detecting microbiologically determined pulmonary tuberculosis in patients, including HIV-negative immunocompromised hosts. We found that the sensitivity for the diagnosis of pulmonary tuberculosis is very high in both tests, and the sensitivity was not significantly confounded by age and gender. ELISPOT sensitivity, but not QFT-G sensitivity, was unaffected by nutritional state as indicated by the serum Alb levels.

This study evaluated the effect of peripheral blood lymphocyte count on QFT-G and ELISPOT and their sensitivity in patients with pulmonary tuberculosis, including immunocompromised hosts. The sensitivity of both tests was affected by lymphocyte counts, with a clear trend of a decrease in sensitivity with decreasing lymphocyte count. This was particularly marked in the case of QFT-G.

Overall sensitivity determined by this study was nearly identical to the results of the meta-analysis by Pai et al (5), which reported sensitivities of 78% for QFT-G and 90% for ELISPOT but appeared inconsistent with other reports (2, 5, 17, 18, 22, 23) in suggesting that ELISPOT is significantly more sensitive than QFT-G.

The correlation between peripheral lymphocyte count and the sensitivity of IGRAs has been reported previously in HIV-positive patients. In some earlier reports, QFT-G sensitivity correlated with CD 4 lymphocyte counts (8, 10, 11, 15, 24) but ELISPOT sensitivity was independent of it (19, 20, 25, 26). In this study, ELISPOT was more than 80% sensitive even when the lymphocyte count was less than 500/μL, whereas QFT-G was less than 70% sensitive when the lymphocyte count was <1,000/μL and less than 39% when the lymphocyte count was <500/μL. In addition, nearly identical tendencies were found in a population of HIV-negative immunocompromised patients.

The present study also indicated that the group of patients with negative and indeterminate results with QFT-G had a lower mean peripheral lymphocyte count, lower than the

