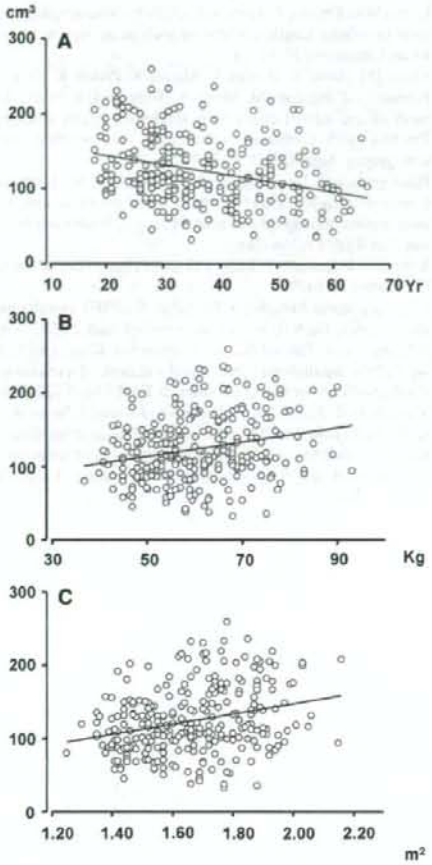

Fig. 2 Scatterplot of the spleen volume and age (**a**), body weight (**b**) and body surface area (**c**). The correlation was significant in each relation. A regression line is superimposed

Table 1 Distribution of the most ventral point of the spleen

Group	Zone in Method N					Zone in Method C		
	1	2	3	4	5	1	2	3
A								
n (%)	12(8)	45(30)	64(43)	21(14)	6(4)	5(3)	72(49)	71(48)
B								
n (%)	19(21)	52(57)	16(18)	4(4)	0(0)	14(15)	60(66)	17(19)

215 cm^3 in a normal population ($n = 140$). De La Grandmasion et al. [3] weighed the spleens of 684 postmortem Caucasian subjects. The subjects were selected from those that died of injury with a short survival time. They reported that mean spleen weight was 156 ± 87 g in males ($n = 355$) and 140 ± 78 g in females ($n = 329$). Spleen size on CT is relatively larger than that measured on postmortem. This may be due to the measurement method. Spleen size is measured after blood had been removed from spleen.

We found that spleen volume estimated by CT was significantly correlated with not only age, but also with BM and BSA. The results were consistent with previous data [2], but were discrepant with the data from another study [14] of autopsy cases. A weak correlation between spleen size and BM or BSA was also reported also by Prassopoulos and associates [13] who calculated the spleen volume by CT. One of the reasons for the discrepancy between our and Prassopoulos et al.'s results might be the subject age distribution. In our study, the ages ranged from 20 to 65 years and the ratio of those under 40 years of age was 62%; in their study, the ages ranged from 20 to 80 and the ratio of those under 40 years of age was only 20%. Meier and associates [12] reported that volume of adult spleen did not change significantly with age. In their report [12], a mean spleen volume age of 18–40 was 234 ml and age of 50–81 was 213 ml although the number of the subjects was small ($n = 57$).

We found the difference of spleen size by gender in Japanese population. The results were consistent with previous data [5]. However, previous data [13, 15] indicated no difference by gender in spleen size of 141 human fetuses and 153 children. The difference of spleen size by gender could be explained by the difference of the body size.

Conclusion

Spleen volume was measured by CT in 238 healthy donors for liver transplantation. Spleen size was significantly correlated with age and body size. Splenomegaly can be evaluated by the simple method on CT although the threshold must be changed by the age of the subject.

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Original Article

Double-dose double-phase use of second generation hepatitis B virus vaccine in patients after living donor liver transplantation: Not an effective measure in transplant recipients

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Aims: Post-transplant active immunization for chronic hepatitis B patients has been attempted in several studies with controversial results. We assessed the effect of a double-dose double-phase vaccination regimen among partial living donor liver recipients.

Methods: Eighteen patients who underwent liver transplantation (LT) for chronic hepatitis B and two non-hepatitis B virus (HBV)-infected patients who received hepatitis B core antibody (HBcAb)-positive donor organs were recruited 18–78 months after LT. All were on hepatitis B immunoglobulin (HBIG) mono-prophylaxis before and throughout vaccination, to maintain hepatitis B surface antibody (HBsAb) titers of more than 100 IU/mL. Recombinant hepatitis B surface antigen vaccine (40 μg) was administered intramuscularly during weeks 0, 4, 8, 24, 28 and 32.

Results: The patients consisted of 15 males and five females with a median age of 52 (39–59) years. None developed a sufficient HBsAb titer above 500 IU/mL by week 48. In two patients whose maximum HBsAb titer increased to above 300 IU/mL, we attempted to skip HBIG, but shortly thereafter the titer dropped below 100 IU/mL and HBIG administration was resumed. Although the HBIG dose was reduced during and after vaccination, cessation of administration was not achieved.

Conclusion: Double-dose double-phase use of second generation recombinant vaccine was not effective in this study population. The selected population should be targeted for a conventional vaccine regimen, and different approaches, such as strong adjuvant or pre-S containing protein, should be further tested in a larger number of patients after LT for chronic hepatitis B.

Key words: Hepatitis B vaccine, HBcAb positive donor, HBIG, lamivudine, liver transplantation, prophylaxis

INTRODUCTION

THE LONG-TERM use of hepatitis B immunoglobulin (HBIG) and/or nucleos(t)ide analog prophylaxis has dramatically improved survival rates after liver transplantation for hepatitis B virus (HBV)-related liver disease.[1] Historically, hepatitis B (HB) recurs in approximately 80% of liver transplant recipients with HBV-related liver diseases. The use of HBIG mono-prophylaxis has improved the rate of recurrent hepatitis to 35%.[2] Long-term use of HBIG therapy, however, is costly and there is insufficient evidence regarding the optimal length of administration. Lamivudine (LAM) mono-prophylaxis is less costly and is useful for decreasing the rate of hepatitis recurrence to less than 40%.[3,4] Emerging resistant strains are a concern in long-term follow-up for transplant recipients, however, and additional nucleos(t)ide analogs such as adefovir and entecavir are required.[5,6] The combination of HBIG and antiviral agents has decreased the rate of hepatitis B recurrence.[7,8]

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Active immunization against hepatitis surface antigens has been attempted in patients after liver transplantation for HB-related liver diseases.[9-11] Repeated vaccination effectively initiates the production of anti-hepatitis B antibodies and is thus followed by HBIG administration withdrawal.[9-11] This idea is theoretically a more economical and simple method compared to passive immunization or nucleos(t)ide analog administration. Contradictory results have been reported, however, and suitable candidates for this type of vaccination have not been determined.[12-15]

At the University of Tokyo, we use HBIG monoprophylaxis.[16] The ultimate goal of vaccination is to achieve sufficient production of anti-hepatitis B immunoglobulin and to discontinue further prophylaxis against recurrent hepatitis B. In the present study, we report the results of an active immunization protocol in chronic hepatitis B-related living donor liver transplantation (LDLT) recipients.

