INTERNAL MEDICINE

□ CASE REPORT □

Three Cases of Fungemia in HIV-Infected Patients Diagnosed Through the Use of Mycobacterial Blood Culture Bottles

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Abstract

We treated three cases of fungemia in HIV-infected patients. These cases were caused by *Candida albicans*, *Cryptococcus neoformans*, and *Penicillium marneffei*, respectively, and all were diagnosed through the use of mycobacterial blood culture bottles. Although the detection of the etiologic agents of fungal infection is difficult, it has been shown that blood culture media for mycobacteria are more effective for the detection of fungemia than media for aerobes and anaerobes. Some reports have shown that Bactec Myco/F lytic bottles were useful for the diagnosis of fungemia in clinical samples. Here, we report the successful use of BacT MB bottles.

Key words: mycobacterial blood culture bottle, BacT MB bottle, fungemia, HIV

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Introduction

The incidence of fungemia, and especially of that caused by *Candida* spp., has recently been increasing. Because diagnosis and treatment with antifungal agents tend to be delayed in such cases, the mortality rate is high (1, 2). Part of the reason for this is that the estimated sensitivity of candidemia detection methods using standard aerobic and anaerobic blood culture bottles is only about 50% (3-6). Blood culture media for mycobacteria, however, are more suitable for detecting fungi *in vitro* than these traditional media are (7). In addition, a few reports have shown that, in clinical situations, Bactec Myco/F Lytic bottles manufactured by Becton Dickinson (Franklin Lakes, NJ, USA) yield better accuracy in detecting fungemia (8, 9). In the cases reported here, we used BacT MB bottles manufactured by bioMérieux (Marcy l'Etoile, France), and showed that they, too, were useful in the diagnosis of three different fungemia cases in human immunodeficiency virus (HIV)-positive patients.

Case Report

Case 1

A 53-year-old Japanese HIV-infected man with a 1-month history of dizziness was referred to us. Four months previously, he had started trimethoprim-sulfamethoxazole for *Pneumocystis* pneumonia and anti-retroviral therapy (lopinavir/ritonavir+ tenofovir/emtricitabine). On admission, his CD4 lymphocyte count was 12/μL and his viral load was under 50 copies/mL. Brain MRI showed a 3-cm ring-enhanced tumor in his cerebellum. After open biopsy, he was diagnosed with malignant lymphoma (diffuse large B cell type). Whole-brain radiation therapy was started. One month later, he was treated with meropenem against extended-spectrum beta-lactamase (ESBL)-producing *Kleb-*

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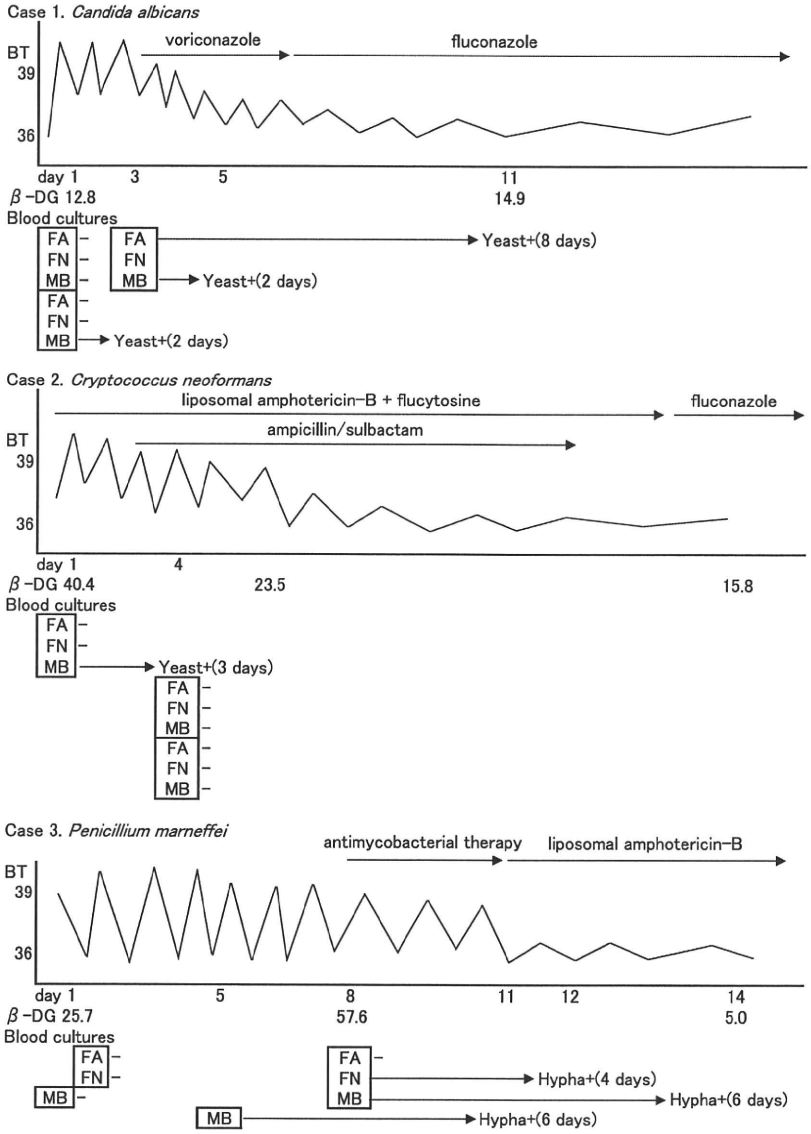

Figure 1. The timing of blood cultures and detection for the three cases. BT: body temperature (℃), β-DG: serum 1 → 3-β-D-glucan (pg/mL), FA, FN, and MB, BacT FA (aerobic bottle), BacT FN (anaerobic bottle), and BacT MB (mycobacterial bottle), respectively. - represents negative in culture. ( ) indicates the number of incubation days.

siella pneumoniae sepsis, and with vancomycin against methicillin-resistant *Staphylococcus epidermidis* (MRSE) sepsis. Initially his fever abated, but after antibiotic treatment he experienced another spike fever (defined hereafter as day 1). We took blood cultures in six bottles: two bottles for aerobes (BacT FA), two for anaerobes (BacT FN), and two for mycobacteria (BacT MB). Two days later (day 3), yeast was growing in one mycobacterial bottle (Fig. 1). We drew another set of blood cultures on day 3 to determine whether the yeast growth indicated a true fungemia or a contamination. Because the patient was in severe distress, we started voriconazole empirically, though it is known to interact with lopinavir/ritonavir. Yeast was found on day 5 in the mycobacterial bottle cultured on day 3, and on day 11 in the aerobic bottle cultured on day 3. All three yeasts were identified as *Candida albicans*. Since the same organism was detected in each of three bottles which had been taken on different days, we considered this case to be a true fungemia. They were sensitive to fluconazole, so we switched the patient from voriconazole to fluconazole. Serum 1→3-β-D glucan was not elevated, measuring 14.9 pg/mL at most. An ophthalmologist confirmed no endophthalmitis. The patient had no central venous catheter, and the entry point of candidemia was unknown. After treatment for candidemia, he was found to have a brain abscess, cellulitis, and a skin abscess at the site of bone marrow examination. He recovered from these serious infections and was discharged home.

Case 2

A 44-year-old Japanese HIV-infected man with a 3-week history of fever and headache was referred to us. He had chronic hepatitis B virus (HBV) infection. His CD4 lymphocyte count was 40/μL, and his viral load was 40,000 copies/

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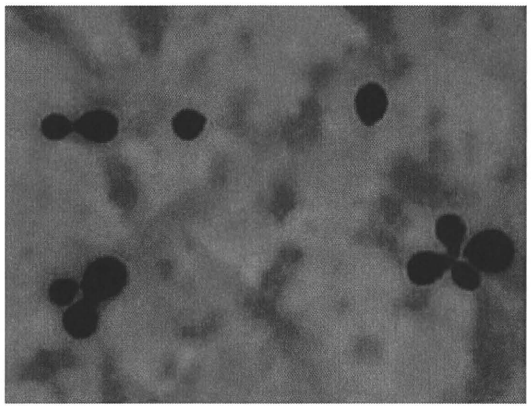

Figure 2. Gram-stained yeasts (*C. neoformans*) from a mycobacterial bottle in Case 2 (×1000).

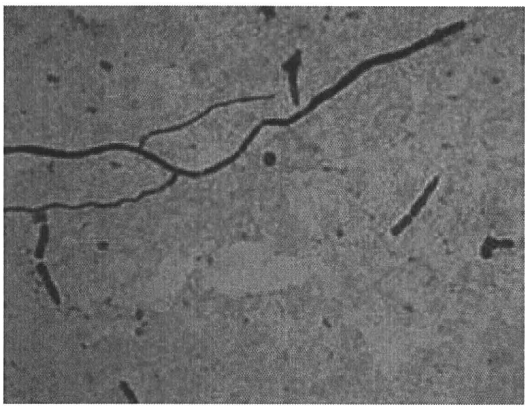

Figure 3. Gram-stained hyphae (*P. marneffei*) from a mycobacterial bottle in Case 3 (×1000).

mL. On admission (defined hereafter as day 1), we took one set of blood cultures including one bottle each for aerobes (BacT FA), anaerobes (BacT FN), and mycobacteria (BacT MB) (Fig. 1). Encapsulated yeasts were detected from the cerebrospinal fluid. We suspected *Cryptococcus* meningitis and accordingly started both liposomal amphotericin-B and flucytosine. We also used ampicillin/sulbactam for aspiration pneumonia. The patient's 1→3-β-D glucan was slightly elevated at 40.4 pg/mL. On day 4, yeasts were found growing in the only mycobacterial bottle (Fig. 2). We took two additional sets of blood cultures, which were all negative, probably because antifungal therapy had already been started. These yeasts were identified as *Cryptococcus neoformans*. After treatment for *Cryptococcus* meningitis, we started anti-retroviral therapy (atazanavir+ritonavir+ tenofovir/emtricitabine), and the patient was discharged home.

