## Author Summary

Amebiasis is usually transmitted by ingestion of contaminated food or water in developing countries. Recently, however, increased risk for amebiasis among men who have sex with men (MSM) due to oral-anal sexual contact was reported in developed countries, resulting in growing concern on amebiasis in HIV-1-infected MSM. The recommended treatment of amebiasis is metronidazole or tinidazole, followed by a luminal agent to eliminate intestinal cyst colonization. However, the efficacy of luminal treatment in preventing recurrence has not been assessed yet. In this study, we analyzed the medical records of 170 patients with amebiasis and HIV-1 co-infection. Treatment with metronidazole or tinidazole was excellent whereas luminal treatment did not reduce the frequency of recurrence of amebiasis. Recurrence was more frequent in those MSM with signs of sexual activity such as syphilis infection. Luminal treatment following metronidazole or tinidazole treatment does not reduce recurrence of amebiasis in high risk populations.


related symptoms, e.g., fever and liver abscess, or tenesmus and diarrhea, 2) high serum titer (>1:100) for antibody against *E. histolytica* in patients with IA-related symptoms in whom microbiological cultures or histological examination of clinical specimens did not identify any pathogen, and who showed improvement of IA symptoms following metronidazole or tinidazole monotherapy [10–12]. The medical records were surveyed for patients' characteristics, presenting forms of clinical IA [e.g., colitis, amebic liver abscess (ALA), and perianal abscess], HIV-1-induced immunocompromised status, and symptoms, laboratory data and serological markers of other sexually-transmitted diseases (STD) including syphilis, hepatitis B and C viruses (HBV and HCV). After completion of treatment for IA, the medical records were followed-up until March 2010, excluding those cases found to have died or lost to follow-up.

### Genotyping of *E. histolytica*

To determine the strains of *E. histolytica* among HIV-1-infected Japanese patients, genotyping of *E. histolytica* was performed in patients who were PCR positive. The PCR method was used for the first time in our clinic for the diagnosis of amebiasis in December 2008, and since then 14 patients had been diagnosed as IA based on a positive PCR. For the PCR, DNAs were extracted from various biological specimens (e.g., stool, colon wash and punctuate-exudate) by using QIAamp DNA stool Mini Kit (Qiagen, Valencia, CA). Polymerase chain reactions were performed with specific sets of primers designed to target each of 6 loci (D-A, S-Q, R-R, A-L, $S^{TGA}$-D, and N-K) of tRNA-linked polymorphic short tandem repeats (STR), as described previously [21]. The PCR product was sequenced by ABI 3130XL Genetic Analyzer (Applied Biosystem, Foster city, CA) in both forward and reverse directions. Phylogenetic analysis and genotyping were performed as described previously [22].

### Statistical analysis

Differences in patients' characteristics and clinical features were examined using the chi-square test or nonparametric test. The cumulative risk for recurrence was analyzed by the Kaplan-Meier method, and differences were tested by the log-rank test. The Cox proportional hazards model was used to assess the impact of luminal treatment on the recurrence rate after adjustment for other factors. The hazard ratio and 95% confidence interval were calculated. *P* values less than 0.05 were considered to denote statistical significance. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL).

## Results

### Clinical data and response to treatment

IA was diagnosed in 170 HIV-1-infected cases between April 1997 and March 2010 (including amebic colitis, n = 102; ALA, n = 63; and perianal abscess, n = 5, Table 1). Thirty-three patients had two of the above three clinical forms of IA. All patients were males and 164/170 (96.5%) were MSM. High rates of positive TPHA (*Treponema pallidum* hemagglutination assay) (71.2%) and HBV exposure (HBs antigen-positive, HBs antibody-positive, or HBc antibody-positive) (60.0%) were observed. No significant differences were seen in CD4 counts, HIV-1 loads, coexisting AIDS definite disease and the proportion of patients treated with antiretrovirals, suggesting that HIV-induced immunocompromised status did not have an impact on the clinical presentation of amebic infection, in agreement with previous data [12]. In cases of amebic colitis (n = 102), diarrhea (69.7%) was the most common symptom followed by dysentery (55.9%) (Table 2). Fever (>37.5°C) was seen in only 20 patients (19.6%), including 5 cases with perforative peritonitis. In cases with ALA (n = 63), fever (95.2%) was the most common symptom followed by abdominal pain (55.6%). Diarrhea (46.0%) and dysentery (19.0%) were only seen in less than half of ALA cases. Single abscess (72.6%) was identified in most cases. Liver abscesses were seen more frequently in the right lobe (70.5%) than the left (9.8%). Nine patients (14.3%) had pleuritis (considered a co-existing disease), as well as abscesses in the right lobe, and 7 of these presented chest pain. Comparison of physical and laboratory data showed higher peak body temperature (BT), leukocyte count and C reactive protein (CRP) in ALA cases (Table 2) and perforative peritonitis cases (data not shown) compared with colitis cases, indicating that high fever, leukocytosis and high CRP could be the signs of extraluminal amebiasis. It is reported that high fever and leukocytosis are also common in ALA patients free of HIV-1 infection, though both parameters were unusually associated with simple amebic colitis [23]. In ALA cases, however, leukocyte count correlated positively with CD4 count (data not shown in tables: Pearson product-moment correlation coefficient 0.36, $p$ value 0.004) and negatively with HIV-RNA load (Pearson product-moment correlation coefficient -0.28, $p$ value 0.03), but CRP correlated neither with CD4 count nor HIV-RNA load (CRP-CD4, $p = 0.81$, CRP-HIV-RNA, $p = 0.32$). There were also no correlations between CD4 count, HIV-RNA load, BT, leukocyte count or CRP and abscess size or number.

All patients were treated with metronidazole (750 mg t. i. d. for 10 days) for IA, with the exception of two who were treated with tinidazole (2 g q. d. for 3 days). Complete remission of all IA symptoms was observed in 165 patients including the two treated with tinidazole. Five cases died within six months after diagnosis of IA; two from complications related to amebic colitis (one peritoneal perforation and one gastrointestinal bleeding), one from malignant lymphoma, one from *Pneumocystis jirovecii* pneumonia, and one from pulmonary thrombosis. The overall mortality rate was 3% in this study, which was comparable to those reported in non-HIV cases [2,23].

### Recurrence after treatment

Luminal agents; paromomycin and diloxanide, are not approved in Japan, and they were not always available in our facility during the study period. After completion of IA treatment with metronidazole or tinidazole, luminal agents were administered when available. Consequently, 83 cases were treated with luminal

**Table 1.** Patient demographics, state of HIV, and serological markers.

| | Colitis (n = 102)[1] | ALA (n = 63)[2] | Perianal abscess (n = 5)[3] | All (n = 170) | *P* value[4] |
|---|---|---|---|---|---|
| Age (years) [IQR] | 38 [32–43] | 37 [31–44] | 45 | 38 [31–44] | 0.58 |
| Male sex (%) | 102 (100) | 63 (100) | 5 (100) | 170 (100) | – |
| Homosexual (%) | 96 (94.1) | 63 (100) | 5 (100) | 164 (96.5) | 0.053 |
| Past History of amebiasis (%) | 16 (15.7) | 9 (14.3) | 1 (20.0) | 26 (15.3) | 0.81 |
| CD4 count (/μl) | 262 [98–398] | 271 [123–411] | 58 | 269 [107–403] | 0.84 |
| HIV-RNA (log copies/ml) | 4.60 [3.89–5.32] | 4.66 [3.91–5.11] | 5.04 | 4.66 [3.93–5.28] | 0.70 |
| AIDS (%) | 18 (17.6) | 8 (12.7) | 2 (40.0) | 28 (16.5) | 0.40 |
| ART initiated (%) | 18 (17.6) | 11 (17.5) | 1 (20.0) | 30 (17.6) | 0.98 |
| TPHA test positive (%) | 77 (75.5) | 40 (63.5) | 4 (80.0) | 121 (71.2) | 0.10 |
| HBV exposure (%) | 59 (57.8) | 41 (65.1) | 2 (40.0) | 102 (60.0) | 0.36 |
| HCV Antibody positive (%) | 3 (2.9) | 3 (4.8) | 0 (0) | 6 (3.5) | 0.42 |

Data are median [interquartile range: IQR] or number (percentage) of patients.
[1]5 cases of perforative peritonitis are included as co-existing diseases. Four cases were diagnosed coincidentally by colonoscopy in asymptomatic patients.
[2]31 cases of colitis, 1 case of perianal abscess, 9 cases of pleuritis, and 2 cases of peritonitis are included as co-existing diseases.
[3]1 case of colitis is included as co-existing diseases.
[4]Chi-square test or non-parametric test was performed for data of colitis and ALA.
UD: undetectable, ART: anti-retroviral therapy, TPHA test: *Treponema pallidum* Hemagglutination Assay test, HBV exposure: HBsAg-positive or HBsAb-positive, and/or HBc-Ab positive.
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agents; 38 cases with promomycin (500 mg t. i. d. for 10 days) and 45 cases with diloxanide furoate (500 mg t. i. d. for 10 days). No significant differences were seen in patients' characteristics, including HIV-1-induced immunocompromised status, serological markers of other STD, and clinical forms and severity of amebiasis between the 83 cases with luminal treatment and 82 cases who did not receive such treatment (Table S1). The median follow-up period after completion of metronidazole or tinidazole treatment was 50 months (inter quartile range: 19–85) in those who received luminal treatment, and 43 months (inter quartile range: 23–98) in those without.

