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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 7.9×10^5 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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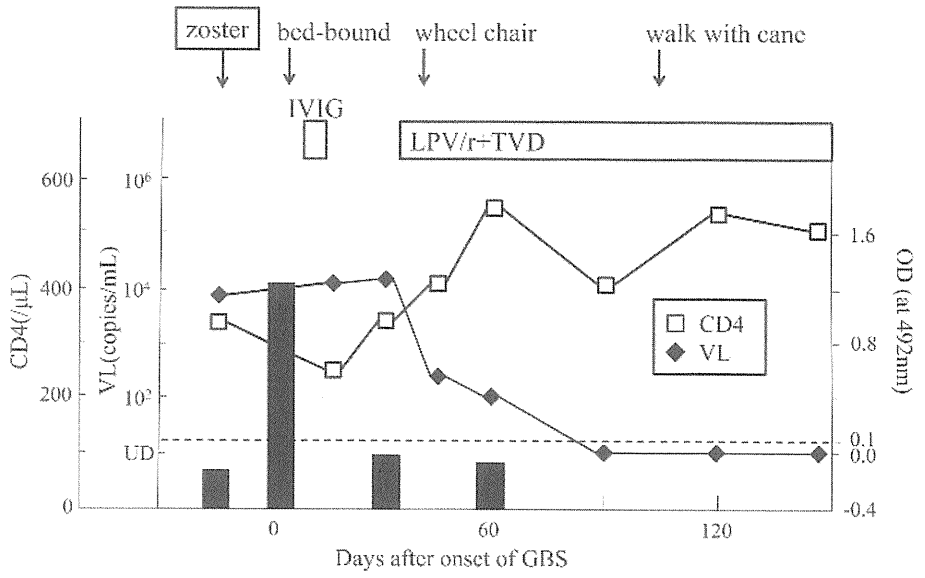

Figure 1. **Clinical course after the onset of GBS. *Solid bars*: titer of serum anti-GM1 IgG antibody measured with ELISA (cut-off value =0.1 OD, dashed line) (5). *Open squares*: CD4 cell count, *solid diamonds*: viral load (VL). Anti-ganglioside antibody became positive at the onset of GBS but it 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: 1.7×10^5 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.

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

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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

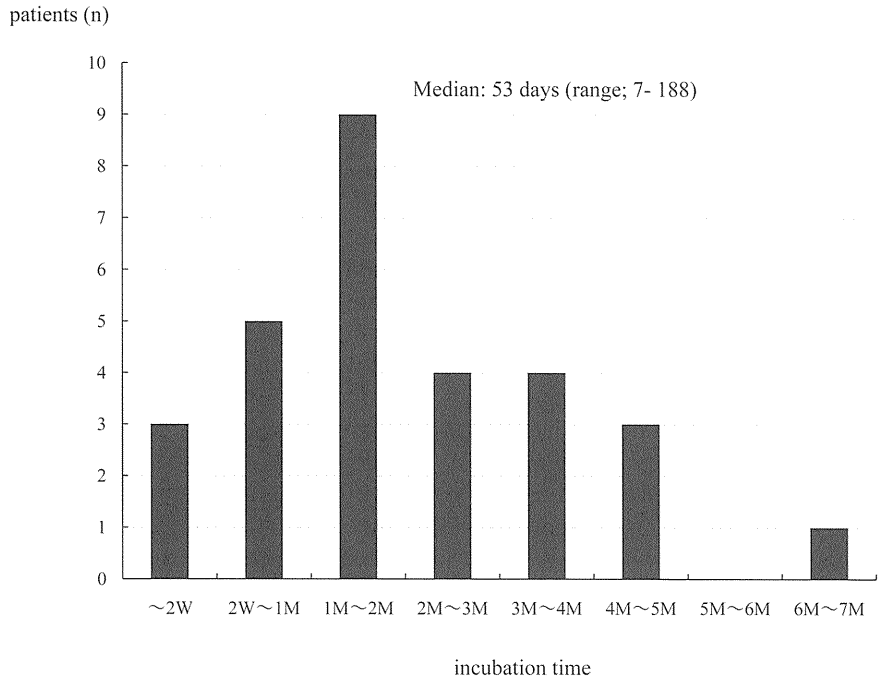

Fig. 1. Incubation period of *Pneumocystis jirovecii* pneumonia (n = 29). W, week; M, month.

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).