Case 3

A 30-year-old Japanese HIV-infected man with a 10-day history of fever, cervical and subclavian lymphadenopathy was referred to us. He had traveled to Thailand several months previously. His CD4 lymphocyte count was 10/μL, and his viral load was 140,000 copies/mL. On the day after admission (defined hereafter as day 1), we took a single blood culture in a mycobacterial bottle (BacT MB) to test for *Mycobacterium avium* complex (Fig. 1). On day 2, we also drew blood cultures into aerobic (BacT FA) and anaerobic (BacT FN) bottles. These three bottles were all negative. Another mycobacterial bottle was taken on day 5, and aerobic, anaerobic, and mycobacterial bottles were taken on day 8. At this point we started antimycobacterial therapy empirically. Hyphae were observed on day 11 growing from the mycobacterial bottle taken on day 5 (Fig. 3), on day 12 from the anaerobic bottle taken on day 8, and on day 14 from the mycobacterial bottle taken on day 8. All of those hyphae were identified as *Penicillium marneffei*. The culture from a subclavian lymph node biopsy tested positive for the same organism. The patient's serum 1→3-β-D glucan was elevated at 57.6 pg/mL. We started liposomal amphotericin-B and he became afebrile. After anti-retroviral therapy (fosamprenavir+tenofovir/emtricitabine), he was discharged home.

Discussion

The incidence of fungemia, especially that caused by *Candida* spp., has recently been increasing (1, 2). The diagnosis of candidemia is frequently difficult, however, because the efficacy of fungemia detection using traditional aerobic and anaerobic bottles is estimated at only 50% (3-6). In general, serum 1→3-β-D-glucan is not sufficiently sensitive or specific to serve as a diagnostic marker for fungemia (4). Delayed diagnosis leads to poor prognosis: the mortality rate is over 40 percent (1, 2, 5). *Cryptococcus* meningitis is somewhat easier to diagnose, because in most cases it can be detected in cerebrospinal fluid. One report, however, has described a case of *Cryptococcus* meningitis that was not detected in cerebrospinal fluid but only through blood culture (8). *Penicillium marneffei* infection is rare in Japan but common in Southeast Asia. In cases of delayed diagnosis, the mortality rate is about 75% (9).

We have described the detection of three different fungal species, *Candida albicans*, *Cryptococcus neoformans*, and *Penicillium marneffei*, through the use of BacT MB bottles. The positivity rates of *C. albicans* detection were 33% (1/3 bottles) using aerobic bottles (BacT FA), 0% (0/3 bottles) using anaerobic bottles (BacT FN), and 67% (2/3 bottles) using mycobacterial bottles (BacT MB) (Table 1). The aerobic bottles (BacT FA) required 8 days of incubation before yielding results, while the mycobacterial bottles (BacT MB) required only 2 days. Mycobacterial bottles therefore exhibited the highest sensitivity and the shortest incubation period. The positivity rates of *C. neoformans* detection were 0% (0/3 bottles) using BacT FA bottles, 0% (0/3 bottles) using BacT FN bottles, and 33% (1/3 bottles) using BacT MB bottles (Table 1); in other words, *C. neoformans* was detected only when a mycobacterial bottle was used. The positivity rates of *P. marneffei* detection were 0% (0/2 bottles) using BacT FA bottles, 50% (1/2 bottles) using BacT FN bottles, and 67% (2/3 bottles) using BacT MB bottles (Ta-

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Table 1. Positivity Rates and Detection Times for Aerobic, Anaerobic, and Mycobacterial Bottles

	Case 1 *Candida albicans*	Case 2 *Cryptococcus neoformans*	Case 3 *Penicillium marneffei*
Positivity rate of aerobic bottle(BacT FA)	0.33 (1/3bottles)	0 (0/3bottles)	0 (0/2bottles)
anaerobic bottle(BacT FN)	0 (0/3bottles)	0 (0/3bottles)	0.5 (1/2bottles)
mycobacterial bottle(BacT MB)	0.67 (2/3bottles)	0.33 (1/3bottles)	0.67 (2/3bottles)
Detection time of aerobic bottle(BacT FA)	8 days	–	–
anaerobic bottle(BacT FN)	–	–	4 days
mycobacterial bottle(BacT MB)	2 days	3 days	6 days

ble 1). The mean number of days required for incubation was 4 days for BacT FN and 6 days for BacT MB. For *P. marneffei*, therefore, the mycobacterial bottle again exhibited the highest sensitivity, while the anaerobic bottle required the shortest incubation period. All three of these cases were completely cured through treatment with appropriate antifungal therapies. No other organisms were found in any other blood culture bottles.

About 200 HIV-positive patients are admitted to our hospital each year. When these HIV patients are febrile, we routinely take six bottles of blood culture, two each for the detection of aerobes, anaerobes, and mycobacteria. The required amounts of blood are 10 mL for each aerobic or anaerobic bottle and 5 mL for each mycobacterial bottle. Our laboratory uses the BacT/ALERT 3D automated blood culture system.

Between 2000 and 2005, we took 552 sets of aerobic and anaerobic blood cultures and 390 sets of mycobacterial blood cultures from 684 HIV-positive patients. The positivity rate among aerobic and anaerobic cultures was 3.81% (21/552 sets); three of the 21 positive results were considered to have been contaminations. The organisms involved in the true-positive cases were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecium*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Cryptococcus neoformans*, and *Candida guilliermondii*. The positivity rate among mycobacterial cultures was 1.74% (7/390 sets); all of these involved only *Mycobacterium avium*.

The main reason for culturing blood in mycobacterial bottles is to detect miliary tuberculosis or *Mycobacterium avium* complex. In the three cases described here, however, the routine use of mycobacterial bottles for these other purposes led to early diagnosis of fungemia, not mycobacteremia. In Case 1, the first *Candida* culture grew in only one mycobacterial bottle, but because we had suspected opportunistic infection, we did not assume that it represented a contamination. Because continuous fungemia was demonstrated in another set of blood cultures, *Candida* was determined to be the etiologic agent. Having noticed that the *Candida* grew well in the mycobacterial bottle, we were not surprised when *Cryptococcus* and *Penicillium* were also detected through the use of mycobacterial bottles in Cases 2 and 3. Before witnessing these three cases, when fungi or bacteria other than mycobacteria grew in a mycobacterial bottle, we had suspected that the bottle had been contaminated.

Two kinds of blood culture bottles for mycobacteria are available: the Bactec Myco/F Lytic bottle manufactured by Becton Dickinson, and the BacT MB bottle manufactured by bioMérieux. Bactec Myco/F Lytic bottles are designed to detect both mycobacteria and fungi, but BacT MB bottles are designed to detect mycobacteria only, and not fungi. Nevertheless, both mycobacterial bottles were superior to aerobic and anaerobic bottles for fungal detection *in vitro* (7). One report has stated that, in a clinical situation, Bactec Myco/F Lytic bottles exhibited higher sensitivity and shorter incubation periods in the detection of *Candida albicans* and *Candida glabrata* than aerobic bottles (Bactec Plus Aerobic/F) did (5). Another report has shown that the routine use of Bactec Plus Aerobic/F, Plus Anaerobic/F and Myco/F Lytic bottles for immunocompromised hosts, such as patients in the ICU, permitted highly efficient *Candida albicans* detection (6). That study examined 1,253 blood culture sets (3,759 bottles) in two years. From these sets, 62 yeasts were isolated. The positivity rates were 7.33% among Plus Aerobic/F bottles (44/600 bottles), 1.13% among Plus Anaerobic/F bottles (5/441 bottles), and 25.4% among Mycocis IC/F bottles (48/189 bottles).

Because the present report includes only three fungemia cases, it may not be appropriate to compare these results with those of their reports, but our data correspond well with those from the larger studies in showing that mycobacterial blood cultures can detect fungi with a higher sensitivity than aerobic or anaerobic cultures offer.

Nevertheless, it is very difficult to estimate the true positivity rate of fungemia detection through the use of these blood cultures. Especially among HIV-positive patients, even if serum 1→3-β-D-glucan is elevated, this is frequently caused by *Pneumocystis* pneumonia, not by fungemia. Thus, the direct detection of fungi from blood cultures is particularly important in this area.

Some antibiotics are included inside Bactec Myco/F Lytic bottles to inhibit the growth of bacteria other than mycobacteria or fungi. No antibiotics are included inside BacT MB bottles. The reason why fungi grow so well in mycobacterial bottles is unclear. Both fungi and mycobacteria grow well in aerobic environments, and fungi grow faster than mycobacteria. Accordingly it is possible that, in cases of co-infection with fungi and mycobacteria, the mycobacteremia will be

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overlooked.

Each mycobacterial bottle requires an extra 5 mL of blood from the patient, as well as laboratory space for its storage. The mycobacterial bottles also cost three times as much as typical aerobic or anaerobic bottles (7, 10). The efficacy of aerobic and anaerobic detection is so low that the regular use of mycobacterial bottles is not recommended in the case of community-acquired infection. Mycobacterial bottles are suitable for patients with high risk of fungemia, including immunocompromised hosts and patients with central venous catheters in place. The prognosis of fungemia is still not very good, but early diagnosis leads to early antifungal treatment which is more likely to result in a complete cure.

We have encountered three cases of fungemia in HIV-infected patients, caused by *Candida albicans*, *Cryptococcus neoformans*, and *Penicillium marneffei*, respectively, all of which were diagnosed through blood culture in BacT MB bottles. Blood culture in aerobic and anaerobic bottles alone would not have been sufficient in these cases. We have found that BacT MB bottles are also useful for the isolation of fungi in clinical situations. More data are required to confirm the usefulness of these mycobacterial bottles for the detection of fungemia in immunocompromised hosts.

