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).

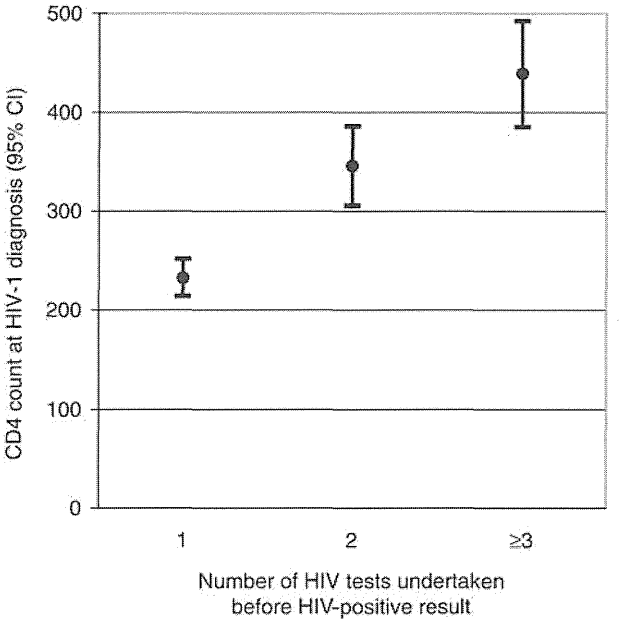

Figure 2 **Relationship between CD4 count at diagnosis and number of HIV tests undertaken prior to HIV diagnosis. CI = confidence interval**

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).

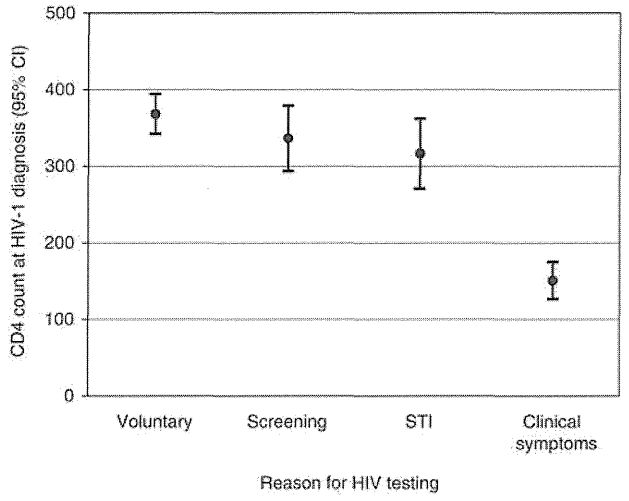

Figure 1 **Relationship between CD4 count at diagnosis and reason for HIV testing. CI = confidence interval; STI = sexually transmitted infections**

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				P value
		CD4 < 200 (n = 275)		CD4 ≧ 200 (n = 379)		
		No	%	No	%	
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.

