2010/9789241599764_eng.pdf). It is expected that the use of TDF will spread rapidly in Asia and Africa in the near future, where patients are more likely to be of small body weight. Thus, at this stage, it is important to establish the relationship between TDF-associated renal dysfunction and body weight. A small body weight is considered a risk factor for TDF-associated renal dysfunction, in addition to old age, high baseline serum creatinine level, low CD4 count, concurrent use of ritonavir-boosted protease inhibitor, and concurrent use of nephrotoxic drugs [4,17,19–21]. To our knowledge, there is almost no report that primarily analyzed the influence of body weight on TDF-associated renal dysfunction. Since Japanese are generally of smaller stature and have a lower median body weight than Whites and African Americans, who mostly comprise the cohorts of studies published to date, it is important to investigate the impact of TDF-associated renal dysfunction in Japanese patients.

Based on the above background, the present study was designed to determine the incidence of TDF-associated renal dysfunction in Japanese patients and analyze the impact of small body weight on TDF-associated renal dysfunction.

Methods

Ethics Statement

This study was approved by the Human Research Ethics Committee of National Center for Global Health and Medicine (Text S1). All patients included in this study provided a written informed consent for their clinical and laboratory data to be used and be published for research purposes. This study has been conducted according to the principles expressed in the Declaration of Helsinki.

Study Design and Settings

We performed a single-center, retrospective cohort study of HIV-infected Japanese patients using medical records at the National Center for Global Health and Medicine, Tokyo, Japan. Our facility is one of the largest clinics for patients with HIV infection in Japan with more than 2,700 registered patients.

Study Subjects

The study population were patients >17 years of age who commenced treatment with standard 300 mg/day of TDF-containing antiretroviral regimen at our clinic between January 1, 2002 to March 31, 2009. Both treatment-naive and patients with experience in antiretroviral treatment but not TDF, with an estimated glomerular filtration rate (eGFR) of >60 ml/min/1.73 m^2 were enrolled. Patients were followed up until September 31, 2009. Patients were excluded if their follow-up period at our facility was less than 24 weeks after commencement of TDF-based therapy, if they had started TDF at other facilities, or if there was evidence of prior TDF use. We only included Japanese patients in order to examine a population with comparatively homogenous basic demographics and background.

Measurements

Outcome measure: TDF-associated renal dysfunction. We defined TDF-associated renal dysfunction as more than 25% decrease in eGFR relative to the baseline [17,22,23]. Baseline eGFR was estimated for each patient from the average of two successive serum creatinine measurements made closest to and preceding the commencement of TDF by no more than 90 days. Changes in eGFR were plotted from the baseline measurement until the value diminished to less than 75% of the baseline or at the end of the follow-up period. The eGFR values at occurrence of TDF-associated renal dysfunction, at censoring, and closest to and preceding 24, 48, and 96 weeks to the diagnosis were collected. Patients generally visited our clinic between every month to every 3 months, and measurement of eGFR was usually conducted on every visit. eGFR was calculated using the equation from the 4-variable Modification of Diet in Renal Disease (MDRD) study [24].

Primary exposure variable. Our primary exposure variables were body weight and body mass index (BMI) at the time of commencement of TDF-containing antiretroviral therapy (ART). BMI was calculated by the equation: BMI = [body weight (kg)/height $(m)^2$].

Other variables: potential risk factors. Potential risk factors for TDF-associated renal dysfunction were determined according to previous studies and collected together with the basic demographics from the medical charts [4,19,20,25]. They included sex, age, baseline laboratory data: CD4 cell count, HIV viral load, and serum creatinine, and other medical conditions (antiretroviral treatment-naïve or experienced, concurrent ritonavir-boosted protease inhibitors, concurrent nephrotoxic drugs such as ganciclovir, sulfamethoxazole/trimethoprim, ciprofloxacin, and NSAIDs, diabetes mellitus, co-infection with hepatitis B defined by positive hepatitis B surface antigen, co-infection with hepatitis C defined by positive HCV viral load, hypertension defined by current treatment with antihypertensive agents, dyslipidemia defined by current treatment with lipid-lowering agents, and current smoking) [26]. We used the data on or closest to and preceding the day of starting TDF-containing ART by no more than 90 days. The data on weight change from the baseline to the end of follow-up period and the frequency of eGFR monitoring for each patient were collected.

Statistical analysis

The time to 25% decline in eGFR from the baseline was calculated from the date of treatment initiation to the date of occurrence of TDF-associated renal dysfunction. Censored cases represented those who discontinued TDF, dropped out, referred to other facilities, or at the end of follow-up period. The time from TDF initiation to 25% decrease in eGFR was analyzed by the Kaplan Meier method for the whole cohort. To estimate the impact of body weight on the incidence of TDF-associated renal dysfunction, we calculated the impact of every 5 kg decrement from the median weight using Cox proportional hazards regression analysis. The impact of every 1 kg/m^2 decrement in BMI on the incidence of TDF-associated renal dysfunction was estimated by the same method. The impact of each basic demographics, baseline laboratory data, and other medical conditions listed above was also estimated with univariate Cox proportional hazards regression.

To estimate the unbiased prognostic impact of weight on TDF-associated renal dysfunction, we conducted three models using multivariate Cox proportional hazards regression analysis. Model 1 was the aforementioned univariate analysis for every 5 kg decrement. Model 2 included sex, age plus model 1 in order to adjust for basic characteristics. In model 3, we added variables with P values<0.05 in univariate analysis for adjustment (these included age per 10 years, serum creatinine >0.8 mg/dl, CD4 count <200/μl, HIV viral load per log10/ml, concurrent nephrotoxic drugs, co-infection with hepatitis C, and current smoking). Concurrent ritonavir-boosted protease inhibitors were also added in Model 3 although their p value was 0.116 in the univariate analysis. This was based on the results of several studies suggesting that concurrent use of ritonavir-boosted protease inhibitors is a risk factor for TDF-associated renal dysfunction

[19,20]. The eGFR was excluded from multivariate analysis because of its multicollinearity with sex, age, and serum creatinine, since eGFR was gained by the equation of those variables. The impact of every 1 kg/m^2 decrement in BMI on the incidence of TDF-associated renal dysfunction was estimated by the same method with Model 1 to Model 3.

Four other analyses were conducted to further examine the relationship between low body weight and TDF-associated renal dysfunction. First, the time from initiation of TDF therapy to 25% decrease in eGFR was analyzed by the Kaplan Meier method for intertertile baseline body weight categories: <59, 59–67, and >67 kg. The log-rank test was used to determine statistical significance. Second, to investigate the impact of changes in muscle mass on changes in the eGFR as calculated by MDRD, we compared weight changes with one-way ANOVA among intertertile baseline weight categories. We also conducted the sensitivity analysis by adding the variable "weight change" in multivariate analysis. Third, the median and interquartile value for the actual fall in eGFR from the baseline to 24, 48, and 96 weeks for the whole cohort and three baseline weight categories, respectively, were calculated. The eGFR value at 24, 48, and 96 weeks included those that were censored before reaching 24, 48, and 96 weeks, respectively, so that we could interpret the data for actual fall in eGFR, including not only survived cases but also censored cases. Fourth, we counted the number of patients whose eGFR decreased to <60 and <10 ml/min/1.73 m^2, and who discontinued TDF with the clinical diagnosis of renal dysfunction due to TDF. Chi-square test was used to determine whether the difference among the weight categories was statistically significant.

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

Results

Between January 1, 2002 to March 31, 2009, 599 patients started TDF-containing ART (Figure 1). Of these, 104 patients were excluded based on the abovementioned criteria. Thus 495 patients were included in the present study (Dataset S1). Table 1 shows the demographics, laboratory data, and medical conditions of the study population at baseline. Two patients received ART with 3 NRTIs, 3 patients received ART with one protease inhibitor (PI), one non-NRTI (NNRTI), and tenofovir/emtricitabine, and the remaining patients had a standard ART with 2 NRTIs and either PI, NNRTI, or integrase inhibitor (INI). The median body weight and BMI were 63 kg and 21.9 kg/m^2, respectively. The median age of the patients was 38 years and 95.2% were males. The eGFR was well maintained (median: 120.9 ml/min/1.73 m^2), and the median baseline CD4 count was 247/μl. Of the total, 208 patients (42%) were antiretroviral treatment naïve, while 287 were treatment-experienced patients. Viral load was suppressed to <50 copies/ml in 162 (32.7%) patients. 403 (81.4%) were on concurrent PIs as the key drug, 367 (74.1%) were on ritonavir-boosted PIs, and only 83 (16.8%) had NNRTIs as the key drug. Smoking was prevalent among the study population, as 240 (48.5%) were identified as a current smoker.

TDF-associated renal dysfunction defined by more than 25% decrease of eGFR from baseline occurred in 97 patients (19.6%), with an estimated incidence of 10.5 per 100 person-years. The median time from commencement of TDF to occurrence of TDF-associated renal dysfunction was 39 weeks (IQR 13.5–99.4 weeks) (range: 1–1,841 days). The total observation period was 924.7 patient-years (median 72 weeks, IQR 38.6–139.3 weeks). Figure 2 shows the Kaplan-Meier survival curve for the occurrence of TDF-associated renal dysfunction for the whole cohort.

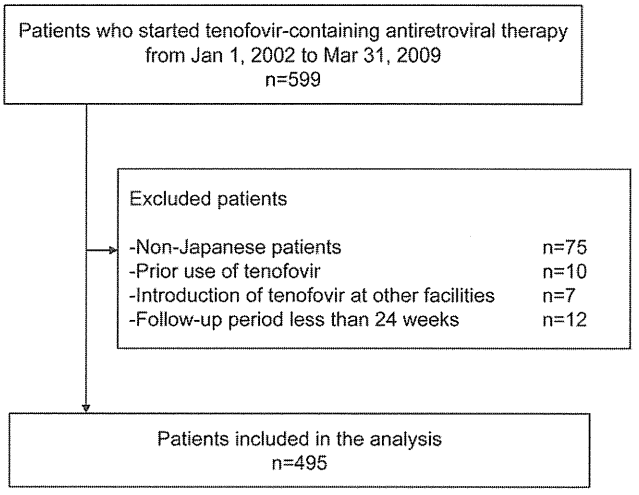

Figure 1. Flow diagram of patient selection.
doi:10.1371/journal.pone.0022661.g001

Univariate analysis showed a significant relationship between TDF-associated renal dysfunction and every 5 kg less than the median body weight (HR = 1.23; 95% CI, 1.10–1.37; p<0.001), and 1 kg/m^2 less BMI than the median BMI (HR = 1.14; 95% CI, 1.05–1.23; p = 0.001) (Table 2). Furthermore, old age, high eGFR, low serum creatinine, low CD4 counts, high HIV viral load, concurrent use of nephrotoxic drugs, presence of chronic hepatitis C, and smoking were associated with TDF-related renal dysfunction. On the other hand, concurrent use of PIs, ritonavir boosted PIs, and LPV/r tended to be associated with TDF-related renal dysfunction, albeit statistically insignificant. Treatment-naïve or Treatment-experienced was not associated with TDF-related renal dysfunction.