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Trends in transmitted drug-resistant HIV-1 and demographic characteristics of newly diagnosed patients: Nationwide surveillance from 2003 to 2008 in Japan

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ABSTRACT

The emergence and transmission of drug-resistant human immunodeficiency virus-1 (HIV-1) compromises antiretroviral treatment for HIV-1. Thus, testing for drug resistance is recommended at diagnosis and before initiating highly active antiretroviral treatment. We conducted an epidemiological study enrolling newly diagnosed patients between 2003 and 2008 in our nationwide surveillance network. In the 6-year study period, the prevalence of drug-resistant HIV-1 among 2573 patients, consisting mainly of Japanese men in their late-30s and infected through male-to-male sexual contacts, followed an increasing trend from 5.9% (16/273) in 2003 to 8.3% (50/605) in 2008. Nucleoside reverse transcriptase inhibitor-associated mutations predominated in each year, with T215 revertants being the most abundant. The predictive factor for drug-resistant HIV-1 transmission was subtype B (OR = 2.36; $p = 0.004$), and those for recent HIV-1 infection were male gender (OR = 3.79; $p = 0.009$), MSM behavior (OR = 1.67; $p = 0.01$), Japanese nationality (OR = 2.31; $p = 0.008$), and subtype B (OR = 5.64; $p < 0.05$). Continued activities are needed to raise awareness of the risks of HIV-1 infection and complications of drug-resistant strains. Continued surveillance is also needed to understand trends in the HIV-1 epidemic.

Abbreviations: HIV-1, human immunodeficiency virus type 1; HAART, highly active antiretroviral therapy; PI, protease inhibitor; HBV, hepatitis B virus; HCV, hepatitis C virus; PR, protease; RT, reverse transcriptase; RT-PCR, reverse transcription polymerase chain reaction; CRF, circulating recombinant form; NRTI, nucleoside RT inhibitor; NNRTI, non-nucleoside RT inhibitor; OR, odds ratio; CI, confidence interval; MSM, men who have sex with men; IDU, intravenous drug user.

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1. Introduction

The emergence of drug-resistant human immunodeficiency virus type 1 (HIV-1) among patients under highly active antiretroviral therapy (HAART) limits the successful suppression of HIV-1 replication. Several years after the introduction of HAART, drug-resistant strains are being detected among newly diagnosed HAART-naïve patients, suggesting the transmission of drug-resistant HIV-1 from the treatment-exposed population. Thus, treatment-naïve patients have been recommended by the US Department of Health and Human Services, International AIDS Society-USA, and other drug-resistance testing guidelines to undergo drug resistance testing at diagnosis and before initiation of HAART (DHHS, 2009; Hirsch et al., 2000, 2008). Indeed, choosing effective antiretrovirals according to the results obtained from this testing has led to successful control of HIV-1 infection. Furthermore, the drug resistance testing at diagnosis helps to understand transmission of drug-resistant HIV-1 in HAART-naïve individuals which in turn may help prevent transmission events.

The prevalence of drug-resistant HIV-1 among treatment-naïve patients has been closely monitored and reported from many countries. Before and early in the HAART era, when only mono or dual therapy was available, the prevalence was as high as 10–20% (Boden et al., 1999; Gómez-Cano et al., 1998; Tambussi et al., 1998). However, after the introduction of antiretrovirals with better pharmacokinetics, such as ritonavir-boosted protease inhibitor (PI), the emergence of drug-resistant viruses seemed to decrease (Gallego et al., 2001; Maia Teixeira et al., 2006).

Furthermore, despite the great number of HIV-1-infected patients, the prevalence tended to be low in developing countries where patients had limited or no access to antiretroviral drugs, e.g., 0–4.2% in Africa (Bártolo et al., 2009; Mintsa-Ndong et al., 2009; Ndembi et al., 2008; Pillay et al., 2008), 1.5% in Cambodia (Nouhin et al., 2009), and 2.6% in Vietnam (Ishizaki et al., 2009). In contrast, in countries where antiretroviral drugs are more accessible, the prevalence has been higher, e.g., 5.2% in Thailand (Apisarnthanarak et al., 2008), 9.4% in Taiwan (Chang et al., 2008), 10.0% in India (Lall et al., 2008), 7.8% in Portugal (Palma et al., 2007), 9.0% in Germany (Sagir et al., 2007), 9.5% in Belgium (Vercauteren et al., 2008), 10.9% in France (Chaix et al., 2009), and 15.9% in the US (Eshleman et al., 2007).

In Japan, since the first HIV-1-infected case was identified in 1985, the annual number of reported cases has been increasing every year, reaching 15 451 by the end of 2008. With more people getting infected, larger numbers of patients are starting anti-HIV-1 treatment and the risk of emerging drug-resistant HIV-1 is increasing. To understand the trends in drug-resistant HIV-1 in Japan, a nationwide surveillance project has been in effect since 2003. In our previous report of surveillance results from 2003 to 2004, the prevalence of drug-resistant HIV-1 in newly diagnosed patients was 4.0% (Gatanaga et al., 2007). We have continued collecting and analyzing data from newly diagnosed HIV-1-infected patients at participating clinical and research facilities in Japan. We report here the prevalence of drug-resistant HIV-1 among newly diagnosed therapy-naïve patients between 2003 and 2008.

2. Materials and methods

2.1. Sample

The study population included all the HIV-1-infected patients newly diagnosed between January 2003 and December 2008 at any of the participating HIV/AIDS clinics. Drug resistance genotypic tests were performed at 12 laboratories including 8 clinical laboratories at HIV/AIDS clinics, 3 public health laboratories, and the National Institute of Infectious Diseases. After patients agreed to participate in our surveillance project and gave informed consent, peripheral blood was drawn with EDTA added, and their demographic and clinical information were collected. Demographic information included age, gender, nationality, and risk behavior. Clinical data included HIV-1 viral loads, $CD4^+$ T cell counts, status of hepatitis B and C virus (HBV, HCV) co-infection, baseline sequence data, and drug-resistant amino acid mutations.

This study was conducted according to the principles in the Declaration of Helsinki, and was approved by the ethical committee of the National Institute of Infectious Diseases, Japan. By Japanese law, HIV-1-infected patients must be reported to the Japanese Ministry of Health, Labour, and Welfare upon diagnosis. The numbers reported to the Ministry are considered the "official numbers" of newly diagnosed HIV/AIDS cases, and were used as comparison controls to evaluate our study population.

2.2. Drug resistance genotypic testing

Drug resistance genotypic testing was performed using in-house protocols. Briefly, viral RNA was extracted from patient plasma samples. HIV-1 protease (PR, 1–99 amino acids) and the N-terminal region of reverse transcriptase (RT, 1–240 amino acids) were amplified in reverse transcription polymerase chain reaction (RT-PCR) followed by nested PCR using in-house primer sets. Subsequently, the amplified PCR products were purified and their sequences were analyzed by direct sequencing method using an automated sequencer. The resulting electropherograms were analyzed using commercially available software. The quality of testing methods used at each participating facility was assessed and confirmed for detection of drug-resistant mutations (Fujisaki et al., 2007). Thus, detection of drug-resistant mutations was consistent among facilities.

2.3. Determination of HIV-1 subtypes and drug-resistant HIV-1

HIV-1 subtypes were determined using the sequences of HIV-1 PR and RT genes obtained in the drug resistance genotypic testing explained above. Each sequence was aligned with the reference sequences of HIV-1 subtypes A through K, and circulating recombinant forms (CRFs), all of which were obtained from the Los Alamos HIV Databases (Los Alamos, 2010), using ClustalW, and phylogenetic trees were constructed using the neighbor-joining method with bootstrap value of 1000.

The resulting sequences were compared to that of HXB2 to judge the presence of amino acid mutations. The drug-resistant mutations were determined according to criteria of the HIV Drug Resistance Database of Stanford University (Bennett et al., 2009). Thus, a sample was considered to harbor drug-resistant HIV-1 if it possessed any of the following mutations: in the PR gene, L23I, L24I, D30N, V32I, M46I/L, I47V/A, G48V/M, I50V/L, F53L/Y, I54V/L/M/A/T/S, G73S/T/C/A, L76V, V82A/T/F/S/C/M/L, N83D, I84V/A/C, I85V, N88D/S, and L90M (indicating PI resistance); in the RT gene, M41L, K65R, D67N/G/E, T69D/insertion, K70R/E, L74V/I, V75M/T/A/S, F77L, Y115F, F116Y, Q151M, M184V/I, L210W, T215Y/F/I/S/C/D/V/E, K219Q/E/N/R (indicating nucleoside RT inhibitor [NRTI] resistance), and L100I, K101E/P, K103N/S, V106M/A, V179F, Y181C/I/V, Y188L/H/C, G190A/S/E, P225H, M230L (indicating non-nucleoside RT inhibitor [NNRTI] resistance).

2.4. BED assay

The time of HIV-1 seroconversion was estimated in randomly selected samples as recent (within 155 days) or not recent using the BED assay (Calypte HIV-1 BED Incidence EIA, BioRad) according to the Manufacturer's instruction. Briefly, 5 μL of plasma was diluted

with 500 μL of sample diluent in the kit, and the proportion of anti-HIV-1 IgG to a total IgG in the sample was measured by optical density.

2.5. Statistical analysis

Statistical analyses were performed using R software (SAS Institute). Chi-square or Fisher's exact probability tests were used to determine associations among patients' demographic characteristics, nationality, BED assay results, and transmission of drug resistance. The odds ratio (OR) and 95% confidence intervals (CI) were calculated for all the variables. Recent and not-recent seroconversion groups were examined for differences in HIV-1 viral loads by analysis of covariance (ANCOVA), with $CD4^+$ T cell count as the covariate.