**Table 2.** Clinical features of amoebic colitis and ALA.

| | Colitis (n = 102) | ALA (n = 63) | *P* value |
|---|---|---|---|
| Symptoms | | | |
| Diarrhea (%) | 71/102 (69.6) | 29/63 (46.0) | 0.003 |
| Dysentery (%) | 57/102 (55.9) | 12/63 (19.0) | <0.001 |
| Abdominal pain (%) | 23/102 (22.5) | 35/63 (55.6) | <0.001 |
| Chest pain (%) | 0/102 (0.0) | 7/63 (11.1) | <0.001 |
| Peak BT (°C) [IQR][3] | 36.8 [36.5–37.4] | 39.0 [38.8–39.5] | <0.001 |
| WBC (/μ l) [IQR][3] | 5,830 [4490–7580] | 11,760 [9460–15170] | <0.001 |
| CRP (mg/dl) [IQR][3] | 0.62 [0.16–3.02] | 19.15 [10.53–24.75] | <0.001 |
| Frequency of diarrhea[1] | | | |
| ≤ 5 times/day (%) | 63/101 (62.4) | – | |
| 6–10 times (%) | 26/101 (25.7) | – | |
| ≥ 11 times (%) | 12/101 (11.9) | – | |
| Size of abscess (mm) | – | 59 (10–180) | |
| Location of abscess[2] | | | |
| Right lobe only | – | 43/61 (70.5) | |
| Left lobe only | – | 6/61 (9.8) | |
| Both lobes | – | 12/61 (19.7) | |
| Number of abscesses[1] | | | |
| Single (%) | – | 45/62 (72.6) | |
| Multiple (%) | – | 17/62 (27.4) | |

[1]Data of one case were not available.
[2]Data of two cases were not available.
[3]Data are median [interquartile range: IQR] or number (percentage) of patients.
BT: body temperature, WBC: White Blood Cell counts, CRP: C reactive protein.
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Within the 12-month post-metronidazole treatment period, recurrence of IA was noted in only two patients who did not receive luminal treatment, suggesting reactivation of residual cysts of *E. histolytica* (Figure 1). However, during the entire follow-up period, six in each group experienced recurrence of IA, with no significant difference in the recurrence frequency by the log-rank chi-square test. Multivariate analysis showed that recurrence did not correlate with past history of IA, CD4 count, TPHA, HBV exposure (HBs antigen-positive or HBs antibody-positive), or the presence of extraluminal IA disease (Table 3). However, a positive HCV antibody was significantly associated with IA recurrence. Recurrence also tended to occur in those who acquired new syphilis infection during the follow-up period, though the difference did not reach statistical significance.

### Genotypes of *E. histolytica*

Genotyping of *E. histolytica* was performed in samples obtained from 14 patients between December 2009 and March 2010 (colitis, n = 8; ALA, n = 4; colitis and ALA, n = 1; and perianal abscess, n = 1; Table S2). Eleven different genotypes were recognized, including five genotypes (J8, J12, J13, J20, and J23) identified previously in Japan [22], and six newly recognized genotypes (J24-J29). There was no significant relation between *E. histolytica* genotype and clinical presentation.

## Discussion

In the present study, retrospective analysis of the medical records of 170 patients with HIV-1-infection and IA showed no

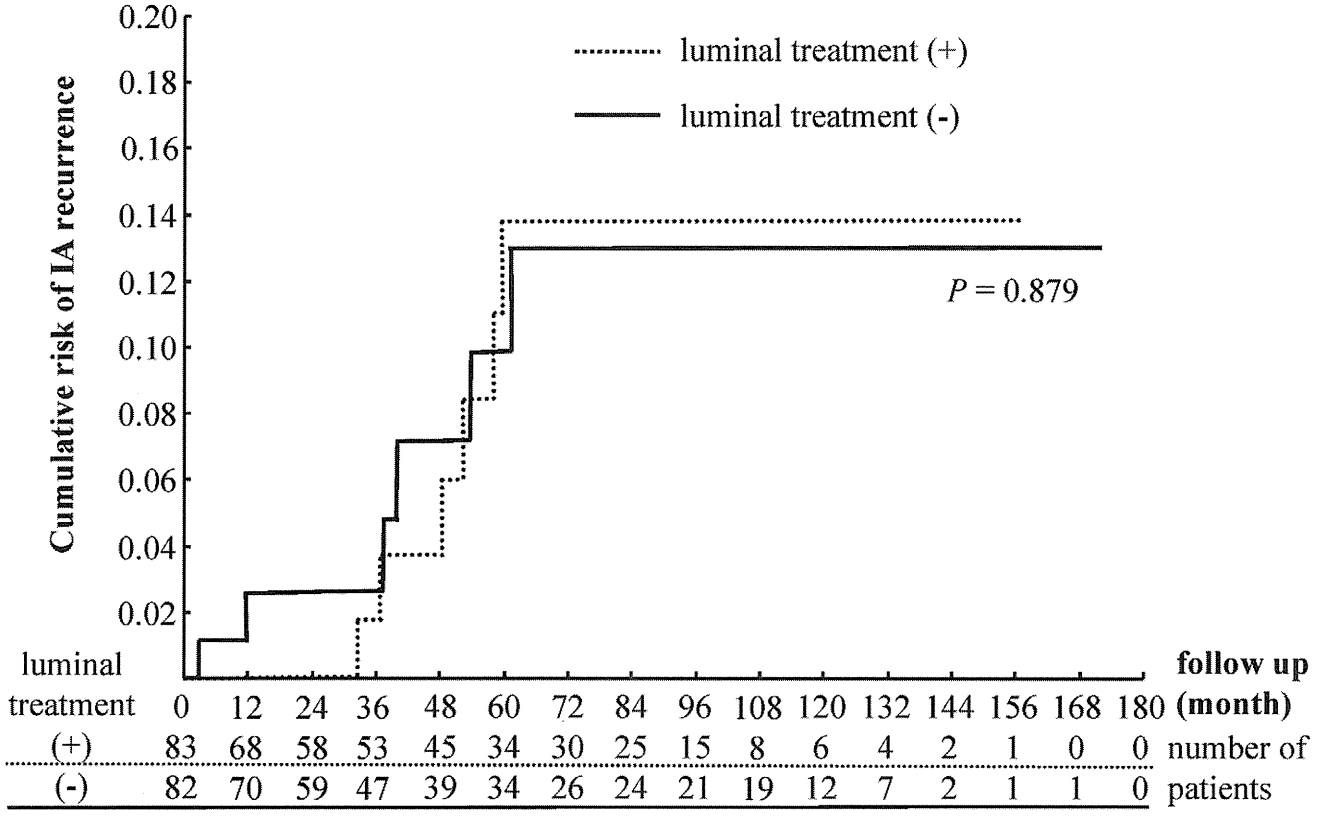


**Figure 1. Kaplan-Meier estimates of time to IA recurrence.** Cumulative probability of IA recurrence after completion of metronidazole or tinidazole treatment with or without subsequent luminal treatment.
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impact for HIV-1-induced immunocompromised status on the clinical forms of amebiasis. The physical and laboratory findings showed that high fever, leukocytosis and high CRP correlated with extraluminal diseases of amebiasis. In ALA cases, however, leukocyte count correlated positively with CD4 count and negatively with HIV-RNA load, indicating that CRP is more sensitive marker for the detection of the extraluminal diseases in advanced immunocompromised patients.