Multivariate analysis showed that every 5 kg less than the median body weight was a significant risk for TDF-associated renal dysfunction after adjustment for sex and age (adjusted HR = 1.21; 95% CI, 1.07–1.36; p = 0.002) (Table 3, Model 2), and also after adjustment for other risk factors (adjusted HR = 1.13; 95% CI, 1.01–1.27; p = 0.039) (Table 3, Model 3). Similarly, every 1 kg/m^2 less than the median BMI was also a significant risk factor for TDF-associated renal dysfunction even after adjustment for sex and age (adjusted HR = 1.13; 95% CI 1.05–1.22; p = 0.002) (Table 4, Model 2), and tended to be a significant factor after adjustment for other variables (adjusted HR = 1.07; 95% CI 1.00–1.16; p = 0.058) (Table 4, Model 3). Old age and current smoking were also independent risk factors in both multivariate analysis for body weight and BMI (Table 3, Model 3 and Table 4, Model 3).

In complementary analyses, First, Figure 3 shows the relation between probability of TDF-associated nephrotoxicity and time from initiation of TDF therapy to 25% decrease in eGFR analyzed by the Kaplan Meier method for intertertile baseline weight categories. Compared to patients with baseline body weight >67 kg, patients with baseline weight <59 kg were significantly more likely to develop >25% decline in eGFR (p = 0.002). On the other hand, the difference in this probability between patients with baseline weight 59–67 kg and those >67 kg was only marginally significant (p = 0.073, log-rank test). Secondly, one-way ANOVA

Table 1. Baseline demographics and laboratory data.

Characteristics		
Median (IQR) weight (kg)	63	(57–69)
Median (IQR) BMI (kg/m^2)	21.9	(20.3–23.8)
Male, n (%)	471	(95.2)
Median (IQR) age	38	(33–46)
Median (IQR) eGFR (ml/min/1.73 m^2)	120.9	(104.8–138.2)
Median (IQR) serum creatinine (mg/dl)	0.72	(0.64–0.81)
Median (IQR) CD4 count (/μl)	247	(159–371)
Median (IQR) HIV viral load (log10/ml)	3.73	(1.60–4.81)
HIV viral load <50 copies/ml, n (%)	162	(32.7)
Antiretroviral therapy naïve, n (%)	208	(42.0)
Key drugs, n (%)*		
PIs	403	(81.4)
Ritonavir-boosted PIs	367	(74.1)
LPV/r	175	(35.4)
ATV/r	131	(26.5)
FPV/r	52	(10.5)
DRV/r	9	(1.8)
FPV	14	(2.8)
ATV	4	(0.8)
NFV	15	(3)
SQV	2	(0.4)
IDV	1	(0.2)
NNRTIs	83	(16.8)
EFV	65	(13.1)
NVP	17	(3.4)
ETR	1	(0.2)
INI		
RAL	10	(2.0)
Concurrent use of nephrotoxic drug, n (%)	131	(26.5)
Diabetes mellitus, n (%)	30	(6.1)
Hepatitis B, n (%)	75	(15.2)
Hepatitis C, n (%)	52	(10.5)
Hypertension, n (%)	28	(5.7)
Dyslipidemia, n (%)	40	(8.1)
Smoking, n (%)	240	(48.5)
Median (IQR) weight change (kg)	0.0	(−2.0–2.25)
Median (IQR) frequency of eGFR monitoring	16	(9.0–27)

(n = 495).
*Two patients did not take any key drugs. Three patients took both PI and NNRTI.
IQR: interquartile range, BMI: body mass index, eGFR: estimated glomerular filtration rate, PI: protease inhibitor, LPV/r: lopinavir/ritonavir, ATV: atazanavir, FPV: fosamprenavir, DRV: darunavir, NFV: nelfinavir, SQV: saquinavir, IDV: indinavir, NNRTI: non-nucleos(t)ide reverse transcriptase inhibitor, EFV: efavirenz, NVP: nevirapine, ETR: etravirine, INI: integrase inhibitor, RAL: raltegravir.
doi:10.1371/journal.pone.0022661.t001

showed that weight changes among the three baseline weight categories were not significantly different (p = 0.206). Sensitivity analysis after adding the variable "weight change" in Model 3 multivariate analysis (Table 3) showed that adjusted hazard ratio for weight per 5 kg decrement hardly changed (adjusted HR 1.131; 95% CI, 1.007–1.271; p = 0.038). Thirdly, Table 5 shows

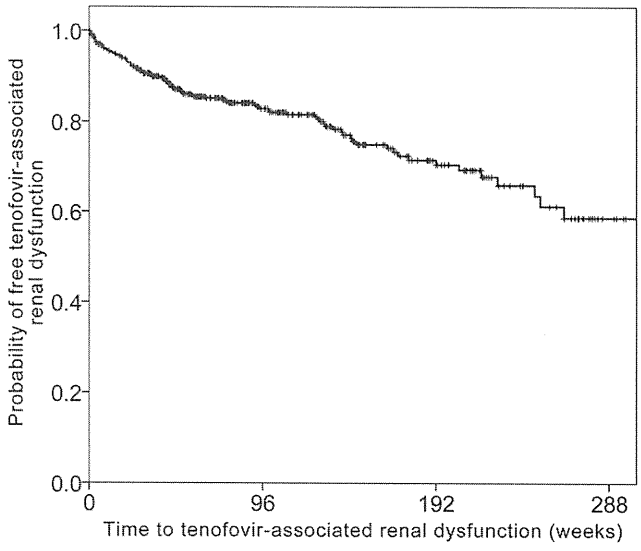

Figure 2. Kaplan-Meier curve showing the time to 25% reduction in eGFR for the whole cohort. eGFR: estimated glomerular filtration rate.
doi:10.1371/journal.pone.0022661.g002

the median and interquartile values for the actual falls in eGFR from the baseline to 24, 48, and 96 weeks. The eGFR decreased gradually in all categories, except for patients with baseline weight

Table 2. Univariate analysis for TDF-associated renal dysfunction.

	HR	95%CI	P value
Weight per 5 kg decrement	1.23	1.10–1.37	<0.001
BMI per 1 kg/m^2 decrement	1.14	1.05–1.23	0.001
Male gender	0.54	0.26–1.11	0.094
Age per 10 years	1.22	1.02–1.45	0.027
eGFR per 10 ml/min/1.73 m^2	1.10	1.05–1.15	<0.001
Serum creatinine >0.8 mg/dl	0.51	0.30–0.88	0.014
CD4 count <200/μl	1.97	1.32–2.93	0.001
HIV viral load per log10/ml	1.15	1.01–1.30	0.037
Antiretroviral therapy naïve	0.98	0.63–1.52	0.927
Concurrent key drugs			
Any PIs	1.52	0.89–2.59	0.124
Ritonavir boosted PIs	1.46	0.91–2.33	0.116
LPV/r	1.45	0.97–2.17	0.072
ATV/r	1.05	0.66–1.68	0.826
Concurrent nephrotoxic drug	1.59	1.04–2.42	0.031
Diabetes mellitus	1.57	0.76–3.24	0.220
Hepatitis B	1.36	0.82–2.24	0.231
Hepatitis C	1.80	1.07–3.04	0.028
Hypertension	1.18	0.51–2.69	0.702
Dyslipidemia	0.97	0.47–2.00	0.932
Smoking	1.57	1.05–2.36	0.028

TDF: tenofovir, HR: hazard ratio, CI: confidence interval, BMI: body mass index, eGFR: estimated glomerular filtration rate, PI: protease inhibitor, LPV/r: lopinavir/ritonavir, ATV: atazanavir.
doi:10.1371/journal.pone.0022661.t002

Table 3. Multivariate analysis to estimate the effect of lower body weight on TDF-associated renal dysfunction.

	Model 1 Crude		Model 2 Adjusted		Model 3 Adjusted	
	HR	95%CI	HR	95%CI	HR	95%CI
Weight per 5 kg decrement¶	1.23	1.10–1.37	1.21	1.07–1.36	1.13	1.01–1.27
Male gender			0.88	0.41–1.89	0.57	0.26–1.26
Age per 10 years¶			1.16	0.98–1.38	1.24	1.04–1.49
Serum creatinine >0.8 mg/dl					0.62	0.35–1.07
CD4 count <200/μl					1.65	0.97–2.79
HIV viral load per log10/ml					1.05	0.90–1.23
Boosted PIs					1.54	0.93–2.54
Concurrent use of nephrotoxic drug					1.23	0.77–1.97
Hepatitis C					1.57	0.92–2.69
Smoking¶					1.65	1.09–2.48

¶P<0.05 in Model 3.
TDF: tenofovir, HR: hazard ratio, CI: confidence interval, PI: protease inhibitor.
doi:10.1371/journal.pone.0022661.t003

>67 kg. Fourthly, the number (percentage) of patients whose eGFR decreased to <60 ml/min/1.73 m^2 was not different among the baseline weight categories (p = 0.229), whereas the number of patients who discontinued TDF with a clinical diagnosis of renal dysfunction due to TDF varied significantly according to body weight (p = 0.001, chi-square test, Table 6). None of the patients showed reduction of eGFR to <10 ml/min/1.73 m^2.

Discussion

In this Japanese cohort, 19.6% of the patients experienced eGFR decline of more than 25% from the baseline after commencement of TDF. The incidence of TDF-associated renal dysfunction was 10.5 per 100 person-years. Multivariate analysis identified smaller body weight and smaller body mass index as significant and almost significant factors, respectively, for TDF-associated renal dysfunction.