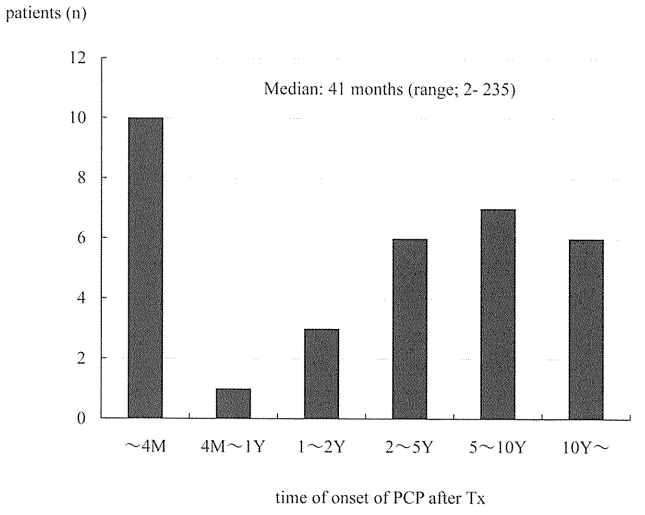

Fig. 2. Time to onset of *Pneumocystis jirovecii* pneumonia (PCP) after renal transplantation (n = 33). Tx, transplantation; M, month; Y, year.

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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Epstein–Barr virus associated colitis in an HIV-infected patient

Epstein–Barr virus (EBV)-associated lymphoma of the gastrointestinal tract is common in HIV-infected patients [1]. However, EBV involvement of the gastrointestinal tract without frank lymphoma is rare [2]. Although a few cases of EBV-associated colitis in both immunocompetent and immunocompromised patients have been reported [2,3], to our knowledge, EBV-associated colitis in HIV-infected patients has never been reported. We report a case of EBV-associated colitis with HIV infection successfully treated by combination antiretroviral therapy (cART).

A 40-year-old homosexual man who had had diarrhea for a few years developed bloody diarrhea. After persistence of the symptom for 2 months, he sought medical advice at a local hospital. He was diagnosed with HIV infection and referred to our hospital for further examination. On admission, the patient was alert with body temperature of 37.6°C. Physical examination showed oral candidiasis but no peripheral lymphadenopathy or abdominal tenderness. Laboratory tests at admission showed low $CD4^+$ cell count (84 cells/μl), anemia (hemoglobin 10.6 g/dl), low serum albumin (2.1 g/dl), and elevated C-reactive protein (3.45 mg/dl). Colonoscopy showed diffuse edematous mucosa with deep ulcers in the rectum, sigmoid colon, and descending colon (Fig. 1a), suggestive of either cytomegalovirus (CMV) colitis or amebic colitis. Based on the clinical suspicion, we initiated empirical treatment of gancicrovir and metronidazole. However, the results for amebic colitis such as stool microscopy, serum antiamebic antibody, and trophozoites in colonic biopsy specimens were all negative. Furthermore, histopathology revealed no inclusion bodies and negative immunological staining for CMV. Then, we suspected inflammatory bowel disease (IBD), and mesalazine 4 g/day was initiated on day 7. Since there was no sign of other opportunistic infections, cART of raltegravir and emtricitabine/tenofovir was initiated on day 13. However, the bloody diarrhea persisted and a repeat colonoscopy was performed on day 19 to investigate the cause. The edematous mucosa and deep ulcers were still observed on colonoscopy. To identify infectious agents, a polymerase chain reaction (PCR) assay for EBV in the biopsy sample was performed, which showed 9000 copies/ml. Histopathological examination showed dense lymphoplasmacytic infiltration and mild neutrophil infiltration (Fig. 1c). In-situ hybridization (ISH) for EBV-encoded small RNA-1 (EBER-1) showed some positive cells (Fig. 1d). Based on these tests, the final diagnosis was established as EBV-associated colitis. The treatment plan included continuation of cART and withdrawal of mesalazine since IBD was considered unlikely. The symptom of bloody diarrhea gradually improved and disappeared by cART alone. At 3 months, the CD4 cell count had increased to 190/μl and the third colonoscopy showed significant improvement (Fig. 1b). PCR for EBV DNA in the biopsy sample showed a decrease to 80 copies/ml, and ISH showed no EBER-1-positive cells.

Although EBV-associated lymphoma of the gastrointestinal tract is common, EBV-associated colitis is very rare. To our knowledge, this is the first study demonstrating EBV-associated colitis in an HIV-infected patient. In