3. Results

3.1. Majority of treatment-naïve patients are Japanese men who have sex with men (MSM) in mid-30s

The demographics of the 2573 newly diagnosed HIV-1-infected patients enrolled between 2003 and 2008 are summarized in Table 1. Male (n = 2397, 93.2%), Japanese (90.1%), and those infected through male-to-male sexual contact (68.9%) predominated, and the median age was 35. For the female cases (n = 170), high-risk heterosexual contact was the major risk factor (n = 152, 89.4%), and approximately half were non-Japanese (n = 63, 41.4%). Further analysis showed a significant association between the transmission route and nationality, i.e., most Japanese patients were infected through male-to-male sexual contact, while non-Japanese patients were infected by other routes (OR = 5.60; 95% CI 4.14–7.63; $p < 0.01$) (Table 2). It should be noted that sexual contacts (92.1%) are the major risk factor for HIV-1 infection in Japan. On the other hand, injecting drug usage, one of the high risk factors in other countries, accounts for only 0.4%.

HBV and/or HCV co-infection, an important clinical factor affecting prognosis and treatment of HIV infection (Ockenga et al., 1997; Piroth et al., 2000), was found to have a prevalence of 8.4% of 2101 patients, and 4.7% of 2071, respectively (Table 1). These prevalence rates did not change significantly throughout the study period (supplementary Table 1). HBV co-infection was found to be significantly associated with subtype B (OR = 2.04; $p < 0.05$) or infection through male-to-male sexual contact (OR = 1.66; $p < 0.05$).

3.2. Subtype B HIV-1 predominates in Japan

Of 2573 plasma samples collected during the study period, the sequences of PR and RT genes were successfully amplified and analyzed in 2536 (98.6%) and 2534 (98.5%) samples, respectively. Of these, we examined sequences of the PR-RT region from 2496 cases by phylogenetic tree analysis to determine the distribution of HIV-1 subtypes in Japan. Subtype B HIV-1 was found to predominate among the study population (n = 2194, 87.9%). The remaining non-B subtypes included 210 (8.4%) CRF01_AE, 30 (1.2%) C, 19 (0.8%) CRF02_AG, 18 (0.7%) A, 9 (0.4%) G, 7 (0.3%) F, 5 (0.2%) D, and 1 (0.04%) CRF08_BC (Table 1). In addition, 1 recombinant case of K/C, A/K, and D/B was detected in 2005, 2006, and 2007, respectively. These non-B subtype viruses were found mostly among the heterosexually infected population (223/302, 73.8%). In contrast, subtype B HIV-1 was found in the vast majority of MSM (1700/1773, 95.9%). In terms of nationality, Japanese patients, most of whom were MSM, were infected with subtype B HIV-1. On the other hand, only about a half of non-Japanese patients harbored subtype B HIV-1, and the remaining half were infected with non-B HIV-1, such as CRF01_AE (OR = 8.85; 95% CI 6.46–12.1; $p < 0.01$) (Table 2). This result is reasonable considering that the predominant HIV-1 subtype differs by country, and our study population included many Thais and Malaysians. In addition, this result suggests that subtype B HIV-1 is transmitted in a closed community of MSM, while non-B subtype strains are spread in wider areas among those infected through high-risk heterosexual contacts.

Table 1
Demographic characteristics of newly diagnosed HIV/AIDS patients.

	6-Year total (2573)	
Age		
Average	37.4	
Median	35	
Mode	35	
Quartile (Q1, Q3)	29, 43	
Nationality	n	(%)
Japanese	2319	(90.1)
Non-Japanese	225	(8.7)
Asian	83	(3.2)
Oceanian	4	(0.2)
North American	17	(0.7)
South American	58	(2.3)
European	10	(0.4)
African	26	(1.0)
Unspecified[a]	27	(1.0)
Unknown	29	(1.1)
Transmission category		
Male	2397	(93.2)
Male-to-male sexual contact	1773	(68.9)
High-risk heterosexual contact	369	(14.3)
Sexual contact	75	(2.9)
IDU	8	(0.3)
Other[b]	26	(1.0)
Unidentified	146	(5.7)
Female	170	(6.6)
High-risk heterosexual contact	152	(5.9)
IDU	3	(0.1)
Other[b]	5	(0.2)
Unidentified	11	(0.4)
Unknown	6	(0.2)
Unidentified	6	(0.2)
Hepatitis co-infection[c]		
HBV		
(+)	176	(8.4)
(−)	1925	(91.6)
Unknown	472	
HCV		
(+)	98	(4.7)
(−)	1973	(95.3)
Unknown	502	
HIV-1 subtype[c]		
B	2194	(87.9)
non-B	302	(12.1)
AE	210	(8.4)
C	30	(1.2)
AG	19	(0.8)
A	18	(0.7)
G	9	(0.4)
F	7	(0.3)
D	5	(0.2)
Other	4	(0.2)
Unidentified	77	

[a] Unspecified individuals in the nationality category were identified only as of non-Japanese origin.

[b] Other transmission categories include mother-to-child, blood products, transfusion, and needle stick.

[c] Prevalence of subtypes, HBV, and HCV was calculated after omitting the unidentified or unknown data. IDU, intravenous drug user; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus type 1.

3.3. Prevalence of drug-resistant HIV-1 is increasing in Japan

A total of 194 cases (7.7%) in the 6-year study period were found to harbor HIV-1 strains with at least one major drug-resistant muta-

Table 2
Characteristics of newly diagnosed Japanese and non-Japanese HIV/AIDS patients.

	Nationality (*n*)			Odds ratio
	Japanese	Non-Japanese	Unknown	
Gender				
Male	2224	151	22	11.45*
Female	95	74	1	
Unknown[b]			6	
Transmission category				
Male-to-male sexual contact	1691	73	9	5.60[a,*]
High-risk heterosexual contact	399	114	7	
Sexual contact	72	4	0	
Other	29	10	2	
Unidentified[b]	128	24	11	
Subtype				
B	2051	118	25	8.85*
Non-B	198	101	3	
Unidentified[b]	70	6	1	
BED assay (*n* = 640)				
Recent	220	13	0	2.31*
Not recent	351	48	8	
Drug-resistant HIV-1				
Detected	173	16	5	1.05
Not detected	2146	209	24	

[a] Odds ratios for the transmission category were calculated between male-to-male sexual contact and other categories which include high-risk heterosexual contact, sexual contact, and other.
[b] Unknown and Unidentified cases were omitted in calculation of odds ratio.
* $p < 0.01$.

tion conferred by PIs, NRTIs, or NNRTIs. The annual prevalence of drug-resistant mutations shown in Fig. 1 had an overall tendency to increase from 5.9% (16/273) in 2003 to 8.3% (50/605) in 2008. The most prevalent mutation in each year was NRTI-associated resistance, with 11 (4.0%), 12 (4.0%), 21 (5.0%), 23 (5.2%), 28 (5.9%), and 23 (3.7%) cases, followed by PI- and NNRTI-associated mutations. PI-resistant major mutations were detected in 63 cases (2.5%), and NNRTI-associated mutations were detected only in 20 cases (0.8%). These data reflect the type of antiretrovirals being prescribed in treated population. In other words, NRTIs have a long history of being prescribed including the period of mono and dual therapy; thus, NRTIs have been more frequently used. As a consequence, NRTI-resistant HIV-1 has emerged and been transmitted more frequently to treatment-naïve patients. Regarding the drug-resistant mutations shown in Table 3, T215 revertants (T215X) (3.2%), M184I/V (0.5%), K103N (0.6%), and M46I/L (1.7%) accounted for the majority of detected mutations in contrast to other muta-

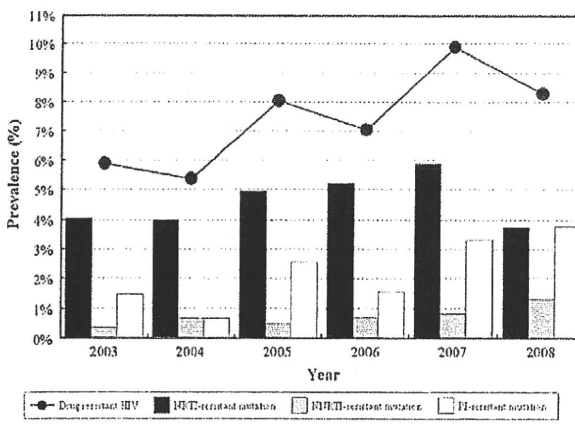

Fig. 1. Annual overall prevalence of drug-resistant HIV-1 (solid circles) in Japan increased in treatment-naïve patients in Japan from 2003 to 2008. The most prevalent mutation in each year was associated with resistance to nucleoside reverse transcriptase inhibitor (NRTI) treatment. Annual prevalence of drug-resistance mutations was categorized by antiretroviral drug class (NRTIs, solid black bars; non-nucleoside reverse transcriptase inhibitors [NNRTIs], horizontally striped bars; protease inhibitors [PIs], solid white bars). Drug-resistant HIV-1 was counted once even when the strain contained multiple drug-resistant mutations. Each drug-resistant mutation was counted even when multiple mutations were detected in one patient.

Table 3
Drug-resistant mutations in newly diagnosed HIV/AIDS patients, by class of antiretroviral drugs.

	6-Year total (2573)	
	n	(%)
NRTI[a]		
M41L	11	(0.4)
K65R	1	(0.0)
D67N/G/E	7	(0.3)
T69D	8	(0.3)
69INS	1	(0.0)
K70R/E	2	(0.1)
L74V/I	3	(0.1)
V75A/M	2	(0.1)
Y115F	3	(0.1)
M184V/I	12	(0.5)
L210W	5	(0.2)
T215X	81	(3.2)
K219Q/E/N/R	4	(0.2)
NNRTI[a]		
L100I	1	(0.0)
K101E	2	(0.1)
K103N	14	(0.6)
V106A/M	1	(0.0)
Y181C/I/V	3	(0.1)
P225H	1	(0.0)
P236L	1	(0.0)
PI[a]		
L24I	1	(0.0)
D30N	5	(0.2)
V32I	3	(0.1)
M46I/L	44	(1.7)
I47V/A	2	(0.1)
V82A/L	2	(0.1)
I85V	5	(0.2)
N88D/S	7	(0.3)
L90M	4	(0.2)

[a] Numbers of cases and the proportions in parentheses are listed.