METHODS

Subject selection

PATIENTS WHO UNDERWENT LDLT at least 18 months before for HBV-related end-stage liver disease or received hepatitis B core antibody (HBcAb)-positive donor livers were enrolled if they were being treated with an HBIG prophylaxis protocol, free of nucleos(t)ide analogs, without co-infection with hepatitis C virus or human immunodeficiency virus, and if they had no evidence of HBV reactivation. Twenty Japanese patients provided informed consent to the study protocol. There were 15 men and five women with a median age of 52, ranging from 39 to 59 years. Etiologies of end-stage liver disease included chronic hepatitis B in 16, fulminant hepatic failure with a history of chronic hepatitis B in two, and end-stage liver disease due to autoimmune hepatitis and primary biliary cirrhosis in one each. Two non-HBV patients and one chronic hepatitis B patient received core antibody-positive donor organs. Nine patients with hepatocellular carcinoma were free from post-transplant recurrence with a median follow-up period of 43 (18–90) months.

The donors were 13 men and seven women ranging in age from 18 to 54 years and weighing 43–75 kg. Their relationship to the patients included 10 children, five spouses, three nephews, one sibling and one cousin. Right liver graft was performed in eight, extended right graft in one, right lateral graft in two, left lobe with caudate graft in seven and left lobe graft in two. Three donors were positive for both HB surface antibody (HBsAb) and HBcAb.

Pre- and post-LDLT follow-up protocol

The post-transplantation immunosuppression regimen consisted of steroid induction with tacrolimus, or cyclosporine in case of tacrolimus intolerance, for maintenance.[17] Among the 18 patients with hepatitis B virus infection, LAM 100 mg/day was given orally prior to LDLT. One patient received LAM for more than 1 year, one for 3 months, and 15 received LAM for less than 4 weeks. Of the 17 patients on LAM, a negative HBV-DNA load was confirmed preoperatively in nine cases. In eight patients whose HBV-DNA was detectable pre-transplant, LAM therapy was continued for 4 weeks after LDLT, and discontinued after confirming negative HBV-DNA. Postoperatively, HBIG (Mitsubishi Tanabe Pharma, Hebsbulin-IH, Tokyo, Japan) was administered to HBV-infected patients and those who received HBcAb-positive donor organs. Details of the HBIG administration protocol and doses are described elsewhere.[16] In brief, HBIG was administered to maintain the anti-HB surface antibody (HbsAb) levels at greater than 1000 IU/L for patients with HBV and greater than 500 IU/L for patients that received HBcAb-positive donor organs. After 1 year, 1000–2000 U was given intravenously indefinitely to maintain HbsAb levels of greater than 100 IU/L.[16,18]

Vaccination protocol

After obtaining informed consent, baseline laboratory tests were performed in all patients. Table 1 shows the baseline patient characteristics and laboratory findings. HBV-DNA was undetectable in all patients. Increased dose, namely "double-dose", of recombinant anti-HB vaccination (40 μg/2 mL; Heptavax II, Banyu Pharm, Tokyo, Japan) was injected bilaterally into the deltoid muscles (half dose each side). HBsAb titers were measured at week 0 and every 4 weeks thereafter. Vaccination was scheduled for two phases of three administration cycles at weeks 0, 4, 8, 24, 28 and 32.

During the protocol period, HBIG was administered 2 weeks before and after vaccination if necessary; either 1000 or 2000 IU was administered intravenously according to the previously measured HBsAb titer, as long as it was maintained above 100 IU/mL.

Study end-points and ethical considerations

The primary end-point of this study was the vaccine response. A significant increase, that is >500 IU/mL, in

Table 1 Patient characteristics

Factors	Values[1]
Age	52 (39–59) years
Men, women	15, 5
HBV-related hepatitis	18
HBcAb positive donor	3
Immunosuppression (Tac + CS), (CyA + CS)	15, 5
Months from LDLT (median)	45 (17–90) months
Pre-LDLT LAM	17
Duration of LAM administration before LDLT: <1 month, 1–12 months, >12 months	15, 1, 1
HBV-DNA positive at LDLT[2]	8
HBsAg titer at entry	138.5 (92–302) IU/mL
Aspartate aminotransferase	18.5 (7–30) IU/mL
Alanine aminotransferase	16 (5–30) IU/mL
Alkaline phosphatase	197 (103–528) IU/mL
γ-glutamyl transferase	33.5 (11–187) IU/mL
Lactate dehydrogenase	178 (99–315) IU/mL
Total bilirubin	0.9 (0.1–1.5) mg/dl

[1]Values are number or median (range). [2]HBV-DNA measured by transcription-mediated amplification methods. Lower detectable limit is 3.7 LEC/mL.
CS, corticosteroid; CyA, cyclosporine; HBsAb, hepatitis B surface antibody; HBcAb, hepatitis B core antibody; HBV, hepatitis B virus; LAM, lamivudine; LDLT, living donor liver transplantation; Tac, tacrolimus.

HBsAb 12 weeks after the last vaccination was considered effective. Secondary end-points were changes in the required HBIG dose.

The study protocol was approved by the institutional review board (No. P2005015-11X) and informed consent was obtained from each patient.

Statistical analysis

The number of units of HBIG administered before and during the study protocol was recorded, and the sum of the number of HBIG units administered at −24 to −1 weeks, 0–23 weeks, 24–47 weeks and 48–71 weeks was compared by the Friedman test.

RESULTS

ALL PATIENTS COMPLETED the full vaccination course. Double-dose vaccination was well-tolerated with the only side effect of local pain not requiring analgesics. During the study period, none of the cases developed active hepatitis or rejection. One patient developed kidney dysfunction during the study and was unable to complete the HBIG administration protocol (case #5).

Pre-, post- and maximum serum HBsAb titers are shown in Table 2. None of the cases developed a sufficient HBsAb titer level of >500 IU/mL (Fig. 1). None achieved cessation of HBIG administration.

Three cases (#2, #3, #20) developed maximum titers of 313, 408 and 469 IU/mL, and HBIG was thus discontinued in two of them (#2 and #3). The titers then decreased below 100 IU/mL in both cases, however, and HBIG administration was resumed (Fig. 2). Case #20 desired to continue HBIG administration despite the elevation of HBsAb titer to 469 IU/mL. Eventually, HBsAb titer dropped to 214 IU/mL and thus the vaccination was considered ineffective.

HBIG administration in four 24-week periods (−24 to −1, 0 to 23, 24 to 47, 48 to 71) in each case are shown in Table 2. In case 11, HBIG administration between weeks 48 and 71 was stopped due to the appearance of HBsAg and HBV-DNA. HBIG doses as a whole, excluding case 11, decreased over time ($P = 0.006$), as illustrated by the box plot (Fig. 3).

After the final vaccination, all patients were followed for a median of 17 (10–18) months. All patients are alive. In two cases (#11 and #13), serum HBsAg and HBV-DNA were observed at 4 and 8 months after the vaccination protocol, and 60 and 58 months after liver transplantation. In these two cases, the minimum HBsAb level was above 100 IU/mL even after our vaccination protocol. These cases are currently on antiviral therapy and are free from signs of active hepatitis.