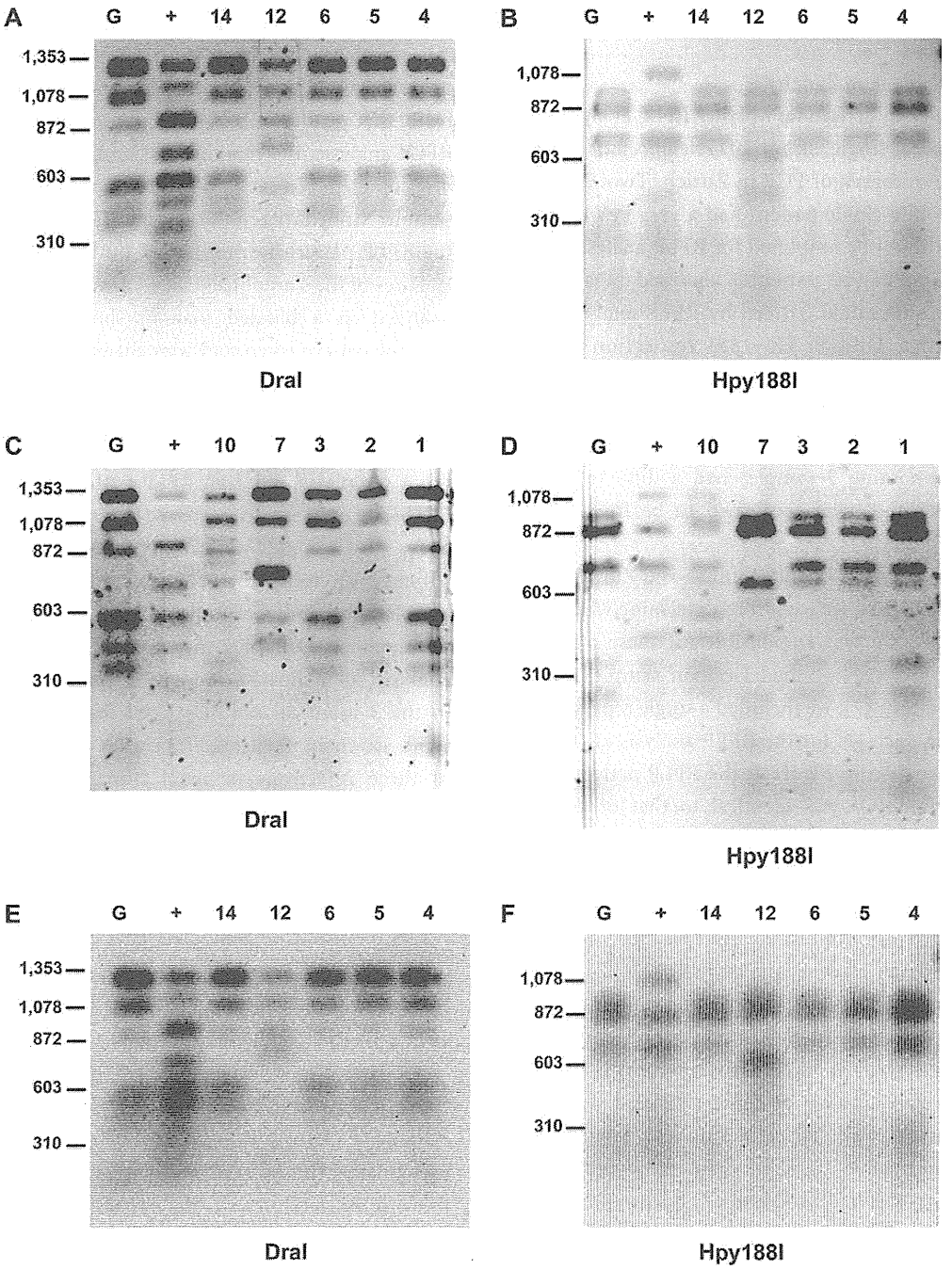

Figure 1. Restriction fragment length polymorphism (RFLP) analysis of *Pneumocystis* samples from Zurich, Switzerland. *A–D*, The RFLP pattern following agarose gel electrophoresis for the 10 samples that could be amplified for analysis. Labels at the top represent the individual samples. *A* and *C*, 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

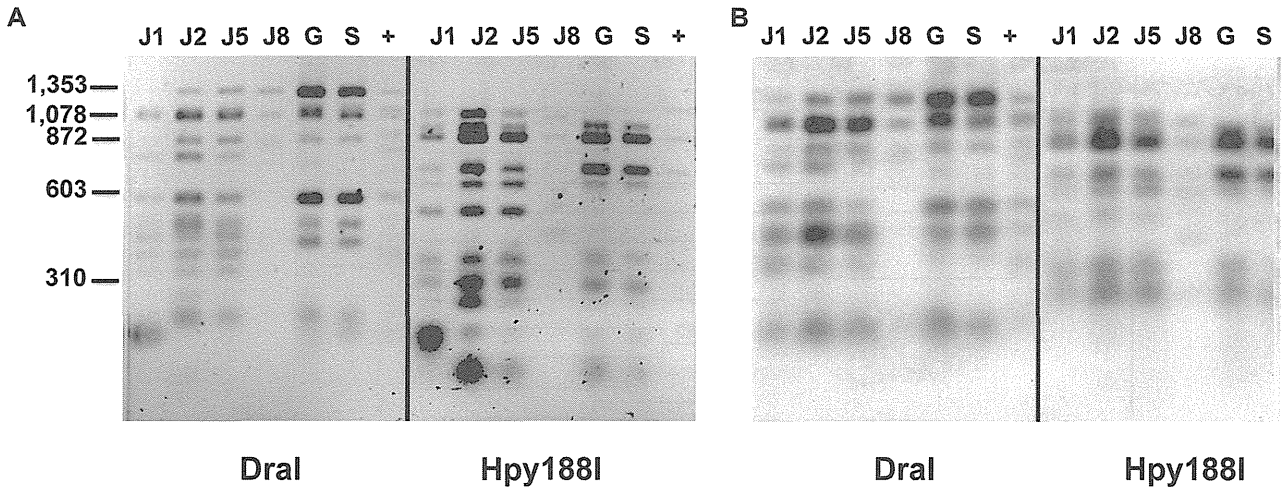

Figure 2. Restriction fragment length polymorphism (RFLP) analysis of *Pneumocystis* samples from Nagoya, Japan. The RFLP pattern following agarose gel electrophoresis (*A*) (following digestion with DraI on the left and Hpy188I on the right) and following Southern blotting (*B*), for the 4 samples that 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