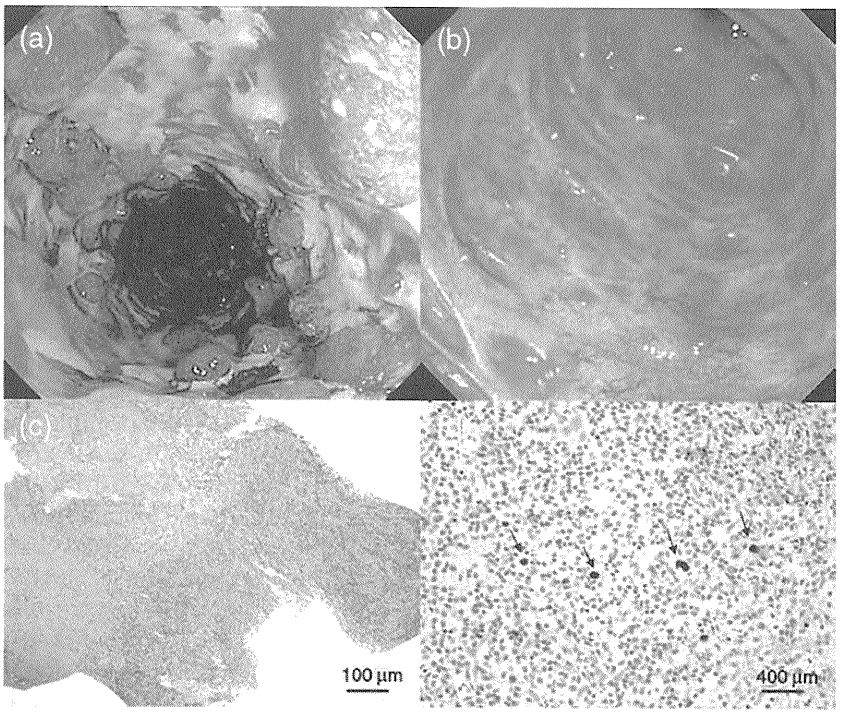

Fig. 1. Endoscopic and microscopic findings. Colonoscopic findings (a) on admission and (b) on 105th day of antiretroviral therapy. (c) Histopathological examination showing granulation tissue in ulcer floor (hematoxylin-eosin staining). (d) Epstein–Barr virus-encoded small RNA-1 in-situ hybridization demonstrated the presence of positive cells (black arrow).

addition, the significant improvement was achieved by cART alone. Several cases of EBV-associated colitis have been reported previously in immunocompromised patients, such as post-transplant patients and patients with IBD treated with immunosuppressants [2,3]. For this reason, EBV reactivation due to impaired immunity is considered to be a major causative factor of EBV-associated colitis.

In this case, EBV-associated colitis was diagnosed by the presence of EBV DNA and EBER-1-positive cells in the biopsy sample, and the improvement of colonoscopic findings associated with a decrease in EBV DNA. Because colonic appearance is grossly indistinguishable from that of CMV colitis, other forms of infectious colitis and IBD, positive EBV DNA and EBER-1 in the colonic specimens are important findings for establishing the correct diagnosis. In the case of delayed recognition of EBV colitis, treatment for IBD with corticosteroids can lead to unfavorable outcome [4]. Thus, EBV-associated colitis should be considered in HIV-infected patients, especially those with low $CD4^+$ cell counts, who present with colitis of unclear cause.

Because there is no established treatment for EBV infection, we treated this case with cART alone without specific treatment for EBV [5]. The loss of CMV viremia by cART in the absence of specific anti-CMV therapy has been reported previously [6]; therefore, it is likely that the suppression of EBV was also achieved by cART alone.

Because most reported cases of EBV colitis occurred from EBV reactivation due to impaired immunity, it is rational that restoration of the immune system by cART allowed the suppression of EBV activation and resulted in the resolution of colitis.

In conclusion, cART was effective against EBV-associated colitis. Clinicians should consider EBV infection in HIV-infected patients who present with colitis of unclear cause.

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Conflicts of interest

There are no conflicts of interest.

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PLoS one

Renal Function Declines More in Tenofovir- than Abacavir-Based Antiretroviral Therapy in Low-Body Weight Treatment-Naïve Patients with HIV Infection

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Abstract

Objective: To compare the rate of decline of renal function in tenofovir- and abacavir-based antiretroviral therapy (ART) in low-body weight treatment-naïve patients with HIV infection.

Design: We conducted a single-center retrospective cohort study of 503 Japanese patients who commenced on either tenofovir- or abacavir-based initial ART.

Methods: The incidence of renal dysfunction, defined as more than 25% fall in estimated glomerular filtration rate (eGFR) from the baseline, was determined in each group. The effect of tenofovir on renal dysfunction was estimated by univariate and multivariate Cox hazards models as the primary exposure. Changes in eGFR until 96 weeks were estimated in both groups with a repeated measures mixed model.

Results: The median body weight of the cohort was 64 kg. The estimated incidence of renal dysfunction in the tenofovir and the abacavir arm was 9.84 per 100 and 4.55 per 100 person-years, respectively. Tenofovir was significantly associated with renal dysfunction by univariate and multivariate analysis (HR = 1.747; 95% CI, 1.152–2.648; p = 0.009) (adjusted HR = 2.080; 95% CI, 1.339–3.232; p<0.001). In subgroup analysis of the patients stratified by intertertile baseline body weight, the effect of tenofovir on renal dysfunction was more evident in patients with lower baseline body weight by multivariate analysis (≤60 kg: adjusted HR = 2.771; 95%CI, 1.494–5.139; p = 0.001) (61–68 kg: adjusted HR = 1.908; 95%CI, 0.764–4.768; p = 0.167) (>68 kg: adjusted HR = 0.997; 95%CI, 0.318–3.121; p = 0.995). The fall in eGFR was significantly greater in the tenofovir arm than the abacavir arm after starting ART (p = 0.003).