Table 4
Predictive factors for transmission of drug-resistant HIV-1.

	Drug-resistant HIV-1 (*n*)		Odds ratio
	(+)	(−)	
Gender			
Male	183	2214	1.92
Female	7	163	
Nationality			
Japanese	173	2146	1.05
Non-Japanese	16	209	
Transmission category			
Male-to-male sexual contact	130	1643	0.91
High-risk heterosexual contact	37	484	
Sexual contact	15	60	
Other	1	40	
Unidentified[a]	11	152	
Subtype			
B	180	2014	2.36*
Non-B	11	291	
Unidentified	3	77	

[a] For calculation of odds ratio, unidentified cases were omitted.
* $p < 0.01$.

tions that were detected only sporadically throughout the study period (supplementary Table 2).

Analysis of possible predictive factors for transmission of drug-resistant HIV-1 showed that individuals infected with subtype B HIV-1 had a significantly higher tendency to harbor drug-resistant HIV-1 than non-B subtypes (OR = 2.36; 95% CI = 1.27–4.88; $p < 0.01$) (Table 4). Other possible predictive factors, including male gender (OR = 1.92; 95% CI = 0.89–4.93; $p = 0.1$), Japanese nationality (OR = 1.05; 95% CI = 0.62–1.92; $p = 1$), and MSM behavior (OR = 0.91; 95% CI 0.66–1.26; $p = 0.57$), were not significant predictive factors in our study population. These results indicate that the chance of getting infected with drug-resistant HIV-1 was the same for anyone regardless of gender, nationality, or risk behavior.

3.4. MSM are diagnosed earlier than heterosexually infected individuals

To examine awareness of HIV infection, especially of risk behavior, and to characterize HIV-testing patterns among the HIV-infected population, we estimated the time of seroconversion by quantifying the amount of anti-HIV antibody in plasma samples. Of 640 randomly selected samples in 2007 and 2008, 233 (36.4%) were classified by BED assay with a cut-off value of 0.8 as recently infected (<155-day seroconversion), while the remaining 407 (63.4%) were classified as not recently infected (Table 5). For the recently and not recently infected groups, the average $CD4^+$ T cell count and HIV-1 viral load were 285 and 215 cells/μL and 5.1×10^5 and 1.4×10^5 copies/mL, respectively. Recently infected individuals were shown by ANCOVA with $CD4^+$ T cell counts as the covariate, to have significantly higher HIV-1 viral loads than not recently infected cases (Fig. 2). These data support that the BED assay had precisely determined early infected cases.

With respect to risk behavior, the highest rate of recent infection was in MSM (39.2%), followed by either homo- or heterosexual contacts (38.9%), and heterosexual contacts (25.0%). No patients infected through a risk behavior other than sexual contacts were categorized as recently infected. Whereas 37.8% of male patients were determined to be recently infected, only 13.8% of female patients were categorized as recently infected. These findings were reinforced by statistical analysis. Recent HIV-1 infection was significantly predicted by male gender (OR = 3.79; 95% CI 1.29–15.17; $p < 0.01$), MSM behavior (OR = 1.67; 95% CI = 1.11–2.54; $p = 0.01$), Japanese nationality (OR = 2.31; 95% CI 1.20–4.76; $p < 0.01$), and infection with subtype B HIV-1 (OR = 5.64; 95% CI = 2.37–16.33; $p < 0.01$) (Table 5). In other words, Japanese males, especially those who were MSM, were more aware of being at high risk of HIV-1 infection and got tested more often than non-Japanese. In contrast, females, individuals of non-Japanese origin, heterosexuals, and non-subtype-B-infected persons, had low awareness of the risks of HIV-1 infection.

Table 5
Predictive factors for recent or not-recent seroconversion determined by BED assay, $n = 640$.

	Seroconversion (*n*)		Odds ratio
	Recent ($n = 233$)	Not recent ($n = 407$)	
Gender			
Male	229	377	3.79*
Female	4	25	
Unknown[b]	0	5	
Nationality			
Japanese	220	351	2.31*
Non-Japanese	13	48	
Unknown[b]	0	8	
Transmission category			
Male-to-male sexual contact	189	293	1.67[a]*
High-risk heterosexual contact	24	70	
Sexual contact	7	11	
Other	0	4	
Unidentified[b]	13	29	
Subtype			
B	224	350	5.64*
Non-B	6	53	
Unidentified[b]	3	4	
Drug-resistant HIV			
Detected	14	37	0.64
Not detected	219	370	

[a] Odds ratio for the transmission category was calculated between male-to-male sexual contact and other categories which include high-risk heterosexual contact, sexual contact, and other.
[b] Unknown or unidentified cases were omitted in calculation of odds ratio.
* $p < 0.05$.
** $p < 0.01$.

Regarding associations between the time of diagnosis and drug-resistant HIV transmission event, time of diagnosis did not differ significantly between those harboring and those not harboring drug-resistant HIV-1 (OR = 0.64; 95% CI = 0.31–1.24; $p = 0.18$) (Table 5), suggesting that transmission of drug-resistant HIV-1 is not a recent trend, but has been ongoing since the first antiretroviral, AZT, was introduced in 1986.

4. Discussion

Our study results show that the proportion of drug-resistant HIV-1 among newly diagnosed cases in Japan increased slightly (by 2.4%) from 2003 to 2008, with fluctuations from year to year. Drug-resistant HIV-1 in HAART-naïve patients are transmitted from HAART-experienced patients with inadequate adherence or from other treatment-naïve individuals with drug-resistant strains, but not yet diagnosed or tested for drug-resistant HIV-1 (de Mendoza et al., 2005). Hence, drug-resistant mutations detected in the naïve population should be tightly related to trends in antiretroviral use in the treated population. Antiretrovirals available in the early days of the HAART era, especially, had short half-lives and low genetic barriers for drug resistance acquisition, making the viruses easily resistance prone. On the other hand, new antiretroviral drugs, such as lopinavir, atazanavir, amprenavir and darunavir, have been developed so that they have improved pharmacokinetics and higher genetic barriers, thus the viruses have less chance of developing drug resistance (Dunn et al., 2008; Lima et al., 2008; Zajdenverg et al., 2009). In the present study, we found that drug-resistant mutations detected among treatment-naïve patients were

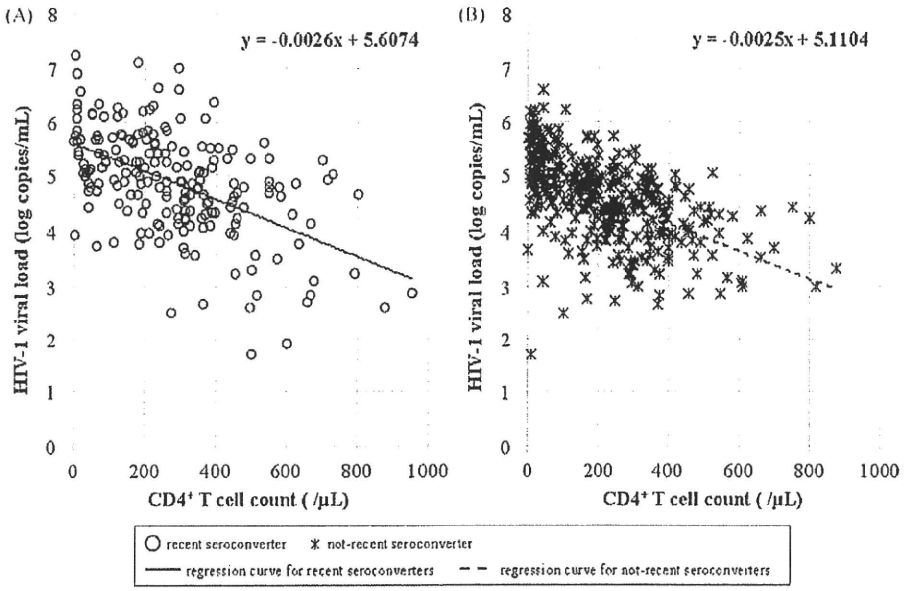

Fig. 2. Scatter plots of viral load and CD4+ T cell counts for (A) recently seroconverted patients (○), and (B) not recently seroconverted patients (*) determined by BED assay. Regression curves and their equations are shown for each group.

associated especially with antiretrovirals used prior to and early in the HAART era. It should be noted that contrary to the reports from the United States and many of European countries (Audelin et al., 2009; Vercauteren et al., 2009; Wheeler et al., 2010), the prevalence of NNRTI-resistant variants have been determined to be low in Japan, less than 1% in the study period 2003–2007 and 1.3% in 2008 being the highest. This difference is due to the situation in Japan that delavirdine had never been used and even nevirapine is only rarely prescribed. Nonetheless, strains with T215X, M46I/L, K103N, and M184V/I mutations were detected every year, suggesting that these strains are stably maintained in individuals and in high-risk populations even under antiretroviral drug-free environments. This finding is supported by the insignificant difference in prevalence of drug-resistant HIV-1 between recently and not recently infected groups. These results raise the concern that such drug-resistant strains may have become some epidemic strains actively transmitted among newly diagnosed HIV/AIDS patients. Furthermore, considering the presence of low frequent variants, the prevalence of drug-resistant mutations in this report may be higher if more sensitive techniques, such as allele-specific PCR and ultra-deep sequencing, are applied to test the samples (Halvas et al., 2010; Varghese et al., 2009). Further studies employing such techniques are needed to understand the detailed epidemic in Japan.

