**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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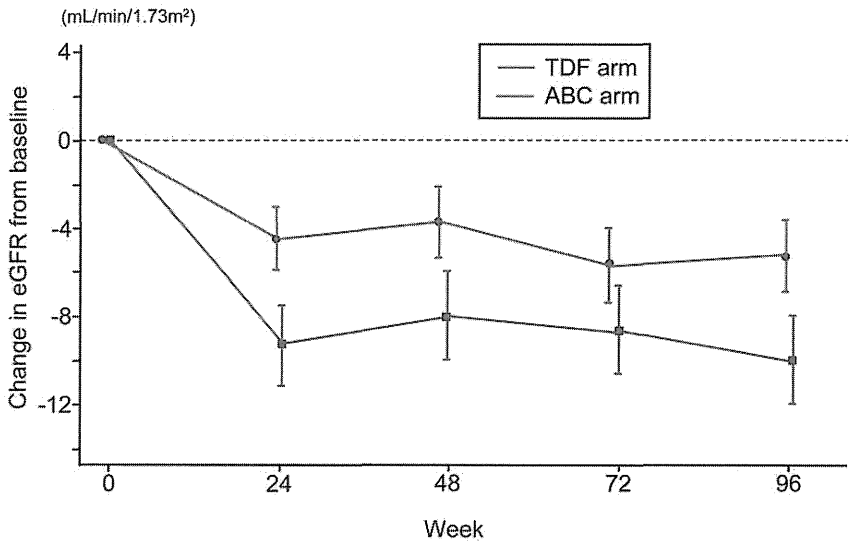


**Figure 2. Changes in eGFR in patients treated with TDF or ABC between baseline and 96 weeks.** The fall in eGFR was significantly greater in the TDF group than the ABC group ($p = 0.003$). Data are adjusted mean ±95% confidence interval. eGFR: estimated glomerular filtration rate, TDF: tenofovir, ABC: abacavir.
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results of the present study on TDF-related nephrotoxicity differ from the findings of randomized clinical trials that demonstrated no major change in renal function of TDF- and ABC-treated patients over 48–96 week follow-up [2,10,11]. The discrepant results might arise from differences between observational cohort and clinical trials, since observational studies tend to express the results in "real world setting" whereas clinical trials include patients who fulfill more strict criteria, therefore with better profile [9]. The discrepant results could be also due to the use of different definitions for renal dysfunction in these studies. However, the discrepant results could also reflect the difference in median body weight between the present study and these clinical trials. The results of our subgroup analysis support this hypothesis by showing that the effect of TDF on renal dysfunction was more evident in patients with low body weight. Apart from being low-body-weighted, the patients in this study did not appear to have many of other established risks for TDF-related nephrotoxicity; they were comparatively young, had relatively stable CD4 count, and had only a few co-morbidities (Table 1). Although the majority concurrently used ritonavir-boosted PIs, which are a probable risk for TDF-related nephrotoxicity, ritonavir-boosted PIs were not significantly associated with renal dysfunction in our cohort (Table 2) [24].

Changes in eGFR in those patients treated with TDF-containing ART were characterized by a rapid decline during the first 24 weeks of therapy, followed by a plateau until 96 weeks (Fig. 2). This finding is consistent with that reported from the Johns Hopkins group [9,28]. Together with the finding that the median time from commencement of ART to the >25% decline in eGFR in the TDF-treated patients was 246 days, these results suggest that careful monitoring of renal function is particularly warranted in the first year of TDF-based therapy. Thus, we suggest that renal function should be monitored by measurement of serum creatinine at least once annually in resource-limited settings and twice annually in resource-rich settings in patients starting TDF-containing ART, especially those with baseline body weight <60 kg.

The Department of Health and Human Services guideline for the treatment of HIV infection in the U.S. lists ABC as an alternative NRTI because it can potentially cause serious hypersensitivity reaction and cardiovascular diseases (URL:http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf). However, some international guidelines consider both TDF and ABC as the preferred NRTIs under the condition that ABC should be used with caution in patients with viral load >100,000 copies/mL, based on the low incidence of ABC-related hypersensitivity among HLA-B*5701-negative population and the controversial association between ABC and cardiovascular diseases [1,29–32] (URL: http://www.europeanaidsclinicalsociety.org/images/stories/EACS-Pdf/1_treatment_of_hiv_infected_adults.pdf) (http://www.haart-support.jp/guideline2011.pdf. in Japanese). The present study, together with our previous analysis that demonstrated preferential TDF-related nephrotoxicity in patients with low body weight, emphasize the advantage of ABC over TDF with regard to prognosis of renal function in low body weight patients [16].

TDF is the prodrug of acyclic nucleotide analog tenofovir, which is excreted by both glomerular filtration and active tubular secretion. Tenofovir is considered to cause mitochondrial damage in proximal renal tubular cells [33]. The concentration of tenofovir in the proximal renal tubules could be augmented with the complex interactions of pharmacological, environmental, and genetic factors, including small body weight, consequently resulting in renal tubular dysfunction [34]. Body weight has been identified as an important factor in TDF-related nephrotoxicity not only in clinical trials, but also in *in vitro* and pharmacokinetic studies [35–37].

The present study has several limitations. First, because of its retrospective nature, it was not possible to control the baseline characteristics of the enrolled patients. Thus, it is possible that patients with potential risk for TDF-related nephrotoxicity were not prescribed TDF. A proportion of patients treated with ABC had low CD4 count and others were hypertensive, both conditions are known risk factors for renal dysfunction [23,25]. However, for these reasons, the incidence of TDF-associated renal dysfunction might have been underestimated. Second, the definition of TDF-related nephrotoxicity, especially the criteria used to evaluate proximal renal tubular damage, is not uniformly established in the field and is different in the published studies. Accordingly, we

decided to adopt changes in eGFR, instead of parameters for proximal renal tubular damage. Using the eGFR as a marker for TDF-associated renal dysfunction, our results might have underestimated the incidence of TDF-related renal tubular dysfunction. However, the result of this study could be informative to resource-limited settings, where it is difficult to evaluate renal tubular markers. The rational and limitation of adopting more than 25% decrement in eGFR as the criterion for renal dysfunction were discussed in detail in our previous study [16]. Third, our cohort was characterized by the high prevalence of ritonavir-boosted PI use, which is considered by some groups a risk for TDF-related nephrotoxicity [24]. While it is difficult to completely exclude the impact of concurrent ritonavir-boosted PI in this study, it should be noted that the use of ritonavir-boosted PIs did not correlate with renal dysfunction in univariate analysis in this cohort (Table 2). Fourth, the study subjects were mainly men (mostly men who have sex with men and very few injection drug users). Further studies are needed to determine whether the findings of this study are also applicable to females, patients with different route of transmissions, and patients of different racial background.

In conclusion, the present study demonstrated a high incidence of renal dysfunction with TDF use, compared to ABC, among treatment-naïve patients with low body weight. TDF use was identified as an independent risk for renal dysfunction in a statistical model that included TDF as a primary exposure. At 96 weeks, patients with TDF showed greater eGFR decrement than patients treated with ABC. TDF is certainly a drug of choice in the treatment of HIV infection, but the importance of close monitoring of renal function in patients with small body weight, especially those with baseline body weight <60 kg should be emphasized for early detection of TDF-related nephrotoxicity. Further studies are warranted to elucidate the long-term prognosis of renal function with TDF use in patients with low body weight.

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## Author Contributions

Conceived and designed the experiments: TN HK HG TS EK JT SO. Performed the experiments: TN HK TS TA KW EK MH. Analyzed the data: TN HK HG TS HH HY K. Tsukada MH K. Teruya YK. Contributed reagents/materials/analysis tools: TA KW HH JT HY K. Tsukada MH K. Teruya YK. Wrote the paper: TN HK HG TS EK SO.

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ORIGINAL RESEARCH ARTICLE

# The relationship between HIV testing and CD4 counts at HIV diagnosis among newly diagnosed HIV-1 patients in Japan

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**Summary:** The aim of this study was to investigate the factors relating to CD4 level at HIV diagnosis and HIV testing behaviour. Participants were newly diagnosed patients ($n = 654$) in Japan from 2000 to 2005. Around 75% of participants were diagnosed at hospital and clinics. Mean CD4 counts at diagnosis through voluntary HIV testing, screening tests and testing due to concomitant sexually transmitted infection (STI) were 368, 336 and 316 cells/μL, respectively. In contrast, the mean CD4 count where testing was due to the presence of HIV-related clinical symptoms was 151 cells/μL ($P < 0.0001$). Compared with those diagnosed at their first HIV test, those who had undertaken multiple HIV tests prior to diagnosis showed CD4 counts that increased significantly ($P < 0.0001$) in relation to the number of tests undertaken: CD4 count at first test was 232 cells/μL, second test 346 cells/μL and third or additional tests 439 cells/μL. According to our results, HIV testing policy that promotes HIV testing in medical settings and among STI patients is needed to facilitate earlier HIV diagnosis in Japan.