Inter Med 49: 1849-1855, 2010 DOI: 10.2169/internalmedicine.49.3659

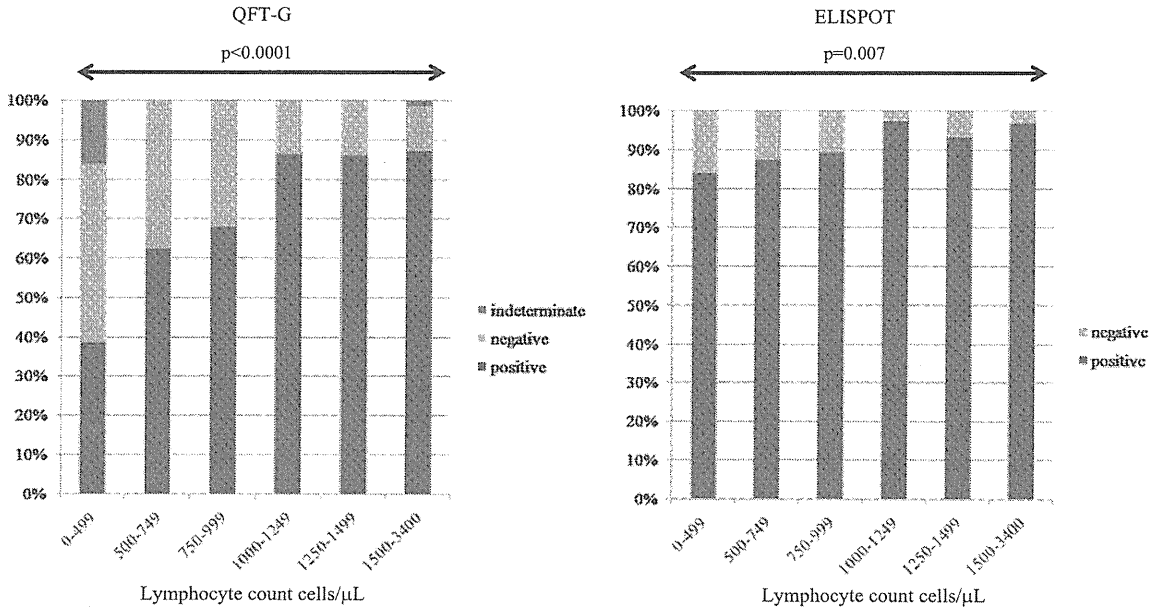

Figure 3. Influence of lymphocyte count on performance of the QFT-G and ELISPOT analysis in pulmonary tubercurosis patients. For all patients the % of positive, negative and indeterminate was grouped by the individual number of lymphocyte count cells/μL. p-values are for chi-square exact for linear trend. The number of patients in each lymphocyte count group was: 0-499:31, 500-749:30, 750-999:28, 1000-1249:37, 1250-1499:29, 1500-3400:60.

Table 3. Association of Risk Factors with a Positive QFT-G Results

Parameter	n	Univariate analysis		Multivariate analysis	
		OR(95% CI)	p	OR(95% CI)	p
lymphocyte count >1000 cells/μL	126	5.167(2.679-9.967)	<0.0001	3.788(1.861-7.708)	<0.0001
Male sex	156	0.751(0.371-1.523)	0.428	0.562(0.234-1.351)	0.198
Age >67 years	107	0.591(0.320-1.090)	0.092	1.180(0.511-2.721)	0.699
Alb >3.3 g/dL	98	3.908(1.971-7.746)	<0.0001	2.507(1.200-5.234)	0.013
CRP >4.72 mg/dL	100	0.395(0.208-0.750)	0.005	0.714(0.284-1.794)	0.714
HbA1c >6.5 %	39	1.555(0.672-3.599)	0.303	2.223(0.793-6.231)	0.129
Immunosuppressive therapy	18	0.517(0.190-1.405)	0.196	0.558(0.176-1.770)	0.322
Bedridden	38	0.400(0.192-0.831)	0.014	0.718(0.273-1.888)	0.502
Malignancies	10	0.329(0.092-1.182)	0.088	0.365(0.086-1.553)	0.173
Cavitation	105	1.572(0.852-2.898)	0.147	1.951(0.918-4.144)	0.082

OR:Odds Ratio. CI:Confidence interval.

Table 4. Association of Risk Factors with a Positive ELISPOT Results

Parameter	n	Univariate analysis		Multivariate analysis	
		OR(95% CI)	p	OR(95% CI)	p
lymphocyte count >1000 cells/μL	126	4.133(1.418-12.045)	0.009	3.314(1.100-9.990)	0.033
Male sex	156	1.054(0.359-3.096)	0.924	0.782(0.215-2.848)	0.709
Age >67 years	107	0.612(0.228-1.641)	0.329	1.760(0.447-6.934)	0.478
Alb >3.3 g/dL	98	3.524(1.110-11.182)	0.033	1.778(0.363-8.702)	0.478
CRP >4.72 mg/dL	100	0.396(0.135-1.167)	0.093	0.619(0.152-2.528)	0.504
HbA1c >6.5 %	39	1.159(0.319-4.204)	0.823	2.626(0.483-14.284)	0.268
Immunosuppressive therapy	18	0.179(0.055-0.579)	0.004	0.202(0.060-0.684)	0.01
Bedridden	38	0.376(0.132-1.076)	0.068	0.465(0.112-1.929)	0.292
Malignancies	10	0.793(0.095-6.635)	0.83	0.645(0.066-6.295)	0.706
Cavitation	105	2.000(0.723-5.536)	0.182	2.174(0.658-7.185)	0.203

OR: Odds Ratio. CI: Confidence interval

Inter Med 49: 1849-1855, 2010 DOI: 10.2169/internalmedicine.49.3659

group with positive results. Previously, HIV-positive patients with a positive QFT-G result were shown to have a significantly higher median CD4 lymphocyte count than those with a negative QFT-G (8, 11). Moreover, as the CD4 lymphocyte counts decreased, the number of indeterminate results increased (8, 10, 11). Our study also showed that patients with indeterminate QFT-G results had a significantly lower median peripheral lymphocyte count than those with determinate QFT-G results, and patients with negative results had a lower median peripheral lymphocyte count than patients with positive results with both QFT-G and ELISPOT.