The incidence of TDF-associated renal dysfunction in patients with small body weight might be higher than previously reported in studies of patients with larger statures. Studies from North America, Europe, and Australia reported an incidence of <1% to 4.3% for TDF-related renal dysfunction, although the definition used for the diagnosis of renal impairment was different among the studies and varied from an increase in serum creatinine from >0.5 to >2 mg/dL from baseline [1–3,5,13]. Several studies conducted in these regions indicated that the range of patients' mean body weight was 69–74 kg, indicating that their patients were heavier than those of the present study with a median weight of 63 kg [2,6,12,14]. The impact of the comparatively lower body weight seems stronger in our patients probably because they do not appear to have many of the other established risk factors for TDF-associated renal dysfunction despite the high incidence of 10.5 per 100 person-years. For example, they were comparatively young with a median age of 38 years, CD4 count was relatively maintained, and approximately 30% had suppressed HIV viral load at baseline (Table 1). Furthermore, they were less likely to have hypertension, dyslipidemia, and diabetes mellitus.

Table 4. Multivariate analysis to estimate the impact of BMI decrement on TDF-associated renal dysfunction.

	Model 1 Crude		Model 2 Adjusted		Model 3 Adjusted	
	HR	95%CI	HR	95%CI	HR	95%CI
BMI per 1 kg/m^2 decrement	1.14	1.05–1.23	1.13	1.05–1.22	1.07	1.00–1.16
Male gender			0.67	0.32–1.38	0.48	0.23–1.03
Age per 10 years¶			1.20	1.01–1.43	1.27	1.06–1.52
Serum creatinine >0.8 mg/dl					0.60	0.35–1.04
CD4 count <200/μl					1.64	0.97–2.79
HIV viral load per log10/ml					1.05	0.90–1.23
Boosted PIs					1.49	0.90–2.45
Concurrent use of nephrotoxic drugs					1.22	0.76–1.94
Hepatitis C					1.62	0.94–2.76
Smoking¶					1.63	1.08–2.46

¶P<0.05 in Model 3.
BMI: body mass index, TDF: tenofovir, HR: hazard ratio, CI: confidence interval, PI: protease inhibitor.
doi:10.1371/journal.pone.0022661.t004

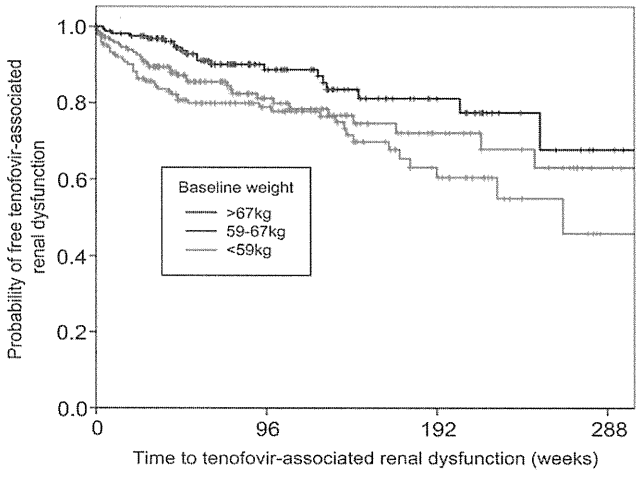

Figure 3. Kaplan-Meier curve showing the time to 25% reduction in eGFR according to baseline weight categories. Compared to patients with body weight >67 kg, those with weight <59 kg were more likely to develop >25% decline in eGFR (P = 0.002), whereas those with weight 59–67 kg showed only a marginal significance (P = 0.073, log-rank test). eGFR: estimated glomerular filtration rate.
doi:10.1371/journal.pone.0022661.g003

The results of multivariate analysis that each 5 kg decrement in body weight was significantly associated with TDF-associated renal dysfunction but not each 1 kg/m^2 decrement in BMI suggests that weight might be more useful and handy information to estimate the risk for TDF-associated renal dysfunction than BMI. Thus, patient's body weight is an important risk factor to consider at the time of TDF prescription.

Our study is one of a few that have examined the impact of TDF-associated renal dysfunction in patients with small body weight, but is the first to examine the impact of small body weight as a primary exposure by creating the model used for multivariate analysis [16–18]. One study from Thailand that included patients with a median weight of 56.5 kg reported a similar incidence of 16.2 per 100 person-years for developing TDF-associated renal dysfunction [17]. They concluded that the small body weight of their patients was probably associated with the high incidence of TDF-associated renal dysfunction. Our study confirmed that conclusion and provided statistically-backed evidence that small body weight is a significant risk factor of TDF-associated renal dysfunction by using a multivariate model with least multi-collinearity to evaluate the impact of small body weight. The results of the present study could be applied to many countries in Asia and Africa, where stature and body weight of the population are comparatively smaller.

This study adopted a decrease in eGFR of >25% as a definition for TDF-associated renal dysfunction. This criterion is one of common methods in evaluating renal function [22,23]. Using this definition, however, does not mean that all patients with >25% fall in eGFR have severe renal dysfunction. However, the definition of renal dysfunction based on a fall in eGFR of >25% is probably more sensitive than that based on eGFR <60 ml/min/1.73 m^2, in patients with comparatively good baseline renal function, such as patients of our study. Adopting this definition could be useful in detecting early renal dysfunction and in the clinical decision making regarding the need for certain interventions, for example, discontinuation of TDF. Early detection of renal dysfunction is particularly important in patients with HIV infection, because kidney disease may be associated with AIDS and death, and TDF-associated renal dysfunction might be irreversible [27,28].

Since the calculation of eGFR using the MDRD formula is based on serum creatinine, age, race and gender, any fall in eGFR is influenced by hypercreatininemia caused by increased muscle mass [29]. It is possible that the muscle mass increases in patients on ART, especially those with low weight at baseline compared to those with higher weight, reflecting reversal of wasting in those patients who were most malnourished. Such increase in muscle mass could then result in a fall in eGFR despite no change in actual renal elimination of creatinine. However, complementary analysis showed that weight change throughout the follow-up period was not significantly different among patients with different baseline weight, and the sensitivity analysis demonstrated that weight change did not alter the significance of every 5 kg decrement.

In the present study, high eGFR and low serum creatinine levels at baseline were identified as risk factors for falls in eGFR of more than 25%, in contrast to several previous studies that showed high serum creatinine and low eGFR were risk factors [4,10,25]. While the exact reason for this discrepancy is unknown at present, it could be related to differences in the definition of TDF-associated renal dysfunction. The aforementioned Thai study used the same definition applied in the present study and a Canadian study that used the definition of 1.5-fold increase in serum creatinine from baseline also reported high eGFR and low serum creatinine level at baseline as risk factors [17,30]. Thus, it is plausible to observe a fall in eGFR when the baseline value is high, since Horberg et al. reported that patients with baseline eGFR of >80 ml/min/1.73 m^2 were likely to show a pronounced fall in eGFR with TDF use [31].

Multivariate analysis also suggested that old age and current smoking are significant risks for TDF-associated renal dysfunction

Table 5. Median and interquartile range of the actual fall in eGFR from the baseline to 24, 48, and 96 weeks, according to body weight.

	Total (n = 495)		<59 kg (n = 167)		59–67 kg (n = 168)		>67 kg (n = 160)	
	fall in eGFR(ml/min/1.73 m^2)		fall in eGFR		fall in eGFR		fall in eGFR	
	median	IQR	median	IQR	median	IQR	median	IQR
to 24 weeks	7.8	(−1.7–18.1)	9.8	(−3.6–22.6)	6.8	(−1.5–17.3)	7.3	(−1.8–15.4)
to 48 weeks	9.0	(−0.7–21.9)	13.0	(−0.2–29.3)	7.2	(−1.2–20.0)	8.1	(−0.6–18.6)
to 96 weeks	9.3	(−0.5–23.1)	13.4	(1.2–33.2)	8.6	(−0.2–21.7)	7.5	(−2.4–19.8)

eGFR: estimated glomerular filtration rate, IQR: interquartile range.
doi:10.1371/journal.pone.0022661.t005

Table 6. Number of patients whose eGFR decreased to <60 ml/min/1.73 m^2 and who discontinued tenofovir with clinical diagnosis of renal dysfunction due to tenofovir.

	<59 kg (n = 167)	59–67 kg (n = 168)	>67 kg (n = 160)	p value
eGFR <60 ml/min/1.73 m^2	4 (2.4%)	1 (0.6%)	1 (0.6%)	0.229
Discontinued tenofovir	16 (9.6%)	8 (4.8%)	1 (0.6%)	0.001
Reasons for discontinuation				
>25% eGFR decrement	8 (4.8%)	4 (2.4%)	0 (0%)	
Urine β2 microglobulin >5000 μg/l	11 (6.6%)	4 (2.4%)	1 (0.6%)	

Among the patients who discontinued tenofovir, both >25% fall in eGFR and urine β2 microglobulin >5000 μg/l were registered in six patients with body weight <59 kg, and in three patients with body weight 59–67 kg.
eGFR: estimated glomerular filtration rate.
doi:10.1371/journal.pone.0022661.t006

(Table 3, Model 3 and Table 4, Model 3). However, these results have to be interpreted with caution, because these multivariate analyses were formulated to primarily evaluate weight decrement, not age or smoking.

The mechanism of TDF-associated renal dysfunction is not fully understood. TDF-associated renal dysfunction probably develops as a result of complex interaction of pharmacological, environmental, and genetic factors, rather than small body weight only [32]. It should be noted, however, that small body weight has been identified as a risk factor for TDF-associated renal dysfunction not only in clinical trials, but also in *in vitro* and pharmacokinetic studies [33–36]. TDF is the prodrug of acyclic nucleotide analog tenofovir, which is excreted by both glomerular filtration and active tubular secretion. *In vitro* studies showed that tenofovir exhibits mitochondrial toxicity in renal proximal tubular cells, and animal studies demonstrated that renal tubular dysfunction was associated with the dose and plasma drug concentrations of TDF [34,35]. Furthermore, pharmacokinetic studies showed that small body weight is associated with reduced plasma TDF clearance and thus high plasma TDF concentrations, which could result in renal tubular dysfunction. [33,36].

There are several limitations to our study. First, because of the retrospective nature of the study, patients with possible risks for TDF-associated renal dysfunction could have not been prescribed TDF. Because of this selection bias, the incidence of TDF-associated renal dysfunction might be underestimated. Second, the study did not compare the incidence of renal dysfunction in a control group (TDF-free ART). Due to the small body weight in Japanese or other factors such as genetics, the use of ART without TDF might cause higher incidence of renal dysfunction as well. Third, as discussed above, the definition of TDF-associated renal dysfunction, 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-associated renal dysfunction.