Only five patients died after the diagnosis of IA; two from IA complications and three from other causes. The results indicate excellent outcome for HIV-1-infected individuals with uncomplicated amebiasis treated with metronidazole or tinidazole, in agreement with previous reports on HIV and non-HIV cases [2,11,12,20,23]. Based on conventional wisdom and written opinion, adequate management of IA should include treatment with a luminal agent following metronidazole or tinidazole treatment, in order to eradicate residual cysts of *E. histolytica* due to the high rate (40–60%) of luminal colonization [2,23–27]. On the other hand, the results of longitudinal observational studies indicated that asymptomatic cyst carriers rarely develop IA, and

**Table 3.** Multivariate analyses for factors associated with frequency of recurrence.

| | No recurrence (n = 153)[1] | Recurrence (n = 12) | Hazard ratio (95.0% CI) | *P* value |
|---|---|---|---|---|
| Past history of IA[2] (%) | 24 (15.7) | 2 (16.7) | 0.914 (0.186–4.478) | 0.911 |
| CD4 counts <200[2] (%) | 57 (37.3) | 3 (25.0) | 0.385 (0.101–1.470) | 0.162 |
| TPHA test positive[2] (%) | 108 (70.6) | 10 (83.3) | 2.435 (0.501–11.827) | 0.270 |
| HBV exposure[2] (%) | 92 (60.1) | 7 (58.3) | 1.248 (0.364–4.277) | 0.725 |
| HCV Antibody positive[2] (%) | 3 (2.0) | 2 (16.7) | 7.664 (1.369–42.890) | 0.020 |
| Extraluminal disease[2] (%) | 66 (43.1) | 4 (33.3) | 0.559 (0.163–1.921) | 0.356 |
| No luminal agent (%) | 76 (49.7) | 6 (50.0) | 1.070 (0.322–3.559) | 0.912 |
| Syphilis during follow-up period (%) | 33 (21.6) | 7 (58.3) | 3.332 (0.961–11.547) | 0.059 |

[1]Five patients died within 6 months from disease onset and their data were excluded from analysis.
[2]Status at diagnosis of IA.
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that cyst form ameba often disappears spontaneously without any treatment [28,29]. There is controversy about the need for cyst eradication following metronidazole or tinidazole treatment, especially in endemic areas where re-infection is frequent. In this study, recurrence of IA within the first year of metronidazole treatment was noted in only two patients of 82 patients who did not receive luminal therapy. Moreover, long-term follow-up indicated IA recurrence also in those who received luminal agents, and the benefits obtained from luminal treatment seemed to have disappeared. IA recurred more frequently in those with HCV infection, which was recently reported to be transmittable sexually among MSM [30], and in those who acquired new syphilis infection during the follow-up period, suggesting that sexually active MSM tend to experience IA recurrence due to re-acquisition of new *E. histolytica* infection. HBV exposure and positive TPHA at IA diagnosis did not correlate with IA recurrence probably because the high prevalence of these two parameters in this study masked the difference between recurrence and non-recurrence cases. Educational approach for safer sex may be more appropriate rather than luminal treatment to prevent IA recurrence after treatment.

Eleven genetic strains of *E. histolytica* were identified in this study and none of them had been reported so far from geographic areas other than Japan [21,22,31,32], indicating that diverse Japan-specific isolates of *E. histolytica* are already prevalent among MSM in Japan. In fact, the *E. histolytica* seropositivity rate in HIV-1-infected MSM in our clinic was as high as 17.9% in 2009 (unpublished data), which is comparable with the seropositivity rate in Japanese MSM reported more than 20 years ago [5]. Unfortunately, we could not compare the genotypes of *E. histolytica* between the incidences of the primary and recurrent IA within the same individuals due to the lack of appropriate stocked samples, which would have probably demonstrated acquisition of new infection.

Considered together, the results emphasize the difficulty of preventing IA recurrence without educational approach to prevent new amebic infection even after successful IA treatment in the high risk groups such as HIV-1-infected MSM. The spread of *E. histolytica* in MSM of other developed countries beyond Asia should be of great concern.

## Supporting Information

**Table S1 Patient demographics with and without luminal treatment.**
(DOC)

**Table S2 Genotyping data of 6 STR loci in 14 clinical samples.**
(DOC)

## Author Contributions

Conceived and designed the experiments: HG JT SO. Performed the experiments: KW AEdC TN. Analyzed the data: KW HG. Contributed reagents/materials/analysis tools: KW HG JT SO. Wrote the paper: KW HG.

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INTERNAL MEDICINE

□ CASE REPORT □

# Primary HIV Infection with Acute Transverse Myelitis

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## Abstract

Primary HIV infection (PHI) is associated with various neurological disorders. However, acute transverse myelitis (ATM) complicating PHI has not been reported after the introduction of the combination antiretroviral therapy (cART). We encountered one patient with known PHI with clinical presentation of ATM. Treatment with cART and corticosteroids successfully improved symptoms, and no recurrence was noted after discontinuation of cART. In conclusion, concurrent use of cART and corticosteroids was effective against PHI accompanied by ATM and could be withdrawn after improvement of ATM.

**Key words:** acute transverse myelitis, primary HIV infection, cART

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## Introduction

Primary HIV infection (PHI) is associated with various neurological disorders (1, 2). However, PHI complicated with acute transverse myelitis (ATM) had only been reported before the availability of combination antiretroviral therapy (cART) (3). We report a case of ATM, successfully treated with cART and corticosteroids.

## Case Report

A 30-year-old homosexual man visited a local hospital with 5-day history of systemic skin eruption, high fever and sore throat. Although these symptoms disappeared spontaneously within one week, PHI was suspected because of the positive result of 4th generation HIV diagnostic test. He was referred to the clinic of our hospital for further management. PHI was confirmed by the negative result of Western blot analysis and high HIV-RNA level (6.38 log copies/mL) with a CD4 count of 601/μL. No treatment for HIV infection was provided because the patient was asymptomatic at that stage. However, he subsequently developed urinary retention and abnormal sensation in the lower limbs, and he returned to the clinic 2 days later. On admission, neurological examination showed normal function of the cranial nerves and no nuchal stiffness. Motor system assessment showed no paresis. The deep tendon reflexes were exaggerated in the lower extremities but normal in the upper extremities. Pathological reflexes such as Babinski reflex and Chaddock sign were not noted. Sensory system examination showed bilateral hypoesthesia and hypalgesia below the level of Th7, and deep sensation was preserved in all extremities. Urinary retention was observed and anal muscle tone was reduced. The cranial and entire spinal magnetic resonance imaging (MRI), with and without gadolinium enhancement, showed no abnormal findings. Examination of the cerebrospinal fluid (CSF) showed mild pleocytosis (cell count: 8.0/μL, mononuclear cells 6.0/μL), a normal protein level (29 mg/dL), and normal IgG index (0.62). The CSF level of myelin basic protein (MBP) was elevated to 1857.3 pg/mL (normal range: <102 pg/mL). Herpes simplex virus, varicella zoster virus, cytomegalovirus and Epstein-Barr virus DNA were negative in the CSF on polymerase chain reaction assay.

ATM was diagnosed by the typical neurological findings with spinal cord inflammation, which was proceeded by PHI. Upon the diagnosis, cART of lopinavir/ritonavir plus abacavir/lamivudine and methylprednisolone pulse treatment (1000 mg for 3 days) were initiated (Fig. 1). The treatment resulted in immediate and rapid improvement of clinical symptoms, and all symptoms disappeared by treatment day 6. The MBP level (less than 31.2 pg/mL) and cell count

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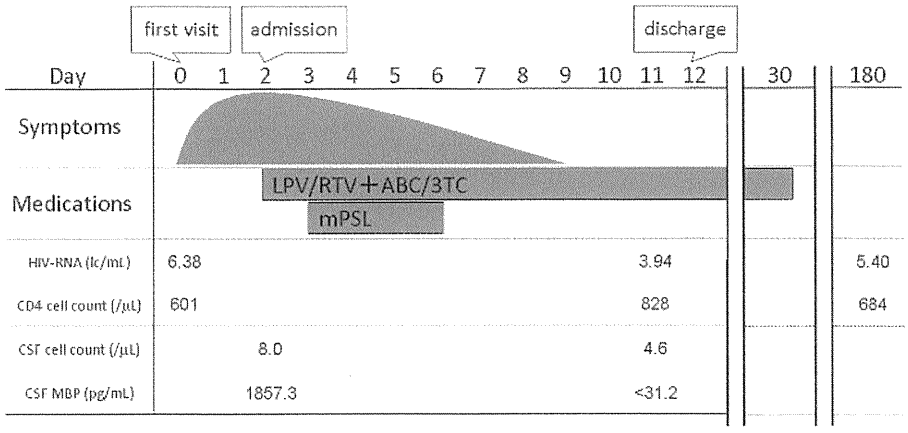


**Figure 1.** **Clinical course. LPV: Lopinavir, RTV: Ritonavir, ABC: Abacabir, 3TC: Lamivudine, mPSL: methylprednisolone, CSF: cerebrospinal fluid, MBP: myelin basic protein**

(4.6/μL) in the CSF decreased to the normal ranges at treatment day 9. Methylprednisolone was given for 3 days and cART was continued for one month, which resulted in no relapse of ATM-related symptoms in the subsequent 6 months (Fig. 1), and also no resistant mutation was seen in Protease and Reverse Transcriptase lesions of HIV (data not shown).