DISCUSSION

ACTIVE IMMUNIZATION FOR chronic hepatitis B has been attempted in patients after liver transplantation. In early studies, commercially available recombinant vaccine was used in patients receiving HBIG mono-prophylaxis and was effective in 64–80% of patients.[9,10] This immune response was sustained for a longer follow-up period of 41 (31–85) months in 14 responders.[10] The use of a potential adjuvant in combination with recombinant vaccine is remarkably effective in 80% of post-transplant recipients, and the HBsAb titer was maintained for a long (8–27 months) follow-up period.[11] Studies by other groups, however, demonstrated unfavorable results in patients who were either on LAM prophylaxis or HBIG mono-prophylaxis.[12–14] Several factors are thought to

Table 2 Characteristics, HBsAb titer and dose requirement of HBIG

Case	Age	Sex	Etiology	Donor HBcAb	Pre-LT HBV-DNA	Pre-LT LAM	Timing of vaccination[†]	IS	Pre-HBsAb[‡]	Max HBsAb[‡]	End HBsAb[‡]	HBIG administration				Outcome[†] (recurrence[¶])
												−24–1 weeks[§]	48–71 weeks[§]	0–23 weeks[§]	24–47 weeks[§]	
1	52	F	PBC	+	Negative	NA	78	Tac	165	253	253	10	7	10	11	104, alive
2	46	F	AIH	+	Negative	NA	51	CyA	92	313	152	3	5	7	3	75, alive
3	54	M	B-FHF	−	Positive	Yes	48	Tac	122	408	136	12	5	8	5	65, alive
4	39	M	B-FHF	−	Positive	Yes	30	Tac	163	227	221	8	6	6	5	56, alive
5	50	M	BLC, HCC	+	Negative	Yes	90	Tac	175	79	48	6	5	6	7	115, alive
6	57	F	BLC	−	Positive	Yes	57	Tac	127	191	107	7	6	9	7	82, alive
7	47	F	BLC	−	Negative	Yes	56	Tac	140	186	132	7	6	6	6	81, alive
8	50	M	BLC, HCC	−	Negative	Yes	55	Tac	101	153	113	5	6	5	4	77, alive
9	50	M	BLC,	−	Positive	Yes	51	Tac	117	114	70	7	6	6	6	75, alive
10	51	M	BLC, HCC	−	Positive	Yes	49	Tac	111	293	138	8	5	6	5	74, alive
11	48	M	BLC, HCC	−	Positive	Yes	48	Tac	281	171	131	11	1	10	4	71, alive, rec 60
12	59	M	BLC	−	Positive	Yes	43	CyA	160	176	162	6	8	6	6	69, alive
13	52	M	BLC, HCC	−	Positive	Yes	43	Tac	209	210	132	8	7	6	6	67, alive, rec 56
14	57	F	BLC, HCC	−	Negative	Yes	41	CyA	109	220	220	12	7	10	8	67, alive
15	56	M	BLC, HCC	−	Negative	None	40	Tac	144	188	93	9	10	12	12	60, alive
16	54	M	BLC	−	Negative	Yes	40	Tac	137	190	190	8	6	5	5	60, alive
17	50	M	BLC	−	Negative	Yes	20	CyA	95	262	140	6	6	6	6	38, alive
18	51	M	BLC, HCC	−	Negative	Yes	19	CyA	135	179	115	11	8	10	9	44, alive
19	52	M	BLC, HCC	−	Negative	Yes	18	Tac	152	245	121	14	7	12	6	43, alive
20	53	M	BLC	−	Negative	Yes	17	Tac	302	469	214	14	10	12	12	37, alive

[†]Number indicates months after liver transplantation. [‡]IU/mL. [§]Number indicates vials of HBIG (1V = 1000 Units) used during the period. [¶]Number indicates months after liver transplantation, when HBV-DNA became positive.

AIH, autoimmune hepatitis; B-FHF, hepatitis B related fulminant hepatic failure; BLC, hepatitis B related liver cirrhosis; CyA, cyclosporine A; FHF, fulminant hepatic failure; HBsAb, hepatitis B surface antibody; HBcAb, hepatitis B core antibody; HBIG, hepatitis B immunoglobulin; HCC, hepatocellular carcinoma; IS, immunosuppression; LAM, lamivudine; LC, liver cirrhosis; LT, liver transplantation; PBC, primary biliary cirrhosis; Tac, tacrolimus.

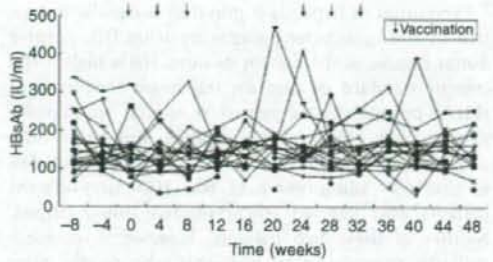

Figure 1 Change in serum hepatitis B surface antibody (HBsAb) titers before and during the vaccination protocol. Heptavax II (40 μg) was administered at weeks 0, 4, 8, 24, 28 and 32. HBsAb levels were between 100 IU/mL and 200 IU/mL in most cases. There was no significant response to vaccination throughout the study.

contribute to a good response, such as younger age, use of an adjuvant to the vaccine and negative HBV-DNA preoperatively. Use of LAM was once speculated to have a negative effect, and, in another study, Angelico *et al.* failed to reveal a favorable effect of mono-prophylaxis with HBIG.[12]

In our study, the double-dose double-phase use of a second generation recombinant hepatitis B vaccine was tested. Our institution applies an HBIG mono-prophylaxis protocol against hepatitis B recurrence. For non-replicate HBV disease, LAM is discontinued at the time of transplantation. During this study, HBIG was administered throughout the vaccination protocol according to Binzele's report,[11] and anti-hepatitis B antibody levels of greater than 500 IU/mL were determined to be effective. Unfortunately, none of the 20 patients developed an adequate anti-hepatitis B antibody titer after two vaccination cycles. Although the dose of HBIG administration decreased during and after the vaccination protocol, we did not consider it a significant change, since none achieved cessation of HBIG administration. The possible factors contributing to vaccination failure among the subjects are that 15 of the 20 patients were aged 50 years or older, eight of 18 chronic hepatitis B patients had a HBV replicative state at the

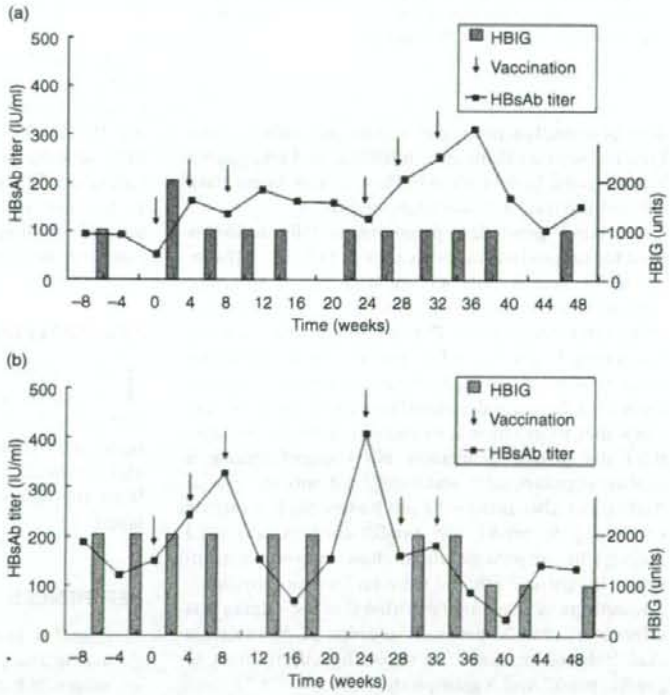

Figure 2 Hepatitis B surface antibody (HBsAb) titers in response to vaccination. (a) Case 2: 46-year-old female with auto-immune hepatitis (AIH), hepatitis B core antibody (HBcAb)-positive donor. (b) Case 3: 54 year-old male with B-fulminant hepatic failure. Hepatitis B immunoglobulin (HBIG) administration was skipped in response to elevated HBsAb level. The HBsAb titer then dropped and HBIG administration was resumed in both cases.