In conclusion, the present study demonstrated a high incidence of TDF-associated renal dysfunction among Japanese patients, a potentially high-risk group due to the low median body weight. The results also identified small body weight as a risk for TDF-associated renal dysfunction in a statistical model that included small body weight as a primary exposure. TDF is certainly a drug of choice for one of the components of the first line therapies for HIV infection. However, the importance of close monitoring for renal function in patients with small body weight should be emphasized for early detection of TDF-associated renal dysfunction.

Supporting Information

Text S1 Letter of Approval from Human Research Ethics Committee of National Center for Global Health and Medicine.
(PDF)

Dataset S1 Raw data of the target population.
(XLS)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: AN TH KU TN MKS. Performed the experiments: AN H. Sakai. Analyzed the data: AN TH KU KO IK H. Sakai. TN MKS. Contributed reagents/materials/analysis tools: H. Suemori H. Sakai. Wrote the manuscript: AN TN MKS.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Clinical Symptoms and Courses of Primary HIV-1 Infection in Recent Years in Japan

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Abstract

Background The natural course of HIV-1 infection includes 10 years of an asymptomatic period before the development of AIDS. However, in Japan, the disease progression process seems faster in recent years.

Methods The study subjects were 108 new patients with primary HIV-1 infection during the period from 1997 through 2007. We evaluated their clinical symptoms and laboratory data, and then analyzed disease progression in 82 eligible patients. Disease progression was defined as a fall in CD4 count below 350/μL and/or initiation of antiretroviral therapy.

Results Ninety percent of the patients were infected via homosexual intercourse. All patients had at least one clinical symptom (mean; 4.75±1.99) related to primary HIV-1 infection, with a mean duration of 23.2 days (±14.8) and 53.3% of them had to be hospitalized due to severe symptoms. The mean CD4 count and viral load at first visit were 390/μL (±220.1) and 4.81 log10/mL (±0.78), respectively. None developed AIDS during the study period. Estimates of risk of disease progression were 61.0% at 48 weeks and 82.2% at 144 weeks. In patients who required antiretroviral therapy, the median CD4 count was 215/μL (range, 52-858) at initiation of such therapy. Among the patients with a CD4 count of <350/μL at first visit, 53% never showed recovery of CD4 count (>350/μL) without antiretroviral therapy.

Conclusion Despite possible bias in patient population, disease progression seemed faster in symptomatic Japanese patients with recently acquired primary HIV-1 infection than the previously defined natural course of the disease.

Key words: HIV-1, primary infection, disease progression

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Introduction

The natural course of HIV-1 infection has been well described in large cohorts from the United States and Europe before the introduction of highly active antiretroviral therapy (HAART); primary HIV-1 infection (PHI) is followed by a clinical latency, usually lasting around 10 years, which precedes the eventual collapse of the immune system (1, 2). However, there is a common feeling among clinicians at present that the natural disease progression of recently infected patients is faster than in previous years (3, 4). Disease progression depends on various factors such as HLA type (5), concomitant infections (6, 7), and available medical resources (8). In addition to these factors, events occurring during PHI could also determine the natural course of the disease. Initial studies suggested that patients with more symptoms related to primary PHI and longer duration of illness exhibit faster rates of progression to AIDS (9-13). Plasma viral load at a set point is also an independent predictor of disease progression (14, 15). However, to determine the viral set point is sometimes difficult. Therefore, for clinicians, the severity of clinical symptoms is the only predictor of subsequent disease progression. The latency be-

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tween the development of PHI and commencement of HAART is also important in the present HAART era.

The main aim of this study was to evaluate the natural disease progression of recently infected Japanese patients. To determine whether or not the disease progression of recently infected patients is accelerated, their CD4 decline was compared with that of hemophiliacs infected before 1985 as the first HIV-1 infection in Japanese.

Furthermore, we also evaluated the correlation between initial CD4 count, viral load, and clinical events and subsequent changes in CD4 and/or time to start HAART in symptomatic Japanese patients with PHI.

Patients and Methods

Study site and patients with PHI

This study was conducted at the AIDS Clinical Center (ACC), National Center for Global Health and Medicine (NCGM: formerly International Medical Center of Japan). The NCGM (925 beds) is a tertiary general hospital located in central Tokyo and the ACC is the main referral clinic for treatment of HIV infected patients in Japan. As part of the follow-up service, HIV-1 infected patients usually visit the ACC on a monthly basis and CD4 count and viral load are measured at each visit. In the present retrospective study, we reviewed the medical records of 108 patients with PHI who were newly diagnosed with PHI between 1997 through 2007 at the ACC. We had conducted a clinical trial of structured treatment interruptions in patients with PHI from November 2000 through December 2002 and 26 patients were enrolled in that trial (16, 17). In terms of the data of these 26 patients, only the initial clinical and laboratory data were included in the present analysis, while all other data, such as time to events, were excluded from this study. To compare the natural CD4 decline of previously and recently infected patients, CD4 counts of 42 Japanese hemophiliacs recorded in the database in 1988 were analyzed as a previous control. Japanese hemophiliacs were infected with HIV-1 through contaminated blood products before 1985 (the estimated mean year of infection was 1983). Therefore, CD4 counts at the end of 1988 were the data at least 3 years after infection. In this comparison, the number of eligible recently infected patients was 59 patients; untreated and CD4 count at 3 years after infection was available.

Definition of PHI

PHI was diagnosed based on the presence of the following three criteria: 1) negative or incomplete western blot finding at the first visit with subsequent change to positive, 2) negative or weakly reactive enzyme-linked immunosorbent assay (ELISA) result for plasma HIV-1 RNA, and 3) confirmed HIV-1 infection on the first visit with documentation of negative ELISA result within 6 months. Symptomatic PHI was defined as PHI accompanied by at least one symptom related to acute retroviral syndrome, such as fever, lymphadenopathy, or skin rash.

Definition of disease progression

Disease progression was defined as fall in CD4 count below 350/μL and/or initiation of antiretroviral therapy. Specifically, patients with an AIDS-defined illness [listed under Centers for Disease Control and Prevention (CDC) category C], patients with AIDS requiring initiation of HAART, and those with severe symptomatic PHI on HAART were defined to have disease progression. The selection of a cutoff value of 350/μL for CD4 count was based on the fact that treatment is generally indicated during the chronic phase of infection when CD4 count falls below 350/μL (18). Patients were considered to be in immunologic progression at the first visit when the initial CD4 count was <350/μL and never subsequently reached 350/μL. For patients who showed a spontaneous increase in subsequent CD4 counts to ≥350/μL (such recovery occurred within 3 months from the first visit in all such patients), disease progression was set to have started at the time when such change in CD4 count occurred.

Statistical analysis

Continuous variables are presented as mean value ± SD. Categorical variables were presented as absolute numbers and proportions. Time to events was analyzed by the Kaplan-Meier survival curves, and compared using log-rank test. For patients who did not experience the events described above, data were censored at their last visit. To evaluate the differences between patients groups, the Student t test and χ^2 test were used when appropriate. The relationships between variables were analyzed by the Spearman rank-over correlation test. Statistical significance was defined as $p<0.05$. Data were analyzed using SPSS for Windows (version 15, SPSS, Inc., Chicago, IL).

Results

Table 1 lists the demographics of the enrolled patients with PHI. All patients had at least one documented symptom consistent with PHI (median 5; range 1-11). Fever, cervical lymphadenopathy, pharyngitis, and rash were found in more than 50% of patients (Table 2). The mean duration of symptoms was 23.2 days (SD ±14.8). Fifty-eight (53.7%) patients had to be hospitalized due to severe clinical symptoms. The initial viral loads in hospitalized patients were significantly higher than those of non-hospitalized patients. A longer duration of symptoms was associated with higher initial viral load (R=0.31, p=0.002) (Fig. 1A), and lower CD 4 count (R=-0.22, p=0.03) (Fig. 1B). Consequently, a higher viral load slightly was correlated with a lower CD4 count at the first visit (R=-0.22, p=0.033) (Fig. 1C).

Disease progression was analyzed in 82 patients. None of the patients had AIDS-defining events. Estimates of the risk of disease progression were 50.6% at 24 weeks, 61.0% at 48 weeks, 67.0% at 96 weeks, and 82.2% at 144 weeks

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Table 1. Baseline Characteristics of 108 Patients with Primary HIV-1 Infection in this Study

Characteristics	Total number or mean (±SD) or %	Hospitalized patients (n = 58)	Non-hospitalized patients (n = 50)	p
Age (year)	31.8±8.48	32±9.07	31±7.82	NS
Sex				
Male	102	56	46	NS
Female	6	2	4	NS
Predisposing factor				
MSM	97	53	44	NS
Heterosexual	8	3	5	NS
IDU	1	0	1	NS
Unknown	2	2	0	NS
PMH of STD	75 (69.7)	44 (40.4)	31 (29.3)	NS
Syphilis	49 (45.5)	27 (25.3)	21 (20.2)	NS
Acute hepatitis A	11 (10.1)	6 (6.1)	5 (4.0)	NS
Acute hepatitis B	36 (33.3)	22 (20.2)	14 (13.1)	NS
Amebiasis	10 (9.1)	9 (8.0)	1 (1.1)	0.035
Others	7 (6.1)	2 (2.0)	5 (4.1)	NS
No. of symptoms	4.75±1.99	4.98±1.94	4.48±2.04	NS
Duration of symptoms (days)	23.2±14.8	27.8±13.1	18.0±15.1	0.001
Laboratory findings				
CD4 count/μL	390.0±220.1	356.1±204.1	443.7±236.0	0.06
HIV RNA $\log_{10}$/mL	4.81±0.78	5.03±0.68	4.48±0.81	0.001
STI trial*	26	12	14	NS

*Patients enrolled in a clinical trial of structured treatment interruptions in recently HIV-1-infected patients. Abbreviations; MSM: men who have sex with men, PMH of STD: past medical history of sexual transmitted diseases, STI: structured treatment interpretations, IDU: intravenous drug user, Others: genital herpes infection, chlamydial urethral infection condyloma acuminata, NS: not significant

Data are presented as mean ± SD or percentage (%) unless otherwise indicated

Table 2. Symptoms and Physical Findings Observed in the Patients with >10% Frequencies (n=108)

Symptoms and physical findings	frequency (%)
Fever	91
Lymphadenopathy	63
Pharyngitis	53
Rash	50
Diarrhea	37
Fatigue	32
Headache	26
Myalgia	20
Weight loss	19
Nausea	16
Appetite loss	14
Neurological sign	13
Hepatomegaly	13
Thrush	12

(Fig. 2). Eighteen of 34 (53.3%) patients with an initial CD 4 cell count below 350 cells/μL had immunologic progression at the first visit. Their CD4 counts never increased above 350/μL until initiation of HAART. Forty-eight (58.5%) required initiation of HAART in this study. The reasons for the initiation of HAART were severe clinical symptoms related to PHI in 16 patients and immunologic progression in 32 patients. The median CD4 count of those patients at initiation of HAART was 215/μL (range, 52-858).