## Discussion

ATM is a segmental spinal cord injury caused by acute inflammation characterized by acute or subacute motor, sensory, and autonomic (genitourinary and digestive systems) spinal cord dysfunction (4, 5). Although preceding infection is noted in 20-50% of cases (4, 6-9) ATM associated with PHI was only reported before the era of cART (3). There is currently no standard treatment for ATM. Although corticosteroids are the first-line treatment due to the probable mechanisms such as molecular mimicry and the development of autoantibodies, approximately 30 to 50% of patients develop severe sequelae (4, 10, 11).

In the present patient, ATM was diagnosed by the rapid development of symptoms, neurological findings, including clearly-defined bilateral sensory deficits below Th7 level, autonomic dysfunction, and MBP elevation, suggestive of spinal cord inflammation (4, 5). Furthermore, PHI was diagnosed just before the onset of ATM, based on the absence of bands in Western blot analysis and a high titer of HIV-RNA level. It was concluded that PHI was the trigger of ATM.

Although direct cytopathic effects of the virus and immune-mediated toxicity are suggested, pathogenesis of PHI is not fully understood (12-14). There are case reports of rapid improvement of PHI-related symptoms after cART initiation, even though the HIV-RNA level was not completely suppressed at the time of clinical resolution (15, 16). Regarding these phenomena, it is assumed that inhibition of viral replication by cART induces the resolution of symptoms. In the present case, complete recovery was achieved by the combination of steroids and cART (4, 11, 12).

In conclusion, concurrent use of cART and corticosteroids was effective against PHI accompanied by ATM. The immediate improvement in ATM allowed the subsequent discontinuation of treatment.

**The authors state that they have no Conflict of Interest (COI).**

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INTERNAL MEDICINE

□ CASE REPORT □

# Antiretroviral Therapy for Treatment-naïve Chronic HIV-1 Infection with an Axonal Variant of Guillain-Barré Syndrome Positive for Anti-ganglioside Antibody: A Case Report

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## Abstract

Guillain-Barré syndrome sometimes manifests as immune reconstitution inflammatory syndrome. We report a treatment-naïve male with chronic HIV-1 infection who presented with an axonal variant of Guillain-Barré syndrome. Antiretroviral therapy commenced one month later and no rapid improvement or deterioration of tetraparesis was noted. This is the first report that describes the detection and serial measurements of anti-ganglioside antibody in a patient with HIV infection. This case suggests a limited role for T-cell immunity in the production of anti-ganglioside antibody and the pathogenesis of axonal variants, since the antiretroviral therapy-induced improvement in T-cell immunity neither re-elevated anti-ganglioside antibody titer nor worsened tetraparesis.

**Key words:** human immunodeficiency virus, Guillain-Barré syndrome, anti-ganglioside antibody, acute motor axonal neuropathy, immune reconstitution inflammatory syndrome

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## Introduction

Guillain-Barré syndrome (GBS) is a well-known but rare complication of primary human immunodeficiency virus (HIV) infection (1). GBS can also manifest as immune reconstitution inflammatory syndrome, a condition sometimes seen in patients with HIV infection after the introduction of antiretroviral therapy, which is characterized by recovery of T-cell immunity with an overwhelming response to pre-existing antigen (usually opportunistic infections) leading to worsening of symptoms (1, 2). Thus, CD4+ T cell-mediated cellular immunity appears to play a role in the pathogenesis of GBS. However, little is known about the effect of CD4+ T-cell immunity activated by antiretroviral therapy on the pathogenesis and clinical course of pre-existing GBS. To our knowledge, there are only two case reports that described such patients (3, 4). Here, we report a case of a treatment-naïve patient with chronic HIV infection who presented with acute motor axonal neuropathy (AMAN), an axonal variant of GBS, after an episode of Herpes zoster.

## Case Report

A 33-year-old Japanese male with treatment-naïve HIV-1 infection visited the emergency room, complaining of progressive tetraparesis. He had been diagnosed with HIV infection five years earlier, and the latest CD4 count was 334/μL with a viral load of $7.9\times10^3$ copies/mL. He reported developing the first episode of Herpes zoster on the chest 12 days earlier, and was treated with famciclovir. There was no history of diarrhea. On admission, the head CT scan and MRI of the head and spinal cord showed no abnormalities. Cerebrospinal fluid (CSF) examination showed a cell count

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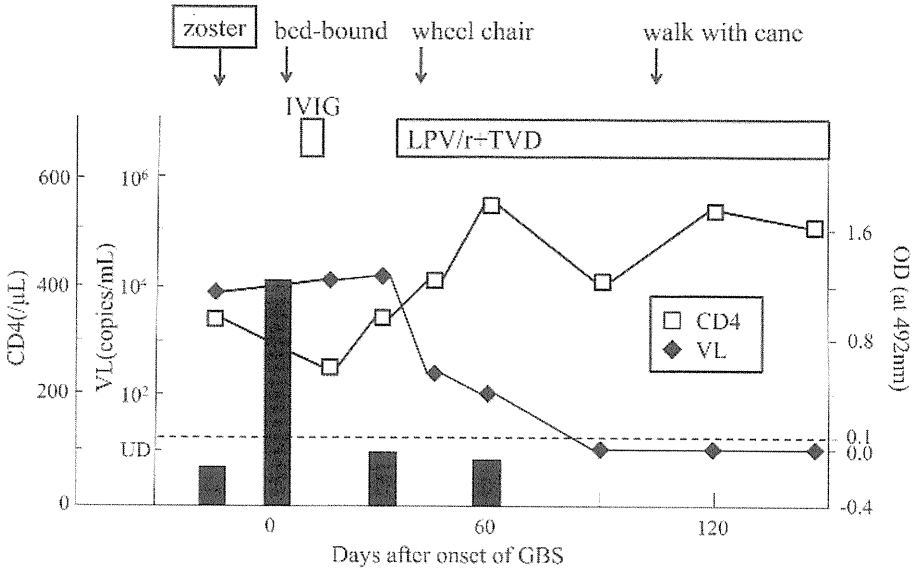


**Figure 1.** **Clinical course after the onset of GBS. *Solid bars*: titer of serum anti-GM1 IgG antibody measured with ELISA (cut-off value =0.1 OD, dashed line) (5). *Open squares*: CD4 cell count, *solid diamonds*: viral load (VL). Anti-ganglioside antibody became positive at the onset of GBS but it was negative thereafter. GBS: Guillain-Barré syndrome, IVIG: intravenous immunoglobulin, LPV/r: lopinavir/ritonavir, TVD: tenofovir/emtricitabine, OD: optical density**

of 6/μL and protein 17 mg/dL, and polymerase chain reaction was negative for varicella zoster virus and herpes simplex virus. Tetraparesis worsened after admission and the patient became bed-bound on day 3. However, the trunk muscles were intact and no respiratory distress was evident. On day 4, a 5-day course of high-dose intravenous immunoglobulin (IVIG) was started with a tentative diagnosis of GBS. Electrophysiological studies on day 5 showed conduction blocks of motor nerves and markedly reduced F-wave frequencies: 0% in the median nerve (normal >40%), 37.5% in the ulnar nerve (>40%), and 0% in the tibial nerve (> 99%). We collected blood samples on day 2 to measure IgG and IgM antibodies to gangliosides GM1, GD1a, GD1b, GT 1b, GQ1b, asialo-GM1, GM2, GM3, GD2, and GD3 with ELISA as described elsewhere (5). Anti-GM1 IgG antibody, a diagnostic marker for AMAN, was positive, whereas other IgG antibodies were negative (6). Neither serum anti-*Campylobacter jejuni* IgG antibody nor stool culture for campylobacter was positive, suggesting that the preceding zoster was the trigger. On day 15, a marked reduction in the compound muscle action potentials of motor nerves was noted: 1.3 mV in the median nerve, 1.2 mV in the ulnar nerve, and 0.09 mV in the tibial nerve, whereas sensory nerve action potentials were intact: 26.5 μV in the ulnar nerve. CSF re-sampling on day 30 showed albuminocytological dissociation (protein: 73 mg/dL, cell count: 7/μL). The clinical symptoms, electrophysiological examination, CSF analysis, and positivity for anti-GM1 IgG antibody confirmed the diagnosis of AMAN (7).