In investigating predictive factors for transmission of drug-resistant strains, we found that the only predictive factor was subtype B HIV-1 (OR = 2.36, $p < 0.01$). The lower transmission risk of drug-resistant strains in non-B HIV-1 can be explained by patients' countries of origin. We observed a significant relationship between non-B subtype HIV-1 and non-Japanese patients, most of whom were from developing countries with limited access to antiretrovirals. Thus, our finding agrees with reports of low prevalence drug-resistant HIV-1 transmission in developing countries (Bártolo et al., 2009; Ishizaki et al., 2009; Mintsa-Ndong et al., 2009; Ndembi et al., 2008; Nouhin et al., 2009; Pillay et al., 2008).

Interestingly, a high proportion of Japanese MSM was diagnosed as recently infected compared to patients of non-Japanese origin, and females determined by BED assay. This result may be due to successful prevention programs targeting the MSM community, so that they have become more aware of their risks of HIV-1 infection. On the other hand, many of non-Japanese patients are seen at hospitals long after HIV infection is established. In addition, women tend to be ignorant of the risks of HIV infection, thus they are often diagnosed upon a prenatal HIV screening test.

Although MSM was not a predictive factor for transmission, this group included 130 cases with drug-resistant HIV-1, the highest prevalence among all the transmission categories. Therefore, those who are involved in prevention programs should take one step further to remind the MSM community about drug-resistant HIV-1 and the limited choice of effective antiretrovirals. HIV-1 transmission has been reported to be prevented in models that assessed the effect of HIV-1 testing for wider populations and immediate initiation of antiretroviral therapy (Granich et al., 2009). Although this model seems very appealing, our results suggest the importance of not forgetting the emergence and transmission of drug-resistant HIV-1 and the limited selection of antiretroviral drugs. It is important to continue surveying newly diagnosed HIV/AIDS patients to keep track of trends in drug-resistant HIV-1 transmission, to reveal high-risk populations with low awareness of HIV infection, to propose effective programs to prevent transmission of drug-resistant HIV-1, and to develop antiretroviral drugs with improved pharmacokinetics/pharmacodynamics. All these efforts may bring us one step closer to eradicating HIV-1.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.antiviral.2010.07.008.

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症　例

旅行者感染症として播種性ペニシリウム症を発症し治療が奏効した邦人HIV感染者の1症例

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序　文

Penicillium marneffei による全身播種性真菌感染症は，タイ・ラオス・中国南部・カンボジアといった東南アジア諸国において，免疫機能の低下したHIV感染者を中心に増加している[1]．本邦ではAIDS指標疾患23種類の内に数えられていないものの，タイではAIDS指標疾患の1つとされている．国内では2008年5月までの感染者数はわずか3例が確認されているのみであるが，その内訳は日本人，ミャンマー人，タイ人であり，いずれもがHIV感染症例であった．近年では，海外旅行は盛んに行われており，かつ日本人HIV感染者が増加していることを考慮すると，今後増加する可能性がある疾患と考える．本症例は，邦人で生前に診断可能であった最初の播種性ペニシリウム症であり，特徴的な経過を示したため報告する．

症　例

患者：30歳男性．

主訴：発熱，全身倦怠感，両側頸部有痛性リンパ節腫脹．

既往歴：特記事項なし．

家族歴：特記事項なし．

海外渡航歴：1998年頃欧州各国・メキシコ，2006年米国，2007年夏インドネシア（バリ島），2008年1月タイ．全て個人旅行であり，1週間ほどの滞在期間であった．尚，タイ旅行では，タイ北西部に位置するチャンマイのリゾートホテルに滞在．

現病歴：2008年5月頃より全身倦怠感・寝汗が出現．同年6月中旬より左頸部に疼痛を伴うリンパ節腫脹を認め，その後同部位は自然軽快したが，新たに右の頸部リンパ節腫脹を認めた．4月から6月までのおよそ2カ月間で約10kgの体重減少も認めた．6月末に近医受診し血液検査のHIV抗体検査結果が陽性であることを指摘され，当院を紹介受診した．AIDS発症が疑われたため，精査加療目的で入院とした．

現症：血圧126/74mmHg，脈拍108/分，体温38.7℃．

意識は清明，眼瞼結膜に貧血なく，眼球結膜黄染なし．胸腹部に異常所見なし．右頸部～鎖骨上窩にかけてΦ5cm×5cm，左頸部～鎖骨上窩にかけてΦ2cm×2cmの圧痛・熱感を伴うリンパ節腫脹を認めた．

入院時検査所見（Table 1）：白血球数は正常であったが，ALP 730U/L及びCRP 9.67mg/dLの上昇を認めた．β-D glucanは25.7pg/mLと上昇しており，HIV-RNA量は1.4×10^5コピー/mL，CD4陽性Tリンパ球数は10/μLであった．

臨床経過（Fig. 1）：発熱・リンパ節腫脹を認め，胆道系酵素ではALPのみの上昇を認め，CD4陽性リンパ球数は低下していた．所見より播種性非結核性抗酸菌症を疑い，抗酸菌ボトルを含めた血液培養（BacT/ALERT社），ならびにリンパ節穿刺液培養を行った．第8病日より前胸部・眼周囲に中心性陥凹・潰瘍を伴う皮疹が出現した（Fig. 2）．第12病日にリンパ節穿刺液培養より糸状菌が検出され（培養9日目），PDA培地でのコロニー形成の形態，cotton blue染色で糸状菌と酵母菌が一体となった菌形態から，第14病日に*P. marneffei*と同定（Fig. 3a，3b）した．また血液培養から同菌の検出が確認されたため，播種性ペニシリウム症と診断した．同日よりliposomal-

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Table 1　Laboratory data on admission

Hematology		Biochemistry			
WBC	5,100 /μL	TP	7.5 g/dL	CRE	0.62 mg/dL
Stab	3.0 %	Alb	3.1 g/dL	Na	137 mEq/L
Seg	84.0 %	T-Bil	1.05 mg/dL	K	3.6 mEq/L
Lym	5.0 %	AST	154 IU/L	Cl	101 mEq/L
Mon	7.0 %	ALT	24 IU/L	CRP	9.67 mg/dL
Eos	1.0 %	ALP	730 U/L	β-D glucan	25.7 pg/mL
CD4 + T lymphocyte	10 /μL	LDH	367 IU/L	HIV-RNA	1.4×10^5 copies/mL
Hb	13.1 g/dL	γGTP	96 IU/L		
PLT	2.5×10^5 /μL	BUN	6.0 mg/dL		

Fig. 1　Clinical course.
Arrow (→): fine-needle lymph node aspiration. Short arrow (>): blood culture

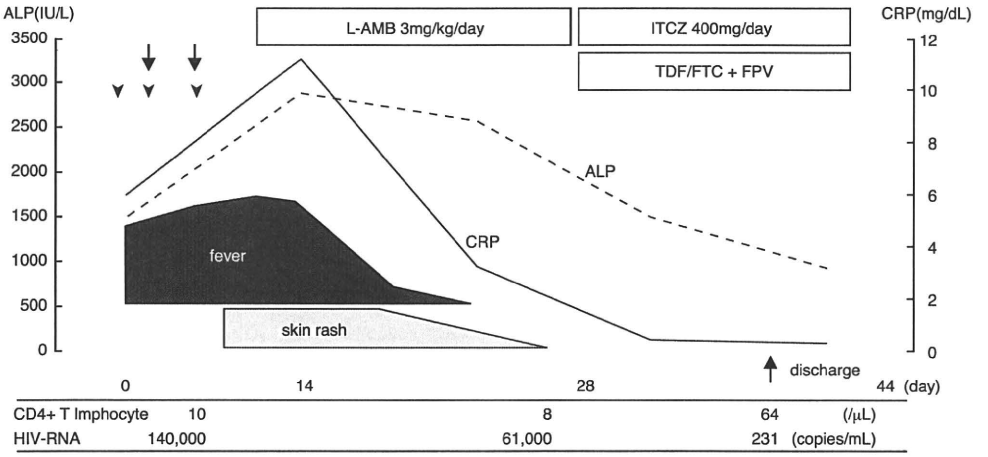


Table 2　HIV subject summaries

Subject No.year	Gender	Nationality	Age	diagnosis
1. 2000	male	Japan	38	Autopsy and histology
2. 2002	male	Myanmar	22	PCR by Skin Biopsy
3. 2008	female	Thailand	41	Blood culture
4. 2009 (our case)	male	Japan	30	Blood and lymph node culture

amphotericin B（L-AMB）3mg/kg/日で加療を開始した．治療開始後は 2 日間で解熱傾向を認め，血液検査上も改善を認めた．13 日間で L-AMB による点滴加療を終了したのち，itraconazole（ITCZ）400mg の経口加療に切り替えた．同時に HIV 感染症に対して tenofovir（TDF）300mg/日，emtricitabine（FTC）200 mg/日，fosamprenavir（FPV）2800mg/日で初回治療を開始した．以後，経過は良好であり，免疫再構築症候群など併発することもなく，第 44 病日に退院．ITCZ に関しては 10 週間に及び 400mg/日での加療を行った後に 200mg/日の維持量に減量したが，播種性ペニシリウム症の再燃は認めず経過している．

考　察

本症例のリンパ節穿刺液ならびに血液培養より分離された *P. marneffei* は，東南アジアを主な流行地とする地域流行型真菌症の病原菌である[1]．HIV 感染者で生じた症例が 1988 年に報告されて以来[2]，アジアを中心として増加の一途をたどっている．現在，タイにおいては，クリプトコッカス症・粟粒結核症についで 3 番目に多い HIV 感染者の日和見感染症となっている[3]．本邦においては 2009 年 8 月までに発症例は本症例を含め 4 例であり[4)~6)]，いずれも基礎疾患として HIV 感染症が存在していた（Table 2）．

P. marneffei の自然宿主は，ヒト以外では bamboo rat（*Rhizomys sinesnsis*）のみが確認されている．わが国が *P. marneffei* の生息域ではないことや，潜伏期間を考慮すると，本症例は発症 4 カ月前のタイ旅行の際に感染したものと考えられた．その後 HIV 感染症に伴

Fig. 2 Skin rash on left neck and scattered around cheeks and forehead.