We analyzed the clinical course in 66 patients (excluding 26 patients who enrolled in a clinical trial of structured treatment interruptions in PHI and 16 patients who received HAART for PHI) to determine the factors associated with disease progression. Half of these patients (33 patients) required hospitalization. As shown in Fig. 3A, the mean time to disease progression of the hospitalized patients [57.4 weeks, 95% confidence interval (95%CI); 34.9-79.8 weeks] was shorter than that of the non-hospitalized (33 patients, 94.4 weeks, 95%CI; 71-117 weeks, p=0.002). Among the 32 patients with CD4 count >350/μL at first visit, 24% had documented disease progression within 1 year, whereas among 34 patients with CD4 count <350/μL at first visit, 76.4% showed disease progression (Fig. 3B). The mean times to disease progression for the two groups were 111.9 weeks (95%CI; 92.8-131) and 39.5 weeks (95%CI; 18.6-60.5), respectively (p<0.001). Disease progression in 39 patients with high viral load (≥5.0 $\log_{10}$/mL) was not significantly different (p=0.41) from that in 27 patients with low viral load (<5.0 $\log_{10}$/mL) (Fig. 3C). The number of symptoms was not significantly different in each group (Fig. 3D). The mean time to disease progression was 69.8 weeks (95% CI; 47.2-92.5) in patients with a high viral load and 80.4 weeks (95%CI; 54.9-105.8) in those with a low viral load.

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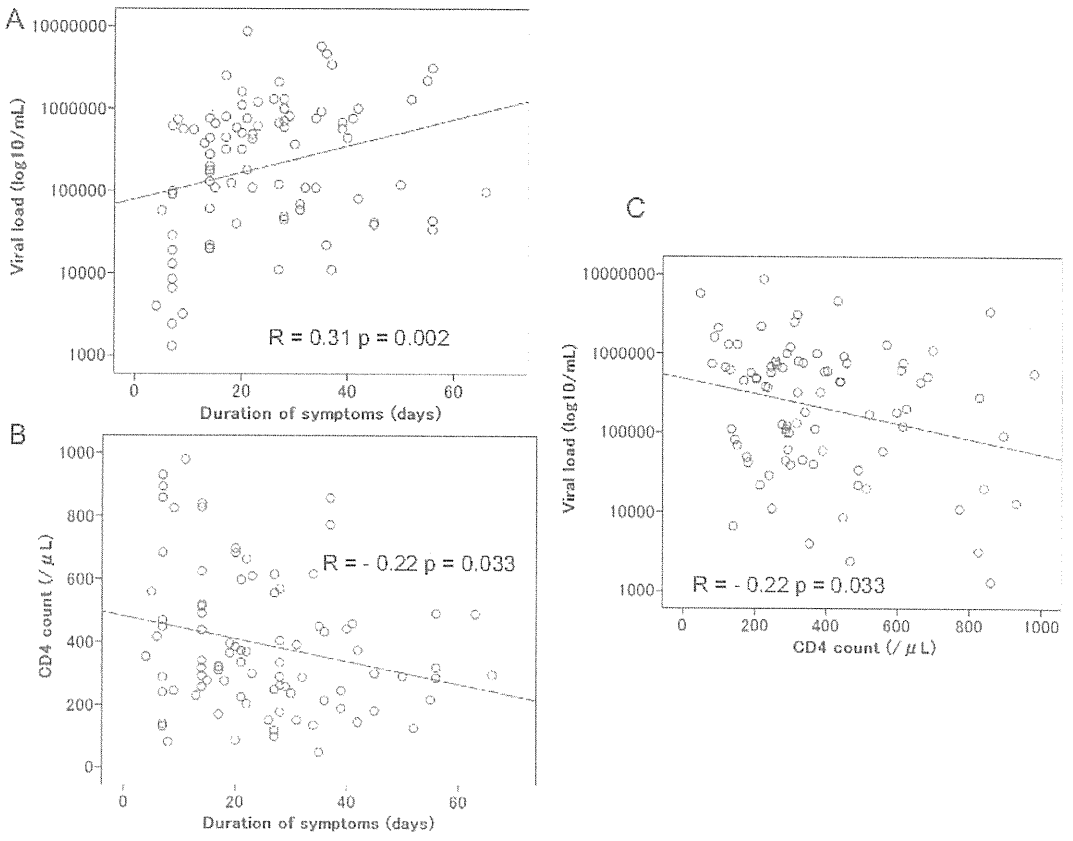

Figure 1. Correlations among plasma viral load, CD4 count, and clinical symptoms. A; Plasma viral load correlated with duration of symptoms (R=0.31, p=0.002). B; CD4 count correlated inversely with duration of symptoms (R=-0.22, p=0.033). C; plasma viral load correlated inversely with CD4 count (R=-0.22, p=0.033).

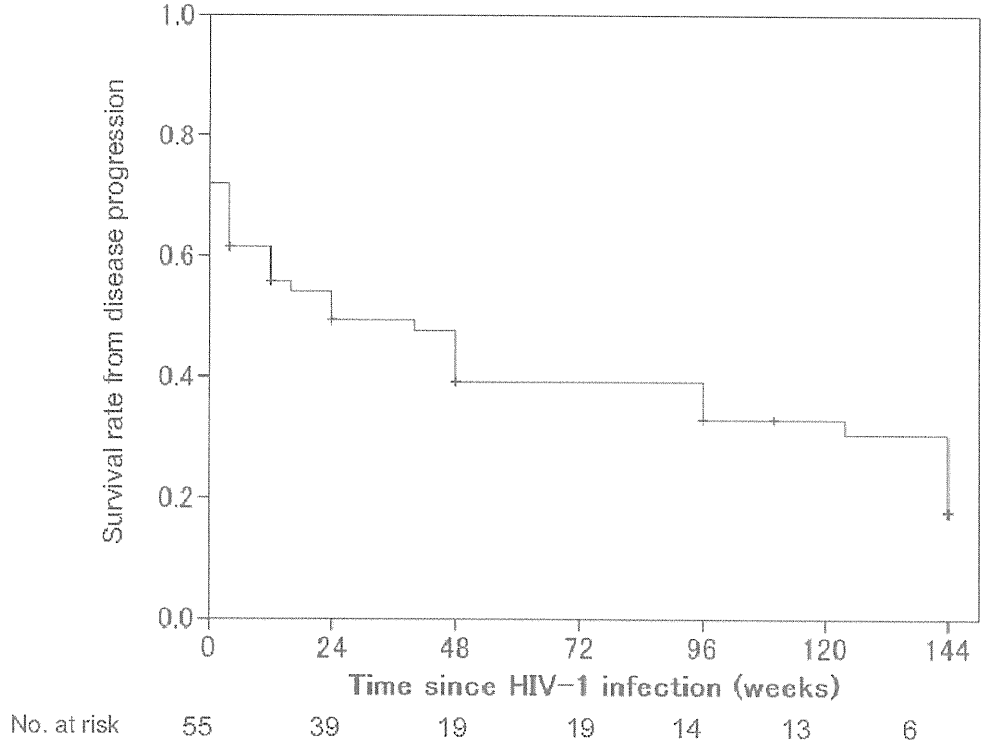

Figure 2. Progression-free survival in 82 patients. Progression was defined as CD4 count <350/μL or initiation of HAART. No. at risk: the number of CD4 count >350/μL or HAART naïve patients

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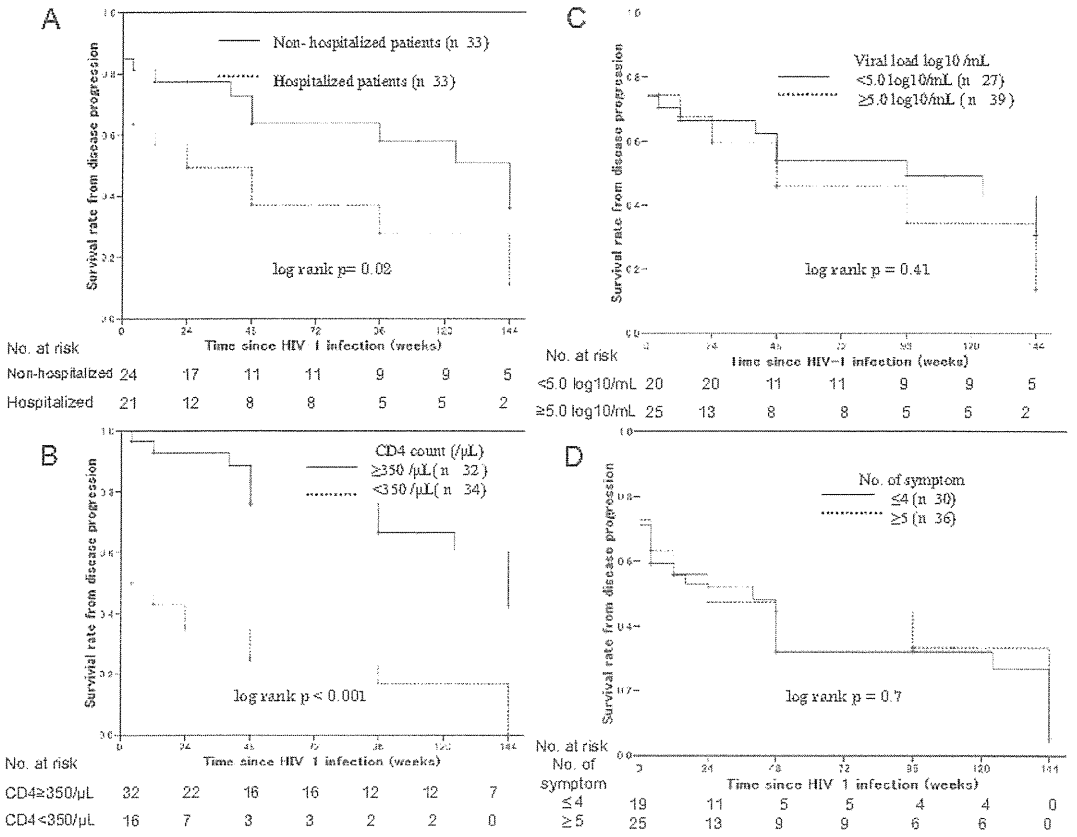

Figure 3. Progression-free survival among 66 patients according to rate of hospitalization, baseline CD4 count, and viral load. No. at risk: the number of CD4 count >350/μL or HAART naïve patients. A; Solid line: patients who required hospitalization due to PHI, dashed line: patients who did not require hospitalization (p=0.02, by log-rank test). B; Solid line: patients with CD4 count >350/μL at first visit, dashed line: patients with CD4 count <350/μL (p<0.001). C; Solid line: patients with viral load <5.0 log_{10}/mL, dashed line: patients with viral load ≥5.0 log_{10}/mL (p=0.41). Disease progression was defined as CD4 count <350/μL or initiation of HAART. D; Solid line: patients with the number of PHI symptoms ≤4, dashed line: patients with the number of PHI symptoms ≥5 (p=0.7, by log-rank test).