Considering patient characteristics, 5 of 6 patients with indeterminate QFT-G results had lymphocyte counts of less than 500/μL. Two of 6 with indeterminate QFT-G results had malignancies, and 1 of these had a terminal gastric cancer. Three of the 4 remaining patients (75%) had low serum Alb levels of 1.8, 2.0, and 2.0 g/dL, despite not having any particular underlying disease. Jones et al (27) reported that the severe tubercular infections were associated with a low volume of serum Alb and CD4 lymphocyte counts in a study that measured CD4 lymphocyte counts of HIV-negative patients. Serum Alb was found to significantly affect the QFT-G sensitivity in the multivariate analysis of the study of Jones et al. However, the effect was not as remarkable as that of the lymphocyte count. This finding suggested that not only malnutrition but also advanced tuberculosis was a factor for indeterminate results (27-29). In contrast, 1 patient had an indeterminate result, despite having a lymphocyte count of 1,640/μL. Although this patient was a 54-year-old male with no underlying disease, he had giant cavities in bilateral lung fields. In advanced tuberculosis with giant cavities, the disease state itself may control lymphocyte function, increasing the expected risk of false negative findings (3, 27, 30-32). Therefore, these factors may have influenced the indeterminate result.

In the 16 patients with negative results for both QFT-G and ELISPOT, 5 (31%) were currently receiving immunosuppressive therapy, and their median lymphocyte count was conspicuously low (616/μL, IQR: 201, 1,776). This result confirmed that the long-term administration of glucocorticoid decreased the T lymphocyte count (33, 34). In contrast, Matulis et al (35) reported that neither corticosteroids nor conventional DMARDs significantly affected IFN-γ responses, but the odds for a positive IFN-γ assay decreased in patients treated with TNFα inhibitors. In the 33 patients, immunosuppressive therapy subjects constituted a smaller proportion among the group with negative results for QFT-G and positive results for ELISPOT than among the group with negative results for both assays. Moreover, all QFT-G-positive patients were positive for ELISPOT. There were few immunocompromised hosts in these subjects.

Thus, this study has indicated that the sensitivity of 2 IGRAs, QFT-G and ELISPOT, has the tendency to correlate with the peripheral lymphocyte count. This is not contradictory to the study by Ariga et al, who also found that sensitivity of QFT-G was influenced by the peripheral lymphocyte count in HIV-negative patients (Ariga et al in preparation). However, in some cases, the results of IGRAs were negative, although lymphocyte counts were not low. In multivariate analysis, the lymphocyte counts and serum Alb significantly affected QFT-G sensitivity, while lymphocyte counts and immunosuppressive therapy affected ELISPOT sensitivity. Based on the above, lymphocyte count alone does not affect the sensitivity of IGRAs and the IFN-γ production ability of lymphocytes may be a contributing factor. The IFN-γ production ability of lymphocytes could be measured in the positive control, and thus future research is expected.

This study also indicates that for maximum sensitivity, ELISPOT is preferred to QFT-G. The difference of sensitivity between ELISPOT and QFT-G is based on the difference in the testing principle (3). A reliable method of detecting LTBI is required for implementing this therapy. Measures that could be taken to improve the performance of IGRAs include re-evaluation of the recommended cut-off (36) for each geographic region and exploration of alternative biomarkers for tuberculosis diagnosis.