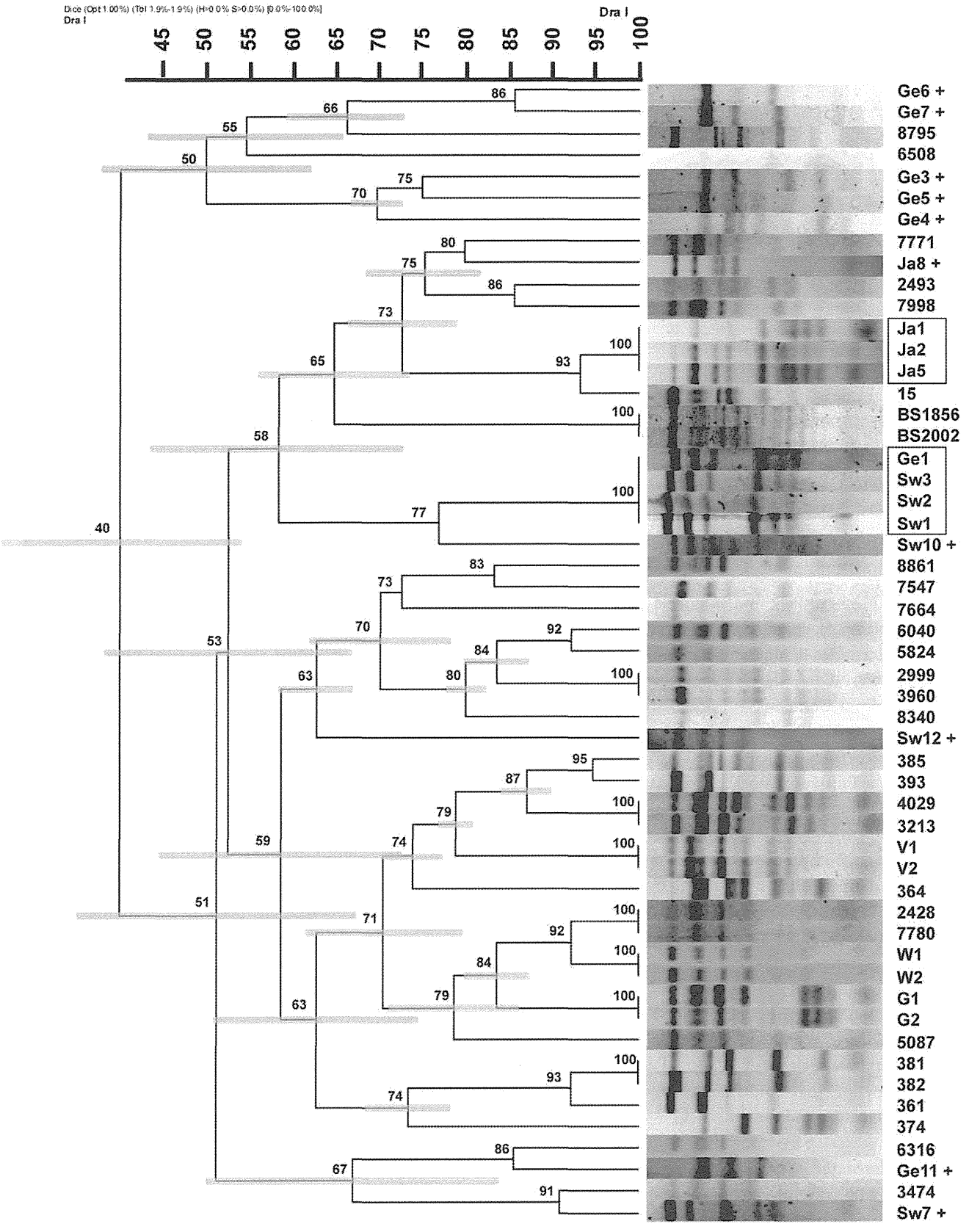

Figure 3. Dendrogram derived by BioNumerics software from restriction fragment length polymorphism (RFLP) analysis of 53 samples following agarose gel electrophoresis. All samples were digested with DraI. Thirty-six are samples from endemic cases of *Pneumocystis* pneumonia that were

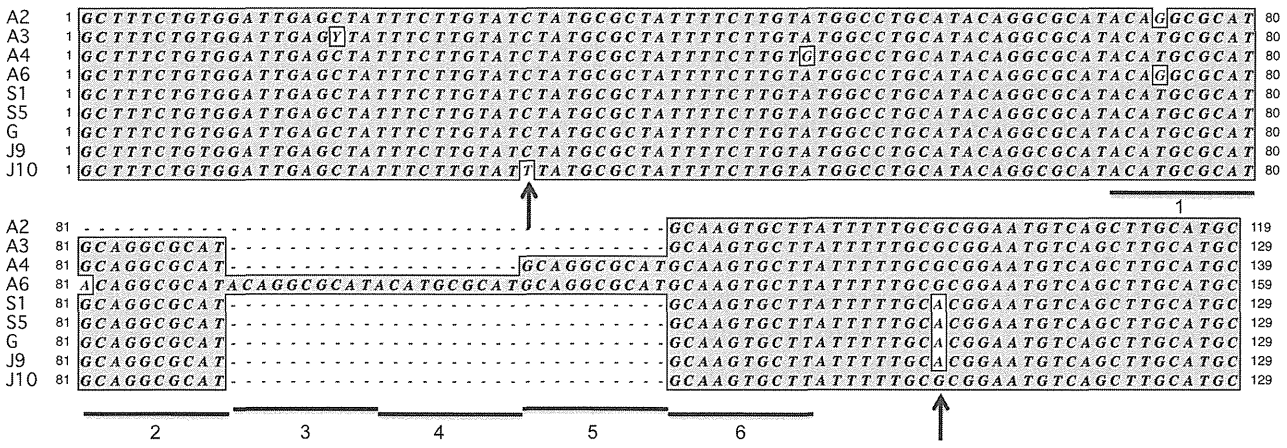

Figure 4. Sequence analysis and alignment of a region in the intron of the *msg* expression site that includes tandem repeats, which are underlined. Shown are results for 2 Swiss samples (S1, S5), a German sample (G), and 2 Japanese samples (J9, J10). Samples J1–J8 (not shown) were identical in sequence 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).

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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