Arrest of symptom progression was noted after day 1 of IVIG; however, it was not clear whether this represented the natural course of the disease or was IVIG-related. Antiretroviral therapy with lopinavir/ritonavir and tenofovir/emtricitabine was started on day 33, one month after the onset of GBS (CD4: 349/μL, viral load: $1.7\times10^5$ copies/mL). The treatment suppressed the viral load within 8 weeks to an undetectable level and increased CD4 count to 414/μL. However, tetraparesis remained stable with no rapid improvement or deterioration after initiation of antiretroviral therapy. Recovery of compound muscle action potentials of motor nerves was also slow: 1.27 mV on day 28, 2.7 mV on day 56, and 2.6 mV on day 98 in the left median nerve. Serum samples obtained 14 days before the onset of GBS, on day 1 of antiretroviral therapy (day 33), and 4 weeks later (day 61) were negative for anti-ganglioside antibodies (AGA) as shown in Fig. 1. The recovery of tetraparesis was slow; the patient needed 15 months of rehabilitation to be able to walk without a cane.

## Discussion

We described a treatment-naïve patient with chronic HIV infection who presented with AMAN due to preceding Herpes zoster. Herpes zoster reactivation is known to sporadically trigger the occurrence of GBS (8). Antiretroviral therapy was introduced one month after the onset of GBS, which resulted in a substantial rise in the CD4 cell count. However, elevated T-cell immunity did not improve or worsen the symptoms of GBS; it was 15 months after onset that the patient could walk independently. HIV-related GBS often occurs as a complication of primary HIV infection or immune reconstitution inflammatory syndrome (1, 2). The uniqueness of this case is that the clinical course of pre-existing GBS, which was triggered by Herpes zoster, not by

HIV *per se*, after the commencement of antiretroviral therapy was described, and that CD4+ T-cell immunity activated by antiretroviral therapy modulated neither the clinical course of pre-existing GBS nor AGA titer.

To our knowledge, this is the first report that describes the detection and serial measurements of AGA in a GBS patient with HIV infection. AGA was negative in a previously reported case of AMAN (4). In the present patient, the AGA titer was highest at the onset of GBS but rapidly decreased afterwards (Fig. 1). This trend is similar to that of patients free of HIV infection (9). In this patient, antiretroviral therapy did not seem to affect either clinical recovery from GBS or AGA titer.

GBS is classified into two forms based on clinical, electrophysiological, pathological, and immunological criteria: axonal variants including AMAN, and acute inflammatory demyelinating polyneuropathy (AIDP) (7). The pathogenesis of axonal variants is thought to be different from that of AIDP. AGA is an antibody against lipopolysaccharide in the cell wall of infectious pathogens and it is known to cross-react with oligosaccharide epitopes of gangliosides in the peripheral nervous tissue. AGA plays a major role in the pathogenesis of axonal variants (7). In contrast, T-cell-mediated cellular immunity was found to play a major role in AIDP, with knowledge earned through experimental autoimmune neuritis, an autoimmune disease with clinical and pathological features similar to AIDP (10). One report described a patient with advanced AIDS and a very low CD4 count (24/μL) who presented with Fisher/Guillain-Barré overlap syndrome and was positive for AGA, suggesting that axonal variants might occur independent of T-cell immunity (11).

Antibody production against lipopolysaccharides is generally T-cell independent (11). Such antibodies produced by B-cells are the IgM isotypes, and the intervention of T-cells has been considered to be essential in switching class to IgG1 and IgG3 subclasses, to which most AGA belongs (12). However, it was recently discovered that subsets of B cells could produce class-switched IgG T-cells independently, with the help of B-cell activating factor and a proliferation-inducing ligand secreted by dendritic cells (13). Although the role of T-cells in the production of AGA in axonal variants of GBS has yet to be determined, it appears that recovery of CD4+ T-cell immunity did not alter the clinical course of pre-existing GBS or AGA titer, at least in this patient.

In conclusion, we reported a treatment-naïve patient with chronic HIV infection who presented with AMAN due to Herpes zoster. Antiretroviral therapy was introduced one month after the onset of GBS and resulted in substantial improvement of CD4+ T-cell immunity, but it had no effect on the prognosis of tetraparesis or trend of AGA titer. It is important to distinguish axonal variants from AIDP especially in patients with HIV infection, because the risk of immune reconstitution inflammatory syndrome after the introduction of antiretroviral therapy might be lower in the axonal variants. The measurement of AGA titer during the acute phase of GBS should help establishing the diagnosis.

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Minireview

# *Pneumocystis jirovecii* pneumonia in kidney transplantation

N. Goto, S. Oka. *Pneumocystis jirovecii* pneumonia in kidney transplantation. Transpl Infect Dis 2011. All rights reserved

**Abstract:** *Pneumocystis jirovecii* pneumonia (PCP) remains an important cause of morbidity and mortality in immunocompromised renal transplant recipients. In recent years, PCP outbreaks in renal transplant centers have been reported in many countries. Person-to-person transmission between PCP patients and other recipients lacking prophylaxis is one of the possible sources of infection. To prevent infection, effective prophylaxis in susceptible patients is recommended. Trimethoprim-sulfamethoxazole (TMP-SMX) is the most effective drug for PCP prophylaxis, but its recommended duration of use after transplantation varies among the different guidelines. The European Renal Association recommends a prophylaxis period of 4 months after transplantation, the American Society of Transplantation (AST) 6–12 months, and the Kidney Disease Improving Global Outcomes guidelines 3–6 months. Lifelong prophylaxis with TMP-SMX is not recommended in renal transplant recipients; however, in many cases, PCP has occurred after the recommended prophylaxis periods after transplantation. In this minireview, we discuss the risk factors including environmental-nosocomial exposure; state-of-the-art diagnosis, treatment, prophylaxis and isolation; and references to the AST 2009 guidelines with the aim of integrating our experience with PCP outbreaks into recent reports, and we discuss how renal transplant recipients can be protected from PCP.

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Key words: *Pneumocystis jirovecii* pneumonia; PCP; kidney transplantation; non-HIV; diagnosis; treatment; prophylaxis; isolation

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*Pneumocystis jirovecii* pneumonia (PCP) is a major cause of morbidity and mortality in persons receiving immunosuppressive therapy.

PCP in patients without human immunodeficiency virus infection (non-HIV) is increasing with widespread immunosuppressive treatment, whereas PCP in HIV is decreasing. The clinical manifestations, diagnosis, treatment, outcome, infection control, and prophylaxis differ between PCP in patients with and without HIV.

PCP in HIV patients is commonly the first opportunistic infection, but it occurs rarely, and is not seen until the CD4 count drops to <200 cells/mm$^3$ (1). The incidence of PCP in HIV patients receiving highly active antiretroviral therapy has decreased dramatically, thanks to very effective prophylaxis (2). The onset of PCP in non-HIV patients more commonly presents with an abrupt respiratory insufficiency than in HIV patients (3). PCP in non-HIV patients may show up with fewer organisms in the lungs during an episode than in HIV patients (4). The outcomes in non-HIV patients treated for PCP are generally worse than those in HIV patients; mortality of PCP in HIV patients is approximately 10–20%, compared with 35–50% in non-HIV patients (5–7).

Historically, approximately 5–15% of patients who underwent solid organ transplantation developed PCP in the absence of prophylaxis. The rates were lowest in renal transplant recipients (8, 9) and highest among lung and heart-lung transplant recipients.

## Epidemiology

*Pneumocystis* organisms were first identified in the early 20th century. *Pneumocystis* organisms in different mammals belong to different species, and strains from one host animal do not infect other animal species. *Pneumocystis carinii* was derived from rats. From the time of its discovery until late in the 1980s, *Pneumocystis* was widely thought to be a protozoan. In 1988, DNA analysis demonstrated that *Pneumocystis* is a fungus (10). According to genetic studies, the initial name *P. carinii* was changed to *Pneumocystis jiroveci* in 1999 (11) and later revised to *P. jirovecii* when it was determined to be a fungus.

The life cycle of *P. jirovecii* remains poorly defined (12). Pneumocystosis is a common childhood respiratory infection. By 4 years of age, two-thirds of normal children affected by the respiratory-aerosol route are found to have antibodies against *P. jirovecii* (13). Immunocompetent hosts clear the infection without obvious clinical consequences, but immunocompromised patients develop the disease as a consequence of reinfection and possibly reactivation of chronic colonization (14). Studies in HIV-positive patients demonstrate that reinfection with different genotypes probably occurs with as much regularity as reactivation of endogenous organisms (15).