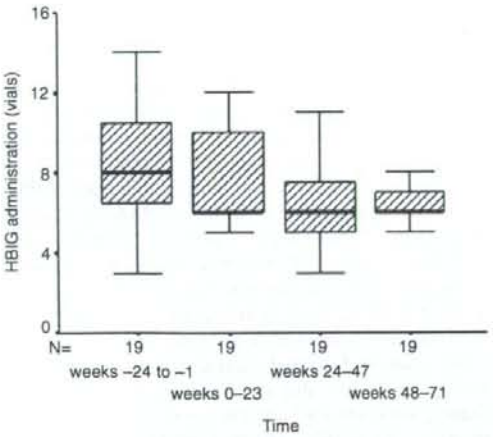

Figure 3 Hepatitis B immunoglobulin (HBIG) administration in four 24-week periods (−24 to −1, 0–23, 24–47, 48–71) is graphically shown by box plot. The dose of HBIG administration decreased over the time period ($P = 0.006$). Box plot explanation: upper horizontal line of box, 75th percentile; lower horizontal line, 25th percentile; horizontal bar within box, median; upper horizontal bar outside box, 90th percentile; lower bar outside box, 10th percentile.

time of transplantation, use of corticosteroids in combination with a calcineurine inhibitor, and one patient was receiving hemodialysis.[20] These factors might have affected the results of this study negatively.

A second generation recombinant HB vaccine is used for prophylaxis in the healthy population. HBsAg-specific T and B cells are induced and a sufficient amount of HBsAb can be produced to neutralize circulating HB virus particles. The recombinant HB vaccines containing S, pre-S2 and/or pre-S1, the so-called the third generation vaccines, have immunogenic advantages over the second generation recombinant HB vaccines; they more efficiently induce an immune response than the second generation HB vaccines among a healthy population,[21,22] and induce not only anti-S antibodies, but also anti-pre-S2 antibodies. Such a vaccine containing S, pre-S1 and pre-S2 antigens was used among Chinese patients who underwent liver transplantation for chronic HB and were on LAM prophylaxis.[23] The authors of that study reported that the vaccine was effective in 10 of 20 patients. An earlier study by Karasu *et al.*,[13] however, failed to show the effectiveness of pre-S1, pre-S2 and S gene products.

Prevention of hepatitis B infection is equally important in HBsAg-negative patients receiving HBc-positive donor organs, as the risk for de novo HB is high.[24] The current standard of care for transplant recipients of HBcAb-positive donor organs is similar to that for patients with chronic HB, including long-term HBIG and/or nucleos(t)ide analog.[18,24–26] In our study, HB vaccine was administered to two HBV non-infected patients who received HBcAb-positive donor organs. Neither of these two patients, however, responded. Pediatric transplantation recipients who receive prior vaccination under an immunization program are likely to achieve a high anti-HB titer by active immunization after LDLT.[27] LAM prophylaxis was withdrawn after 2 years if an adequate anti-hepatitis B titer was achieved. Soejima *et al.*[28] recently reported that 6 of 11 Japanese patients receiving liver transplants for fulminant hepatitis B or for non-HBV diseases with HBcAb-positive donor organs had seroconversion. The tested patients were younger, with a median age of 33 years. These patients were on combination prophylaxis with LAM and HBIG, and HBIG was withdrawn after vaccination. The question remains, however, as to whether LAM therapy can be discontinued in the future among this population.

Vaccination may be promising in selected populations, such as in younger recipients or in those with fulminant hepatitis or HBsAg-negative recipients receiving HBcAb-positive donor organs. For patients older than 50 with vaccination failure or chronic hepatitis B patients, a different approach may prove optimal, such as the use of a pre-S containing vaccination. The research findings for the use of such vaccines are controversial, however, warranting further study.

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Persistent, Undetected *Trichomonas vaginalis* Infections?

To the Editor—In a recent large, randomized, controlled trial [1], 64 participants received a diagnosis of infection with *Neisseria gonorrhoeae, Chlamydia trachomatis,* or *Trichomonas vaginalis* during intervals in which they reported not having sex. We considered the problems that might lead to this paradoxical situation, including errors in laboratory testing [2] and patient reporting [3] and treatment failure [4]. Our findings regarding *N. gonorrhoeae* and *C. trachomatis* infection were consistent with these scenarios; however, the findings regarding infection with *T. vaginalis* were surprising.

The HIV prevention trial was performed in 3 clinics that specialize in the treatment of sexually transmitted diseases [1]. At the initial visit, participants were counseled, examined, and tested for sexually transmitted diseases, including HIV infection. Infections were treated according to the treatment guidelines of the Centers for Disease Control and Prevention [4]. Outcomes were measured at 3, 6, 9, and 12 months after enrollment.

Nucleic acid amplification tests for *C. trachomatis* and *N. gonorrhoeae* infection were performed on urine specimens. *T. vaginalis* was cultured (from women only) using the InPouch TV test (BioMed Diagnostics) or modified Diamond's medium. Sensitivity has been estimated to be 82.4% for the InPouch TV test and 87.8% for culture on modified Diamond's medium; specificity for both culture methods is nearly 100% [5, 6]. At follow-up visits, vaginal swab specimens were obtained by the participant (at the Denver, CO, and Long Beach, CA, clinics) or a clinician (at the Newark, NJ, clinic).

Persons who reported having no sex partners were considered to have had no sex during that 3-month interval. Infections were considered to be new if the person had a negative test result at the beginning of the trial or had been treated for another infection at least 14 days before the infection was detected. Each participant could contribute up to 4 three-month intervals to the analysis. We measured associations with each new sexually transmitted infection by multivariate logistic regression models with generalized estimating equations with use of SAS (SAS Institute). [7].

Six hundred sixty-eight persons reported having had no sex during 1125 three-month intervals; 64 new infections were diagnosed among 59 of these persons during these intervals. Among persons who reported having no sex, test results were more likely to be positive for *T. vaginalis* (3.9%; the test for *T. vaginalis* had the highest reported specificity), compared with *N. gonorrhoeae* (1.4%; $P < .01$) or *C. trachomatis* (2.4%; $P = .1$).

The risk of new infection with *T. vaginalis* was nearly identical for women who did (4.2%) and did not (3.9%) have sex during the 3 months before receiving the diagnosis. New *T. vaginalis* infection was more likely to occur in women who were aged 26–39 years (6.2%), compared with women who were aged 16–25 years (2.0%; adjusted OR, 3.4; 95% CI, 1.2–9.5). Infection was more likely to occur in women who had a sexually transmitted infection at baseline (9.3%), compared with women who did not (2.0%; adjusted

Figure 1. *Trichomonas vaginalis* infections detected among women in intervals during which they were not having sex. Each row represents the history of 1 woman. Shaded areas are intervals during which the woman reported not having sex. Positive *(+)* and negative *(−)* test results for *T. vaginalis* are indicated for each woman.