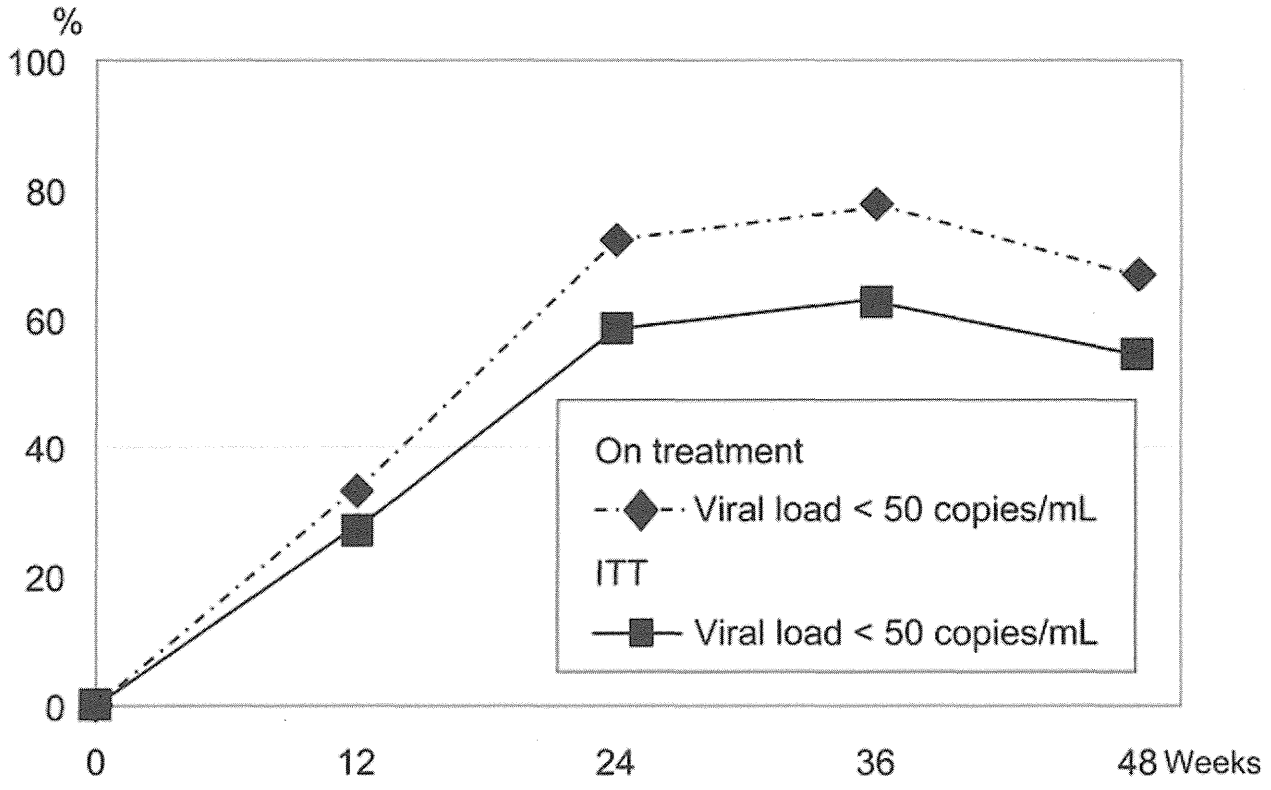

Fig. 1. Proportions of patients with viral load <50 copies/mL at 48 weeks with on-treatment and intention-to-treat (ITT) analysis.

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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Risk Factors for Intestinal Invasive Amebiasis in Japan, 2003–2009

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Learning Objectives

Upon completion of this activity, participants will be able to:

- Describe yearly change in prevalence of amebic colitis, based on a Japanese study of persons who underwent endoscopy
- Describe independent risk factors for amebic colitis, based on a Japanese study of persons who underwent endoscopy
- Compare risk factors for amebic colitis between HIV-positive and -negative patients, based on a study of Japanese persons who underwent endoscopy.

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We determined yearly change in prevalence and risk factors for amebic colitis caused by intestinal invasive amebiasis among persons who underwent endoscopy and assessed differences between HIV-positive and HIV-negative persons in Japan. A total of 10,930 patients were selected for analysis, of whom 54 had amebic colitis. Prevalence was in 2009 (0.88%, 12/1360) compared with 2003 (0.16%, 3/1904). Male sex (odds ratio [OR] 8.39, 95% CI 1.99–35.40), age <50 years (OR 4.73, 95% CI 2.43–9.20), history of syphilis (OR 2.90, 95% CI 1.40–5.99), and HIV infection (OR 15.85, 95% CI 7.93–31.70) were independent risk factors. No differences in risk factors were identified between HIV-positive and HIV-negative patients. Contact with commercial sex workers was a new risk factor among HIV-negative patients. Homosexual intercourse, rather than immunosuppressed status, appears to be a risk factor among HIV-positive patients.

Amebiasis is caused by the protozoan *Entamoeba histolytica*. Each year, this disease develops worldwide in ≈40–50 million persons and causes 40,000 deaths (*1*,*2*). There are several amebic species of protozoans; *E. histolytica* is a pathogenic ameba that can cause invasive intestinal and extraintestinal disease (*1*–*3*). The most

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frequent manifestations of invasive amebiasis are colitis and liver abscess (*1,3–5*). Many persons with *E. histolytica* infection are asymptomatic, but invasive disease develops in 4%–10% of persons with symptomatic *E. histolytica* infections over a 1-year period (*1,6–8*).

Areas with high incidences of amebic infection include India, Africa, Mexico, and parts of Central and South America (*1,2,9*). In countries with low incidence, such as Taiwan, South Korea, and Australia, invasive amebiasis is uncommon, but reports have indicated that amebiasis is an emerging parasitic infection, particularly among men who have sex with men (MSM) (*10–13*). Although epidemics of amebiais have not occurred in Japan, reports from 2001 indicate that invasive amebiasis is common in middle-age men, MSM and HIV-infected patients (*8,14,15*). In Japan, the prevalence of amebiasis has been increasing according to data from the National Epidemiologic Surveillance of Infectious Diseases (*16*). However, the reasons amebiasis is increasing and the actual prevalence of amebic colitis in daily clinical practice have not been fully clarified. Moreover, some studies in Japan have examined risk factors, but most of these studies have reported case series or case reports without control patients (*14,15,17,18*).

Several studies have indicated that HIV infection is a risk for invasive amebiasis, but no consensus has been reached on this issue (*10–12,19*). Furthermore, some researchers have suggested that severe invasive amebiasis may develop in HIV-positive patients (*20–22*). Susceptibility and clinical factors differ between HIV-positive and -negative patients because of differences in immune status. However, the effect of HIV infection on these risk factors for invasive intestinal amebiasis remains unclear.

To address these issues, we clarified annual changes in prevalence and risk factors for amebic colitis among persons who had undergone endoscopy. These factors were then compared between HIV-positive and HIV-negative patients.

Methods

Study Design

We retrospectively reviewed endoscopy records for 14,923 consecutive patients who underwent colonoscopy at the National Center for Global Health and Medicine (NCGM) (Tokyo, Japan) during 2003–2009. Indications for endoscopy included screening for fecal occult blood test; colorectal cancer; anemia; examinations for symptoms such as constipation, loose stool, diarrhea, hematochezia, and abdominal pain; or therapies for colorectal adenoma, early colorectal cancer, and diverticular bleeding.

We excluded patients who had not been tested for HIV infection, syphilis, or hepatitis B virus (HBV) infection. Patients who underwent endoscopic observation only of the anorectal area and those <15 years of age were excluded. A total of 10,930 patients were selected for analysis.

NCGM has 900 beds and is the largest referral center for HIV/AIDS in Japan. Written informed consent for procedures was obtained from all patients before endoscopy and biopsy. The study protocol was approved by the ethics committee of NCGM.