## Acquisition of *P. jirovecii*

*Pneumocystis jirovecii* cannot be grown outside an infected host (16). *P. jirovecii* can be transmitted by the airborne route. The agent of infection is suspected to be airborne spores, but these have not been identified yet. There are 3 routes of acquisition; one is person-to-person transmission from an infected patient, which is the most likely mode of acquiring new infections (17), second is from environmental exposure (18–22), and third is from asymptomatic carriers (23). *P. jirovecii* DNA can be detected transiently in immunocompetent individuals following close contact with PCP, indicating that temporary asymptomatic carriage of *P. jirovecii* in immunocompetent persons can occur (15, 24–27). Many transplant recipients are complicated with PCP soon after transmission of *P. jirovecii*. But some become asymptomatic carriers without clinical manifestation of PCP for quite some time, as is the case with immunocompetent individuals. The estimated median incubation period of PCP is 53 days (range 7–188 days) (Fig. 1) (28).

## Risk factors

Generally, T-cell depletion or blocking therapies increase the risk of PCP. The most significant risk factors for PCP in non-HIV patients are glucocorticoid use and defects in cell-mediated immunity (3, 29, 30). In addition to glucocorticoid, mycophenolate mofetil (MMF), calcineurin inhibitors, and sirolimus are risk factors for PCP, as well as immunosuppressive agents. Furthermore, combined use of these agents strongly suppresses immune functions. The risk is also increased in phases where immunosuppression is increased, such as during rejections therapies.

Rejection therapies involve immunosuppressive drugs. MMF has anti-*Pneumocystis* effects *in vitro* and in animal models. But the effective clinical relevance has not been confirmed in prospective clinical trials (31). In a case–controlled study, cases of PCP increased among MMF-treated recipients (32). The incidence of PCP in cyclosporine-treated recipients was lower than in those treated with tacrolimus (33). Analysis of the United States Renal Data System showed that the incidence of PCP was high in patients who were on sirolimus (34).

However, there are other risk factors for PCP aside from immunosuppressive drugs. An increase in incidence of rejection has been shown to increase the risk of PCP. In a case–controlled study, treatment of 1, 2, and 3 rejections was associated with 2-, 5-, and 10-fold increases in PCP, respectively (35). Cytomegalovirus (CMV) may be an independent risk factor for PCP (36). Failure of primary trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis may occur in association with some of these risk factors (37). Close contact with a PCP cluster, which sometimes causes PCP outbreaks, is a risk factor for recipients. Reports of these transmissions recently have been increasing (9, 29, 38, 39). Asymptomatic carriage plays a role in the transmission of *P. jirovecii* and may pose a risk for developing PCP (15, 23).

## Clinical manifestations

PCP in HIV patients is slowly progressive in onset, and non-specific symptoms, such as fever, non-productive cough, and dyspnea, are common (40). In contrast, PCP in non-HIV patients sometimes lacks these symptoms, because immunosuppressive agents suppress these clinical findings (41) by suppressing immune response. In just a few days, which is generally more rapid than in HIV, PCP develops into

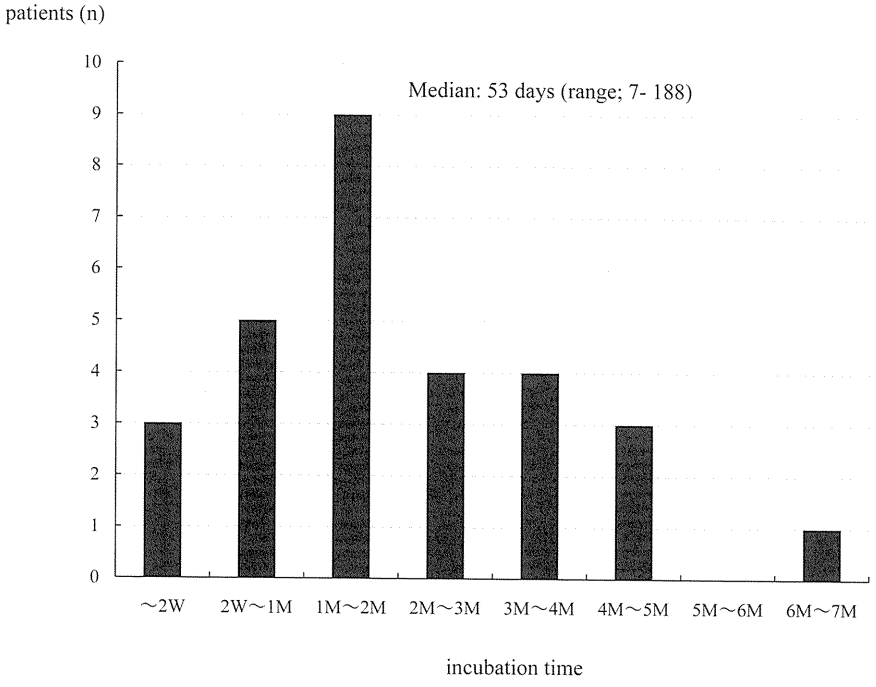


*Fig. 1.* Incubation period of *Pneumocystis jirovecii* pneumonia ($n$ = 29). W, week; M, month.

symptomatic disease with severe dyspnea and hypoxemia.

Serum level of lactate dehydrogenase is significantly higher but non-specific, and C reactive protein (CRP) is not high in PCP patients (42). Therefore, PCP in recipients is sometimes misdiagnosed as a common cold in the early stage, because of the low level of CRP and suppressed fever.

## Diagnosis

A typical radiographic feature of PCP is the presence of bilateral peripheral interstitial infiltrates (43). High-resolution computed tomography scans (HRCT) are more sensitive than chest radiography and may show ground glass opacities sparing the lung periphery. However, these abnormalities are non-specific. Despite several attempts, it has not been possible to culture *Pneumocystis* organisms *in vitro* (16). Identification of *P. jirovecii* from sputum, bronchoalveolar lavage (BAL) fluid, or lung tissue is needed for the definitive diagnosis of PCP. If the initial specimen of induced sputum is negative, then bronchoscopy with BAL should be performed. Transbronchoscopic or surgical lung biopsy is rarely needed (44).

*P. jirovecii* has 2 predominant life-cycle forms, the trophozoite and the cyst form. During infection, the trophozoite is more dominant than the cyst form (45). The trophozoite can be detected with modified Papanicolaou, Wright–Giemsa, or Gram–Weigert stains. Cysts can be stained with Gomori methenamine silver, cresyl echt violet, toluidine blue O, or calcofluor white (12). Immunofluorescent staining techniques are available, which could provide increased specificity and sensitivity (46).

PCP in non-HIV-infected patients presents with significantly lower numbers of *P. jirovecii* organisms and substantially higher neutrophils in BAL fluid samples than in HIV-infected patients (4). Polymerase chain reaction (PCR) with sputum and BAL fluid samples has a high sensitivity and specificity for the detection of the organism, but lacks sensitivity in diagnosing PCP, as PCR cannot differentiate colonization from infection. A high proportion of immunocompromised patients, both HIV positive and HIV negative, are colonized with *P. jirovecii*, which is a disadvantage for PCR testing. However, PCR is helpful in excluding PCP in HIV-negative patients (47). Clinical judgment is essential in cases of negative staining and positive PCR. Treatment for PCP should be initiated if the clinical suspicion is high (12). For these patients, PCR may be useful (48).

Plasma (1 → 3) beta-D-glucan (beta-glucan) is one of the major components of the cystic wall of *P. jirovecii* (49). PCP in mice was successfully treated on this

basis with a beta-glucan inhibitor (50). Detection of plasma beta-glucan had a high sensitivity (96.4%) and specificity (87.8%) for the diagnosis of PCP in HIV patients, where *P. jirovecii* densities are usually higher than those in other immunocompromised situations (51). Although clinical relevance of this marker for the sensitive diagnosis of PCP in HIV-negative patients has yet to be elucidated, the detection of beta-glucan in plasma raises a strong suspicion of PCP even in HIV-negative patients. Plasma beta-glucan is an adjunctive non-invasive diagnostic marker for PCP in HIV-negative patients (52). In fact, plasma beta-glucan is widely used as a laboratory diagnostic test in Japan, not only for deep-seated mycosis but also for PCP, irrespective of HIV status. However, it does not correlate with disease severity and is not suitable for monitoring response to treatment (51, 53).

## Treatment

TMP-SMX is the first choice for the treatment of PCP, even in non-HIV patients (54). It has excellent oral bioavailability. If the patients have a functioning gastrointestinal (GI) tract, a comparable serum level is achieved either by intravenous (IV) or oral (p.o.) administration. The standard dose of TMP-SMX is 15 mg/kg of the TMP component per day IV in divided doses every 6–8 h, according to the renal function. For non-severe patients who are able to take p.o. medications, 2 double-strength tablets can be given p.o. every 8 h.