**Keywords:** HIV, testing, early diagnosis, Japan, CD4 count

## INTRODUCTION

Combination antiretroviral therapy has dramatically reduced the morbidity and mortality of HIV-1 infection and has led to the extension of long-term survival rates for people with HIV and AIDS.[1-3] Late diagnosis is currently the major factor that increases the risk of morbidity and mortality related to HIV infection. Short-term mortality (death within a year of diagnosis) has been reported to be 6.1% for patients diagnosed late (CD4 counts <200 cells/μL) and 9.7% for patients who start therapy with CD4 counts <50 cells/μL.[4,5] An increase in the proportion of people with HIV who are aware of their serostatus can also contribute to the prevention of HIV infection transmission;[6] thus earlier diagnosis has benefits for the patient as well as for public health promotion.

Incidence and factors relating to the late diagnosis of HIV-1 infection have been investigated in a number of countries,[7-11] and factors identified as contributing to late diagnosis include being male, heterosexual, older in age and from minority racial/ethnic groups. Greater access to HIV testing for those infected is necessary in order to reduce late diagnosis.[12] In 2001, the Centers for Disease Control and Prevention (CDC) in the USA proposed routine testing for high-risk groups, such as patients with sexually transmitted infections (STIs) or tuberculosis, men who have sex with men (MSM), injecting drug users and people with multiple sexual partners.[13]

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Furthermore, in 2006, new CDC recommendations have promoted 'opt-out' screening for HIV-1 infection, in which medical professionals offer HIV testing as a part of routine clinical care for all patients aged 13-64 years in all health-care settings. Recommendations also state that health-care providers should facilitate screening for HIV-1 infection at least annually for all people likely to be in a high-risk category.[14] Actually, the number of people who have undergone HIV testing has increased 3.4-6.8 times due to routine testing or opt-out screening.[15,16] Opt-out screening in antenatal care has led to a reduction in undiagnosed maternal infection, and to higher CD4 counts at diagnosis in pregnant women than in non-pregnant women.[17] Cost and consequence evaluation has found that opt-out screening may lead to increased diagnosis of patients with unknown HIV infection.[18] However, due to insufficient investigation, it is not yet known whether opt-out screening is effective for earlier detection and improved prognosis of HIV-1 infection in general populations.

Japanese Ministry of Health surveillance data report 13,894 cases of HIV infection to the end of 2007, which translates to the cumulative number of HIV/AIDS patients per 100,000 population as 10.9 nationally, and 38.7 in Tokyo. The number of newly infected HIV/AIDS patients has been increasing annually and in 2007 the number of reported new HIV diagnoses was 1500 cases. Among new cases reported in 2007 approximately 30% had developed AIDS at the time of diagnosis. Incidence modelling indicates that the actual number of people with undiagnosed HIV is estimated to be 4.2 times higher than the reported number of cases.[19] Given the large number of patients who had developed AIDS at the time of

diagnosis and the high level of undiagnosed HIV-1 infection in Japan, it is a priority to identify factors associated with early and late HIV-1 diagnosis. Therefore, the aim of this study was to clarify the factors for early and late diagnosis by investigating when and why HIV patients undertake HIV testing, their CD4 counts at diagnosis, and the number of previous HIV tests undertaken prior to diagnosis.

## METHODS

### Study population

Patients with newly diagnosed HIV-1 infection who attended the AIDS Clinical Center at the National Center for Global Health and Medicine from April 2000 to March 2005 were included in this study. The AIDS Clinical Center is the largest national HIV treatment and research centre in Japan, treating approximately 40% of HIV patients in Tokyo and the surrounding regions.

The study was approved by the Ethics Committees of the National Center for Global Health and Medicine and the University of Tsukuba. Written consent from respondents was not required because the study was a retrospective observational study. The study protocol was publicized on the University Hospital Medical Information Network-Clinical Trials Registry database and followed the ethical guidelines for epidemiological studies set by the Japanese Ministry of Health, Labor and Welfare.

As the study aimed to analyse the risk behaviours of patients with HIV-1, patients with perinatally and occupationally acquired HIV were excluded. Study participation was limited to Japanese nationals diagnosed with HIV infection in Japan because the HIV testing experiences of non-Japanese people is affected by visa type, health insurance status, and accessibility to HIV information and testing services.

### Data collection

Data collection was conducted from a medical record review of patient medical charts. Data were collected on patient demographics (age, gender, sexual orientation), CD4 count at diagnosis, stage of HIV-1 infection, place of HIV testing, reasons for undertaking the HIV test and the number of HIV tests undertaken before diagnosis. Patients with CD4 counts <200 cells/μL at diagnosis were defined as 'late testers' in this study. CD4 count at diagnosis was defined as the count closest to, and within six months of, the date of diagnosis. AIDS patients were defined according to the CDC category C disease listing,[20] regardless of CD4 count at diagnosis.

### Reasons for HIV testing

Reasons for HIV testing were classified into four categories: (1) voluntary, (2) screening for other reasons, (3) concomitant STI-related testing and (4) clinical symptoms of HIV-1 infection. The voluntary group included patients who undertook the test voluntarily at any site (for example, public health centres, HIV testing centres, private clinics and self-conducted home testing). The screening group included patients who undertook tests before surgery, before blood donation, as part of a health check-up, and for antenatal screening. The STI group consisted of patients who were advised to undertake HIV testing by a medical provider following an STI diagnosis. Patients in the fourth group had clinical signs or symptoms suggesting HIV-1 infection (for example, *Pneumocystis Pneumonia*, oropharyngeal or oesophageal candidiasis, aseptic meningitis, fever of unknown origin and lymphadenopathy) and were offered HIV testing by a medical provider on the basis of these signs or symptoms.

### Statistical analyses

The mean CD4 counts at diagnosis were compared according to the reason for testing and number of tests undertaken, using one-way analysis of variance and a Tukey multiple comparison test. The characteristics of patients with CD4 counts <200 cells/μL at diagnosis were examined using the chi-square test. Logistic regression analysis was used to assess associations between CD4 counts <200 at diagnosis and patient characteristics. All statistical analyses were performed by using SPSS v.14.0 (SPSS Inc, Chicago, IL, USA). $P$ values of <0.05 were considered statistically significant.

## RESULTS

### Patient characteristics

A total of 830 new patients visited the AIDS Clinical Center for consultation from April 2000 to March 2005. Among these, 110 foreigners, four patients with perinatal HIV transmission, one patient with occupational exposure and 16 patients diagnosed outside Japan were excluded from the study. Additionally, 45 patients were excluded because of insufficient data regarding CD4 count within the six months following HIV-1 diagnosis, sexual orientation, reason for HIV testing and/or number of tests taken previously. As a result, 654 patients were identified as being eligible for the study. Participant characteristics are shown in Table 1. The participant characteristics in this study

Table 1 **Characteristics of newly diagnosed HIV-1 patients from April 2000 to March 2005 ($n$ = 654)**

| Characteristics $n$ = 654 | $n$ | % |
|---|---|---|
| Gender | | |
| Men | 603 | 92.2 |
| Women | 51 | 7.8 |
| Age (years) | | |
| 19–29 | 199 | 30.4 |
| 30–39 | 245 | 37.5 |
| 40–49 | 102 | 15.6 |
| ≧50 | 108 | 16.5 |
| Sexual orientation | | |
| Homosexual/bisexual | 538 | 82.3 |
| Heterosexual | 116 | 17.7 |
| HIV stage | | |
| AIDS | 189 | 28.9 |
| Non-AIDS | 391 | 59.8 |
| Primary infection | 74 | 11.3 |
| CD4 count at HIV-1 diagnosis | | |
| 0–49 | 144 | 22.0 |
| 50–199 | 132 | 20.2 |
| 200–349 | 156 | 23.9 |
| 350–499 | 122 | 18.6 |
| ≧500 | 100 | 15.3 |
| Place of HIV testing for HIV diagnosis | | |
| Hospital | 425 | 65.0 |
| Clinic | 77 | 11.8 |
| Public health centre/HIV testing site | 130 | 19.9 |
| Other* | 22 | 3.4 |

*Other included 18 subjects for blood donation, three for mail-order test and one for prison intake

show similar patterns to national HIV surveillance data, in that 82.3% of patients were homosexual/bisexual, 67.9% were in the 20–30 years old age distribution range and 28.9% of patients had AIDS at the time of diagnosis.

### Reasons for HIV testing, the number of HIV tests previously undertaken and CD4 count at the time of diagnosis

The relationship between the reason for HIV testing and CD4 count at diagnosis is shown in Figure 1. The mean CD4 count at the time of HIV diagnosis was highest in patients who were diagnosed through voluntary testing (368 cells/μL, 95% confidence interval (CI): 342–394) followed by screening tests (336 cells/μL, 95% CI: 294–379), testing performed due to a concomitant STI (316 cells/μL, 95% CI: 271–362) and testing performed due to the existence of clinical symptoms (151 cells/μL, 95% CI: 127–175). The level of CD4 count for voluntary, screening and concomitant STI testing categories did not differ significantly. In contrast, the CD4 count for those testing due to the existence of clinical symptoms was significantly lower than that found in the other categories ($P < 0.0001$:Tukey multiple comparison test).