OR, 6.2; 95% CI, 2.5–15.4). There was no increase in the risk of *T. vaginalis* infection among women who were infected with *T. vaginalis* during the immediately preceding interval (4.4%), compared with women who were not (3.9%). However, 13 (62%) of 21 new infections occurred in women who had been previously infected with *T. vaginalis*, and 11 (85%) of 13 had negative test results during the immediately preceding interval (figure 1).

Some of the women might have acquired infections during sexual contact that they did not report, and some might have had infections that were not detected at the baseline visit. However, many women were treated for infection, had negative test results, and then had positive test results again, which suggests that *T. vaginalis* was undetected by testing but still present for months after treatment. The possibility of long-term asymptomatic carriage is consistent with the age distribution of infected women; *T. vaginalis* is found more often in older women [8, 9]. This pattern is different from the pattern for bacterial sexually transmitted diseases but similar to that for incurable viral infections, such as herpes simplex virus type 2 [10]. Trials have suggested cure rates of >90%, but most have tested women once within a few weeks after treatment [11]. When women were tested again a few months after treatment, some of the previously cured women had infection detected again [11], and none of the studies continued testing women beyond a few months. Cultures might not detect infections if the concentration of *T. vaginalis* is low, which would be expected in asymptomatic infections [6, 12, 13]. Nucleic acid amplification tests may be better, but reports are inconsistent and the tests are not commercially available in the United States [14]. Similarly, self-obtained vaginal swab specimens occasionally miss infections, but the sensitivity of tests performed with self-obtained specimens has compared favorably with that of tests performed with clinician-obtained specimens [15].

Treatment failure could explain many of our findings, because 13 women had a documented preceding infection. However, our results were not simply attributable to treatment failure. Most of the women ($n = 11$) had an intervening negative test result before having a positive result during an interval when they reported not having sex. This suggests that, after treatment, *T. vaginalis* infection can become nondetectable for months and then reappear. Because these findings were unexpected and obtained with a small number of participants, additional studies are needed to confirm or refute these observations.

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Detection of HIV Type 1 Load by the Roche Cobas TaqMan Assay in Patients with Viral Loads Previously Undetectable by the Roche Cobas Amplicor Monitor

To the Editor—In March 2008, the Roche Cobas TaqMan assay replaced the Roche Cobas Amplicor Monitor, version 1.5, for measuring plasma HIV type 1 (HIV-1) load in Japan. This has resulted

in the detection of an HIV-1 load >50 copies/mL in some of the patients whose HIV-1 load had been undetectable (<50 copies/mL) by the Amplicor Monitor over several years and for whom antiretroviral treatment regimens had not been changed [1, 2].

A total of 1387 HIV-1–infected patients visited our outpatient clinic from March through June 2008, and their HIV-1 load was measured by the TaqMan assay. Among these patients, 876 regularly visited the clinic (once every 1–3 months) and had an undetectable HIV-1 load by the Amplicor Monitor at the last visit. Surprisingly, the TaqMan assay detected an HIV-1 load >50 copies/mL in 253 (28.9%) of the 876 patients, although antiretroviral treatment had not been modified for these patients. Furthermore, another 22 patients (2.5%) were found to have an HIV-1 load >40 copies/mL with use of the TaqMan assay. The same assay also detected HIV-1 RNA at levels lower than the linear range of the assay (<40 copies/mL) in 128 (14.6%) of the 876 patients.

We analyzed the relationship between TaqMan detectability and time during which the HIV-1 load was undetectable by the Amplicor Monitor. This time was defined as the period from the first HIV-1 load undetectable by the Amplicor Monitor to the viral load first measured by the TaqMan assay, without any HIV-1 load rebound or blip detected during the period. Interestingly, among the patients who had a viral load undetectable by the Amplicor Monitor for <1 year, 43.7% had an HIV-1 load >50 copies/mL detected by the TaqMan assay; among the patients who had a viral load undetectable by the Amplicor Monitor for >4 years, 18.5% had an HIV-1 load >50 copies/mL detected by the TaqMan assay (figure 1). Conversely, 37.3% of patients who had a viral load undetectable by the Amplicor Monitor for <1 year had HIV-1 RNA undetectable by the TaqMan assay, and 70.2% of patients who had a viral load undetectable by Amplicor Monitor for >4 years had an HIV-1 load undetectable by the TaqMan assay. Thus, the proportion of patients who had an HIV-1 load >50 copies/mL was inversely correlated with the duration that the viral load was undetectable ($R^2 = 0.895$), and the proportion of patients with undetectable viral load was positively correlated with the duration that the viral load was undetectable ($R^2 = 0.979$). These findings indicate that the longer the effective treatment, the greater the number of patients with HIV-1 RNA undetectable by the TaqMan assay.

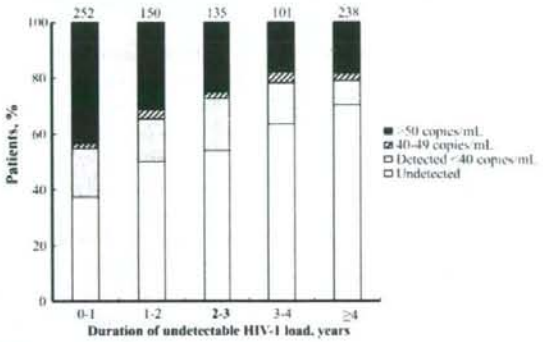

Figure 1. Results of the TaqMan assay and duration of undetectable HIV-1 load in 876 patients whose HIV-1 load was undetectable (<50 copies/mL) when the last Amplicor Monitor was performed. The number of patients is shown above each bar.

We observed significant discrepancy of HIV-1 detectability between the TaqMan assay and the Amplicor Monitor [3–5]. The TaqMan assay detected HIV-1 RNA in a significant percentage of treated patients with HIV-1 loads previously undetectable by the Amplicor Monitor; this is confusing to clinicians and patients and may be a critical problem in ongoing clinical trials of antiretroviral treatment. To determine the permissible range of detectable HIV-1 load during successful antiretroviral treatment, year-long clinical follow-up of treated patients is necessary. Our observation revealed that the detection rate of HIV-1 RNA with use of the TaqMan assay was inversely correlated with the previous duration of undetectable HIV-1 load, suggesting that long-term antiretroviral treatment can further suppress HIV-1 load even after it has decreased to below the detection limit of the Amplicor Monitor.

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Use of Active Surveillance Cultures in Intensive Care Units

To the Editor—I appreciated the systematic review by McGinigle et al. [1] of active surveillance cultures (ASCs) for methicillin-resistant *Staphylococcus aureus* (MRSA) in the intensive care unit (ICU) but question their conclusions about the lack of enough robust evidence to provide definitive recommendations for the use of ASCs in the control of MRSA infection. The authors included 20 studies, but only 13 of these studies seem to be original intervention studies that assess the effect of ASCs on the rate of MRSA infection. In addition, as the authors indicate, the methodology and/or robustness of many of these studies are not optimal.