Sexually Transmitted Diseases

We collected laboratory data for sexually transmitted diseases (STDs), such as HIV infection, syphilis, and HBV infection, before endoscopy. Histories of HBV infection and syphilis were defined as presence of antibody against as hepatitis B surface antigen and positive results in a *Treponema pallidum* hemagglutination test, respectively. In Japan, because only health care workers are vaccinated against hepatitis B, positive results for antibody against hepatitis B surface antigen were attributable to vaccination in few cases.

For HIV-positive patients, we determined CD4 cell counts within 1 week of endoscopy. We categorized CD4 cell counts into 4 groups: >300 cells/μL, 201–300 cells/μL, 101–200 cells/μL, and <100 cells/μL Routes of infection were determined by medical staff who questioned each patient at their first visit to the hospital. Routes were classified into 6 categories: homosexual, bisexual, heterosexual, drug use, untreated blood products, and unknown. We defined sexual preference into 2 categories: MSM and heterosexual. Patients who were not homosexual or bisexual were regarded as heterosexual.

Diagnosis of Amebic Colitis Caused by *E. histolytica* Infection

We performed a biopsy and aspirated intestinal fluid from lesions endoscopically when abnormal findings were seen by endoscopy. Amebic colitis was suspected on the basis of endoscopic findings, such as erythema, edematous mucosa, erosions, white exudates, and ulcers (Figure 1) (*22,23*). Negative results for intestinal fluid cultures for bacterial species or acid-fast bacillus were confirmed. Amebic colitis was defined as amebic trophozoites in biopsy specimens stained with both hematoxylin and eosin (Figure 2, panel A) and periodic acid–Schiff (Figure 2, panel B), negative intestinal fluid cultures for other species, negative histologic features for other colonic diseases, and a positive clinical response to metronidazole. Trophozoites showed characteristic hemophagocytosis, which is specific for *E. histolytica* infection (Figure 2, panel A).

Routes of Amebic Infection

When amebic colitis was diagnosed, the physician asked the patient directly for information about the route

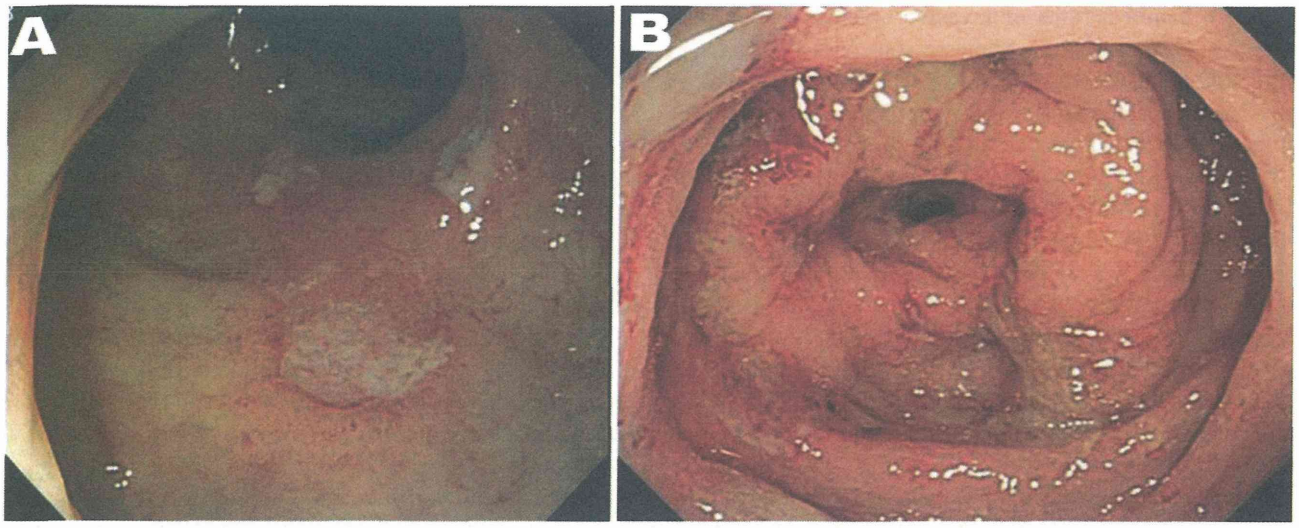

Figure 1. Endoscopic features of amebic colitis, Japan, 2003–2009. A) Colonoscopy showing ulcers in the rectum. B) Colonoscopy showing multiple erosions with exudates surrounded by edematous mucosa in the sigmoid colon.

of amebic infection. The physician confirmed whether the patient had traveled in tropical areas, resided in a facility for the intellectually disabled, was a male or female commercial sex worker (CSW), or had contact with a CSW or MSM. For travel exposure, history of overseas travel in the past year was elicited. Patients to whom none of the above applied were treated as unknown.

Statistical Analysis

We assessed changes in annual prevalence by using the χ^2 test for linear trends. We summarized descriptive data for patients with and without amebic colitis. To determine risk factors for amebic colitis, we estimated the odds ratio (OR) between amebic colitis and clinical factors including age, sex, sexual preference, and history of STDs. We divided patients into 2 age groups, $\geq$50 years and <50 years. We used a multiple logistic regression model with factors that showed $p<0.2$ by univariate analysis. A final model was then developed by backward selection of factors that showed $p<0.05$. The adequacy of this model was evaluated by using the Hosmer-Lemeshow goodness-of-fit test and a receiver operating characteristic area under the curve.