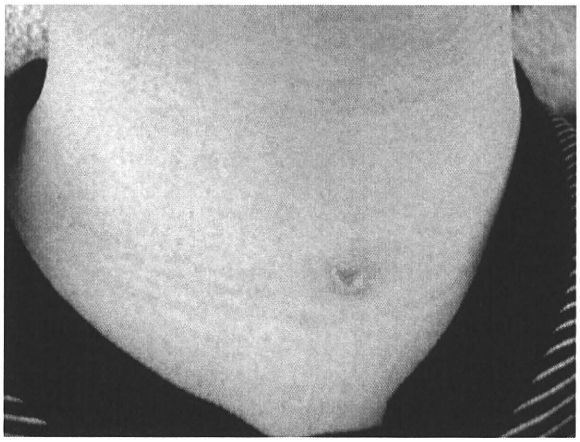
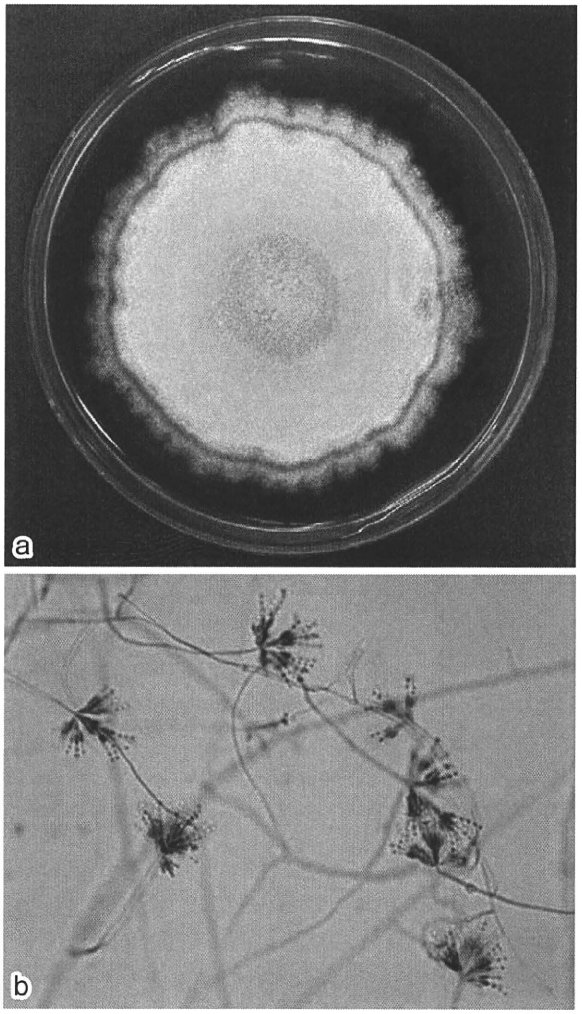


Fig. 3 a) Colony on potato dextrose agar (27℃, 20 days)
b) Microscopic slide culture × 400 cotton blue staining



う免疫力の低下が進行することで，全身性播種を起こし，顕在化したものと考えられた．

主な症状としては発熱，貧血，体重減少，皮膚症状，リンパ節腫脹，肝脾腫，下痢などである[7]．そのうちの皮膚症状は，典型的なものでは顔・頸部を中心とした中心性陥凹を伴った結節性皮疹であり[8]，本症例でも同様な皮疹を経過中に認めた．診断は各種培養により行われ，抗原検査やPCR法などはいずれも実用化には至っていない[9]．

本症例では，血液培養およびリンパ節穿刺液培養を行ったが，菌が分離されたのは抗酸菌ボトルと嫌気性ボトルからであり，好気性ボトルからは分離されなかった．真菌を直接血液培養ボトルに注入し，培養する観察においても，抗酸菌ボトルが最も培養され易いということの報告もあり[10]，本症例の診断においては，嫌気性ボトルに加えて，抗酸菌ボトルの培養も有用であった．本邦で確認されている播種性ペニシリウム症では，培養で菌が証明されたものは3例目の症例のみであったが，その症例では好気性ボトルからの分離であり，好気性の条件が培養に有効であったと著者らは結論づけている[5]．我々の症例と異なる結果となった原因は明らかではないが，培養における病原体検出は重要であり，特に免疫機能の低下したHIV感染者では，複数の感染症を合併している症例が多く，多種の感染症を想定した検体採取が必要であると考えられた．

治療としては，本菌はamphotericin B，itraconazole，ketoconazoleに感受性があり，fluconazoleには耐性である[11][12]．またvoriconazoleが治療に効果的であったという報告もある[13]．本症例では抗HIV薬を使用することを考慮し，その相互作用の面からまずはL-AMBによる加療を行った後に，ITCZによる維持療法に移行した．本症例の治療に対する反応は良好であり，速やかな症状の改善を認めた．播種性ペニシリウム症は的確な加療が行われない場合，致死的な疾患となるため，的確な微生物学的診断を行うことが重要であると考えられた．

本症例と同様に，欧州諸国でも近年東南アジア渡航後の播種性ペニシリウム症の報告が散見されるようになっている[14]．現在のところ本邦では稀な疾患であるが，HIV感染者数の増加に加え，海外渡航が盛んとなっている本邦の状況を考えあわせると，今後日和見感染症の鑑別として注目すべき疾患であり，十分な注意を払う必要があると思われる．

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文　献

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A Diagnosed, Cured Case of an HIV-infected Japanese Subject Developing Disseminated Penicilliosis After Thailand Travel

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Disseminated penicilliosis-an AIDS-indicator disease in Southeast Asian countries -but not Japan- is a systemic fungal infection caused by *Penicillium marneffei*. A 30-year-old HIV-positive Japanese man visiting Southeast Asia three months before admission and reporting fever, general fatigue, and enlarged lymph nodes lasting over one month was admitted for detailed tests. Blood culture and fine-needle aspiration lymph node biopsy a led to a diagnosis of disseminated penicillioisis, later cured by several anti-fungal agents. Caution is thus recommended regarding the possibility of this disease, given the large number of travelers visiting overseas, geographical proximity to Southeast Asia, and increasing numbers of HIV patients in Japan.

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 The Journal of AIDS Research

研究ノート

ロピナビル・リトナビル配合剤（LPV/r）の1日2回から1日1回投与へのスイッチ臨床試験結果

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目的：LPV/r を BID で治療を開始し，LPV のトラフ濃度が 6.00μg/mL 以上であった患者を対象に，服薬方法を QD に変更した後の有効性と安全性について検討することを目的に臨床試験を実施した。

対象および方法：当院で LPV/r を BID で服薬し，LPV のトラフ血中濃度が 6.00μg/mL 以上の患者 8 例を対象とした。QD 変更後 4，8，12，16，20，24 週目に有害事象，トラフ濃度，HIV-RNA 量，CD4 細胞数，T-Cho，HDL-Cho，TG を確認し，BID 服用時と比較検討した。

結果：対象患者 8 例の LPV のトラフ濃度（mean±S.D.）は，10.99±2.75μg/mL（range：7.46-14.94）であった。QD スイッチ 4 週後，LPV トラフ濃度の平均値は 2.28±1.72μg/mL（range：0.41-5.85）に低下したが，HIV-RNA 量は臨床試験を実施した 24 週間を通じて，全例感度未満を持続した。CD4 細胞数，T-Cho，HDL-Cho，TG の変動は認められなかった。新たな有害事象として，便秘，嘔気等の消化器症状が出現したが，下痢の増加は軽微であった。

考察：LPV の血中濃度が比較的高く，臨床経過が安定している患者を QD に変更した場合の 24 週間における安全性と有効性を確認することができた。今回の試験結果が，本邦における QD 投与の有用性確認の第一歩となったものと考える。

キーワード：HAART，LPV，RTV，血中濃度，QD

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序　文

ロピナビル・リトナビル配合剤（LPV/r）は，米国で 2000 年 9 月に HIV 感染症の治療薬として承認されたプロテアーゼ阻害薬（PI）である。米国において承認された LPV/r の用法・用量は，1 回 2 錠の 1 日 2 回投与（BID）に加え，未治療患者または初回治療の LPV/r 服薬患者に限り，1 回 4 錠の 1 日 1 回投与（QD）が認められている[1)]。2008 年 11 月 3 日付で改訂された米国 DHHS（Department of Health and Human Services）ガイドラインは，LPV/r の QD 投与を代替処方から推奨処方に変更した[2)]。

QD 承認の基礎となった試験では，BID 群と QD 群を投与期間 48 週で比較し，HIV-RNA 量が感度未満となった割合に有意な差を認めず，QD 群では下痢の頻度が高かったとしている[3)]。この臨床試験における薬物動態では，BID 群のトラフにおける LPV の血中濃度（トラフ濃度）は約 6μg/mL で，QD 群では約 3μg/mL に減少したものの[3,4)]，米国 DHHS ガイドラインが推奨する LPV の目標トラフ濃度 1.00μg/mL を上回っていた[2)]。

我々は LPV/r を BID で治療を開始し，治療効果が安定し，LPV のトラフ濃度が 6.00μg/mL 以上であった患者を対象に，服薬方法を QD に変更した後の，有効性と安全性について検討するための臨床試験を実施した。なお本試験は，国立病院機構大阪医療センターの倫理委員会に相当する受託研究審査委員会の承認を得た（承認番号：0724）。