Because I have been interested in this subject for many years, I have collected the literature on another 7 published nonpediatric and nonneonatal ICU studies that merit inclusion in the systematic review by McGinigle et al. [1–8], as well as another 6 neonatal and/or pediatric ICU studies (not referenced). It would be interesting to understand why these adult ICU studies were not included in the systematic review by McGinigle et al. [1]. Three of these studies were interrupted-time series, and 1 was a controlled before-and-after study; both of these methodologies are fairly robust. It is true that not all of the studies included weekly ASCs, but this seems to be a questionable exclusion criteria if a reduction in the rate of MRSA infection was still reported. However, the consistency of positive findings in the adult ICU studies is worth emphasizing (i.e., ASCs can aid in the control of MRSA infection in the ICU, particularly when ASCs are combined with at least 1 of the following: patient and environmental decontamination and hand-hygiene initiatives).

It is noteworthy that, of the 20 studies (13 in the systematic review and the 7 aforementioned adult ICU studies), only 3 do not mention use of additional hygiene and/or decontamination procedures (4 of 26 studies, if the neonatal and/or pediatric ICU studies are also considered). Moreover, although all but 1 study reported a reduction in the rate of MRSA infection after introduction of ASCs, this 1 study was notable for its poor hand-hygiene compliance, late isolation of MRSA-positive patients, and absence of any decontamination or disinfection.

Finally, the rating of high-quality interrupted-time series as only "fair" evidence by McGinigle et al. [1] is debatable. The most important difference between my interpretation of the data and that of McGinigle and colleagues is my observation of the consistency, strength, temporal relationship, and plausibility of the evidence; this insight led me to conclude that ASCs should be recommended as standard practice, particularly in high-risk areas, such as ICUs, where there is a high rate of hospital-acquired MRSA infection and a great risk of MRSA infection.

Incidentally, my colleagues and I conducted a study [9] (which was incorrectly referenced in the systematic review) that demonstrated a two-thirds reduction in the rate of MRSA infection (a decrease from >15% to ~5% of ICU admissions, not the 11% reduction stated in the systematic review by McGinigle et al. [1]). Moreover, this reduction was entirely attributable to a reduction in the number of MRSA isolates from clinical specimens, not screening specimens. Although the number of MRSA isolates is only a surrogate for infection, it is more closely indicative of infection than colonization; that the number of MRSA isolates is a surrogate marker of colonization was wrongly implied by Milstone and Perl [10] in their accompanying editorial commentary. In support of the number of MRSA isolates being a surrogate marker of infection, there was a significant reduction in both length of stay and glycopeptide use associated with the introduction of ASCs.

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INTERNAL MEDICINE

ORIGINAL ARTICLE

Clinical and Radiological Features of *Pneumocystis* Pneumonia in Patients with Rheumatoid Arthritis, in comparison with Methotrexate Pneumonitis and *Pneumocystis* Pneumonia in Acquired Immunodeficiency Syndrome: A Multicenter Study

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Abstract

Objective To elucidate the clinical and radiological features of *Pneumocystis* pneumonia (PCP) in patients with rheumatoid arthritis (RA), compared with methotrexate (MTX) pneumonitis in RA and *Pneumocystis* pneumonia in acquired immunodeficiency syndrome (AIDS).

Subjects and Methods Retrospective analysis of 14 PCP cases in RA (RA-PCP), 10 MTX pneumonitis cases in RA (MTX-P) and 11 PCP cases in AIDS (AIDS-PCP) from 9 centers in the Kanto area in the last 6 years.

Results Compared with AIDS-PCP, both RA-PCP and MTX-P developed more rapidly, showing higher serum CRP and lower plasma β-D-glucan levels, and more severe oxygenation impairment. In most of the RA-PCP cases, a high dose of corticosteroid was administered as adjunctive therapy, resulting in a favorable outcome. The mortality was 14% in RA-PCP, 0% in AIDS-PCP and 0% in MTX-P cases. In RA-PCP patients the CD4 cell count showed only mild suppression, not reaching the predisposing level for PCP in HIV infection, suggesting that there are risk factors for RA-PCP other than immunosuppression. Radiologic analysis revealed some characteristic patterns of each disease. In MTX-P, diffuse homogeneous ground glass opacity (GGO) with sharp demarcation by interlobular septa (type A GGO) was found in 70%, while in AIDS-PCP diffuse, homogeneous or nonhomogeneous GGO without interlobular septal boundaries (type B GGO) was predominant (91%). In RA-PCP, type A GGO was found in 6 cases and type B GGO in 5 cases, showing the complex nature of this disease.

Conclusion RA-PCP differed considerably from AIDS-PCP clinically and radiologically. Clinically it occurred without severe immunosuppression, and showed characteristic aspects, with more intense inflammation and less parasite burden. Radiologically it mimicked MTX-P in some cases sharing the conspicuous CT features of MTX-P, rendering the distinction of these two disorders difficult.

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Key words: rheumatoid arthritis, *Pneumocystis* pneumonia, methotrexate pneumonitis, β-D-glucan, CT, acquired immunodeficiency syndrome (AIDS)

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Introduction

Pneumocystis pneumonia (PCP) is one of the uncommon but serious, life-threatening complications in patients with rheumatoid arthritis (RA) receiving treatment with methotrexate (MTX) (1-3). However it is often difficult to establish a definitive diagnosis, because the clinical and radiological presentations closely resemble those of MTX induced pneumonitis (MTX-P). Both are characterized by acute, progressive respiratory symptoms and diffuse bilateral infiltrates on chest radiography. The clue enabling a distinction lies in the detection of *Pneumocystis jirovecii* (*P. jirovecii*). However it is well known that traditional staining is often not sensitive enough in PCP in non-HIV conditions (4). Recently polymerase chain reaction (PCR) has been widely used for detection of this organism, with satisfactory sensitivity (5-7), but this method alone has the problem of false-positivity (8, 9). The subsidiary role of serology, especially measurement of β-D-glucan, has not received much attention.

We conducted a retrospective multicenter study to elucidate the clinical and radiological characteristics of RA-PCP, comparing it with MTX-P and also with AIDS-PCP , in order to discuss the problem of the differential diagnosis of these diseases.

Materials and Methods

Fourteen cases of PCP during treatment for RA were identified at 7 participating centers in Tokyo and its suburbs by practicing rheumatologists or pneumologists from April 2001 to August 2006. Ten cases of MTX-P were also identified at these centers during the same period. For comparison with RA-PCP, 11 cases of AIDS-PCP were randomly selected at two AIDS centers in Tokyo from March 2001 to December 2005. All of these cases were enrolled in the study after confirming that they had sufficient clinical information and imaging materials obtained before the beginning of definitive treatment for pulmonary events. Among them, 32 cases had thin section CT images of less than 2 mm collimation, while the other 3 cases had CT images using 5 mm collimation, both of good quality.

A diagnosis of PCP (both in RA and AIDS) was based on satisfaction of all of the following criteria; a) symptoms such as fever, cough and progressive dyspnea, associated with diffuse bilateral infiltrates on chest radiography, b) detection of *P. jirovecii* by traditional staining (Grocott or Diff-Quik or Giemsa staining) or by PCR in respiratory specimens, c) significantly elevated plasma (1→3)-β-D-glucan (β-D-glucan) level.

β-D-glucan was measured either with the β-glucan test WAKO (Wako Pure Chemical Industries, Tokyo, Japan) or with the FUNGITEC G test MK (Seikagaku Corp., Tokyo, Japan).

MTX-P was diagnosed based upon the same clinical presentations mentioned above and exclusion of infection, especially PCP, through intensive diagnostic procedures such as bronchoscopy or examination of sputum and measurement of plasma β-D-glucan. Clinical improvement following corticosteroid therapy was also taken into account.