We also conducted subgroup analysis concerning HIV infection. We investigated interactions between the effect of HIV infection and risk factors for amebic colitis. In HIV-positive patients, the relationship between prevalence of amebic colitis and CD4 cell counts in 4 categories was evaluated by using the χ^2 test for linear trends. All statistical

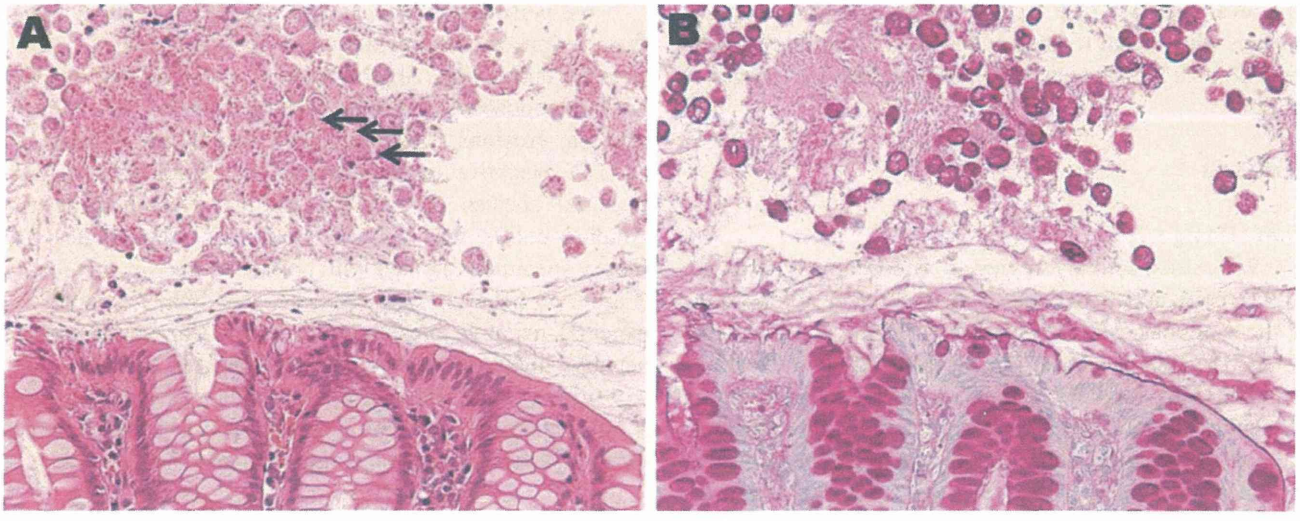

Figure 2. Histologic analysis of amebic colitis, Japan, 2003–2009. A) Trophozoites of *Entamoeba histolytica* ingesting erythrocytes (arrows) (hematoxylin and eosin stain). B) Numerous amebic trophozoites on the mucosal surface (periodic acid–Schiff stain). Original magnification ×200.

analyses were performed by using Stata version 10 software (StataCorp LP, College Station, TX, USA).

Results

Annual Prevalence of Amebic Colitis

Among 10,930 patients, 54 (0.5%) showed development of amebic colitis. Prevalence was 0.16% in 2003 but tended to increase over time ($p<0.01$ by trend test) (Figure 3). Prevalence was 5.6-fold higher in 2009 than in 2003.

Patient Characteristics

HIV-infected patients constituted 248 (2.3%) of 10,930 patients, and they had a median age of 43 years (interquartile range [IQR] 35–55 years) (Table 1). These HIV-infected patients were predominantly male (91.5%, 227/248). Median CD4 cell count was 230 cells/μL (IQR 89.5–401 cells/μL). Routes of HIV infection included homosexual (58.9%, 146/248), heterosexual (12.5%, 31/248), bisexual (10.5%, 26/248), unknown (12.1%, 30/248), untreated blood products (6.0%, 15/248), and drug use (0%).

Patients with a history of HBV infection constituted 184 (1.7%) of 10,390 patients, and they had a median age of 61 years (IQR 47.5–69 years). These patients were also predominantly male (69.0%, 127/184).

Patients with a history of syphilis constituted 266 (2.4%) of 10,390 patients, and they had a median age of 64 years (IQR 48–74 years). These patients were also predominantly male (76.3%, 203/266).

Risk Factors for Amebic Colitis

Risk factors for amebic colitis were age <50 years (OR 11.4, 95% CI 6.1–22.4), male sex (OR 18.5, 95% CI 4.9–156.7), HIV infection (OR 66.2, 95% CI 36.6–120.7), history of HBV infection (OR 9.0, 95% CI 3.4–20.4) and history of syphilis (OR 19.6, 95% CI 10.2–36.2) (Table 1). Multivariate analysis showed that age <50 years (OR 4.73, 95% CI 2.43–9.20, $p<0.001$), male sex (OR 8.39, 95% CI 1.99–35.40, $p<0.01$), HIV infection (OR 15.85, 95% CI 7.93–31.70, $p<0.01$), and history of syphilis (OR 2.90, 95% CI 1.40–5.99, $p<0.01$) were independent risk factors for amebic colitis. This logistic regression model was evaluated by using the Hosmer-Lemeshow test ($p = 0.44$) and receiver operating characteristic area under the curve (0.90).

Comparison of HIV-Positive and HIV-Negative Patients

Annual Prevalence of Amebic Colitis

Numbers of HIV-positive and HIV-negative patients have been increased annually during 2003–2009 in Japan (Figure 4). Among HIV-positive patients, the prevalence in 2009 increased by 2.1-fold over that in 2003 (Figure 4, panel A). Among HIV-negative patients, the prevalence in 2009 increased by 7.1-fold over that in 2003 (Figure 4, panel B).

Risk Factors for Amebic Colitis

Among HIV-positive patients, age <50 years, history of syphilis, and MSM status were risk factors for amebic colitis (Table 2). Immunosuppressed status, such as CD4 cell count <100 cells/μL, was not associated with amebic colitis among HIV-positive patients (Table 2). As CD4 cell counts decreased, the prevalence of amebic decreased (OR 0.3; $p = 0.08$ by trend test).

Among HIV-negative patients, age <50 years, male sex, history of HBV infection, and history of syphilis were risk factors for amebic colitis (Table 2). No interactions were apparent between HIV infection and risk factors, such as age, sex, history of syphilis, and history of HBV infection.