A relationship was observed for patients who had undertaken HIV testing prior to their diagnosis: compared with the mean CD4 count for diagnosis at the first test of 232 cells/μL (95% CI: 213–252), CD4 count when HIV was diagnosed at the second HIV test was 346 cells/μL (95% CI: 306–386) and CD4 count when HIV was diagnosed at the third or subsequent tests was 439 cells/μL (95% CI: 386–493). CD4 counts at diagnosis increased significantly as the number of HIV tests undertaken prior to diagnosis increased ($P < 0.0001$: Tukey multiple comparison test) (Figure 2).

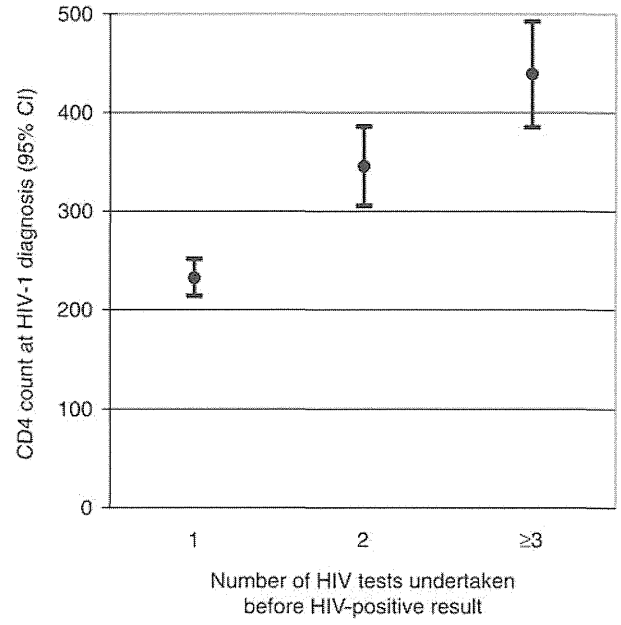


Figure 2 **Relationship between CD4 count at diagnosis and number of HIV tests undertaken prior to HIV diagnosis. CI = confidence interval**

### Characteristics of late testers from logistic regression analysis

The characteristics of late testers, who were defined by CD4 counts <200 cells/μL at diagnosis, are summarized in Table 2. The proportion of late testers increased significantly ($P < 0.0001$) with age and was correlated with heterosexual transmission. After adjusting for demographic factors in the multiple logistic regression model, diagnosis related to voluntary, screening and concomitant STI testing, or increasing number of previously undertaken HIV tests were independently associated with a lower rates of late testing (Table 3).

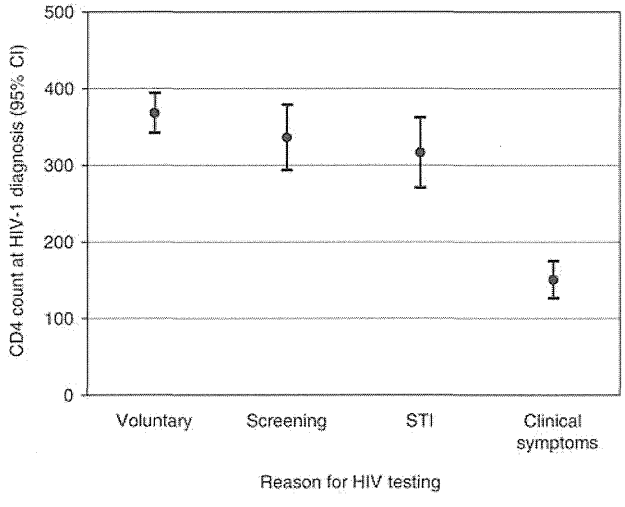


Figure 1 **Relationship between CD4 count at diagnosis and reason for HIV testing. CI = confidence interval; STI = sexually transmitted infections**

## DISCUSSION

This study showed two major new findings regarding the relationship between CD4 level at diagnosis and HIV testing behaviour. Firstly, this study identified that the reason for undertaking HIV testing had some influence on early or late diagnosis. However the relationship between reason for HIV testing and CD4 count at diagnosis has not undergone detailed examination worldwide. One report found that 65% of patients who developed AIDS within one year of their HIV diagnosis took the HIV test because of illness, whereas early testers took the test because of self-perceived risk or because they wanted to know their HIV status.[21] In addition, Lyons *et al.* (2008)[22] found higher CD4 counts in patients diagnosed with HIV infection in partner testing and universal screening. These studies are consistent with our results in indicating that screening and voluntary testing facilitate early diagnosis of HIV infection.

The second major finding in this study was that CD4 count at diagnosis increased significantly as the number of HIV tests undertaken prior to diagnosis increased. A previous study showed that patients with prior experience of HIV testing had higher CD4 counts at diagnosis compared with patients having no previous HIV testing history.[23] This study showed for the first time that CD4 count at diagnosis increases in proportion to the number of tests previously undertaken. This indicates that repeated HIV testing should be promoted for early diagnosis.

Table 2 **Patient characteristics and CD4 count at HIV-1 diagnosis**

| Characteristics | Total ($n$ = 654) $n$ | CD4 count at HIV-1 diagnosis: CD4 < 200 ($n$ = 275) No | % | CD4 ≧ 200 ($n$ = 379) No | % | $P$ value |
|---|---|---|---|---|---|---|
| Gender | | | | | | |
| Men | 603 | 257 | 42.6 | 346 | 57.4 | 0.31 |
| Women | 51 | 18 | 35.3 | 33 | 64.7 | |
| Age (years) | | | | | | |
| 19–29 | 199 | 42 | 21.1 | 157 | 78.9 | <0.0001 |
| 30–39 | 245 | 94 | 38.4 | 151 | 61.6 | |
| 40–49 | 102 | 63 | 61.8 | 39 | 38.2 | |
| ≧50 | 108 | 76 | 70.4 | 32 | 29.6 | |
| Sexual orientation | | | | | | |
| Homosexual/bisexual | 538 | 205 | 38.1 | 333 | 61.9 | <0.0001 |
| Heterosexual | 116 | 70 | 60.3 | 46 | 39.7 | |
| Reason for HIV testing | | | | | | |
| Voluntary | 230 | 41 | 17.8 | 189 | 82.2 | <0.0001 |
| Screening | 86 | 26 | 30.2 | 60 | 69.8 | |
| Concomitant STI | 75 | 20 | 26.7 | 55 | 73.3 | |
| Clinical symptoms | 263 | 188 | 71.5 | 75 | 28.5 | |
| Number of HIV tests undertaken previously | | | | | | |
| One | 483 | 244 | 50.5 | 239 | 49.5 | <0.0001 |
| Two | 110 | 27 | 24.5 | 83 | 75.5 | |
| Three or more | 61 | 4 | 6.6 | 57 | 93.4 | |

STI = sexually transmitted infection

In Japan, free and anonymous voluntary HIV testing is available at approximately 600 public health centres and HIV test centres nationwide and HIV testing policy primarily occurs through these centres. Our results suggest that early detection is related to voluntary testing, but according to the AIDS Newsletter published by the Tokyo Metropolitan Government in 2006, only 33% of HIV cases in Tokyo were detected at public health centres and HIV testing centres. This indicates that there is a need to increase voluntary testing provision and utilization for high-risk people. The reason for underutilization may be due to the fact that many centres offer HIV testing only a few times a month and testing periods are limited to one to two hours during the daytime. In order to improve testing accessibility it is necessary to increase the time available for HIV testing.

Table 3 **Multiple logistic regression analysis on the association between patient characteristics and CD4 count <200 cells/μL at HIV-1 diagnosis**

| | AOR | 95% CI | $P$ value |
|---|---|---|---|
| Gender | | | |
| Men | Reference | | |
| Women | 0.31 | 0.12–0.81 | 0.017 |
| Age (years) | | | |
| 19–29 | Reference | | |
| 30–39 | 2.01 | 1.23–3.28 | 0.005 |
| 40–49 | 3.92 | 2.13–7.21 | <0.001 |
| ≧50 | 4.63 | 2.05–8.55 | <0.001 |
| Sexual orientation | | | |
| Homosexual/bisexual | Reference | | |
| Heterosexual | 2.01 | 1.41–6.07 | 0.004 |
| Reason for HIV test | | | |
| Clinical symptoms | Reference | | |
| Voluntary | 0.13 | 0.08–0.21 | <0.001 |
| Screening | 0.18 | 0.10–0.31 | <0.001 |
| Concomitant STI | 0.21 | 0.11–0.39 | <0.001 |
| Number of HIV tests undertaken previously | | | |
| One | Reference | | |
| Two | 0.49 | 0.28–0.84 | 0.01 |
| Three or more | 0.12 | 0.04–0.37 | <0.001 |

AOR = adjusted odds ratio; CI = confidence interval; STI = sexually transmitted infection

Our results also showed that HIV screening tests and HIV testing due to concomitant STI were related to early diagnosis in medical settings. While this result is encouraging, unfortunately, in Japan, HIV testing policies for patients diagnosed with STIs and screening tests for those other than pregnant women are not performed consistently. In the UK, there were almost one million sexual health screens carried out at genitourinary medicine clinics in 2006. Two-thirds of the sexual health screenings in the UK included an HIV test. About 70% of HIV-infected people were diagnosed at a genitourinary, sexual health or HIV clinics.[24] Diagnosis as part of a routine screening and at testing clinics were associated with early detection.[25] Promotion of HIV testing in medical settings, especially at STI clinics, for people with high-risk behaviours should be considered an urgent task to be undertaken in Japan.