Conclusion

The sensitivity of 2 IGRAs, QFT-G and ELIPSPOT, is partly dependent on peripheral lymphocyte counts. With low lymphocyte counts, the clinically acceptable sensitivity was better maintained with ELISPOT than QFT-G. Since the ELISPOT sensitivity is not affected by age, gender, and the nutritional state, ELISPOT is superior to QFT-G for detecting active tuberculosis in HIV-negative immunocompromised patients.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

The Value of Fiberoptic Bronchoscopy in Culture-Positive Pulmonary Tuberculosis Patients Whose Pre-Bronchoscopic Sputum Specimens were Negative both for Smear and PCR Analyses

Atsuhisa Tamura[1], Masahiro Shimada[1], Yoshinori Matsui[1], Masahiro Kawashima[1], Junko Suzuki[1], Haruyuki Ariga[1], Nobuharu Ohshima[1], Kimihiko Masuda[1], Hirotoshi Matsui[1], Hideaki Nagai[1], Naohiro Nagayama[1], Emiko Toyota[1], Shinobu Akagawa[1] and Akira Hebisawa[2]

Abstract

Objective This study assessed the diagnostic rate of pulmonary tuberculosis (PTB) using fiberoptic bronchoscopy (FBS) in patients with suspected PTB, and negative pre-bronchoscopy smear and polymerase-chain reaction (PCR) in sputum.

Patients and Methods We retrospectively reviewed 201 culture-positive PTB patients that underwent FBS because both smear and PCR results in sputum were negative. The positive rates of smear for acid fast bacilli, PCR for *Mycobacterium tuberculosis*, the presence of granuloma in transbronchial biopsy (TBB), and culture of *M. tuberculosis* were analyzed. In addition, the radiographic features, contribution of FBS to rapid and/or definitive diagnosis of PTB, and drug susceptibility results of *M. tuberculosis* were also reviewed.

Results There were 136 males and 102 patients under the age of 40 years; non-cavitary (156 cases) and minimal disease (119 cases) on radiographs predominated. The positive rates of FBS were: 44% (smear), 62% (PCR), 61% (TBB), and 87% (culture). These rates increased in smear and PCR examinations when taken from wider spread shadows on radiographs. The combination of the various bronchoscopy samples increased the diagnostic rate to 92% when all examinations were combined. Positive culture results depended on FBS procedures in 80 cases. Twenty-one cases showed resistance to at least one of the major antituberculous agents.

Conclusion This analysis revealed high positive rates of PTB from bronchoscopy samples, providing rapid and definitive ability for PTB diagnosis, and details of drug susceptibility. Therefore, FBS is an important diagnostic procedure in patients with suspected PTB whose sputum specimens were negative both for smear and PCR analyses.

Key words: pulmonary tuberculosis, fiberoptic bronchoscopy, radiographic findings, rapid diagnosis, definitive diagnosis, drug susceptibility

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Introduction

Pulmonary tuberculosis (PTB) is one of the most prevalent infectious diseases in Japan. Although its incidence has decreased, it still affects a significant number of individuals. In 2007 the incidence was 19.8 per 100,000 people (1), which places Japan in the middle ranks of countries affected

[1]Department of Respiratory Diseases, National Hospital Organization Tokyo National Hospital, Kiyose and [2]Department of Pathology, National Hospital Organization Tokyo National Hospital, Kiyose
Received for publication July 14, 2009; Accepted for publication September 7, 2009
Correspondence to Dr. Atsuhisa Tamura, tamura-in@tokyo-hosp.jp

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by this infectious disease. Many PTB cases were diagnosed and reported based only on clinical findings and/or chest radiographic findings without detection of acid-fast bacilli (AFB) in smears of sputum or gastric aspirate, although some cases later show AFB-positive culture results.

Several powerful laboratory-based diagnostic examinations, such as the polymerase-chain reaction (PCR) (2) and the second generation QuantiFeron-TB (QFT2G) (3), have recently been introduced. However, the former is a tool for rapid diagnosis, and QFT2G, a technique which has proved highly accurate in the detection of a *M. tuberculosis* infection, is influenced by several clinical conditions, such as immunosuppressive diseases, aging, and past infection of tuberculosis, and the presence of active tuberculosis cannot be diagnosed or excluded by the QFT2G result (4).