Comparison of percentage of recently infected patients with CD4 counts >350/μL at 3 years after infection and that of hemophiliacs as the first HIV-1 infected population in Japanese is shown in Fig. 4. The percentage (13.5%) of recently infected patients was significantly lower than that (47.6%) of Japanese hemophiliacs (p<0.001), clearly indicating the rapid decline of CD4 count in recently infected patients.

Discussion

In this study, we demonstrated rapid disease progression of symptomatic PHI Japanese patients in this decade. However, when we divided our study subjects into two groups according to the first half (1997-2002) and the latter half (2003-2007), disease progression of each group was not different (data not shown). In contrast, disease progression surrogated with natural CD4 decline of recently infected patients was significantly accelerated compared with Japanese hemophiliacs infected with HIV-1 before 1985. However, there are two quite different backgrounds; one is the route of infection and the other is the year of infection. Almost all hemophiliac patients are also co-infected with hepatitis C but do not have other sexually transmitted diseases (STDs). In contrast, most patients in the present study were infected via homosexual intercourse with many other STDs that may facilitate acceleration of the disease progression (7). In the present study, 69.7% patients had a past medical history of STDs, and the mean number of STDs was 1.08/patient (0: 31.3%, 1: 37.4%, 2: 23.2%, 3: 8.1%). In this regard, most published data on disease progression were obtained from men who have sex with men (MSM) cohorts (1, 2). Therefore, it is unlikely that the recent rapid disease progression is due to Japanese MSM. Whether or not the rapid disease progression in the recently HIV-1-infected Japanese can be generalized is to be elucidated in future studies.

Some HLA types are protective against disease progression such as HLA-B57 (19) and HLA-B51 (20) because HLA-restricted cytotoxic T lymphocytes (CTLs) play an important role on viral control. On the other hand, virus can easily escape from CTLs (17, 21). In some prevalent HLA types, escape virus can transmit and accumulate in the population (21). In this situation, some HLA types are no more

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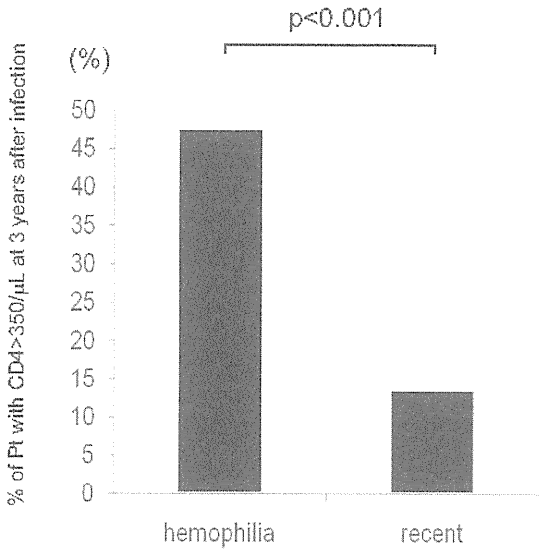

Figure 4. Comparison of percentage of previously and recently infected patients with CD4 counts >350/μL at 3 years after infection. In this analysis, Japanese hemophiliacs (designated "hemophilia" in the figure) were regarded as a previously infected patient, because they were infected with HIV-1 before 1985. The number of hemophiliacs was 42 patients. The eligible number of recently infected patients (designated "recent" in the figure) was 59 patients; infected with HIV-1 after 1997, untreated, and CD4 count at 3 years after infection.

protective. The HLA distribution is different in Americans compared to Japanese. Another possible hypothesis for the different disease progression is that Japanese hemophiliacs were exposed to HIV-1 through contaminated blood products imported from US as the first Japanese population infected with the virus around 1983. However, in recent years, most HIV-1 infection in Japanese is transmitted from Japanese patients. It can be postulated that current HIV-1 in Japan has adapted to the Japanese population, indicating acquisition and accumulation of escape virus from immune pressure of the otherwise protective HLA in Japanese population (21). From a negative point of view, the situation is similar to the epidemic of drug-resistance virus in treatment of naïve patients (22). The clinical relevance of the prevalence of immune escape virus in Japanese is a potentially serious matter in terms of the natural course of HIV-1 infection.

In the present study, all patients have had at least one symptom associated with PHI. During the follow-up period, no patient developed AIDS, whereas around 70% of the patients experienced immunologic progression as defined by a CD4 count <350/μL. It is noteworthy that the majority of these patients exhibited immunologic progression within 3 years and, surprisingly, >60% of them were documented within the first year. HAART was initiated in nearly 60% of patients during this period, including initiation for PHI-related severe symptoms in 20% of these patients. Previous studies on PHI have suggested that the number, duration, and/or severity of symptoms can predict faster disease progression to AIDS (23, 24). Our findings are compatible with these previous studies. Considered together, these results suggest that the duration of illness rather than the number of symptoms is more likely to be a major determinant of immunological progression. The estimated risks of disease progression were more than 50% by week 24 and 80% by week 144. Comparison with those observed elsewhere during the natural course of HIV-1 infection (24), these disease progression rates are surprisingly high. Among the patients with CD4 counts >350/μL at first visit, a quarter of them showed disease progression within 1 year. In contrast, in patients with CD4 count <350/μL, three quarters of them showed disease progression within the same period. Goujard et al (25) suggested possible recovery of CD4 count after the primary infection phase even in patients with very low count because it fluctuates during that period. In contrast, our results suggest that patients with a CD4 count of <350/μL during primary infection should be monitored carefully because spontaneous recovery of CD4 cell count during primary infection was rare. This cautionary remark could also apply to patients with a CD4 count of >350/μL because they exhibited nearly 60% risk of disease progression within 3 years. These observations may allow more targeted clinical monitoring and timely initiation of HAART. The impact of a short-term HAART during symptomatic primary infection on the subsequent disease progression needs to be elucidated in future study.

Although we included all recent seroconverters during the study period, it could be argued that this study carries some institution bias (i.e., a high proportion of cases with severe disease). However, the present finding of a surprisingly rapid disease progression in our patient population is new. Whether or not the natural course of disease progression has recently become accelerated in other countries or other cohorts is a matter of great interest.

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

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Fever-based antibiotic therapy for acute cholangitis following successful endoscopic biliary drainage

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Abstract
Background The current management of acute cholangitis consists of antibiotic therapy in combination with biliary drainage. However, the optimal duration of antibiotic therapy after the resolution of clinical symptoms by biliary drainage is unclear. We aimed to evaluate whether discontinuing antibiotic therapy for acute cholangitis immediately after the resolution of clinical symptoms, achieved by endoscopic biliary drainage, was safe and effective.
Methods This prospective study included patients with moderate and severe acute cholangitis. Cefmetazole sodium and meropenem hydrate were used as initial antibiotic therapy for patients with moderate and severe acute cholangitis, respectively. All patients underwent endoscopic biliary drainage within 24 h of diagnosis. When the body temperature of <37°C was maintained for 24 h, administration of antibiotics was stopped. The primary endpoint was the recurrence of acute cholangitis within 3 days after the withdrawal of antibiotic therapy.
Results Eighteen patients were subjected to the final analysis. The causes of cholangitis were bile duct stone ($n = 17$) and bile duct cancer ($n = 1$). The severity of acute cholangitis was moderate in 14 patients and severe in 4. Body temperature of <37°C was achieved in all patients after a median of 2 days (range 1–6) following endoscopic biliary drainage. Antibiotic therapy was administered for a median duration of 3 days (range 2–7). None of the patients developed recurrent cholangitis within 3 days after the withdrawal of antibiotics.
Conclusions Fever-based antibiotic therapy for acute cholangitis is safe and effective when resolution of fever is achieved following endoscopic biliary drainage.

Keywords Acute cholangitis · Endoscopic biliary drainage · Antibiotic therapy

Abbreviations

CRP	C-reactive protein
CMZ	Cefmetazole sodium
MEPM	Meropenem hydrate
ERCP	Endoscopic retrograde cholangiopancreatography
ENBD	Endoscopic nasobiliary drainage
EST	Endoscopic sphincterotomy
EPBD	Endoscopic papillary balloon dilation
WBC	White blood cell
SD	Standard deviation
ASA	American Society of Anesthesiologists
AZT	Aztreonam

Introduction

Acute cholangitis is a morbid condition characterized by acute inflammation and bacterial infection of the biliary system [1]. Two factors are prerequisites for the development of acute cholangitis, i.e., biliary obstruction and

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bacterial growth in the bile duct. Although choledocholithiasis is the leading cause of acute cholangitis, patients with benign or malignant biliary strictures, biliary-enteric anastomotic stenosis, or indwelling biliary stent malfunction may present with acute cholangitis [2, 3].

Mild acute cholangitis improves with antibiotic therapy [4–8], but patients who do not respond to conservative treatment or those with moderate to severe cholangitis are likely to show rapid exacerbation of the condition, leading to considerably high morbidity and mortality [9, 10]. This group of patients should be offered early biliary decompression along with systemic antibiotic treatment [11–16]. Currently, endoscopic biliary drainage is considered as the first-choice method, because it is less invasive than percutaneous or surgical approaches and provides favorable results [17].