The clinical background and preceding disease course of each patient was assessed with special attention to the dose and duration of antirheumatic drugs and also to the underlying disease. Clinical data at the recognition of the event, the clinical course and its outcome were evaluated.

Chest radiography and computed tomography (CT) were reviewed by two diagnostic radiologists. CT findings were categorized into three patterns: a) diffuse ground glass opacity (GGO) distributed in a panlobular manner, that is, GGO was sharply demarcated from the adjacent normal lung by interlobular septa (type A GGO) (Fig. 1A, Fig. 1B), b) diffuse GGO homogeneous or somewhat not homogeneous in distribution but without sharp demarcation by interlobular septa (type B GGO) (Fig. 2A, Fig. 2B), c) another pattern such as mixed consolidation and GGO (type C) (Fig. 3). The occurrence of each pattern was assessed in each group. The clinical features of each group and also their relationship with CT patterns were analyzed statistically using the Mann-Whitney-U test or Fisher's exact test.

Results

Patient characteristics

Table 1 shows the epidemiologic features of these patients. The RA-PCP group consisted of 14 patients, 2 men and 12 women, and they had a mean age of 66.5 years. *P. jirovecii* was detected in bronchoscopic specimens in 5 cases, in sputum examination in 9, by traditional staining in 3, by PCR in 11 cases. RA had been diagnosed for 11 years (mean). All had a history of receiving corticosteroid therapy and 13 patients were receiving MTX therapy (mean duration of 36.3 months) at the evolution of the lung events. Four patients were concomitantly receiving anti-TNF agents (three cases infliximab and one case etanercept). Six patients had

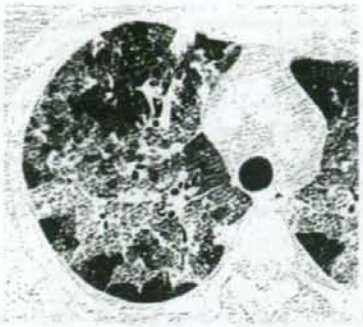

Figure 1A. Type A ground glass opacity (GGO): GGO sharply demarcated from adjacent normal lung by interlobular septa. Methotrexate pneumonitis (MTX-P) was revealed in a 57-year-old woman who had received MTX therapy for 9 years for rheumatoid arthritis (RA). CT image shows homogeneous GGO which is clearly demarcated from adjacent normal lobules by interlobular septa.

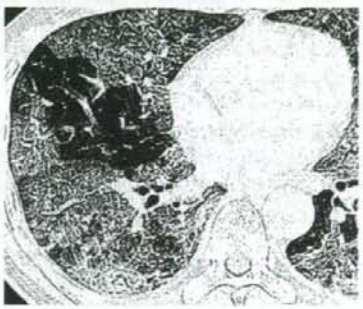

Figure 1B. Type A GGO. A case of MTX-P in a 61-year-old man. He had received MTX therapy for 7 years. He had severe respiratory distress on admission, was treated with mechanical ventilation and resulted in favorable outcome. CT shows homogeneous GGO sharply demarcated from non-affected lung by interlobular septa.

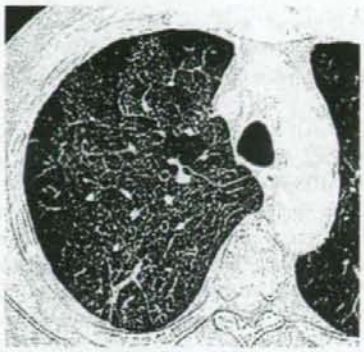

Figure 2A. Type B GGO: homogeneous or nonhomogeneous GGO without sharp demarcation. An 83-year-old man had been treated for RA for 9 years with prednisolone (PSL) and MTX. MTX-P was diagnosed through exclusion of infection with bronchoscopy. CT shows nonhomogeneous GGO without sharp demarcation.

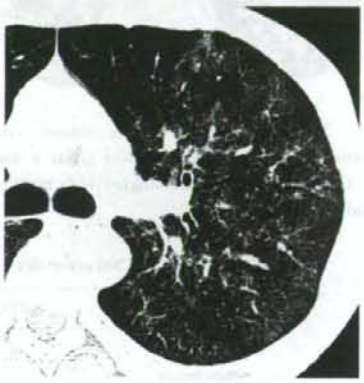

Figure 2B. Type B GGO. A 53-year-old man had been diagnosed as HIV positive for 6 years. *Pneumocystis* pneumonia (PCP) was confirmed through positive staining for *Pneumocystis jirovecii* (*P. jirovecii*) in his sputum. CT shows diffuse, nonhomogeneous GGO without obvious demarcation.

chronic interstitial lung disease (ILD) defined by the presence of honeycombing in CT.

The AIDS-PCP group included 11 cases, 10 men and 1 woman, with a mean age of 39.8 years. All were seropositive for the human immunodeficiency virus (HIV) antibody. *P. jirovecii* was detected by traditional staining in 5 cases and by PCR in 9.

The MTX-P group included 10 patients, 3 men and 7 women, had a mean age of 67.4 years. The diagnosis was made through exclusion of infection, especially PCP, by negative staining or PCR for *P. jirovecii* in 11 cases, and by low plasma β-D-glucan level in 2 cases. They had suffered from RA for 12 years (mean), and 7 of them had a history of corticosteroid therapy. MTX had been given for a duration of 31.0 months (mean). Two patients were concomitantly receiving anti-TNF agents (one case infliximab only once, one case etanercept). None of the patients of this group had ILD.

Clinical features

The clinical features of these three groups are shown in Table 2. Fever, cough and progressive dyspnea were predominant symptoms among all three groups. These symptoms preceded the diagnosis of the event with a period of 8.0±6.0 days in the MTX-P group, 7.6±6.4 days in the RA-PCP group, and 37.9±24.3 days in the AIDS-PCP group. The disease development was significantly faster in the MTX-P and the RA-PCP groups than the AIDS-PCP group. The serum CRP level was significantly higher in RA-PCP and MTX-P group than AIDS-PCP group (Fig. 4). The

plasma β-D-glucan level of AIDS-PCP was significantly higher (965.4 pg/ml, mean) than that of RA-PCP (98.5 pg/ml, mean). The value was below the cut-off level in MTX-P cases.

The CD4 cell count was 780.0±497.1/μl in the MTX-P group, 793.2±274.8/μl in the RA-PCP group, and 62.9±79.5/μl in the AIDS-PCP group, respectively. Taking the preserved serum immunoglobulin G (IgG) level into account, RA-PCP patients, as with MTX-P patients, showed a slight to moderate degree of immunosuppression, which was markedly different from AIDS-PCP patients (Fig. 5). PCP is usually considered to be an opportunistic infection under immunosuppressed conditions, but the immunological status was not greatly impaired in RA-PCP group. Severe hypoxemia necessitating oxygen supplementation was seen in 8 (80%) MTX-P cases, 11 (78.6%) RA-PCP cases and 3 (27.8%) AIDS-PCP cases. In summary, RA-PCP patients, along with MTX-P patients, showed more rapid clinical development, had significantly higher CRP level, lower β-D-glucan level, and worse oxygenation than AIDS-PCP patients.