Conclusion: The incidence of renal dysfunction in low body weight patients treated with tenofovir was twice as high as those treated with abacavir. Close monitoring of renal function is recommended for patients with small body weight especially those with baseline body weight <60 kg treated with tenofovir.

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Introduction

Tenofovir disoproxil fumarate (TDF) and abacavir sulfate (ABC) are widely used nucleot(s)ide reverse transcriptase inhibitors (NRTIs) as part of the initial antiretroviral therapy for patients with HIV infection in the developed countries (URL:http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf) (URL: http://www.europeanaidsclinicalsociety.org/images/stories/EACS-Pdf/1_treatment_of_hiv_infected_adults.pdf). TDF is generally preferred to ABC, since ABC is reported to cause serious hypersensitivity reaction in 5–8% of the patients and its efficacy in viral suppression is reported to be inferior to TDF among patients with baseline HIV viral load of >100,000 copies/ml [1,2]. On the other hand, renal proximal tubular damage and renal dysfunction are well-known adverse effects of TDF [3–9]. A meta-analysis study that compared TDF and other NRTIs concluded that the decline in renal function with TDF use is significant but modest, and the ASSERT study conducted in Europe compared randomly-selected treatment naïve patients who commenced treatment with either TDF or ABC with efavirenz and showed no difference in estimated glomerular filtration

rate (eGFR) between the two groups at 48 weeks [9,10]. To date, the nephrotoxicity of TDF have been regarded as mild and tolerable [2,5–7,9–11].

However, the TDF-related nephrotoxicity has hardly been evaluated in patients with small body weight, who are potentially at higher risk for larger drug exposure and thus, more severe toxicity [12–15]. Indeed, some recent studies including ours reported a higher incidence of TDF-related renal dysfunction among Asian patients with low body weight compared with previous studies on mostly Whites and African Americans with larger body weight [13,16]. Thus, it is important to provide more evidence in support of TDF-associated nephrotoxicity in patients with low body weight since such data can elucidate whether TDF-related nephrotoxicity is as mild in low-body-weighted patients as previously reported in Europe and the USA. This is also important because there is increasing use of TDF in resource-limited settings, where patients are often of relatively small body weight, following the revised 2010 WHO guidelines that recommend TDF as one of the components of first line therapies (URL:http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf)[13,16–19]. To our knowledge, there are no studies that compared renal function in treatment naïve Asian patients who commenced treatment with TDF or ABC.

Based on the above background, the present study was designed to compare the incidence of renal dysfunction and change in eGFR between treatment-naïve Japanese patients with low body weight who started either TDF or ABC as part of the antiretroviral regimen.

Methods

Ethics Statement

This study was approved by the Human Research Ethics Committee of National Center for Global Health and Medicine, Tokyo. All patients included in this study provided a written informed consent for their clinical and laboratory data to be used and published for research purposes. This study has been conducted according to the principles expressed in the Declaration of Helsinki.

Study Subjects

We performed a retrospective, single-center cohort study of HIV-infected Japanese patients using the medical records at the National Center for Global Health and Medicine, Tokyo, Japan. Our facility is one of the largest clinics for patients with HIV infection in Japan with more than 2,700 registered patients. The study population was treatment-naïve patients with HIV infection, aged >17 years, who commenced treatment with either the recommended 300 mg/day dose of TDF or 600 mg/day dose of ABC-containing antiretroviral regimen at our clinic between January 1, 2004 and March 31, 2009. During this inclusion period, all except two patients at our clinic started ART with either ABC or TDF. Patients with an eGFR of >60 ml/min/1.73 m^2 were enrolled. Patients were followed up until March 31, 2011. They were excluded if they started ART with both TDF and ABC, their follow-up period at our facility was less than 24 weeks after commencement of ART, or if they had started ART at other facilities. Only Japanese patients were included in order to examine a population with comparatively homogenous basic demographics and background. The attending physician selected either TDF or ABC at baseline, and the use of these two drugs was based on the Japanese guidelines, which place both ABC and TDF as the preferred NRTIs (http://www.haart-support.jp/guideline2011.pdf. in Japanese). The attending physician also selected the key drug [non-nucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor (PI), or integrase inhibitor (INI)]. All patients received standard ART with two NRTIs combined with either PI, NNRTI, or INI.