In the majority of patients who receive endoscopic biliary drainage for acute cholangitis, clinical manifestations (i.e., fever and abdominal pain) are improved within a few days after the procedure. Antibiotic therapy, however, is usually administered for 7–10 days despite the amelioration of acute cholangitis following endoscopic biliary drainage [18–20]. No study has yet provided substantial evidence supporting the need for long-term antibiotic therapy in patients with acute cholangitis that has subsided clinically with biliary drainage [21]. Because unnecessary prolonged antibiotic therapy may predispose patients to an increased risk of adverse reactions to antibiotics and facilitate the emergence of antibiotic-resistant bacteria, it is preferable to discontinue antibiotics as soon as possible. Therefore, we conducted the present study to evaluate the safety and efficacy of discontinuation of antibiotic therapy for acute cholangitis immediately after the resolution of clinical symptoms such as fever and abdominal pain following endoscopic biliary drainage.

Patients and methods

This was a prospective, single-arm, exploratory study. The protocol was approved by the Institutional Review Board of the University of Tokyo. All patients or their family members provided written informed consent before participation in the study, which complied with the Declaration of Helsinki. This study was registered with the University Hospital Medical Information Network-Clinical Trials Registry (UMIN-CTR), which was accepted by the International Committee of Medical Journal Editors (No. UMIN000001705).

Patients

Patients who were hospitalized for acute cholangitis at the Tokyo University Hospital were evaluated for inclusion in this study. Patients were eligible if they had moderate to severe acute cholangitis, which was diagnosed according to the *Tokyo Guidelines for the management of acute cholangitis and cholecystitis* [1]. We excluded patients who met any of the exclusion criteria listed in Table 1.

Table 1 Exclusion criteria

(1) Age <20 years
(2) Most severe cholangitis requiring catecholamine administration, mechanical ventilation, or hemodialysis
(3) Acute pancreatitis
(4) Active concomitant infections
(5) Inadequate drainage due to remaining biliary strictures
(6) Biliary stent occlusion
(7) Sclerosing cholangitis
(8) Previous choledochojejunostomy
(9) Previous heart valve replacement
(10) Severe cardiovascular disease
(11) Receiving maintenance hemodialysis
(12) History of antibiotic hypersensitivity
(13) Receiving cancer chemotherapy
(14) Receiving steroid or immunosuppressive agents
(15) Antibiotic administration in the 4 weeks preceding the observation period

Study design

Before endoscopic biliary drainage

Before endoscopic biliary drainage, all patients underwent hematological tests, including a complete blood cell count and evaluation of prothrombin time, hepatobiliary enzymes, C-reactive protein (CRP), amylase, and renal function. Abdominal ultrasonography or computed tomography was also performed. Following the collection of blood culture samples, intravenous antibiotic therapy was immediately initiated.

The antibiotic choice for patients with acute cholangitis was based on the *Tokyo Guidelines for the management of acute cholangitis and cholecystitis*: cefmetazole sodium (CMZ; 1 g, twice daily), a cephamycin-group antibiotic available in Japan, was used for moderate acute cholangitis, while meropenem hydrate (MEPM; 1 g, twice daily) was used for severe cases [1].

Endoscopic biliary drainage

Endoscopic retrograde cholangiopancreatography (ERCP) was performed as soon as practicable by an experienced therapeutic endoscopist. Unless the general condition of a patient did not enable them to receive conscious sedation, diazepam and pethidine hydrochloride were administered

intravenously. Throughout the procedure, blood pressure, pulse rate, and oxygen saturation were routinely monitored. Following successful cannulation of the bile duct, bile was aspirated for bacteriological culture, and low-osmolar non-ionic contrast medium was carefully injected to ascertain the cause of cholangitis. A 7-Fr endoscopic nasobiliary drainage (ENBD) tube or a 7- or 8.5-Fr plastic stent was then inserted without endoscopic sphincterotomy (EST) or endoscopic papillary balloon dilation (EPBD). The choice between ENBD and stent placement was at the discretion of the endoscopist, according to a patient's condition, as well as according to the bile characteristics.

Follow-up

Vital signs were checked every 8 h. Blood tests were assessed 1 and 3 days after drainage. Blood cultures were performed 1 and 2 days after drainage. The use of antipyretics was not allowed during the study. When a body temperature of <37°C was maintained for 24 h, administration of antibiotics was stopped. Blood tests were assessed 1, 3, and 5 days after the withdrawal of antibiotic therapy. In addition, blood culture was performed at the time of the withdrawal of antibiotics. In patients in whom the body temperature was ≥37°C for 4 days or more following the start of antibiotic therapy, a change of antibiotics was considered in consultation with an infectious disease specialist. If a body temperature of ≥37°C continued for 7 days or more, the patient was excluded from the study because the persistent fever was likely related to diseases other than cholangitis.

Definitive treatment for biliary obstruction (stone extraction or permanent stent placement) was performed at intervals of 3 days or more after a patient became afebrile, as previously reported [22, 23]. After hospital discharge, patients were required to visit our outpatient clinic at the end of the follow-up period (i.e., 4 weeks after the normalization of body temperature). Patients were also advised to immediately contact one of the study investigators if fever recurred. Blood examinations and abdominal ultrasonography were performed when necessary.

Outcome measures

The primary endpoint of the study was the recurrence of acute cholangitis within 3 days after the withdrawal of antibiotic therapy. Recurrent cholangitis was defined as a combination of fever (body temperature, ≥37°C) with recurrent elevation of the serum white blood cell (WBC) count and hepatobiliary enzymes. Secondary endpoints of the study were: (1) the period between initiation of therapy and normalization of serum inflammation markers (WBC and CRP); (2) the incidence of complications related to cholangitis (such as liver abscess and sepsis); and (3) the rate of re-administration of antibiotics.

Statistical analysis

Continuous variables are reported as means and standard deviation (SD); for non-parametric data, medians and ranges are shown. The patient characteristics and outcomes were compared using the χ^2 test or Fisher's exact test for categorical variables, Student's *t*-test for parametric data, and the Mann–Whitney *U*-test for non-parametric data. All analyses were performed by using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA).

Results

Between September 2007 and August 2009, the diagnosis of acute cholangitis was made in 117 patients at our hospital; of these, 20 patients were enrolled in this study (Fig. 1). After enrollment, 2 patients were found to be ineligible because of concomitant severe congestive heart failure and a history of transduodenal papilloplasty. Therefore, in all, the data for 18 patients were subjected to analysis. During the study period, no patients were excluded from the study because their body temperature of ≥37°C continued for 7 days or more. The characteristics of the patients are shown in Table 2. The majority of the patients were elderly [mean age, 73 (12) years], and 5 (28%) patients had severe underlying diseases [American Society of Anesthesiologists (ASA) score I in 3, II in 10, and III in 5]. All but one patient (who had bile duct cancer) had developed acute cholangitis because of bile duct stones. The severity of acute cholangitis was moderate in 14 patients and severe in 4 patients. One patient presented with shock, 2 presented with consciousness disturbance, and 1 had both symptoms (Table 3).

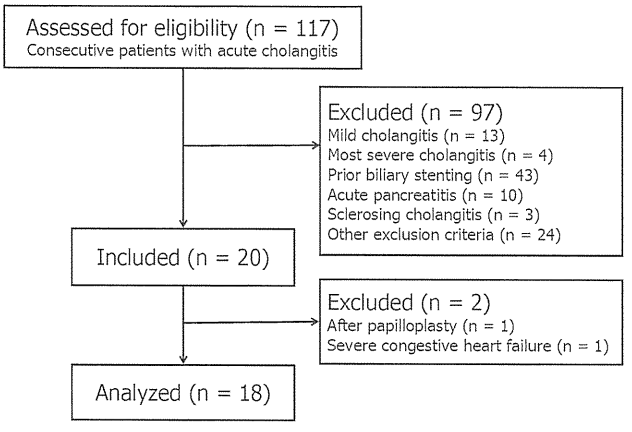

Fig. 1 Study flow chart

Table 2 Patient characteristics

No. of patients	18
Sex (male/female)	12/6
Age, years, mean (SD)	73 (12)
Disease severity (*n*)	
Moderate	14
Severe	4
Causes (*n*)	
Common bile duct stone	17
Bile duct cancer	1
ASA score (*n*)	
1	3
2	10
3	5
Comorbidity (*n*)	
Diabetes mellitus	4
Ischemic heart disease	4
Atrial fibrillation	2
Chronic lung disease	2
Asthma	1
Pulmonary embolism	1

ASA American Society of Anesthesiologists

Table 3 Clinical manifestations and patient laboratory data on admission

Body temperature, mean (SD)	38.4 (0.8)°C
WBC count, mean (SD)	11.2 (3.3) × 10^3/mm^3
CRP, mean (SD)	6.1 (7.5) mg/ml
Total bilirubin, mean (SD)	3.8 (1.9) mg/ml
ALT, mean (SD)	218 (96) IU/l
ALP, mean (SD)	747 (431) IU/l
Platelet count, mean (SD)	18.1 (6.9) × 10^4/mm^3
Shock, *n* (%)	2 (11)
Disturbance of consciousness, *n* (%)	3 (17)
Bacteremia, *n* (%)	6 (33)

WBC white blood cell, *CRP* C-reactive protein, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase

Table 4 Drainage method, bacterial species, and antibiotics

Drainage method (*n*)	
Plastic stent	11
ENBD	7
Blood culture (*n*)	
Klebsiella pneumoniae	3
Escherichia coli	2
Klebsiella oxytoca	1
Enterobacter aerogenes	1
Enterococcus faecium	1
Streptococcus bovis	1
Aeromonas caviae	1
Bile culture (*n*)	
Klebsiella species	11
Escherichia coli	7 (ESBL + 1)
Enterococcus species	7
Enterobacter species	3
Clostridium species	2
Streptococcus species	2
Others[a]	4
Antibiotics (*n*)	
CMZ	12
MEPM	3
CMZ → MEPM[b]	1
CMZ → SBT/ABPC[b]	1
CMZ → AZT[c]	1

ENBD endoscopic nasobiliary drainage, *MEPM* meropenem hydrate, *CMZ* cefmetazole sodium, *SBT/ABPC* sulbactam/ampicillin, *AZT* aztreonam, *ESBL* extended-spectrum β-lactamase antibiotic

[a] *Aeromonas hydrophila* (1), *Pseudomonas aeruginosa* (1), *Bacteroides* (1), gram-positive rods (1)

[b] MEPM and SBT/ABPC were chosen according to the identified causative bacteria and their sensitivity to antibiotics

[c] The antibiotic change was due to a suspected drug eruption

The mean (SD) duration of fever before admission was 29 (13) h. The mean (SD) time interval between the diagnosis of acute cholangitis and biliary drainage was 6 (3) h. The mean (SD) common bile duct diameter before drainage was 12 (5) mm. Seven patients underwent an ENBD tube insertion and 11 underwent a plastic stent placement (Table 4). Body temperature became normal after a median of 2 days (range 1–6) after biliary drainage; 10 patients became afebrile within 2 days after drainage, whereas only 1 patient remained febrile for more than 5 days after drainage. Fourteen patients (78%) had abdominal pain before drainage, and the pain disappeared within 2 days after drainage in all the patients. No early ERCP-related complications, which were defined according to the consensus guidelines [24], occurred.