While opt-out screening has been promoted at emergency departments and other health-care settings in the USA, we argue that a similar policy may not be practical in Japan. Firstly, the cumulative number of HIV/AIDS patients per 100,000 population is still quite low at 10.9 nationally; thus opt-out screening for all patients is not economically realistic. Secondly, the existing public medical insurance system makes the promotion of HIV testing in medical settings difficult. In Japan, HIV testing is covered by public health insurance only in the case of suspected HIV infection or STI.

However, if health insurance coverage were widened to include HIV screening tests this could increase the opportunities for patients to undertake HIV testing without having to pay out-of-pocket costs. We acknowledge that any scaling up of HIV screening and adoption of practitioner-initiated testing needs adequate provision for those wishing to decline HIV testing, and improvements in HIV-related counselling services. This is particularly salient in the Japanese context due to research indicating that there are a significant number of people who do not wish to undertake HIV testing due to fear and not wanting to know the results of such testing.[26]

As this study was performed at a single site, it may be difficult to generalize the findings to other medical settings. However, patterns of patient characteristics in this study are similar to recent Japanese surveillance data indicating that our survey sample is generalizable with the sample of reported HIV cases in Japan. The study site, located in central Tokyo, which has the highest concentration of HIV cases in Japan, treats approximately 40% of HIV patients in Tokyo and the surrounding regions. Therefore, we are confident that our results reflect, at minimum, the situation in Tokyo, but because the study is cross-sectional, improvement of prognosis and prevention of secondary infection were not evaluated. Further studies are needed in order to evaluate early detection, including reduction in development of AIDS, decreased mortality and prevention of secondary infection.

According to our results, existing HIV testing policy and practices need to change in order to improve late diagnosis in Japan. In particular, HIV testing policy that promotes HIV testing in medical settings and among STI patients is needed to facilitate earlier HIV diagnosis in Japan.

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# Outbreaks of *Pneumocystis* Pneumonia in 2 Renal Transplant Centers Linked to a Single Strain of *Pneumocystis*: Implications for Transmission and Virulence

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**(See the Editorial Commentary by de Boer, on pages 1445–7.)**

***Background.*** There have been numerous reports of clustered outbreaks of *Pneumocystis* pneumonia (PCP) at renal transplant centers over the past 2 decades. It has been unclear whether these outbreaks were linked epidemiologically to 1 or several unique strains, which could have implications for transmission patterns or strain virulence.

***Methods.*** Restriction fragment length polymorphism (RFLP) analysis was used to compare *Pneumocystis* isolates from 3 outbreaks of PCP in renal transplant patients in Germany, Switzerland, and Japan, as well as nontransplant isolates from both human immunodeficiency virus (HIV)–infected and uninfected patients.

***Results.*** Based on RFLP analysis, a single *Pneumocystis* strain caused pneumonia in transplant patients in Switzerland (7 patients) and Germany (14 patients). This strain was different from the strain that caused an outbreak in transplant patients in Japan, as well as strains causing sporadic cases of PCP in nontransplant patients with or without HIV infection.

***Conclusions.*** Two geographically distinct clusters of PCP in Europe were due to a single strain of *Pneumocystis*. This suggests either enhanced virulence of this strain in transplant patients or a common, but unidentified, source of transmission. Outbreaks of PCP can be better understood by enhanced knowledge of transmission patterns and strain variation.

*Pneumocystis jirovecii* continues to be an important, often fatal, cause of *Pneumocystis* pneumonia (PCP) in a wide spectrum of immunosuppressed patients including patients with human immunodeficiency virus (HIV) infection and patients who have received human stem cell or solid organ transplants [1, 2]. Although prophylaxis has been very effective in preventing PCP in HIV infection, identification of patients who are at risk for PCP and thus suitable candidates for prophylaxis in non-HIV populations can be more difficult. Notable outbreaks of PCP have occurred, especially in renal transplant patients over the past 2 decades, primarily from centers in Europe and Japan [3–9]. Renal transplant patients in the recent era may well have been susceptible to PCP because of inconsistent use of anti-*Pneumocystis* prophylaxis at many centers in the context of changing immunosuppressive regimens. However, the dramatic occurrence of clusters that are geographically and temporally distinct suggests that special circumstances may exist where renal transplant patients are uniquely susceptible to infection, possibly due to epidemiologic factors, such as dedicated clinics

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for transplant patients, or to a unique, potentially more virulent strain of *Pneumocystis*.

We have recently developed a typing technique using restriction fragment length polymorphism (RFLP) analysis that has allowed us to demonstrate substantial diversity among *Pneumocystis* isolates, both in HIV-infected and uninfected patients [10]. A remarkable feature of our studies is the tremendous variability seen in the RFLP patterns: no 2 patients with sporadic cases of PCP showed the same pattern, suggesting that each case was caused by a unique strain of *Pneumocystis*. However, in contrast to this experience with sporadic cases, using this technique we were able to confirm that an outbreak of PCP in Germany in 2006 was caused by a single *Pneumocystis* strain [7, 10]. These studies support the high discriminatory power of this typing technique. The availability of samples from additional outbreaks in renal transplant centers in Zurich, Switzerland (2006–2007) [5], and Nagoya, Japan (2004–2008) [8], provided an opportunity to study strain differences among patients and centers and to compare strains causing disease within Europe with those outside of Europe.

## MATERIALS AND METHODS

### Patients

The epidemiology, patient characteristics, and molecular analysis of *P. jirovecii* isolates using single-nucleotide polymorphism (SNP) or multilocus sequence typing (MLST) analysis for the outbreaks of PCP in Munich, Zurich, and Nagoya and RFLP analysis for the Munich outbreak have been previously reported [5, 7, 8, 10]. Extracted DNA that included samples from patients who were identified as being part of the outbreak as well as local nonoutbreak (control) PCP samples were provided to the National Institutes of Health (NIH) as coded samples. RFLP analysis was performed in a blinded manner, and the code from each center was not broken until the analysis from that center was complete. Samples from all 11 patients from Zurich (7 outbreak and 4 control) and all 10 from Nagoya (9 outbreak and 1 control) that had previously undergone molecular typing analysis were made available for our studies. To allow confirmation of the results for the latter, a second, recoded aliquot of the same 10 samples was provided and again analyzed in a blinded manner. Our previous analysis of samples from Munich included 13 of the 16 outbreak patients who had undergone molecular typing analysis as well as 6 control samples [10]. The guidelines of the US Department of Health and Human Services and the NIH were followed in the conduct of these studies.

### Polymerase Chain Reaction Amplification and RFLP Analysis

As a first step, the *msg* gene copy number for each DNA sample was quantified by a previously described real-time quantitative polymerase chain reaction (qPCR) assay [11]. In previous studies we have shown that for reproducibility, a minimum of approximately 1000 *msg* copies needs to be used per RFLP PCR reaction [10]. Subsequently, *msg* variable region (~1.3 kb) was amplified by a seminested PCR as previously described [10], using primers GK 472, GK 452, and GK 195. A minimum of 1000 *msg* copies per reaction was used whenever possible. The PCR was performed using HotStart Taq DNA polymerase (Qiagen), and the conditions were 15 minutes at 95°C followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 60°C, and 4 minutes (for the first round) or 2 minutes (for the second round) at 72°C, with a final extension of 10 minutes at 72°C.

RFLP analysis was performed as previously described [10]. Agarose gel electrophoresis was used to verify that amplification was successful. PCR products were purified using the QuickStep 2 PCR Purification Kit (Edge BioSystems, Gaithersburg, Maryland), digested with DraI and Hpy188I restriction enzymes for 6 hours at 37°C, and analyzed on a 1% or 2% tris-borate-ethylenediaminetetraacetic acid agarose gel following staining with SYBR green (Molecular Probes, Eugene, Oregon), as well as by Southern blotting. For the latter, the blot was hybridized with a digoxigenin-labeled DNA probe (PCR DIG Probe Synthesis Kit; Roche, Indianapolis, Indiana) of ~1.3 kb that was an equal mixture of PCR products from 4 *P. jirovecii* isolates; hybridization signal was detected using alkaline phosphatase–conjugated anti-digoxigenin antibody and CDP-Star (Roche) and a Kodak Image Station 440CF (PerkinElmer, Waltham, Massachusetts). Each run included Lambda/HindIII molecular weight markers or a single clinical sample (sample number 385) as an internal control.

The gels were analyzed using BioNumerics software version 4.01 (Applied Maths, Austin, Texas) as previously described [10]. The pattern of banding among different gels/blots was normalized using Lambda/HindIII molecular weight markers. The Dice coefficient was used to analyze the similarity of the patterns of bands with a position tolerance of 1.9% [12]. The unweighted pair group method with average linkages was used by the BioNumerics software for cluster analysis. DNA samples with banding patterns with 100% similarity (Dice coefficient = 1) were considered to be identical. Standard deviations of the branches in the cluster were obtained using the BioNumerics "Clustering/Calculate error flags" setting and represent the reliability and internal consistency of the branch.