Route of Amebic Infection

Among HIV-positive patients, all 31 patients with amebic infection were male (Table 3). Of these patients, 28 were MSM and 2 were male CSWs. No patients reported contact with CSWs. The route of infection was unknown for 3 patients.

Among HIV-negative patients, 2 patients were female and 21 were male. Both female patients were CSWs. Of the 21 male patients, 8 had had sexual contact with a female CSW and 7 patients were MSM (2 bisexual and 5 homosexual). The route of infection was unknown for 6 patients.

Discussion

Endoscopic examination combined with biopsy sample collection is a valuable method for confirming suspected amebic colitis, which is often misdiagnosed as inflammatory bowel disease or other forms of infectious colitis caused by the similarity of associated gastrointestinal symptoms (e.g.,

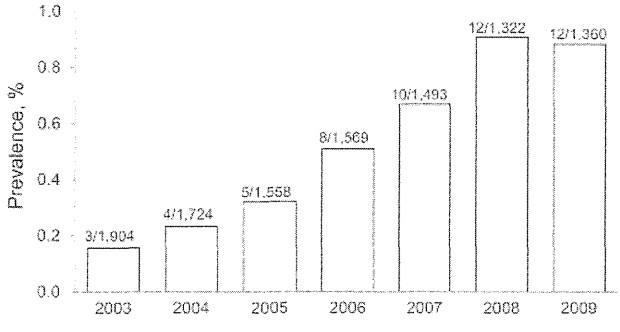

Figure 3. Annual prevalence of amebic colitis, Japan, 2003–2009. Values above bars are no. positive/no. tested.

Table 1. Characteristics and risk factors for 10,930 patients with amebic colitis, Japan, 2003–2009*

Characteristic	All, n = 10,930	Amebic colitis, n = 54	No amebic colitis, n = 10,876	Odds ratio (95% CI)
Median age (IQR)	64 (54–73)	41 (36–52)	65 (54–73)	NA
Age, y				
≥50	8,875 (81.2)	15 (27.7)	8,860 (81.5)	Referent
<50	2,055 (18.8)	39 (72.2)	2,016 (18.5)	11.4 (6.1–22.4)
Sex				
F	4,522 (41.4)	2 (3.7)	4,520 (39.1)	Referent
M	6,408 (58.6)	52 (96.3)	6,356 (58.4)	18.5 (4.9–156.7)
HIV infection				
Negative	10,682 (97.7)	23 (42.5)	10,659 (98.0)	Referent
Positive	248 (2.3)	31 (57.4)	217 (2.0)	66.2 (36.6–120.7)
HBV infection				
Negative	10,746 (98.3)	47 (87.0)	10,699 (84.0)	Referent
Positive	184 (1.7)	7 (13.0)	177 (1.6)	9.0 (3.4–20.4)
Syphilis				
Negative	10,664 (97.6)	37 (68.5)	10,627 (97.7)	Referent
Positive	266 (2.4)	17 (31.5)	249 (2.3)	19.6 (10.2–36.2)

*Values are no. (%) except as indicated. IQR, interquartile range; NA, not applicable; HBV, hepatitis B virus. p values for all comparisons were <0.05, by Mann-Whitney U test.

diarrhea, hematochezia, and abdominal pain) (*14,22,23*). However, only a few studies have included patients who had undergone endoscopy (*17,22,23*). In the present study, we performed a large number of endoscopic examinations. The prevalence of patients with amebic colitis was 0.5% (54/10,930) in this 7-year study. This prevalence was far lower than results from serum prevalence studies, which have shown prevalence in children of 8.4% in Mexico (*24*) and 4.2% in Bangladesh (*25*). However, the annual prevalence of the disease showed a tendency to increase to nearly 1% in recent years, and we assume the prevalence will continue to increase in the future.

In the past, amebic infection in Japan was reportedly caused by overseas travel to countries where epidemics occurred or where amebic infection was found in residents of facilities for the intellectually disabled (*16,26*). However, patients with these characteristics were not observed in this study. Multivariate analysis indicated that risk factors for amebic colitis in this study were male sex, age <50 years, and histories of syphilis and HIV infection.

The reason male sex was a risk factor might be related to specific sexual preference (*8,10–15*) because 52 male patients with amebic colitis often had contact with MSM (n = 35) or female CSWs (n = 8). In this study, MSM constituted 90% of men (OR 4.7 for patients with HIV infection), which is consistent with results of previous reports (*8,10–15*). However, HIV-negative male patients included heterosexual patients, and ≈35% of them had had contact with CSWs. We included CSWs as routes of infection for amebiasis because amebiasis among female CSWs has been reported in Japan (*27*). Therefore, new infection routes other than MSM, which has been considered a risk because of a diversity of sexual activities, should be considered.

Consistent with results of past reports (*8,14,15*), younger age was a risk factor. One possibility is that younger age represents a risk factor because younger persons are more sexually active, although this was not clarified in the present study.

Histories of syphilis or HIV infection have been noted as risk factors in previous case series (*7,15,28*). The present study included many patients with HIV infection or history of syphilis, which supports the hypothesis that these factors increase the risk for amebic colitis.