The procedure of fiberoptic bronchoscopy (FBS) is generally thought to be of importance in diagnosing pulmonary diseases (5). Since FBS has proved to be highly accurate in detecting *Mycobacterium tuberculosis*, the gold standard for the diagnosis of tuberculosis, FBS has been widely used to diagnose PTB in sputum smear-negative cases (6-11). FBS is also useful for the differential diagnosis and for the treatment of tuberculosis via the drug susceptibility test if *M. tuberculosis* can be obtained. However, the number of cases of smear, culture, PCR, and transbronchial biopsy (TBB) findings using FBS in previous Japanese reports is relatively small (6-8, 12-14). Furthermore, the usefulness of FBS in patients whose pre-bronchoscopy samples showed both AFB-smear and *M. tuberculosis*-PCR negative results has been uncertain.

Under the current Japanese law, hospitalization is recommended for patients with suspected infectious tuberculosis based on clinical judgment alone; a positive sputum smear result for AFB is not necessarily required. Therefore, it is very important to differentiate other diseases and obtain a rapid diagnosis of PTB in patients with suspected PTB in order to ensure their prompt hospitalization for treatment. Here, we assessed the diagnostic accuracy of FBS in patients with culture-positive PTB whose sputum smears for AFB and sputum PCR for *M. tuberculosis* were both negative when performing FBS.

Materials and Methods

When reviewing the usefulness of a procedure for diagnosing certain diseases in suspected cases, objectives must be clearly defined in advance, and the value of the test is usually evaluated by calculating the sensitivity, specificity, and positive predictive value, etc. This can be done by recording the number of true-positive findings, false-positive findings, true-negative findings, and false-negative findings in a 2×2 table, and then comparing the various incidences. However, if AFB is not detected in sputum samples, then PTB is often clinically diagnosed by chest radiographic findings, the presence of the epithelioid cell granuloma in a TBB, and more recently, by the positive results of the QFT2G examination alone, followed by empirical anti-PTB treatment while waiting for culture results. Furthermore, since cases of pulmonary non-tuberculous mycobacteriosis are included among the suspected cases of PTB, positive findings of epithelioid cell granuloma and sputum smear-positive for AFB could also be indicative of this non-tuberculous condition, thus making it difficult to develop an accurate protocol in a prospective study based on the 2×2 table as described above.

Therefore, in many studies investigating the usefulness of FBS for PTB diagnosis, objectives have been defined based on a final diagnosis of PTB (6-8, 10, 12-15). As in those studies, the current study focused on the ability of FBS to make a diagnosis of PTB in cases that were ultimately proven to be *M. tuberculosis*-culture positive.

A total of 4,769 culture-positive tuberculosis patients were admitted to this hospital in the 13 year period from 1996 to 2008. The medical records of 201 PTB patients who met the following criteria for inclusion in this study were retrospectively reviewed: 1) smear-negative for AFB on admission (one to three times in sputum, and zero to twice in gastric aspirate), 2) PCR-negative for *M. tuberculosis* on admission (once in sputum and zero to once in gastric aspirate), 3) FBS for making a diagnosis was performed before treatment for PTB, and 4) final diagnosis of PTB was based on the identification of *M. tuberculosis* cultured from at least one of the following samples: sputum and/or gastric aspirates on admission (pre-bronchoscopy samples), bronchial washing and/or aspirate (bronchoscopy samples), and post-bronchoscopy sputum samples. Since previous reports (6, 7) have shown post-bronchoscopy sputum examinations to be a reliable method for detecting *M. tuberculosis*, the examination of post-bronchoscopy sputum has now become a routine procedure, and in this study post-bronchoscopy sputum smear and culture data were automatically included if pre-bronchoscopy and bronchoscopy samples showed negative data. Cases with miliary tuberculosis and/or endobronchial tuberculosis were excluded, even if FBS was performed for the diagnosis of tuberculosis.