IV pentamidine is a second-line agent, but it is also highly toxic. Primaquine in combination with clindamycin is commonly used in several centers, as is recommended in other guidelines (Table 1). Other therapeutic options exist, but the 3 above are used quite often, because they are effective and have good safety profiles (55). Dapsone, in combination with TMP or atovaquone, is used only for mild-to-moderate PCP.

Adjunctive glucocorticoids are recommended in HIV patients with moderate or severe PCP (56). Prednisone 40 mg is administered p.o. twice daily for 5 days, followed by 40 mg p.o. once daily for 5 days, and 20 mg p.o. once daily for 11 days.

On the other hand, no clear evidence of efficacy has been shown for adjunctive glucocorticoids in the treatment of PCP in non-HIV patients. One retrospective study suggests their use in non-HIV patients (57), but the dose, duration, and timing of steroids have not been fully studied in transplantation. Recently published guidelines of the American Society of Transplantation (AST) suggest that prednisone 40–60 mg is administered p.o. twice daily and tapered after 5–7 days over a period of 1–2 weeks (55), which is a high initial dose, but is tapered early to avoid over immunosuppression. Corticosteroids are best administered within 72 h in the setting of hypoxia ($PaO_2 < 70$ mmHg). In HIV patients, corticosteroids should be administered along with TMP-SMX.

The optimal duration of therapy for PCP in HIV-negative patients has not been fully studied. Because of the low number of organisms and faster clinical evolution, antimicrobial therapy is needed for at least 14 days. Therapy for severe PCP may be required for 21 days, as is the case for HIV patients (56). No data verify that immunosuppression should be continued, reduced, or stopped during treatment of PCP. But as a general measure, reduction should be encouraged (58).

## Outcome

The outcome of PCP in non-HIV patients is generally poorer than that in HIV patients (59). The most likely explanation for this difference is that the host inflammatory response is assumed to be more intense in non-HIV patients with PCP, in spite of the presence of a lower number of organisms, contributing to severe lung injury. The outcome of PCP is inversely correlated with intensity of immunosuppression. In HIV patients, mortality is 6.6%, and 5.7% require mechanical ventilation. In non-HIV patients, mortality is 39%, and 59% require mechanical ventilation (5). Mortality in the absence of TMP-SMX prophylaxis is 5–33% in the current immunosuppressive era of renal transplantation (28, 37–39). PCP infection leads to increased graft and patient loss in renal transplantation (34). Mortality is high (32–33%) in PCP complicated with connective tissue disease where the immunological status may not be as severely impaired as in transplant recipients (6, 60).

## Infection control

It is important to control nosocomial patient-to-patient transmission of *P. jirovecii*, which may currently be a predominant transmission route. The transmission of PCP can be highest before onset of clinical symptoms of PCP until the end of the first week of anti-*Pneumocystis* therapy (37).

**Treatment for *Pneumocystis jirovecii* pneumonia**

| Agents | Dosing | Common adverse reactions |
|---|---|---|
| Trimethoprim-sulfamethoxazole (TMP-SMX) | 15–20 mg TMP/kg/day IV divided in 3–4 divided doses For non-severe patients, 2 double-strength tablets can be given p.o. every 8 h | Gastrointestinal upset, bone marrow suppression, rash, hepatitis, elevation of serum, serum creatinine (reversible inhibition of the tubular creatinine secretion), and hyperkalemia |
| Pentamidine | 3–4 mg/kg/day IV | Graft function impaired |
| Primaquine and clindamycin | Primaquine 30 mg once daily p.o. in combination with clindamycin 600–900 mg IV or p.o. every 6–8 h | Primaquine-induced hemolytic anemia (in patients with G6PD deficiency[1]) |
| Dapsone and TMP | Dapsone 100 mg/day once daily in combination with TMP 5 mg/kg 3 times daily | Hemolytic anemia |
| Atovaquone | 750 mg twice daily with fatty food | Gastrointestinal upset, rash |

[1]Screening for G6PD deficiency should be considered before this treatment.
IV, intravenously; p.o., orally.

*Table 1*

Among outpatients, those with a suspected or confirmed PCP diagnosis should wear a mask as soon as possible when sharing a waiting room with other recipients (61). Close contact should be avoided. For all recipients that share a waiting room with a PCP patient, starting transient prophylaxis with TMP-SMX for 6 months may be effective to avoid repeated outbreaks by infectious asymptomatic carriers (28).

Hospitalized patients with PCP should be assigned to standard precautions. Certain authorities recommend that they should not be placed in the same room with immunocompromised patients, including other recipients (62). However, this recommendation is based on animal studies and anecdotal human experience. Data to support this recommendation as standard practice are lacking. In the absence of an isolation bed, prophylaxis with TMP-SMX for all hospitalized immunocompromised patients in the same ward should be considered before the admission of PCP patients.

## Prophylaxis

In HIV patients, CD4 T-cell count is a useful marker that can be classified based on the risk of developing PCP (63). Primary prophylaxis against PCP is provided for patients with CD4 cell counts $<200/mm^3$ (64). However, in non-HIV patients, there are no useful markers to monitor the immunological status.

TMP-SMX is also the drug of first choice for PCP prophylaxis. The dose of TMP-SMX can be 80 mg TMP/400 mg SMX daily or 160 mg TMP/800 mg SMX p.o. (single or double strength) daily or 3 times weekly (55). TMP-SMX prophylaxis also prevents *Toxoplasma* and *Listeria* species, which are respiratory, urinary, and GI pathogens. TMP-SMX reduces urinary tract infection and possibly GI and respiratory infection in transplant patients. Side effects, which are often dose-related, are less common with the prophylaxis dose. Breakthrough PCP infection with TMP-SMX prophylaxis is rare. The second treatment option is dapsone (55). The most common side effects are hemolytic anemia and methemoglobinemia. Atovaquone (1500 mg p.o. qd) and aerosolized pentamidine (300 mg administered through aerosolized nebulizer q 4 weeks) are other options for prophylaxis.

No universal consensus exists on the optimal duration of prophylaxis. The European Renal Transplant Guidelines recommend PCP prophylaxis for at least 4 months after transplantation (65), whereas the AST recommends 6–12 months (55). The Kidney Disease Improving Global Outcomes guideline recommends 3–6 months after transplantation (66). These guidelines are based on the fact that the risk is considered the highest within the first 6 months post-transplant. However, in our experience (Fig. 2), PCP has occurred not only within 12 months but even at >10 years after transplantation. Our hospital had not used PCP prophylaxis until the first index case occurred in July 2004. The incidence within the first 12 months after transplantation is 33% of all PCP cases, whereas PCP at >10 years after transplantation was 18% (28).

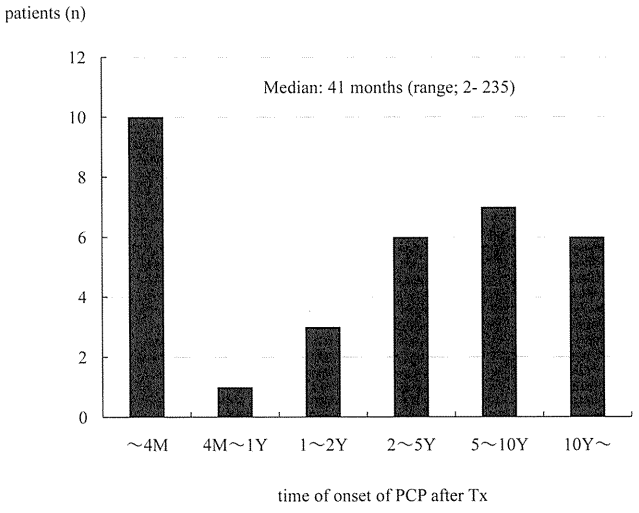


*Fig. 2.* Time to onset of *Pneumocystis jirovecii* pneumonia (PCP) after renal transplantation ($n$ = 33). Tx, transplantation; M, month; Y, year.

Local approaches to PCP prophylaxis vary widely. From a survey of US renal transplant centers, 84% of centers use PCP prophylaxis, but 16% do not. The duration of prophylaxis also varies widely, with 43% of centers using prophylaxis for 6 months or less, and 22% maintaining prophylaxis beyond a year (67). PCP prophylaxis in patients with a previous history of PCP (secondary prophylaxis) is needed after treatment of PCP. Prophylaxis can be discontinued if the CD4 cell count rises to >200/mm$^3$ for >3 months in HIV patients (68). No clear data indicate when secondary prophylaxis can be discontinued in non-HIV patients. For all transplant recipients with a history of prior PCP infection, lifelong prophylaxis may be indicated (55).