対象および方法

国立病院機構大阪医療センター免疫感染症科に通院し，LPV/r を含む HAART で治療を開始し，HIV-RNA 量が 12 週以上感度未満を維持し，LPV のトラフ濃度が 6.00μg/mL 以上で，問診により血中濃度測定前 1 週間の服薬率が 100% と見込まれた患者に対して，本試験の趣旨説明を行い，試験参加の同意を文書で得た。同意取得後，LPV/r を BID から QD に変更した。LPV の血中濃度に影響を及ぼ

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すと考えられる高脂血症治療剤等の投与を受けた患者は本試験の対象外とした。調査期間は2008年3月1日から2009年2月28日までとした。

血中濃度測定は以下の方法で行った。ヘパリンナトリウムを添加した試験管に，1回5 mLの血液を採取し，10℃以下3000回転10分間遠心分離し，ポリプロピレン製のスクリューキャップ付きチューブに血漿を2 mL分注し，分析開始まで−80℃で凍結保存した。測定はHPLC法を用い，株式会社BMLにて実施した。また，血中濃度測定は，厚生労働科学研究費補助金「抗HIV薬の血中濃度に関する臨床研究」により実施した。

LPV/rをQDへ変更後4，8，12，16，20，24週目に採血を実施し，LPVのトラフ濃度，HIV-RNA量，CD4細胞数，T-Cho，HDL-Cho，TGを測定し，有害事象発現の有無を問診にて確認した。QD変更前後の24週間について比較し，T-Cho，HDL-Cho，TGについては，一元配置分散分析法を用いて解析した。

結　果

LPVのトラフ濃度が6.00 μg/mL以上の患者は18例であった。そのうち，選択基準を満たし同意が得られた8例をBIDからQDに変更した。平均年齢（mean±S.D.）は43±12歳（range：27-60）で，男性7例，女性1例であった。併用した核酸系逆転写酵素阻害薬（NRTI）は，テノホビル/エムトリシタビン合剤（TVD）4例，アバカビル/ラミブジン合剤（EZC）3例，ジドブジン/ラミブジン合剤（COM）1例であった。試験期間を通じて中止例は認められなかった。

治療変更前後の血中濃度を表1，臨床検査値を表2に示す。LPV平均トラフ濃度（mean±S.D.）は10.99±2.75 μg/mL（range：7.46-14.94）であった。QDへの変更後4週目に，患者のLPV血中濃度は2.28±1.72 μg/mL（range：0.41-5.85）に低下し，その後24週までほぼ一定の値を示した。HIV-RNA量は，臨床試験を実施した24週間を通じて，全例感度未満を持続し，CD4細胞数は，24週までほぼ一定の値を示した。変更後のT-Cho，HDL-Cho，TGの変動には有意差を認めなかった（p＝0.933，p＝0.607，p＝0.954）。

QD変更後の新たな有害事象として，嘔気，胃部不快感，便秘などの消化器症状が認められた。下痢の回数の変化について確認したところ，軽微な増加は認めたが，グレード2以上の症状は認められなかった（表3）。

表1　LPV trough plasma concentration (μg/mL)

patient	Baseline	week 4	week 8	week 12	week 16	week 20	week 24
#1	9.23	2.10	1.73	0.80	1.34	1.68	1.43
#2	14.94	2.43	3.94	1.65	2.78	2.62	2.49
#3	14.86	0.80	1.09	1.09	1.67	1.07	1.18
#4	9.63	1.10	0.41	0.40	0.40	0.41	0.40
#5	11.19	5.85	4.63	3.17	4.24	3.04	5.47
#6	7.46	2.32	2.89	2.71	3.46	3.41	3.36
#7	11.75	0.41	0.70	0.49	0.33	1.37	1.14
#8	8.89	3.24	6.58	6.22	7.08	6.02	6.28
Mean (S.D.)	10.99 (2.75)	2.28 (1.72)	2.75 (2.17)	2.07 (1.96)	2.66 (2.27)	2.45 (1.77)	2.72 (2.16)

表2　Laboratory parameters

Parameter	n	Baseline	week 4	week 8	week 12	week 16	week 20	week 24
T-Cho (mg/dL)	8	197.3 (33.5)	185.6 (26.6)	191.1 (15.6)	186.5 (15.3)	189.0 (21.8)	190.6 (20.9)	193.6 (24.7)
HDL-Cho (mg/dL)	8	56.2 (9.9)	50.0 (6.7)	54.8 (7.7)	53.8 (7.8)	55.3 (8.1)	59.3 (11.5)	60.4 (9.8)
TG (mg/dL)	8	207.5 (115.0)	174.6 (50.6)	206.4 (110.8)	201.1 (105.6)	230.8 (139.6)	201.1 (114.9)	170.1 (63.6)
CD4 (cells/mm^3)	8	360 (156.4)	340 (140.9)	340 (132.3)	382 (157.1)	375 (163.5)	348 (164.5)	358 (165.7)
HIV-RNA (copies/mL)	8	<50	<50	<50	<50	<50	<50	<50

Data expressed as Mean (S.D.)

考　　察

LPV/r は，大きな IQ (C_{min}/IC_{50} : Inhibitory Quotient) 値を示すことで強力な抗ウイルス作用を示し[5]，高い認容性，持続的なウイルス抑制作用[6]を併せ持つ PI である。PI のウイルス学的効果は血中濃度と相関することから[7,8]，ウイルス学的有効性を考えれば，より高く血中濃度を維持することは有用であると考えられる。しかし，血中濃度が高いと副作用の発現率が増加することから[9]，すべての PI の血中濃度を高く保つことは不可能である。また，有効な抗 HIV 薬も服薬アドヒアランスが低下すれば，服薬の中断あるいは耐性獲得による治療失敗となる[10]。近年，抗 HIV 薬の改良が重ねられ，QD が可能な抗 HIV 薬が複数承認されたことにより，QD は HAART の主流となりつつある。患者は，服薬方法を QD にすることで，BID に比べ服薬回数や服薬時間等の制約が軽減され，服薬アドヒアランスや QOL の向上が期待できる[11]。従って，LPV/r の QD と BID が同等の安全性と有効性を持つことを示すことができれば，臨床的な意義は大きいものと考える。

今回我々は，国内で承認されていない用法用量である QD による臨床試験を行うにあたり，臨床試験の安全性を担保するために，初回治療から LPV/r を BID で服用患者において，HIV-RNA 量が 12 週以上感度未満を維持し，さらに LPV のトラフ濃度が 6.00 μg/mL 以上を示す患者を QD 変更の対象とした。対象患者のトラフ濃度を 6.00 μg/mL 以上にした設定根拠は，海外臨床試験において，BID のトラフ濃度が 6.56±3.71 μg/mL であり，QD では 3.22±2.07 μg/mL とした報告に基づき[3]，DHHS のガイドラインが推奨する[2] LPV 目標トラフ濃度 1.00 μg/mL を維持するためには，トラフ濃度が 6.00 μg/mL 以上が必要であると仮定した。また，当院で LPV/r を BID で服用した患者 36 例の平均トラフ濃度が 6.85±4.13 μg/mL であったことから，日本人におけるトラフ濃度は先の海外報告と同様であると考え，今回の臨床試験の設定根拠とした（図 1）。

LPV/r を BID から QD へ変更 4 週後の平均トラフ濃度は 2.28±1.72 μg/mL であった。トラフ濃度の平均値は今回の試験期間 24 週間を通じて 1.00 μg/mL 以上を維持したことから，対象患者のトラフ濃度を 6.00 μg/mL 以上に設定したことは適切であったと考えられた。しかし，本臨床試験における QD の平均トラフ濃度は，海外報告[3]よりも低値であり，表 1 に示したように，測定した患者の血中濃度には目標トラフ濃度を下回ったデータもあった。臨床試験を実施した 24 週間における患者の HIV-RNA 量は，全例検出限界未満を維持していたものの，QD へ変更するための LPV トラフ濃度の設定に関しては，今後さらに検討が必要と考える。また目標トラフ濃度を複数回，下回った症

表 3　下痢回数の変化/日

患者	変更前 (BID)	変更後 (QD)
#1	5 回	3 回
#2	2-3 回	3-4 回
#3	無	2-3 回
#4	4-5 回	4-5 回
#5	無	無
#6	3 回	1-2 回
#7	無	無
#8	2-3 回	2-3 回

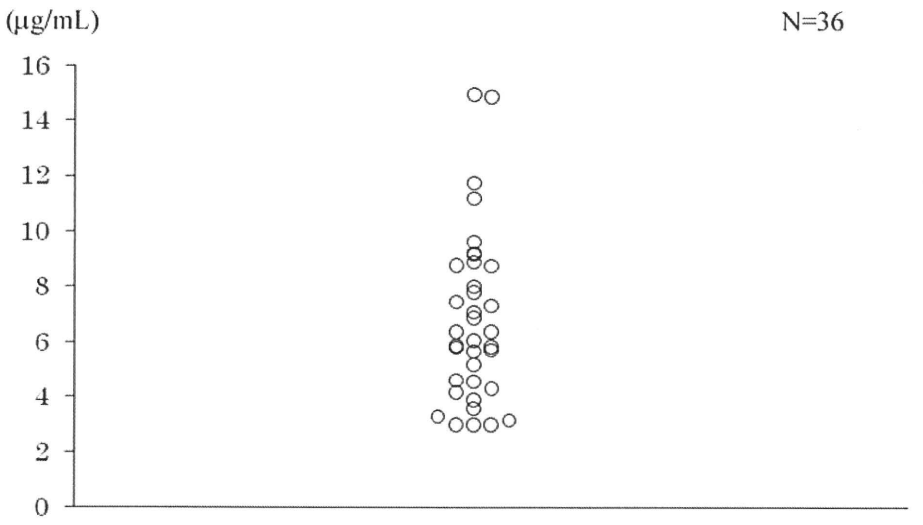

図 1　LPV BID trough plasma concentration (μg/mL)