All FBS investigations performed by experienced physicians were done via the transoral or transnasal route under local anesthesia and sedation, and according to standard procedures: 1) complete inspection of the tracheobronchial trees including subsegmental bronchi and collection of a bronchial aspirate (BA) if bronchial secretions existed; 2) sampling of bronchial brushings (BB, once to three times) from the bronchial segments at which the lesion had been radiographically located; 3) TBB from the same segments; and 4) collection of a bronchial washing (BW) at the orifices of the same segment.

The evidence of a bronchoscopy positive sample was defined as follows: i) a positive sample from at least one among BA, BB, BW, and a mixture of BA and BW in smear examination for AFB; ii) a positive sample which is either among BA or BW, and a mixture of BA and BW in a PCR examination for *M. tuberculosis*; iii) the presence of

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Table 1. Baseline Demographics and Radiographic findings

Factors	Number of Patients (n=201)
Demographics	
Sex	
Male/female	136/65
Age	
~39 yrs (~29 yrs)	102 (51)
40~69 yrs	71
70 yrs~	28
Radiographic findings*	
Type	
II	45
III	156
Spread	
1	119
2	80
3	2

*: according to the classification of pulmonary tuberculosis designated by the Japanese Society for Tuberculosis (Gakkai Classification)[16]

epithelioid cell granuloma or AFB in TBB tissue; iv) a positive sample from at least one among BA, BW, and a mixture of BA and BW in culture examination for *M. tuberculosis*. When spontaneous sputum was not obtained, sputum was induced by either inhalation of a hypertonic, or normal saline, or a bronchodilator plus normal saline. However, the medical files showed that no distinction was made in clinical practice between spontaneous and induced sputum, and therefore these samples were grouped together into the category of pre-bronchoscopy sputum samples. Post-bronchoscopy sputum examination was performed once, after the FBS procedure in all 201 cases, either on the same day as the FBS or on the following day.

This study analyzed the rates of positive smear for AFB, PCR of *M. tuberculosis*, presence of epithelioid cell granuloma or AFB in TBB, culture of *M. tuberculosis* in bronchoscopy samples, culture of *M. tuberculosis* in pre-bronchoscopy samples, and smear for AFB and culture of *M. tuberculosis* in post-bronchoscopy samples. We also examined the drug susceptibility of *M. tuberculosis* identified by culture.

The findings were analyzed according to the classification of pulmonary tuberculosis designated by the Japanese Society for Tuberculosis (Gakkai Classification (16)) in order to consider how the radiographic findings influenced the diagnostic ability of FBS. The Gakkai classification consists of 3 Types and 3 Spreads on plain chest X-rays; Type I: extensively cavitary, Type II: non-extensively cavitary, Type III: non-cavitary, Spread 1: minimal disease, Spread 2: intermediate disease, Spread 3: extensive disease.

Drug susceptibility tests before November 2001 were performed by the absolute concentration method using 1% Ogawa medium; the standard proportional method using WELLPACK was adopted in December 2001. Beginning in February 2003, the cultured *M. tuberculosis* was subjected to the standard method only when the obtained *M. tuberculosis* showed resistance to at least one of the following: isoniazid (INH), rifampicin (RFP), streptomycin sulfate (SM), and ethambutol (EB), by using the Mycobacteria Growth Indicator Tube method (BACTEC MGIT 960). This change was introduced because of reports of discrepancies in the results of drug susceptibility of *M. tuberculosis* between the standard and the MGIT methods (17), and because several reports had indicated that BACTEC MGIT 960 showed a higher proportion of INH resistance than the standard method (18).

The χ^2 test or Fisher's exact test (if adequate) were used for the statistical analysis of the frequency of various factors, and differences were considered to be significant at the $p<0.05$ level.