### 26S Ribosomal RNA and Tandem Repeat Analysis

Amplification and sequencing of the 26S ribosomal RNA (rRNA) gene and tandem repeats in the intron of the *msg* expression site were performed as previously described [5, 7, 13, 14].

## RESULTS

### Analysis of the Outbreak in Zurich

Our initial goal was to determine whether RFLP analysis could demonstrate that a single strain of *Pneumocystis* was responsible for the outbreak of PCP in Zurich. Two *P. jirovecii* DNA samples from a single patient had a very low *msg* copy number and could not be amplified for RFLP analysis. Of the remaining 10 samples (10 patients) analyzed in a blinded manner, 7 had an identical pattern by RFLP analysis when digested with either DraI or Hpy1881 restriction enzymes and evaluated by either agarose gel electrophoresis or Southern blotting (Figure 1). After breaking the code, these 7 patients were confirmed to be part of the renal transplant outbreak. The remaining 3 samples had a different pattern with each enzyme and were confirmed to be from control, nonoutbreak patients.

Given that the outbreaks in both Munich and Zurich were in renal transplant patients, we sought to determine whether the same *P. jirovecii* strain was responsible for these outbreaks. Because all 14 previously studied German samples gave an identical RFLP pattern [7], we included a single representative German isolate in each gel for the RFLP analysis of the Swiss isolates. As can be seen in Figure 1, the RFLP pattern for the German isolate (lane G) was identical to that of the Swiss outbreak isolates with both restriction enzymes. Thus, the same *P. jirovecii* strain was apparently responsible for 2 separate and geographically distinct outbreaks in renal transplant patients.

In the original reports of the 2 outbreaks, MLST was performed using the same set of 4 gene targets [5, 7]. For 3 of the 4 genes, the same allele was identified in transplant patient isolates in both centers: alleles B, 7, and 1 for ITS1, mt26S, and β-tubulin, respectively. For the fourth gene, 26S rRNA, each center reported identification of a new allele, designated as allele 4 [7] and allele 5 [5]. To determine if these alleles were identical, we sequenced 1–2 isolates from each outbreak. We found that both isolates had an identical sequence that differed from the reference, allele 1, at positions 301–306: allele 1 had TACTCT in these positions, while the outbreak isolates had ACTCTT. Thus, MLST analysis provided further evidence that the 2 outbreaks were caused by a single strain. Sequencing of a limited number of subcloned *msg* genes from Swiss and German isolates provided additional support that they are the same strain (data not shown).

We were not able to undertake a formal epidemiologic investigation and thus do not know if there was any link between either patients or healthcare providers at the 2 centers.

### Analysis of the Outbreak in Nagoya

Given that 2 outbreaks in renal transplant patients in Europe were caused by a single *P. jirovecii* strain, we wanted to determine whether renal transplant patients were uniquely susceptible to this strain by examining isolates from a third outbreak that occurred in Nagoya, Japan. We obtained 10 DNA samples from this outbreak [8], but only 4 could be amplified for RFLP analysis; the remaining 6 samples had very low (<20) *msg* copies/μL. In each experiment we included representative samples from Switzerland (S) and Germany (G) to compare the RFLP pattern from different outbreaks.

Three of the 4 amplifiable DNA samples from Japan that were analyzed in a blinded manner showed an identical RFLP banding pattern when they were digested with DraI or Hpy188I (Figure 2). One sample (38 *msg* copies/μL, ~1000 *msg* copies per assay) showed a different RFLP pattern both with DraI and Hpy188I when compared with the other samples. None of the 4 samples showed an RFLP pattern that was identical to the Swiss or German pattern (Figure 2). After breaking the code, all 4 samples were found to be from renal transplant patients. To verify these results, a second aliquot of all 10 samples (recoded) was sent for RFLP analysis, again in a blinded manner. Only 3 samples could be amplified for RFLP analysis; all 3 showed an identical pattern to each other and to the 3 identical samples from the first round. Thus, the same strain of *P. jirovecii* appears to be responsible for 3 of these infections in renal transplant patients, but this strain is different from the strain that caused the 2 European outbreaks.

Figure 3 shows a dendrogram of samples from the current study (representative outbreak as well as control samples) together with samples from endemic cases included in a prior publication [10]. The cases from the European and Japanese outbreaks cluster together but separately from each other as well as from the endemic cases.

To extend our observations we examined 1 representative German sample and 2 representative Swiss samples from renal transplant patients using a second typing method based on variation in the number and sequence of tandem repeats in the *msg* expression site [14]. In addition, we were able to amplify all 10 Japanese samples for this analysis, presumably because the region being amplified was shorter than that required for RFLP analysis, which allows a higher amplification efficiency. All 13 samples had 3 tandem repeats with an identical sequence. Thus, RFLP analysis provided greater discrimination than tandem repeat analysis for distinguishing among the strains. However, although 9 Japanese samples were identical throughout the sequenced region (~250 bp), the 10th sample, which was from the nontransplant patient (and which could not be amplified for RFLP analysis), had 2 SNPs outside the tandem repeat region that differed from the other samples (Figure 4). This is consistent with disease resulting from infection with a strain different from the primary outbreak strain in Japan.

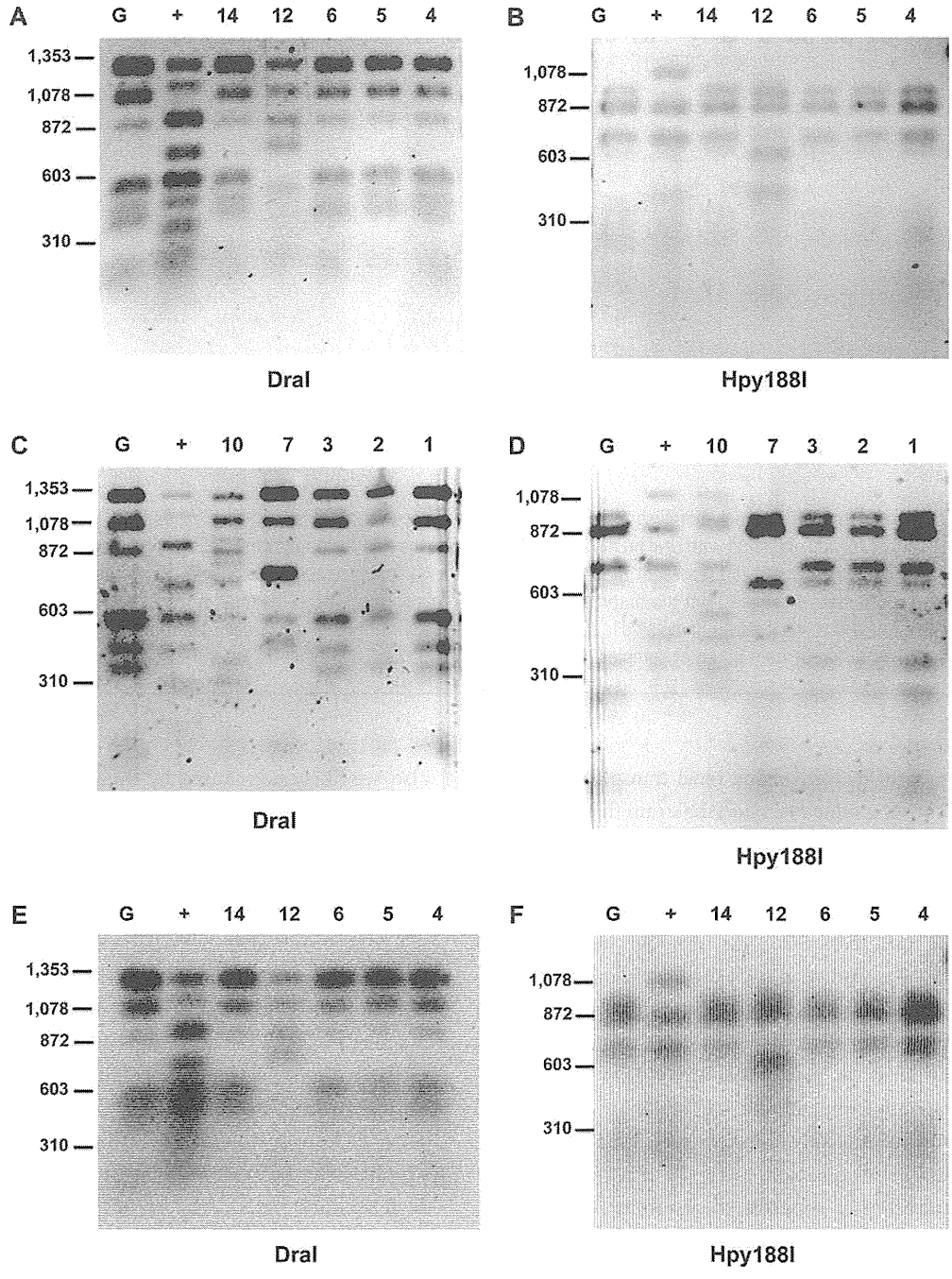


**Figure 1.** Restriction fragment length polymorphism (RFLP) analysis of *Pneumocystis* samples from Zurich, Switzerland. *A–D*, The RFLP pattern following agarose gel electrophoresis for the 10 samples that could be amplified for analysis. Labels at the top represent the individual samples. *A* and *C*, Gels were run following digestion with DraI. *B* and *D*, Gels were run following digestion with Hpy188I. Samples 1–6 and 14 are from renal transplant patients, and samples 7, 10, and 12 are from control patients. The letter G denotes a representative sample from the outbreak in Munich, Germany; + is a positive control. With both enzymes, the RFLP patterns of the renal transplant patients are identical to each other and to the German sample, whereas the control patients showed patterns that were different from each other as well as from the transplant patients. *E* and *F*, Southern blots of the gels from panels *A* and *B*, confirming the results of the gel analysis. Molecular weight markers are indicated on the left.