Initially, 15 patients received CMZ and the remaining 3 received MEPM (Table 4). Antibiotic therapy was administered for a median duration of 3 days (range 2–7) (Fig. 2). The duration of antibiotic therapy in patients with moderate and severe cholangitis was 3 (range 2–7) days and 3.5 (range 2–4) days, respectively. No significant difference was observed in terms of the median period of antibiotic therapy between the 6 patients with bacteremia and the 12 patients without bacteremia [4 (range 2–7) days vs. 3 (range 2–7) days, $p = 0.6$]. In 2 patients, the antibiotic was changed according to the identified causative bacteria and their susceptibility to antibiotics (3 days after the start of initial antibiotic treatment in both cases). These patients became afebrile 1 and 3 days, respectively, after

the switching of antibiotics. In 1 patient, CMZ was changed to aztreonam (AZT) because of a suspected drug eruption.

The time-course of the mean WBC count and serum CRP levels in the 18 patients with acute cholangitis is shown in Fig. 3a. The mean WBC count decreased slightly before and 1 day after biliary drainage and rapidly returned to reference range on the day of the withdrawal of antibiotic therapy. Three days after the withdrawal of antibiotic therapy, the mean WBC count remained within the normal limits. In contrast, the mean CRP levels showed a marked increase before and 1 day after biliary drainage but decreased rapidly afterwards. No patient, however, had achieved normalization of CRP levels 3 days after the withdrawal of the antibiotic therapy. Serum hepatobiliary enzymes and bilirubin levels decreased steadily after biliary drainage despite the discontinuation of antibiotics (Fig. 3b).

Three days after the withdrawal of antibiotics, no recurrent cholangitis or cholangitis-related complications had occurred; that is, subsequent re-administration of antibiotics was not needed in any patient. In addition, during the follow-up period of 4 weeks, none of the 18 patients developed a recurrence of cholangitis or cholangitis-related complications.

The bacterial species isolated in blood and bile cultures are shown in Table 4. Six patients had positive blood cultures on admission, but blood cultures had become negative in all patients 1 day after biliary drainage and remained negative on the day of withdrawal of antibiotics. Bile cultures were obtained in 17 patients; multiple organisms were identified in 10 patients, and a single organism and no organism was found in 5 and 2 patients, respectively. *Klebsiella* species, *Escherichia coli*, and *Enterococcus* species were the most frequent bacterial species isolated from bile cultures.

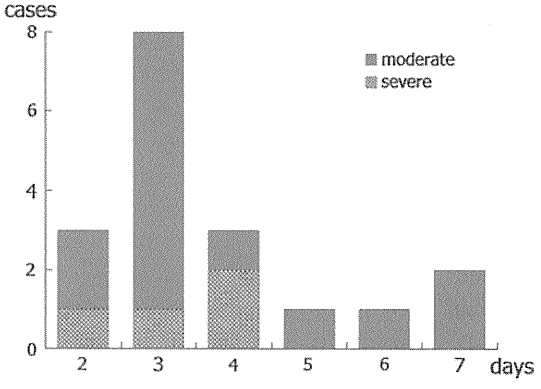

Fig. 2 Duration of antibiotic therapy in patients with moderate and severe acute cholangitis

Discussion

To date, according to our PubMed search (January 1951–April 2011) using the term "Cholangitis"[Mesh] AND "Anti-Bacterial Agents"[Mesh], there has been no prospective study evaluating the duration of antibiotic therapy in patients with acute cholangitis. Therefore, this is the first prospective study investigating the duration of antibiotic therapy in patients with moderate to severe acute cholangitis who received adequate endoscopic biliary drainage; here, we showed that discontinuation of antibiotic therapy 1 day after the abatement of fever was safe and effective. None of our patients developed recurrent acute cholangitis or infectious complications related to cholangitis after discontinuation of the antibiotic therapy.

The *Tokyo Guidelines for the management of acute cholangitis and cholecystitis* recommend the administration of antibiotics for 5–7 days in patients with acute cholangitis [1]. However, a low body of evidence supports this recommendation. In the present study evaluating a new approach, i.e., fever-based antibiotic discontinuation

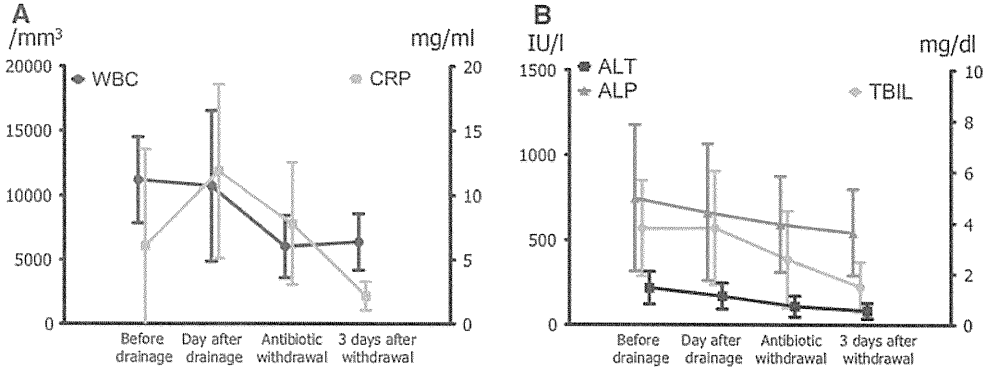

Fig. 3 **a** Time-course of serum white blood cell (*WBC*) count and concentrations of C-reactive protein (*CRP*) in patients with acute cholangitis. Data are expressed as means, SD. **b** Time-course of serum concentrations of total bilirubin (*TBIL*), alanine aminotransferase (*ALT*), and alkaline phosphatase (*ALP*) in patients with acute cholangitis. Data are expressed as means, SD

therapy, the median duration of antibiotic treatment was 3 days, which is consistent with the findings of a previous retrospective study [21]. In addition, the antibiotic treatment could be discontinued within 4 days in 14 out of the 18 patients (78%) included in our study. The remaining 4 patients needed 5–7 days of antibiotic therapy. In these patients, the causative bacteria were found to be resistant to the initial antibiotic therapy. Thus, close attention should be paid to the causative organism's susceptibility to antibiotics, especially when the fever persists for 5–7 days following endoscopic biliary drainage.

We observed bacteremia on admission in 6 patients, who satisfied the diagnostic criteria for sepsis [25]. The Surviving Sepsis Campaign recommends antibiotic administration for 7–10 days in patients with sepsis [25]. Again, no study has provided high-quality evidence to confirm this recommendation. In the present study, fever-based antibiotic therapy led to short-term administration (2–5 days) in 5 of the 6 septic patients. None of these patients developed sepsis-related complications during the 4 weeks after discontinuation of the antibiotic therapy, and their blood cultures remained negative following successful biliary drainage. The remaining 1 patient required 7 days of antibiotic therapy because the identified causative bacteria were resistant to the initial antibiotics. These results suggest that, even in patients with sepsis because of acute cholangitis, fever-based antibiotic therapy is sufficient, provided that early and adequate biliary drainage is performed. Because the number of patients with sepsis in our study was small, further investigations are needed to confirm this new concept.

The *Sanford guide to antimicrobial therapy 2009* [26] and the *Tokyo Guidelines for the management of acute cholangitis and cholecystitis* [1] recommend the use of broad-spectrum penicillin/β-lactamase inhibitors (e.g., ampicillin/sulbactam and piperacillin/tazobactam) in acute cholangitis. These drugs, however, are not covered by health insurance in Japan for the management of cholangitis. Therefore, we employed CMZ for moderate cases and MEPM for severe cases, on the basis of past usage experience. In accordance with the findings of reported series [11, 18–21, 27, 28], *Klebsiella* species and *E. coli* were the most commonly isolated bacteria from bile and blood in our patients with moderate cholangitis, whereas anaerobes and *Enterococcus* species were frequently isolated in cultures from patients with severe cholangitis. The selection of initial antibiotics in this study was considered to be appropriate.

There are several limitations to the present study. First, the sample size of 18 patients is small. This is due to the exploratory nature of our study. Nevertheless, in its prospective design with a strict follow-up protocol, it is noteworthy that none of our patients developed recurrent cholangitis or infectious complications after the discontinuation of antibiotic therapy. Second, our study included only 4 patients with severe cholangitis, making it difficult to evaluate the safety and efficacy of our antibiotic therapy in this group of patients. Third, our study lacked a comparison group (i.e., patients with acute cholangitis who received antibiotic therapy for 7 days after endoscopic biliary drainage irrespective of clinical improvement). Randomized trials would be needed to confirm our findings. However, it would be difficult to conduct a randomized trial because of the low incidence of recurrent cholangitis in our study. Fourth, the majority of our patients had bile duct stones as the cause of acute cholangitis; whether our findings can be extrapolated to patients with acute cholangitis due to other causes (e.g., stent malfunction, malignant biliary obstruction) is uncertain. We believe that our results warrant a further study with the same strategy in a larger group of patients. Last, we cannot rule out a synergistic effect of endoscopic biliary drainage and antibiotic treatment. Although a randomized controlled trial of endoscopic biliary drainage alone versus endoscopic biliary drainage plus antibiotic therapy would answer the question of whether antibiotic therapy can hasten clinical improvement in patients with acute cholangitis, we wonder if such a trial is justifiable.

In conclusion, fever-based antibiotic therapy appears to be sufficient when fever is abated by concomitant and adequate endoscopic biliary drainage in patients with moderate acute cholangitis. Because of the small patient population and the limited range of disease types in our study, we consider that a prospective multicenter trial of fever-based antibiotic therapy for a large number of patients with moderate to severe acute cholangitis of various causes is mandatory to confirm our results.

Conflict of interest The authors declare that they have no conflict of interest.

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