Measurements

We defined renal dysfunction as more than 25% decrease in eGFR relative to the baseline [13,16,20,21]. The baseline eGFR was estimated for each patient from the average of two successive serum creatinine measurements made closest to and preceding the commencement of antiretroviral therapy by no more than 90 days. Changes in eGFR were plotted from the baseline measurement until the average value of two successive measurements diminished to less than 75% of the baseline, discontinuation of TDF or ABC, or at the end of the follow-up period. Discontinuation of TDF and ABC was the choice of the attending physician, and was based on virologic failure or ART-related side effects other than renal dysfunction. Before the initiation of ART and until suppression of HIV-1 viral load, patients visited our clinic every month. However, after viral load suppression, the visit interval was extended up to every three months. Serum creatinine and eGFR were measured in every visit, and the frequency of measurements was similar in patients on TDF and ABC. eGFR was calculated using the equation from the 4-variable Modification of Diet in Renal Disease (MDRD) study, eGFR = $186\times$ [serum creatinine]$^{-1.154}\times$ [age]$^{-0.203}\times$ [0.742 if patient is female] $\times$ [1.212 if patient is African American] [22]. In this study, the primary exposure variable was TDF use over ABC as part of the initial ART.

The potential risk factors for renal dysfunction were determined according to previous studies and collected together with the basic demographics from the medical records [15,23–25]. They included age, sex, body weight, body mass index, (BMI) = {body weight (kg) / [(height (m)]^2}, baseline laboratory data (CD4 cell count, HIV viral load, and serum creatinine), and presence or absence of other medical conditions (concurrent use of ritonavir-boosted protease inhibitors, concurrent nephrotoxic drugs such as ganciclovir, sulfamethoxazole/trimethoprim, and non-steroidal anti-inflammatory agents, diabetes mellitus defined by using anti-diabetic agents or fasting plasma glucose >126 mg/dl or plasma glucose >200 mg/dl on two different days, co-infection with hepatitis B defined by positive hepatitis B surface antigen, co-infection with hepatitis C defined by positive HCV viral load, hypertension defined by current treatment with antihypertensive agents or two successive measurements of systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg at the clinic, dyslipidemia defined by current treatment with lipid-lowering agents, and current smoking). At our clinic, weight and blood pressure were measured on every visit whereas other variables were measured in the first visit and at least once annually. We used the data on or closest to and preceding the day of starting ART by no more than 90 days.

Statistical analysis

The time to 25% decline in eGFR from the baseline was calculated from the date of commencement of treatment to the date of diagnosis of the above-defined renal dysfunction. Censored cases represented those who discontinued ABC or TDF, dropped out, were referred to other facilities, or at the end of follow-up period. The time from the start of ART to >25% decrease in eGFR was analyzed by the Kaplan Meier method for patients who started TDF (TDF arm) and ABC (ABC arm), and the log-rank test was used to determine the statistical significance. The Cox proportional hazards regression analysis was used to estimate the

impact of TDF use over ABC on the incidence of more than 25% decrease in eGFR relative to the baseline. The impact of each basic demographics, baseline laboratory data, and other medical conditions listed above was also estimated with univariate Cox proportional hazards regression.

To estimate the unbiased prognostic impact of TDF use over ABC for renal dysfunction, we conducted three models using multivariate Cox proportional hazards regression analysis. Model 1 was the aforementioned univariate analysis for TDF use over ABC. Model 2 included age and weight plus model 1 in order to adjust for basic characteristics. In model 3, we added variables with P values <0.05 in univariate analysis for adjustment (these included age per 1 year, weight per 1 kg decrement, CD4 count per 1 /μl decrement, HIV viral load per log10/ml, serum creatinine per 1 mg/dl, concurrent use of nephrotoxic drug(s), hepatitis B infection, and diabetes mellitus). The eGFR and the BMI were excluded from multivariate analysis because of their multicollinearity with age and serum creatinine, and weight, respectively, since eGFR and BMI are gained by the equation of those variables [22,26]. We chose to add weight instead of BMI because our previous work showed that weight was more useful and handy information to estimate the risk for TDF-related nephrotoxicity than BMI [16].

As a sensitivity analysis, creatinine clearance was similarly calculated with Cockcroft-Gault equation for each patient, creatinine clearance = [(140 - age) × weight (kg)] / (serum creatinine×72)(×0.85 for females) [27]. Actual body weight was used for the calculation. The impact of TDF use over ABC for >25% decrement of creatinine clearance from the baseline was estimated in univariate analysis and multivariate analysis adjusted with the before mentioned variables with Cox proportional hazards model.

To estimate the impact of weight on TDF-related nephrotoxicity, we did subgroup analysis for intertertile baseline body weight categories: ≤60, 61–68, and >68 kg. Then, the abovementioned multivariate analysis with eGFR was conducted for each subgroup.

We also used a repeated measures mixed model to estimate and compare changes in eGFR between ABC and TDF from baseline to 2 years after initiation of ART by 6-month intervals adjusted for baseline eGFR and weight [10]. For each patient, the eGFR values at closest to and preceding 24, 48, 72 and 96 weeks after commencement of ART were collected. In this analysis, censoring occurred at discontinuation of TDF or ABC, leaving care, or reaching the end of the observation period before 96 weeks. Sensitivity analysis with creatinine clearance calculated by Cockcroft-Gault equation was similarly conducted.