## DISCUSSION

RFLP analysis provides an important new tool for studying the epidemiology of *Pneumocystis* infection. In general, each case of sporadic PCP, whether in HIV-infected patients or in other immunosuppressed patients, is caused by a unique strain of *Pneumocystis* as determined based on RFLP analysis. However, in the current study, we have demonstrated that 2 geographically

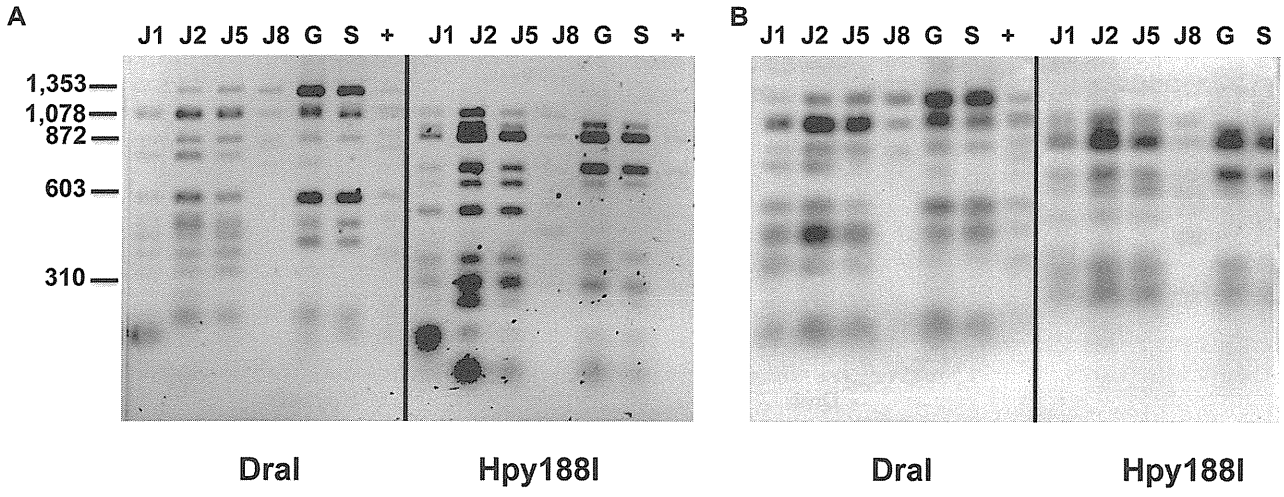


**Figure 2.** Restriction fragment length polymorphism (RFLP) analysis of *Pneumocystis* samples from Nagoya, Japan. The RFLP pattern following agarose gel electrophoresis (*A*) (following digestion with DraI on the left and Hpy188I on the right) and following Southern blotting (*B*), for the 4 samples that could be amplified for analysis. Labels at the top represent the individual samples. All 4 samples (J1, J2, J5, and J8) are from renal transplant patients. The letter G denotes a representative sample from the outbreak in Munich, Germany; the letter S denotes a representative sample from the outbreak in Zurich, Switzerland; + is a positive control. Samples J1, J2, and J5 showed a pattern identical to each other but different from the G and S samples, whereas sample J8 was different from all other samples. Molecular weight markers are indicated on the left.

distinct outbreaks of PCP involving renal transplant recipients were due to a single, unique *Pneumocystis* strain that we had not previously identified in other populations [10]. In line with our previous observations, the 9 contemporaneous nonoutbreak isolates (6 from Germany [10] and 3 from Switzerland) all showed unique RFLP patterns. Thus, although the number of nonoutbreak isolates studied at each site is small, the outbreaks caused by the European Renal Transplant (ERT) strain do not appear to simply represent infection with a predominant, locally circulating strain. Additional analyses of larger numbers of isolates, both from endemic and epidemic cases, as well as colonized or subclinically infected individuals, will more definitively answer this question.

MLST analysis further supports these results: we have reconciled the differences originally reported in 26S rRNA alleles by showing that isolates from both outbreaks had the same allele. Original sequencing data from the German outbreak confirmed this as well. Thus, isolates from both outbreaks have an identical allele in all 4 genes. We were unable to find any information that epidemiologically linked patients at the German center and the Swiss center, which are >300 km apart.

We explored the possibility that this *Pneumocystis* isolate might have a unique association with renal transplant recipients in general, but found that 4 cases in a renal transplant center in Japan had disease due to a different strain. Thus, the ERT strain is not the only strain to cause disease in renal transplant recipients. Two outbreaks recently reported from northwest England also are likely not caused by the same strain, given that they have different mt26S alleles [15]. Similarly, a 2010 outbreak reported from Australia also appears to be caused by a different strain based on MLST, although RFLP analysis of these isolates would be needed to definitively confirm this [16].

Chemoprophylaxis with trimethoprim-sulfamethoxazole or an alternative agent [17] would likely have prevented a substantial fraction of these cases. However, due in part to the low incidence of PCP in the period preceding the outbreaks, none of the patients in these outbreaks received PCP prophylaxis (although a subset of patients in 1 study received short courses of trimethoprim-sulfamethoxazole for urinary tract infection prophylaxis). Following the institution of routine prophylaxis at all 3 centers, the incidence of PCP decreased markedly [5, 7, 8]. Guidelines for the management of renal transplant patients currently incorporate routine anti-PCP prophylaxis [18].

Recently developed typing methods have led to important advances in our understanding of the epidemiology of *Pneumocystis*. Many patients appear to be infected with multiple strains of *Pneumocystis* simultaneously [14, 19]. Although it was long thought that PCP represented reactivation of latent infection that had occurred much earlier in life, possibly during infancy, recent studies have suggested that many sporadic cases in HIV-infected patients result from recently acquired infection [20].

The demonstration that outbreaks of PCP at 1 or more renal transplant centers were caused by a single strain of *Pneumocystis* provides unambiguous evidence that disease can result from