## Conclusion and perspective

PCP is rare in the modern era of prophylaxis. The incidence of PCP has been markedly reduced with TMP-SMX prophylaxis to <1% in renal transplant recipients (9). The highest risk for PCP is within 1–6 months after transplantation, and most guidelines recommend PCP prophylaxis for this period. However, as mentioned earlier, occurrence of PCP even >10 years after transplantation has been documented (28). Under this circumstance, each renal transplant recipient is at risk of PCP occurring at any time after transplantation. PCP prophylaxis should be considered after the classical 6–12 months for those patients who have risk factors, such as the need for increasing immunosuppression in the face of rejection, recurrent or chronic active infection with CMV, prolonged courses of higher dose corticosteroid therapy (e.g., >20 mg daily of prednisone for at least 2 weeks), prolonged neutropenia, flares of autoimmune disease (54), or close contact with a PCP patient (28).

Symptoms of PCP in recipients resemble the common cold in its early stage. Without x-ray or HRCT, the condition has sometimes been misdiagnosed and patients with PCP return several times to the hospital with complaints but go without appropriate treatment for PCP. Patients highly suspected of having PCP and not on prophylaxis should be isolated until the diagnosis of PCP has been excluded.

There are 2 important reasons for initiating PCP therapy as soon as possible in recipients. First, PCP in transplant recipients presents with an abrupt onset of respiratory distress, unlike the course in HIV patients. Despite the low number of organisms, immunological response causes severe lung injury. Late diagnosis and treatment may increase respiratory failure and death. Second, a delay in diagnosis and treatment may lead to an increase in the number of reservoir patients, who may pose a risk of a PCP outbreak or may transmit the infection to other recipients.

If prophylaxis of TMP-SMX is discontinued according to the AST 2009 guideline and PCP occurred in one of the outpatients, we should act quickly not only to treat the patient but also to protect other recipients, who often wait together in small medical waiting rooms for long periods, from becoming reservoirs. To prevent infection, a possible effective prophylaxis in susceptible patients may be considered, rather than strict hospital segregation of immunocompromised patients with PCP (54). The increased number of recent reports of PCP outbreaks in renal transplant units suggests that lifelong prophylaxis may be required for renal transplant recipients, as well as for lung and small bowel transplant recipients (53). Alternatively, once a single case of PCP occurs in an outpatient clinic, 6 months of prophylaxis with TMP-SMX for all other recipients may be effective to avoid repeated colonization of *P. jirovecii* (28).

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## Reply to 'pharmacokinetic concerns related to darunavir/ritonavir plus raltegravir combination therapy trial'

Gervasoni and Cattaneo [1] point out that a potential pharmacokinetic drug–drug interaction between raltegravir and darunavir ultimately affecting the results of the AIDS Clinical Trials Group (ACTG) A5262 trial cannot be ruled out. We agree with this statement, as A5262 was never designed to be a drug–drug interaction study. Indeed, we refrained from categorically rejecting the possibility of significant interactions and simply presented our finding that trough concentrations observed in A5262 were within the range reported in an intensive pharmacokinetic study of darunavir 800/100 mg daily [2].

In A5262, the darunavir troughs were different in the nonvirologic failure (1649 ng/ml) and virologic failure groups (1042 ng/ml), and undetectable raltegravir trough concentrations were associated with increased virologic failure. Importantly, A5262 was not designed to elicit optimal drug exposure variables, nor was it our intention to use the results as a basis for therapeutic drug monitoring (TDM). In applying TDM, the therapeutic range of the drug must first be defined, and this has not been adequately done for raltegravir or darunavir. Along this line, the suggestion by Gervasoni and Cattaneo [1] that darunavir area under the curve (24 h) is the relevant pharmacokinetic parameter raises important questions. What is the optimum exposure of darunavir in HIV-infected patients? Are troughs more important or is the area under the curve? Is there consensus on this exposure variable, and were those data generated from well designed dose-ranging studies or retrospective pharmacokinetic cohort analyses? Clearly, these issues are yet to be resolved in the literature. Without knowing what the ideal darunavir exposure target is, neither our team nor Gervasoni and Cattaneo [1] can state whether virologic failure occurred due to low darunavir troughs, regardless of whether raltegravir caused low darunavir exposure or not. There are in-vitro data to suggest the darunavir protein-binding-corrected 95% inhibitory concentration might be as low as 25 ng/ml [3], which is well below the darunavir trough concentrations in both virologic failure and nonvirologic failure participants in A5262. Previous pharmacokinetic studies of once-daily darunavir found no relevant relationships between darunavir pharmacokinetics and virologic efficacy or safety [4,5].

Gervasoni and Cattaneo [1] also state that no association between raltegravir pharmacokinetics and clinical outcome can be reasonably expected. They apparently based this assertion on raltegravir's pharmacokinetic variability and long residence time on the pre-integration complex, which exceeds the half-life of the complex itself. This presumably makes raltegravir inhibition of the enzyme complex irreversible, which was extrapolated to explain why raltegravir troughs are not related to response. According to Gervasoni and Cattaneo [1], it is unlikely that raltegravir trough concentrations can *per se* directly affect response to therapy of patients enrolled in the ACTG trial. Therefore, they posited that other ways in which raltegravir could indirectly impact on patient outcome, such as an interaction with darunavir, should be advocated. Although there may be a drug–drug interaction, it should be emphatically reiterated that A5262 was not designed to assess this. More importantly, the phase III study of the safety and efficacy of once daily versus twice daily raltegravir in combination therapy for treatment-naïve HIV-infected patients (QDMRK) clearly showed that raltegravir 800 mg once daily was inferior to 400 mg twice daily at 48 weeks [6]; the geometric mean trough concentration$_h$ was 83 nmol/l for once-daily vs. 380 nmol/l for twice-daily dosing. The QDMRK study was a large dose fractionation study, and as such we now know that the raltegravir trough concentrations are indeed an important determinant of therapeutic response. The half-life of drug binding to the integration complex may be an important variable for certain dosing intervals but, if the dosing interval exceeds some threshold, then the integration complex can form and integrate during the period when raltegravir concentrations are low or absent. This is further demonstrated by data with dolutegravir (same mechanism of action as raltegravir), which exhibited a concentration–response relationship when a wide range of doses (25-fold) was used in early development [7]. A recent article demonstrates the dissociative half-life of dolutegravir is 71 h, considerably longer than the 8.8 h for raltegravir [8]. Thus, a concentration–response relationship was still determined for dolutegravir, even though the enzyme complex off-rate was eight times slower. Gervasoni and Cattaneo [1] are incorrect in assuming that the raltegravir trough concentrations do not affect response to therapy in A5262, and we cannot confirm nor rule out a drug–drug interaction between darunavir and raltegravir in this particular study.

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### Conflicts of interest

E.P.A. has served as a consultant to Tibotec and Merck. B.T. has served as an advisor and received research support

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## Pharmacokinetic concerns related to the AIDS Clinical Trial Group (ACTG) A5262 trial

Taiwo *et al.* [1] have recently reported results of the AIDS Clinical Trial Group (ACTG) A5262 trial, specifically designed to investigate a two-drug, reverse transcriptase inhibitor-sparing regimen of darunavir/ritonavir (DRV/r) with raltegravir (RAL) for initial antiretroviral therapy. The proposed regimen met the protocol definition of 'acceptable virologic efficacy', but only 60% of participants reached viral load less than 50 copies/ml at week 48 in spite of an unanticipated high incidence of virologic failure and integrase resistance especially in patients with baseline viral load more than 100 000 copies/ml.

Taiwo *et al.* [1] have attempted to find out potential explanations for these unexpected results. Particularly, they have explored the potential contribution of DRV and RAL pharmacokinetics on the study findings. Average DRV and RAL trough concentrations were not significantly different for patients with and without virologic failure. However, sensitivity analyses evidenced a significant role of DRV levels, which were significantly lower in patients with virologic failure compared with those without virologic failure (1042 vs. 1649 ng/ml, $P = 0.017$). This scenario was further complicated by findings from Cox models, showing that having RAL trough concentrations below the assay detection limit immediately before or at one or more previous visit was also highly significantly associated with increased hazard of virologic failure. So, which conclusions on the value of DRV and RAL therapeutic monitoring can be drawn from this study?

We and others [2,3] have previously shown that RAL trough concentrations are associated with large inter-individual variability and, most importantly, largely failed to correlate with RAL area under the time–concentration curve $(AUC)_{0\text{-}12}$, taken as the golden standard pharmacokinetic parameter for the quantification of daily