Statistical significance was defined at two-sided p values <0.05. We used hazard ratios (HRs) and 95% confidence intervals (95% CIs) to estimate the impact of each variable on renal dysfunction. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 17.0 (SPSS, Chicago, IL).

Results

The study subjects were 199 patients in the TDF arm and 304 patients in the ABC arm who fulfilled the abovementioned criteria. Table 1 shows the demographics, laboratory data, and medical conditions of the study population at baseline. The majority of the study population was males, comparatively young and had a small stature (median weight, 64 kg, median BMI, 22.2 kg/m^2). More than 80% of the patients in the two arms had ritonavir-boosted PI. In the ABC arm, patients had significantly lower CD4 count (p = 0.006), were significantly more likely to have hypertension (p<0.001), and tended to use more nephrotoxic drugs (p = 0.109). On the other hand, in the TDF arm, patients had marginally higher baseline eGFR (p = 0.098) and were significantly more likely to have hepatitis B virus infection (P<0.001). However, all other major background parameters were similar in the two groups (Table 1).

More than 25% decrement in eGFR from baseline occurred in 44 patients (22.1%) in the TDF arm and 41 (13.5%) in the ABC arm, with an estimated incidence of 9.84 and 4.55 per 100 person-years, respectively. Figure 1 shows the time from ART initiation to >25% decrease in eGFR by the Kaplan Meier method in the two groups. Patients who started TDF-containing ART were significantly more likely to develop renal dysfunction, compared to the ABC group (p = 0.001, Log-rank test). The median time from commencement of ART to occurrence of >25% decrement in eGFR was 246 days (range, 1–1,339 days) for the TDF arm and 501 days (range, 7–2,022) for ABC arm. The total observation period was 447.2 patient-years [median, 839 days, interquartile range (IQR), 357–1137 days] for the TDF arm and 901.7 patient-years (median, 1,119 days, IQR, 660.5–1509 days) for the ABC arm.

Univariate analysis showed a significant relationship between TDF use and >25% decrement in eGFR (HR = 1.747; 95%CI, 1.152–2.648; p = 0.009) (Table 2). Furthermore, old age, small body weight, low baseline CD4 count, high HIV viral load, high eGFR, low serum creatinine, concurrent use of nephrotoxic drugs, hepatitis B infection, and diabetes mellitus were associated with renal dysfunction. On the other hand, concurrent use of ritonavir boosted PIs was not associated with renal dysfunction (HR = 1.220; 95%CI, 0.663–2.244; p = 0.523). Multivariate analysis identified TDF use as a significant risk for >25% decrement in eGFR after adjustment for age and weight (adjusted HR = 1.893; 95%CI, 1.243–2.881; p<0.003) (Table 3, Model 2), and also after adjustment for other risk factors (adjusted HR = 2.080; 95%CI, 1.339–3.232; p<0.001) (Table 3, Model 3). We also conducted a sensitivity analysis using BMI decrement instead of weight as a variable in Table 3, Model 3. The results were almost identical; TDF use over ABC use was a risk for renal dysfunction (adjusted HR 1.957, 95% CI 1.262–3.036, p = 0.003).

Sensitivity analysis with creatinine clearance confirmed the abovementioned findings: both univariate and multivariate analyses showed that TDF use was significantly associated with >25% decrement in eGFR (univariate analysis: HR = 2.212; 95%CI, 1.340–3.653; p = 0.002) (multivariate analysis: adjusted HR = 2.544; 95%CI, 1.493–4.335; p = 0.001).

Subgroup analysis of the patients stratified by intertertile baseline body weight showed that the lower the baseline body weight, the more evident the impact of TDF on renal dysfunction (≤60 kg: adjusted HR = 2.771; 95%CI, 1.494–5.139; p = 0.001) (61–68 kg: adjusted HR = 1.908; 95%CI, 0.764–4.768; p = 0.167) (>68 kg: adjusted HR = 0.997; 95%CI, 0.318–3.121; p = 0.995) (Table 4). These findings suggest that there is the effect modification by baseline body weight on TDF-associated renal dysfunction.

Data analysis by repeated measures mixed models showed a significant decrease in adjusted mean eGFR from the baseline to 96 weeks in both groups (TDF: -9.984 ml/min/1.73m^2, 95%CI -12.05 to -7.914 ml/min/1.73m^2, p<0.001; ABC: -5.393 ml/min/1.73m^2, 95%CI -7.087 to -3.699 ml/min/1.73m^2, p<0.001) (Figure 2). There was a statistically significant interaction between the two arms over time (p = 0.003), indicating that adjusted mean eGFR decreased more significantly in the TDF group than in the ABC group after initiation of ART. Analysis of eGFR in each group demonstrated a rapid decrease during the first 24 weeks,

Table 1. Baseline demographics and laboratory data of patients who received tenofovir- and abacavir-based antiretroviral therapy (n = 503).