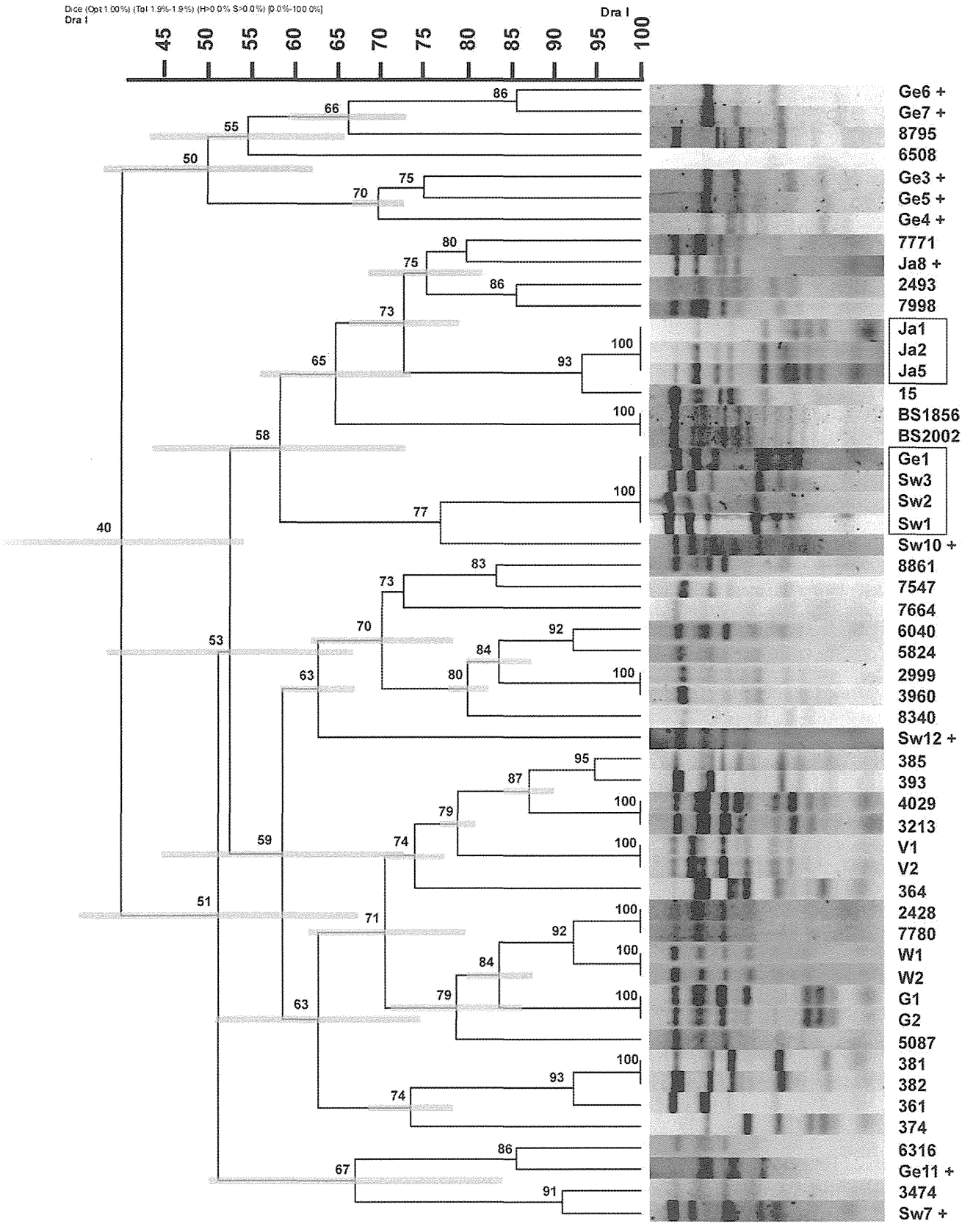


**Figure 3.** Dendrogram derived by BioNumerics software from restriction fragment length polymorphism (RFLP) analysis of 53 samples following agarose gel electrophoresis. All samples were digested with DraI. Thirty-six are samples from endemic cases of *Pneumocystis* pneumonia that were

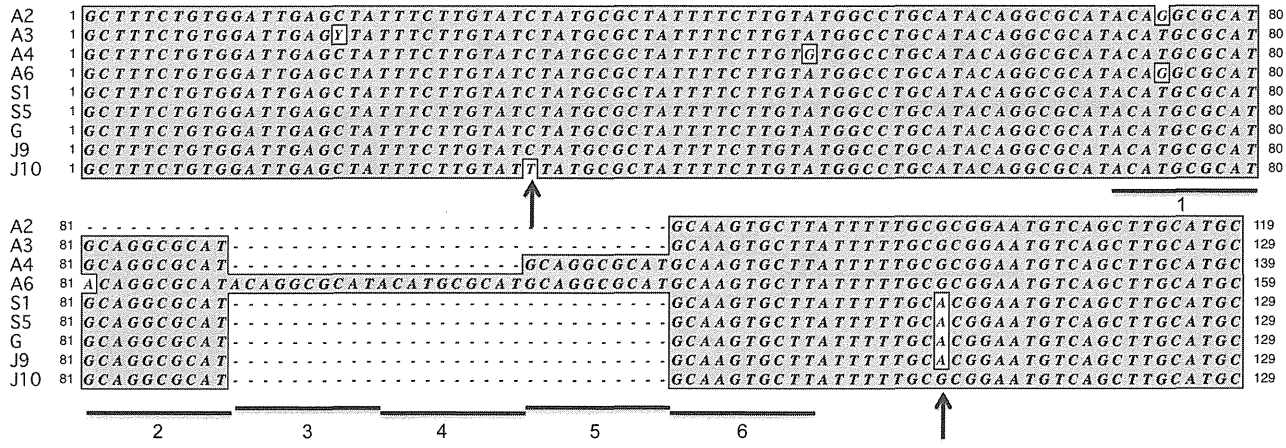


**Figure 4.** Sequence analysis and alignment of a region in the intron of the *msg* expression site that includes tandem repeats, which are underlined. Shown are results for 2 Swiss samples (S1, S5), a German sample (G), and 2 Japanese samples (J9, J10). Samples J1–J8 (not shown) were identical in sequence to sample J9. For comparison are 4 sequences with 2, 3, 4, or 6 tandem repeats (A2–A6) obtained from a single patient from the United States [14]. Although restriction fragment length polymorphism analysis identified differences between the Japanese and European isolates, in this region the sequences from all renal transplant patients from the 3 countries were identical. The isolate from a nontransplant Japanese patient (J10) differed from the transplant isolates at 2 positions indicated by the arrows.

recent infection. The alternative explanation, that all individuals were infected during infancy with the same *Pneumocystis* strain that subsequently reactivated during immunosuppression, appears highly unlikely given the tremendous strain diversity we have previously found by RFLP typing [10].

What is the mechanism of transmission of *Pneumocystis* in these outbreaks? Animal studies have demonstrated that transmission is via the respiratory route, and *Pneumocystis* organisms have been identified in the air near infected patients and animals [21–23]. *Pneumocystis* species have a strict host specificity, and thus human infection does not represent a zoonosis. To date, there is no convincing evidence for an environmental source of infection, although such a source cannot be ruled out definitively at present. For all 3 outbreaks included in this study, the initial reports were able to identify potential contacts between infected patients [5, 7, 8]. Thus, it seems likely that the organism was transmitted from other infected patients or alternatively that a healthcare worker or patient may have been persistently colonized or had a subclinical infection that allowed transmission to a more susceptible population. The fact that at least 21 cases in 2 centers in Europe (amplifiable DNA was unavailable for additional outbreak cases) were due to a single strain raises the possibility that this strain is unusually virulent for the renal transplant population, although the occurrence of outbreaks caused by apparently different strains makes this less likely. The outbreaks may result from a combination of these factors, which are not mutually exclusive.

Whether respiratory isolation of infected patients would decrease the risk of transmission is unknown, because in animals the incubation time following exposure to development of severe infection may be 2–3 months [24]. Nonetheless, given the clear demonstration that infection can be transmitted among susceptible patients, potentially susceptible patients should not be exposed to patients with active PCP to minimize the risk of such transmission. Alternatively, such patients may be provided with anti-*Pneumocystis* prophylaxis. However, given the difficulty in clearly defining risk for *Pneumocystis* pneumonia in many non-HIV populations, it does not seem feasible to provide all such patients with timely prophylaxis.

The link between the 2 European outbreaks is unidentified at present. Additional studies comparing the strains responsible for outbreaks in renal transplant patients at other centers both in

*Figure 3 continued.* included in a prior publication [10]. Seventeen samples are from the current study and include 4 representative samples from the outbreaks and the 9 control samples from Switzerland (Sw) and Germany (Ge), as well as the 4 outbreak samples from Japan (Ja). The Dice coefficient was used to calculate similarities, and unweighted pair group method with average linkages was used for cluster analysis. The position tolerance was 1.9%. The percent similarity scale is shown above the dendrogram and indicated by the numbers at the individual nodes. SDs of the branches are indicated by the gray bars. For branches without a bar, the SD was 0. The samples from the outbreaks in Europe and Japan form unique clusters that are boxed. The control samples from Europe and the outbreak sample from Japan that had a different RFLP pattern are indicated by a +. As previously reported, 6 of the paired samples with 100% identity represent samples from the same patient collected at different times [10].

Europe and elsewhere, as well as outbreaks in other susceptible populations, are needed to better define the role that the ERT strain plays in causing disease in susceptible populations. It will be important to determine if this strain has biological properties that allow it to uniquely infect renal transplant patients and, if so, to better understand what these properties are.

Outbreaks of life-threatening disease can have a potentially devastating impact on immunosuppressed populations. These outbreaks emphasize the need to develop better parameters for determining susceptibility to PCP so that prophylaxis can be continued during periods of enhanced susceptibility. These outbreaks also emphasize the importance of expanding our knowledge of biological factors that might enhance organism virulence and transmission factors that might increase the risk that susceptible patients will develop disease.

## Notes

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# Research Letter

*AIDS* 2012, **26**:000–000

## Efficacy and safety of once-daily ritonavir-boosted darunavir plus abacavir/lamivudine for treatment-naïve patients: A pilot study

Takeshi Nishijima[a,b], Kunihisa Tsukada[a], Katsuji Teruya[a], Hiroyuki Gatanaga[a,b], Yoshimi Kikuchi[a] and Shinichi Oka[a,b]

**The efficacy and safety of once-daily darunavir/ritonavir plus fixed-dose abacavir/lamivudine was examined in 22 treatment-naïve patients with HIV-1 infection. Three patients discontinued antiretroviral therapy due to mild adverse events. Among 18 patients who continued therapy, 66.7% had viral load <50 copies/ml at week 48. Only two patients experienced virologic failure with the emergence of resistant virus. This pilot study demonstrated the viral efficacy and safety of darunavir/ritonavir plus abacavir/lamivudine.**

## Introduction

Only little information is available on the efficacy and safety of the combination antiretroviral therapy (ART) of ritonavir-boosted darunavir (DRV/r) plus fixed-dose abacavir/lamivudine (ABC/3TC) [1]. DRV/r is a protease inhibitor (PI) with proven efficacy and safety as well as with a high barrier to drug resistance [2,3]. ABC/3TC is an alternative choice of nucleoside reverse transcriptase inhibitor (NRTI) backbone in the American Department of Health and Human Services (DHHS) Guidelines and is the other preferred backbone regimen for treatment-naïve patients in other international guidelines [4,5]. In this pilot study, we evaluated the efficacy and safety of DRV/r plus ABC/3TC for treatment-naïve patients in a single-center, observational cohort.