Results

The baseline demographics and radiographic findings of the 201 patients (136 males, 65 females) are shown in Table 1. The majority of patients (102/201, 51%) were aged 39 or under; only 28 patients (14%) were 70 years or older despite the fact that this age group represents over 50% of tuberculosis patients in Japan. According to the radiographic findings, as based on the Gakkai classification, Type III (non-cavitary, 156 cases) and Spread 1 (minimal disease, 119 cases) accounted for the majority of cases, both of which are thought to be less frequently associated with a positive sputum smear result than the other categories of

Inter Med 49: 95-102, 2010 DOI: 10.2169/internalmedicine.49.2686

Table 2. Rate of Positive Cases Detected by the Various Diagnostic Procedures

Samples	Positive cases / examined cases
Bronchoscopy samples	
Smear (BB, BA, BW)	88/201 (44%)
PCR for *M. tuberculosis* (BW, BA)	107/173 (62%)
Pathologic findings (TBB)*	75/123 (61%)
Culture (BW, BA)†	175/201 (87%)
Pre-bronchoscopy samples**	
Culture†	121/201 (60%)

Bronchoscopy = fiberoptic bronchoscopy, BB: bronchial brushing, BA: bronchial aspirate, BW: bronchial washing, PCR: polymerase-chain reaction, TBB: transbronchial lung biopsy, *: presence of epithelioid cell granulomas or acid-fast bacilli, **: sputum and/or gastric aspirate, †: The culture results were determined a few weeks after performing fiberoptic bronchoscopy.

Table 3. Relationship between the Procedures and Radiographic Findings of Pulmonary Tuberculosis

	Positive cases / examined cases			
	Types*		Spreads*	
Samples	Type II (n=45)	Type III (n=156)	Spread 1 (n=119)	Spread 2 (n=80)
Bronchoscopy samples				
Smear (BB, BA, BW)	22/45 (49%)	66/156 (42%)	41/119 (34%)†	47/80 (59%)†
PCR for *M.tuberculosis*	25/37 (68%)	82/136 (60%)	54/102 (53%)‡	52/69 (75%)‡
Culture (BW, BA)	37/45 (82%)	138/156 (88%)	105/119 (88%)	69/80 (86%)
Pathologic findings (TBB)**	22/29 (76%)	53/94 (56%)	44/71 (62%)	30/50 (60%)
Pre-bronchoscopy samples***				
Culture	30/45 (67%)	91/156 (58%)	69/119 (58%)	51/80 (64%)

Bronchoscopy = fiberoptic bronchoscopy
BB: bronchial brushing, BA: bronchial aspirate, BW: bronchial washing, PCR: polymerase-chain reaction, TBB: transbronchial lung biopsy,
*: according to the classification of pulmonary tuberculosis designated by the Japanese Society for Tuberculosis (Gakkai Classification)[16],
: presence of epithelioid cell granulomas or acid-fast bacilli, *: sputum and/or gastric aspirate
†: $p<0.001$, ‡: $p<0.005$

Types and Spreads. The percentage of positive cases from the FBS and pre-bronchoscopy samples are summarized in Table 2. FBS provided a positive-smear in 44% (88/201 cases) of the 201 patients who were negative for smear and PCR in their pre-bronchoscopy samples, and a positive-PCR in 62% (107/173 cases). One to four TBB specimens were obtained, and the positive rate reached 61% (75/123 cases). Although there are obvious limitations in the statistical comparison of the culture results of pre-bronchoscopy and bronchoscopy samples, the culture-positive rate in the bronchoscopy samples was high (87%, 175/201 cases) in comparison to the culture-positive rate in the pre-bronchoscopy samples (60%, 121/201 cases), suggesting the additional utility of FBS for detecting *M. tuberculosis* in patients with suspected PTB who have no bacterial evidence of PTB.

Table 3 shows the relationship between the original baseline radiographic features and the positive rates detected with the various procedures. These results indicated that, with TBB, there was a tendency for a higher positive rate with cavitary (Type II) cases in comparison to non-cavitary (Type III) cases, although this did not reach statistical significance. However, intermediate disease (Spread 2) cases showed a significantly higher positive rate in smear ($p<0.001$) and in PCR ($p<0.005$) examinations of the bronchoscopy samples in comparison to minimal disease (Spread 1) cases, although there were no significant differences in the positive rates between Spread 1 and 2 based on culture or TBB of bronchoscopy samples. There was no correlation between baseline demographics and radiographic findings or with the positive rates of the various diagnostics procedures.