	TDF (n = 199)	ABC (n = 304)	P value
Sex (male), n (%)	196 (98.5)	296 (97.4)	0.539
Median (IQR) age	36 (31–44)	37 (31–43)	0.436
Median (IQR) weight (kg)	64 (58–69)	64 (58.0–70.9)	0.426
Median (IQR) BMI (kg/m^2)	22.1 (20.4–23.9)	22.2 (20.3–24.6)	0.321
Median (IQR) eGFR (ml/min/1.73m^2)	119.4 (103.0–135.0)	115.6 (102.4–132.2)	0.098
Median (IQR) serum creatinine (mg/dl)	0.74 (0.67–0.84)	0.75 (0.68–0.83)	0.250
Median (IQR) CD4 count (/μl)	199 (109–272)	178.5 (75.3–234.8)	0.006
Median (IQR) HIV RNA viral load (log10/ml)	4.63 (4.20–5.20)	4.74 (4.23–5.20)	0.731
Ritonavir-boosted protease inhibitors, n (%)	173 (86.9)	256 (84.2)	0.441
Protease inhibitors (unboosted), n (%)	5 (2.5)	20 (6.6)	0.038
NNRTIs, n (%)	16 (8.0)	26 (8.6)	0.848
INIs, n (%)	5 (2.5)	2 (0.7)	0.119
Hypertension, n (%)	5 (2.5)	53 (17.4)	<0.001
Dyslipidemia, n (%)	4 (2.0)	4 (1.3)	0.718
Diabetes mellitus, n (%)	8 (4.0)	12 (3.9)	1.000
Concurrent use of nephrotoxic drugs, n (%)	65 (32.7)	121 (39.8)	0.109
Hepatitis B, n (%)	35 (17.6)	9 (3.0)	<0.001
Hepatitis C, n (%)	7 (3.5)	7 (2.3)	0.421
Current smoker, n (%)	93 (46.7)	149 (49.3)	0.585

TDF: tenofovir, ABC: abacavir, IQR: interquartile range, BMI: body mass index, eGFR: estimated glomerular filtration rate, NNRTI: non- nucleoside reverse transcriptase inhibitor, INI: integrase inhibitor.
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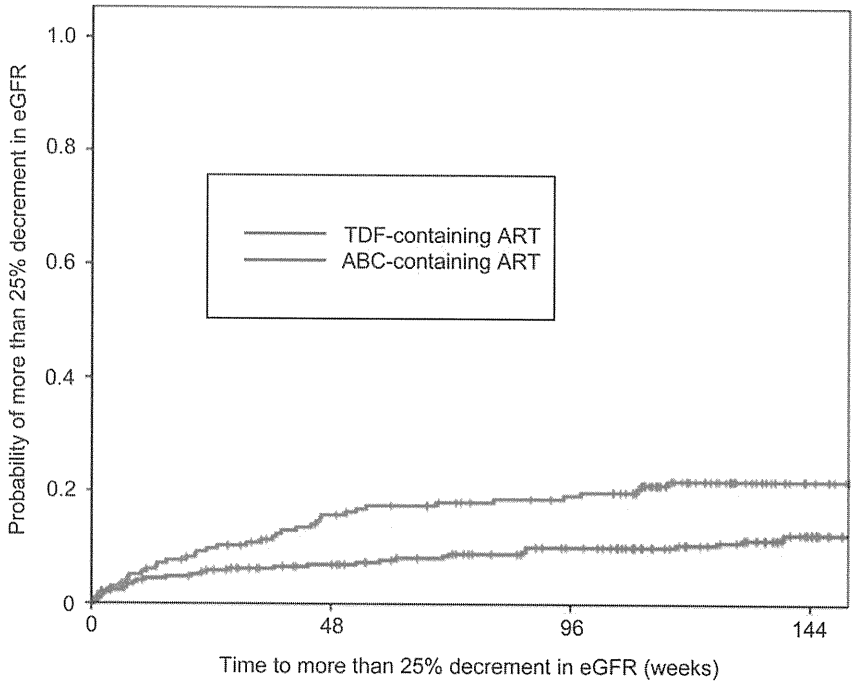

Figure 1. Kaplan-Meier curve showing the time to renal dysfunction in patients treated with TDF or ABC. Compared to treatment-naïve patients who commenced treatment with ABC, those on TDF were more likely to develop >25% fall in eGFR (p = 0.001, Log-rank test). TDF: tenofovir, ABC: abacavir, ART: antiretroviral therapy, eGFR: estimated glomerular filtration rate.
doi:10.1371/journal.pone.0029977.g001

Table 2. Univariate analysis to estimate the risk of various factors in inducing more than 25% fall in eGFR.