## Methods

The subjects of this retrospective study were all treatment-naïve patients with HIV infection who commenced once-daily DRV/r plus fixed dose ABC/3TC from November 2009 (when the first patient commenced such regimen at our clinic) to November 2010 at our clinic (AIDS Clinical Center, Tokyo, Japan). All patients were followed for at least 48 weeks after commencement of treatment at our facility. Baseline data, including age, sex, mode of infection, ethnicity, CD4 count, and HIV viral load, were collected from the medical charts. The Cobas TaqMan HIV-1 real-time PCR version 1.0 assay (Roche Diagnostics, NJ) was used to measure HIV-1 viral load throughout the research period. For those who discontinued either DRV/r or fixed dose ABC/3TC before reaching 48 weeks, the reasons for discontinuation were collected. All patients provided written informed consent for the data to be published. Primary outcomes were the proportion of patients with viral load <50 copies/ml at 24 and 48 weeks. Safety parameters through 48 weeks were also collected.

## Results

The study included 22 patients [1 (4.6%) female] of East Asian origin, with a median age of 34.5 years [interquartile range (IQR) 27.5-43.8]. The route of transmission was homosexual intercourse 86.3%, heterosexual 9%, and unknown in one patient. HLA was examined in 20 patients and all were HLA-B*5701-negative. Twenty one patients had HIV-1 drug-resistant testing before commencement of ART and none had resistant mutations related to NRTIs, PIs, or non-NRTIs. At baseline, the median CD4 count was 47/μl (IQR 27.5-187-8) while the HIV viral load was 5.61 log10 copies/ml (IQR 4.57-6.01 log10 copies/ml). In 3 patients, ART was either changed or discontinued during the study due to adverse events [skin rash (n = 1), vomiting (n = 1), and limb paresthesia (n = 1)] and one patient changed the regimen due to concern with drug interactions with antipsychotics before 48 weeks. The skin rash was due to darunavir, because the rash disappeared after switching darunavir to raltegravir, while continuing ABC/3TC. This patient was HLA-B*5701-negative. None presented with ABC-associated hypersensitivity or with grade 3 or 4 liver enzyme elevation.

On-treatment analysis of the 18 patients (excluding the above 4 patients who discontinued the regimen) showed 72.2% had viral load <50 copies/ml at week 24 (88.9% viral load <200 copies/ml), and 66.7% had viral load <50 copies/ml at week 48 (88.9% viral load <200 copies/ml). Intention-to-treatment analysis showed 59.0% with viral load <50 copies/ml at week 24 (77.3% viral load <200 copies/ml), and 54.6% with viral load <50 copies/ml at

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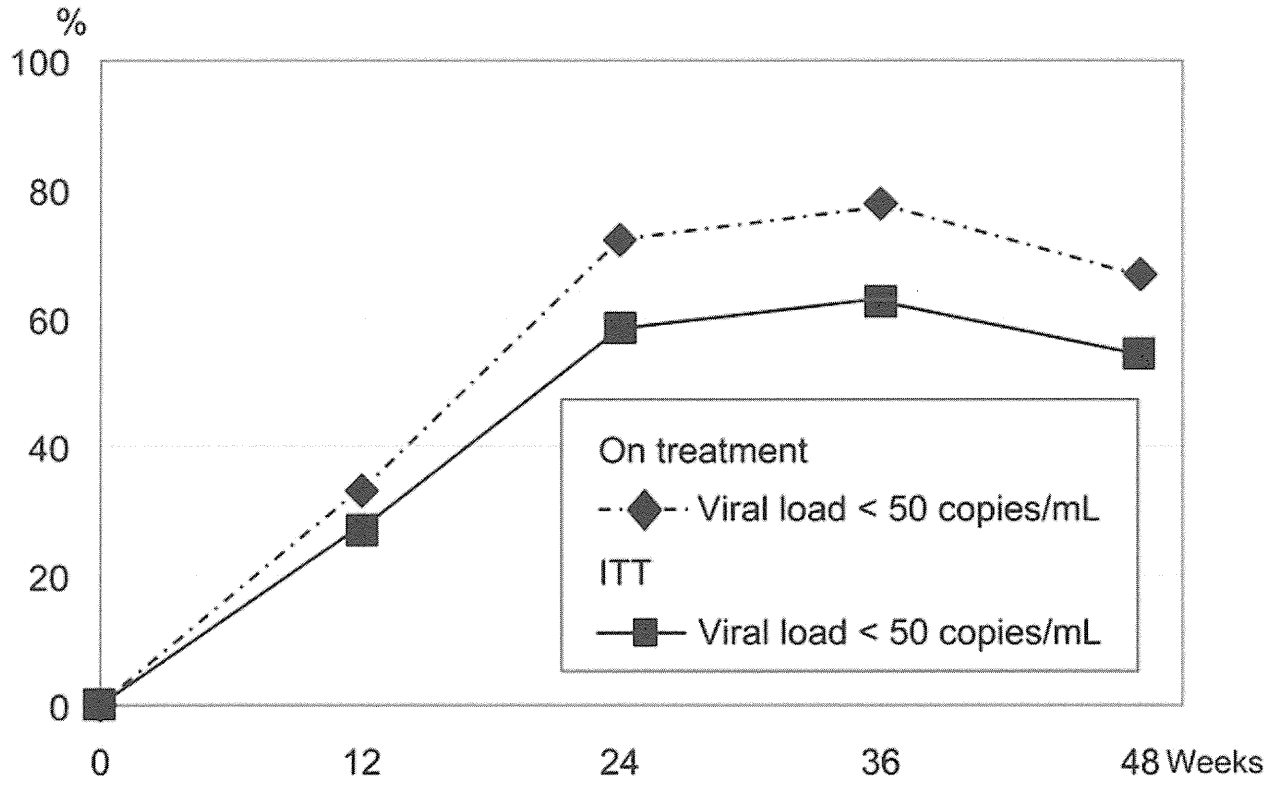


**Fig. 1. Proportions of patients with viral load <50 copies/mL at 48 weeks with on-treatment and intention-to-treat (ITT) analysis.**

week 48 (72.7% viral load <200 copies/ml) (Fig. 1). Four patients showed rebounds >200 copies/ml (<1000 copies/ml) after 24 weeks; two of them were single rebounds and considered blips. The other two patients showed two consecutive viral load >200 copies/ml, fulfilling the criteria of virological failure (11.1% at 48 weeks). The latter two patients underwent genotypic resistance test that detected in one case the reverse transcriptase mutation M184V and in the other the protease mutation M46I.

In the 12 patients with baseline viral load >100,000 copies/ml, on-treatment analysis showed viral load of < 200 copies/ml at 24 weeks in 10 (83.3%) patients, and <50 copies/ml at both 24 and 48 weeks in 7 (58.3%). In comparison, all 6 patients with baseline viral load <100,000 copies/ml showed suppression of the load to < 50 copies/ml at both 24 and 48 weeks. The median increment in CD4 count at 48 weeks was 187/μl (IQR 82.5-264.5/μl).

## Discussion

To our knowledge, this is the first published report on the efficacy and safety of the combination of once-daily DRV/r plus fixed dose ABC/3TC in treatment-naïve patients. This combination ART resulted in viral suppression although the baseline viral load was >100,000 copies/ml in 66.6% of the patients. Only 13.6% discontinued this regimen due to adverse events before 48 weeks and none of the adverse events was serious. Considering that most patients in this cohort were at advanced stage of HIV infection with low median baseline CD4 count of 47/μl, we conclude that DRV/r plus ABC/3TC is a safe and efficacious combination ART.

The DHHS guidelines for the treatment of HIV infection in the U.S. list ABC/3TC as alternative NRTIs since abacavir can potentially cause serious hypersensitivity reaction in 5-8% of the patients and its viral efficacy in patients with baseline viral load of >100,000 copies/mL is inferior to fixed-dose tenofovir/emtricitabine (TDF/FTC) when used with efavirenz or ritonavir-boosted atazanavir as a key drug [4,6]. However, the incidence of ABC-related hypersensitivity is low among HLA-B*5701-negative population, such as the Japanese [7,8]. Moreover, HEAT study demonstrated that the viral efficacy of ABC/3TC was not inferior to that of TDF/FTC when used with lopinavir/ritonavir for treatment-naive patients [9]. Taking this background into account, once-daily DRV/r plus ABC/3TC could be a good alternative, especially in patients with low prevalence of HLA-B*5701 who cannot tolerate tenofovir due to its nephrotoxicity [10].

In conclusion, this single-center pilot study demonstrated the viral efficacy and safety of once-daily DRV/r plus ABC/3TC in treatment-naïve patients with HIV-1 infection. This regimen could be a suitable alternative to DRV/r plus tenofovir/emtricitabine or other first line regimens. Nevertheless, the number of patients in this cohort is too small to allow firm conclusions and further studies of larger samples, ideally a clinical trial that compares the viral efficacy of TDF/FTC to ABC/3TC with once-daily DRV/r, are needed to elucidate this issue.

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### Conflict of Interest and Source of Funding

Author contributions: All of the authors contributed to the conception and design of the study and/or the analyses and interpretation of the data. The manuscript was drafted by T.N., H.G.and S.O. and was critically reviewed and subsequently approved by all authors. The authors declare no conflict of interest.

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