As shown in Table 4, the step-wise combination of results from the various examinations increases the detection rate of positive cases from 44%, based on a smear in bronchoscopy sample alone, to 92% (152/166 cases) when the results of all bronchoscopy samples and post-bronchoscopy sputum samples were combined. This should translate into the

Inter Med 49: 95-102, 2010 DOI: 10.2169/internalmedicine.49.2686

Table 4. Rapid Diagnostic Ability for Pulmonary Tuberculosis according to the Cumulative Combination of Data from the Various Bronchoscopic Examinations

Combination of examinations	Positive cases/ examined cases
(a) smear in bronchoscopy samples	88/201 (44%)
(a) smear in bronchoscopy samples or (b) smear in post-bronchoscopy sputum samples	94/201 (47%)
(a) smear in bronchoscopy samples or (b) smear in post-bronchoscopy sputum samples or (c) PCR for *M. tuberculosis* in fiberoptic bronchoscopy samples	137/186 (74%)
(a) smear in bronchoscopy samples or (b) smear in post-bronchoscopy sputum samples or (c) PCR for *M. tuberculosis* in bronchoscopy samples or (d) pathologic findings*	152/166 (92%)

Bronchoscopy = fiberoptic bronchoscopy

PCR: polymerase-chain reaction, *: presence of epithelioid cell granulomas or acid-fast bacilli

Table 5. Drug Susceptibility Results of Culture-identified *M. tuberculosis*

Sensitivity	Bronchoscopy group (n=80)	Pre-bronchoscopy group (n=121)	Total (n=201)
All sensitive	72	108	180
RFP resistance	0	3	3
INH resistance	1	2	3
EB resistance	1	0	1
SM resistance	5	6	11
INH, SM resistance	1	1	2
INH, RFP resistance	0	1	1

Bronchoscopy = fiberoptic bronchoscopy

RFP: rifampicin, INH: isoniazid, EB: ethambutol, SM: streptomycin sulfate

timely start of PTB treatment in PTB-suspected patients. The definitive diagnostic ability of bronchoscopy for pulmonary tuberculosis is shown in Fig. 1. Cultures positive for *M. tuberculosis* were obtained in 40% of cases (80/201 cases) using bronchoscopy samples and post-bronchoscopy sputum samples, as opposed to pre-bronchoscopy samples. Of the 80 patients whose PTB diagnosis depended on FBS procedures, 51 were male (64%), 47 were under 40 years of age (59 %), 65 cases manifested non-cavitary disease (Type III, 81%), and 50 cases manifested minimal disease (Spread 1, 63%). There were no significant differences in the positive rates of the above factors between the 80 bronchoscopy-proven PTB patients and the remaining 121 patients who were subsequently diagnosed with PTB from a positive culture result of a pre-bronchoscopy sample, even if FBS had not been performed, but the radiographic evidence of bronchoscopy-proven PTB patients tended to show non-cavitary and minimal disease.

Finally, the drug susceptibility of the cultured *M. tuberculosis* (Table 5) was reviewed. The majority of cases (180/201, 90%) were susceptible to all four major anti-tuberculous drugs, but the remaining 21 cases (10%) showed resistance to at least one of the anti-tuberculous drugs, for example, 13 cases were resistant to SM (10 μg/mL), 6 to INH (0.2 μg/mL), 4 to RFP (40 μg/mL), and 1 to EB (2.5 μg/mL), either alone or in combination with other drugs. No significant differences were observed in drug susceptibility between the bronchoscopy-proven PTB patients and the remaining PTB patients.

This hospital is one of the selected hospitals for tuberculosis treatment. This FBS laboratory has been operating under depressurized ventilation conditions with high-efficiency filters since 1999; and all staff performing FBS procedures are required to wear an N95 mask. There have been no cases of tuberculosis, including latent tuberculosis, in the medical staff involved with FBS examinations. Seven out of