	Hazard ratio	95% CI	P value
TDF vs. ABC use	1.747	1.152–2.648	0.009
Female gender	0.048	0.000–16.93	0.310
Age per 1 year	1.031	1.011–1.051	0.002
Weight per 1 kg decrement	1.047	1.023–1.072	<0.001
BMI per 1 kg/m^2 decrement	1.152	1.066–1.244	<0.001
CD4 count per 1 /μl decrement	1.006	1.004–1.008	<0.001
HIV viral load per log10/ml	1.562	1.179–2.071	0.002
Ritonavir-boosted protease inhibitors	1.220	0.663–2.244	0.523
Baseline eGFR per 1 ml/min/1.73m^2	1.009	1.005–1.014	<0.001
Baseline serum creatinine per 1mg/dl	0.016	0.003–0.086	<0.001
Concurrent nephrotoxic drug	2.134	1.417–3.214	<0.001
Hepatitis B	1.866	1.038–3.356	0.037
Hepatitis C	1.721	0.631–4.695	0.289
Diabetes mellitus	2.558	1.181–5.540	0.017
Hypertension	0.865	0.448–1.669	0.664
Current smoking	0.989	0.657–1.489	0.958

eGFR: estimated glomerular filtration rate, CI: confidence interval, TDF: tenofovir, ABC: abacavir, BMI: body mass index.
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followed by a plateau until 96 weeks. In sensitivity analysis with creatinine clearance calculated by Cockcroft-Gault equation, the result was the same; a significant decrease from the baseline to 96 weeks in both groups (TDF: -10.62 ml/min, 95%CI -13.78 to -7.458 ml/min; ABC: -4.325 ml/min, 95%CI -6.893 to -1.756 ml/min) and significantly more eGFR decrement in the TDF group (p = 0.019).

Discussion

In this observational Japanese cohort, treatment-naïve patients who started TDF-containing ART experienced eGFR decline of >25% approximately twice as likely compared to those treated with ABC-containing regimen. Univariate and multivariate analyses identified TDF use as an independent risk factor for renal dysfunction. Subgroup analysis showed that the effect of TDF on renal dysfunction was more evident in patients with lower body weight. Furthermore, eGFR decrement was significantly larger in the TDF group than in ABC group over the 2-year observation period.

In our previous study, we demonstrated a high incidence of TDF-associated nephrotoxicity in patients with low body weight, and the use of a robust statistical model indicated a greater decline in renal function in patients of low body weight treated with TDF [16]. The results of the present study further emphasize the importance of low body weight as a risk factor for TDF-related nephrotoxicity by showing that in a cohort of patients with low body weight, the incidence of renal dysfunction was twice higher with TDF use than with ABC use.

Among the studies designed to compare renal function after the commencement of TDF and ABC-containing ART for treatment-naïve patients, our cohort had the lowest median body weight (64 kg). This is lower than the median body weight of patients of the ASSERT study conducted in European countries (72 kg) [10]. The

Table 4. Multivariate analysis to estimate the risk of TDF-over ABC-based antiretroviral therapy in the induction of more than 25% fall in eGFR according to baseline body weight.

	Adjusted HR	95% CI	P value
Baseline body weight ≤60 kg (n = 171)			
TDF vs. ABC use	2.771	1.494–5.139	0.001
Baseline body weight 61–68 kg (n = 167)			
TDF vs. ABC use	1.908	0.764–4.768	0.168
Baseline body weight >68 kg (n = 165)			
TDF vs. ABC use	0.997	0.318–3.121	0.995

TDF use was adjusted with the same variables indicated in Model 3, Table 3: age per 1 year, weight per 1 kg decrement, CD4 count per 1 /μl decrement, HIV viral load per log10/ml, serum creatinine per 1 mg/dl, concurrent use of nephrotoxic drugs, hepatitis B infection, and diabetes mellitus.
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Table 3. Multivariate analysis to estimate the risk of TDF- over ABC-based antiretroviral therapy in inducing more than 25% fall in eGFR.

	Model 1 Crude		Model 2 Adjusted		Model 3 Adjusted	
	HR	95% CI	HR	95%CI	HR	95%CI
TDF vs. ABC use†	1.747	1.152–2.648	1.893	1.243–2.881	2.080	1.339–3.232
Age per 1 year			1.029	1.010–1.048	1.020	1.000–1.040
Weight per 1 kg decrement†			1.046	1.022–1.071	1.028	1.005–1.052
CD4 count per 1 /μl decrement†					1.004	1.002–1.007
HIV viral load per log10/ml					1.048	0.749–1.466
Serum creatinine per 1 mg/dl†					0.053	0.009–0.304
Use of nephrotoxic drug					1.309	0.825–2.077
Hepatitis B					1.070	0.573–2.000
Diabetes mellitus					1.565	0.684–3.582

†P<0.05 in Model 3.
TDF: tenofovir, ABC: abacavir, eGFR: estimated glomerular filtration rate, HR: hazard ratio, CI: confidence interval.
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