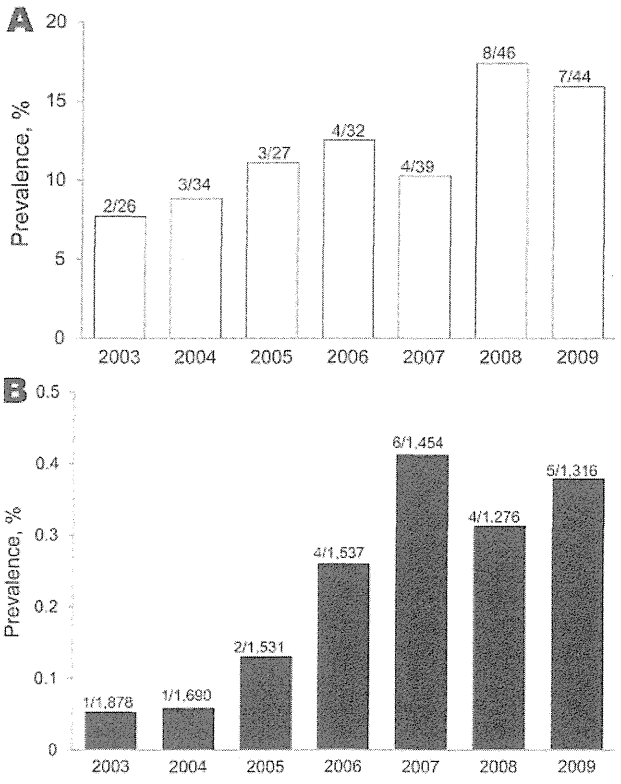

Figure 4. Annual prevalence of amebic colitis in persons with or without HIV infection, Japan, 2003–2009. A) HIV-positive patients. B) HIV-negative patients. Values above bars are no. positive/no. tested.

Table 2. Risk factors for amebic colitis among HIV-positive and -negative patients, Japan, 2003–2009*

Risk factor	HIV-positive patients: Amebic colitis, n = 31	HIV-positive patients: No amebic colitis, n = 217	HIV-positive patients: OR (95% CI)	HIV-positive patients: p value	HIV-negative patients: Amebic colitis, n = 23	HIV-negative patients: No amebic colitis, n = 10,659	HIV-negative patients: OR (95% CI)	HIV-negative patients: p value	p value for interaction
Age, y									
≥50	6	83	Referent		9	8,777	Referent		
<50	25	134	2.6 (1.0–8.0)	0.04	14	1,882	7.3 (2.9–19.0)	<0.01	0.11
Sex									
F	0	21	Referent		2	4499	Referent		
M	31	196	4.6† (0.8–∞)	0.11†	21	6,160	7.7 (1.9–67.5)	<0.01	0.56
HBV infection									
Negative	26	189	Referent		21	10,510	Referent		
Positive	5	28	1.3 (0.4–3.8)	0.62	2	149	6.7 (0.8–27.9)	<0.01	0.07
Syphilis									
Negative	16	163	Referent		21	10,464	Referent		
Positive	15	54	2.8 (1.2–6.5)	<0.01	2	195	5.1 (0.6–21.1)	0.01	0.48
Sexual preference									
Heterosexual	3	73	Referent		ND	ND	ND	ND	ND
MSM	28	144	4.7 (1.4–25.0)	<0.01	ND	ND	ND	ND	ND
CD4 cell count/μL									
>300	14	82	Referent	ND	ND	ND	ND	ND	ND
201–300	5	31	0.9 (0.3–2.8)	ND	ND	ND	ND	ND	ND
100–200	9	39	1.35 (0.5–3.4)	ND	ND	ND	ND	ND	ND
<100	3	65	0.3 (0.07–1.0)	0.15	ND	ND	ND	ND	ND

*OR, odds ratio; HBV, hepatitis B virus; ND, no applicable data; MSM, men who have sex with men.
†Analysis by using exact logistic regression model because number in cell was 0.

Among STDs, HIV infection showed the highest risk ratio, a ≈16-fold increase. HIV infection has been identified as a risk factor for invasive amebiasis in many studies (*10–12,21*), although many details of this risk remain unclear (*19,29*).

We presumed that compromised immune function increased the susceptibility of patients to invasive diseases. However, no relationship was seen between low CD4 cell counts and development of amebic colitis. Under existing conditions, the reason for HIV infection representing a risk factor for amebic colitis is considered the preference for oral–anal sex as a common risk factor for both infectious conditions.

We compared prevalence and risk factors between amebic colitis patients with and without HIV infection. An incidence of 0.1% (4/5,193) has been reported in studies of HIV-negative patients with positive results for occult blood in feces (*17*), and our results were similar. However, annual prevalence increased in 2009 (0.38%, 5/1,316) compared with 2003 (0.05%, 1/1,878), and the rate of increase was higher than that for HIV-positive patients. This result calls for careful attention in hospitals in which patients with HIV infection are not commonly encountered. In terms of risk factors, ORs for age, sex, and history of HBV infection or syphilis in our study did not vary according to HIV infection status.

Table 3. Route of amebic infection for 54 persons, Japan, 2003–2009*

Route	HIV positive, no. (%), n = 31	HIV negative, no. (%), n = 23
Travelers from tropical areas	0	0
Residents of facilities for intellectually disabled	0	0
MSM, male CSW	28, 2 (90.3)	7 (30.4)
Female CSW	0	2 (8.7)
Contact with female CSW	0	8 (34.8)
Unknown	3 (9.7)	6 (26.1)

*MSM, men who have sex with men; CSW, commercial sex worker.

Some limitations need to be considered in this study. First, Japan has not had epidemics of amebiasis, and data in this study were obtained from a metropolitan area. In addition, our hospital treats the largest number of patients with HIV infection in Japan. Second, selection bias was present because participants were patients who had undergone endoscopic examinations, which are highly likely to be performed for healthy patients. In addition, patients suspected before examination of having amebiasis might have been more likely to be actively included in the study. Third, the number of patients with amebic colitis was small; thus, the statistical power of the study might have been low. Fourth, a retrospective design was used for this investigation. With regard to HBV infection or history of syphilis, judgments had to be made for using results of serologic testing in some cases. In addition, determination of sexual preferences and overseas travel had to be based on the self-reports of patients.

In recent years, infectious diseases caused by *E. histolytica* and HIV have been increasing in Japan (*15,16,30*). HIV infection is a particularly serious problem because its incidence is consistently increasing in Japan while decreasing in western countries (*30,31*).

Numbers of patients with both infectious diseases studied are predicted to increase because little is known about measures to prevent infection in association with