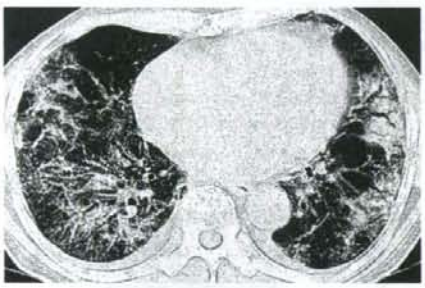

Figure 3. **Type C : other type, mixed GGO and consolidation. A 69-year-old man was given a diagnosis of MTX-P. CT shows GGO intermingled with multiple foci of consolidation.**

Patient outcome

All RA-PCP patients were treated with Trimethoprim-Sulfamethoxazole (TMP-SMX), together with corticosteroids (pulse therapy using methyl-prednisolone 500-1000 mg/day for 3 days in 4 cases, pulse therapy+oral prednisolone in 9 cases and oral prednisolone in 1 case). Eleven cases needed oxygen supplementation but none required mechanical ventilation. Two cases died despite intensive treatment, while the other 12 cases recovered completely within 3 or 4 weeks after admission (Table 2).

Eleven cases of the AIDS-PCP cases were treated with TMP-SMX. Adjunctive corticosteroids were given in 5 cases (oral prednisolone for 2 weeks). Three cases needed oxygen supplementation but none required mechanical ventilation. All patients recovered.

All MTX-P patients received steroid pulse therapy followed by 30-60 mg/day oral prednisolone as an initial dose with tapering. Although two cases required mechanical ven-

Table 1. **Patient Characterstics**

	MTX-P	RA-PCP	AIDS-PCP
number	10	14	11
male:female	3; 7	2;12	10; 1
age‡	67.4(46~88)	66.5(52~80)	39.8(29~58)
Detection of *P jirovecii* organism			
bronchoscopy	(7)†	5	4
sputum		9	7
traditional staining		3(Grocott 1, Diff-Quik 2)	5(Grocott 5, Diff-Quik 3)
PCR		11	9
duration of RA(years)‡	12(8-28)	11(1-26)	
Corticosteroids user	7	14	none
Methotrexate user	10	13	none
Mtotrexate duration(mo)‡	31.0(4~104)	36.3 (1 to 78)	
anti-TNF agents	1 infliximab, 1 ethanercept	3 infliximab, 1 ethanercept	none
lung comorbidity	0	6 chronic ILD	0

† done and resulted in negative study

‡ data are shown at median (with range)

abbreviations: MTX-P = methotrexate pneumonitis, RA-PCP = *Pneumocystis* pneumonia in rhuematoid arthritis, AIDS-PCP = *Pneumocystis* pneumonia in AIDS, AIDS = acquired immunodeficiency syndrome , *P jirovecii* = *Pneumocystis jirovecii* , Grocott = Grocott methenamine silver staining, Diff-Quik = Diff-Quik staining, PCR = polymerase chain reaction

Table 2. Clinical Features

	MTX-P(n=10)	RA-PCP(n=14)	AIDS-PCP(n=11)
duration of symptoms(days) before diagnosis	8.0±6.0	7.6±6.4	37.8±24.3
cough	5(50%)	5(42%)	7(64%)
fever	4(40%)	9(75%)	8(73%)
dyspnea	8(80%)	10(83%)	6(55%)
Alb (g/dl)	2.95±0.43	3.20±0.43	3.36±0.46
LDH (IU/l)	427.1±158.5	435.1±141.6	430.4±150.0
CRP (mg/dl)	11.6±6.2	8.6±4.8	2.3±2.2
KL-6 (U/ml)	814.3±757.5	1204.0±827.0	2490.8±1853.3
β-D-glucan (pg/ml)	below cut off level	98.5±94.8	969.5±1064.6
Leukocyte count(/μl)	7913.3±1851.8	8126.4±3284.3	7154.5±3433.4
Lymphocyte count (/μl)	1096.3±792.9	1028.7±599.6	963.2±684.9
CD4 cell count (/μl)	780.0±497.1	793.2±274.8	62.9±79.5
IgG (mg/dl)	1551±367	1056±340	n.d.
O_2 supplementation needed	8(80%)	11(78.6%)	3(27.8%)
ventilater needed	2(20%)	0	0
use of adjunctive corticosteroids	10(100%)	10(71.4%)	5(27.2%)
outcome(number of death)	0	2(14.3%)	0

* data are presented at median(with standard deviation)

abbreviations : Alb = serum albumin, LDH = lactate dehydrogenase, CRP = C-reactive protein, β-D-glucan = (1→3)-β-D-glucan, IgG = immunogloblin G

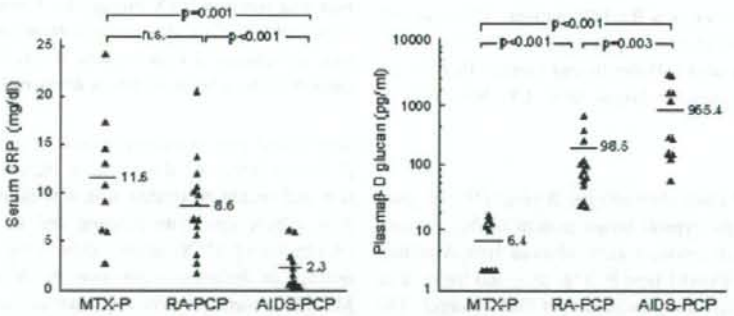

Figure 4. Serum CRP and plasma β-D-glucan in the three groups. CRP is significantly higher in MTX-P and *Pneumocystis* pneumonia in RA patients (RA-PCP) than *Pneumocystis* pneumonia in AIDS patients (AIDS-PCP), while β-D-glucan is significantly lower in RA-PCP than AIDS-PCP.

tilation, all recovered well.

Radiologic features

All patients showed diffuse bilateral infiltrates on chest radiography which, by itself, is neither specific nor pathognomonic for any of these three disorders. Through the analysis of CT images, we found three patterns of opacities, as mentioned above. The occurrence rates of these three patterns in each group are shown in Table 3. In the MTX-P group, the type A pattern predominated, noted in 7 cases (Fig. 1A, Fig. 1B), while type B was found in 2 (Fig. 2A), and type C in 1 case (Fig. 3). Type A was the most predominant image pattern for MTX-P. On the other hand, in the AIDS-PCP group, type A was found only in 1 case,

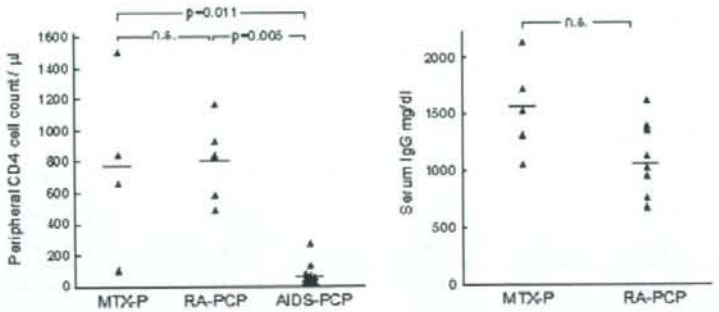

Figure 5. Immunological status of each group represented by peripheral CD4 cell count (measured in every group) and serum IgG (not measured in AIDS-PCP group). Both RA-PCP and MTX-P show a relatively preserved immunological condition in contrast with AIDS-PCP.

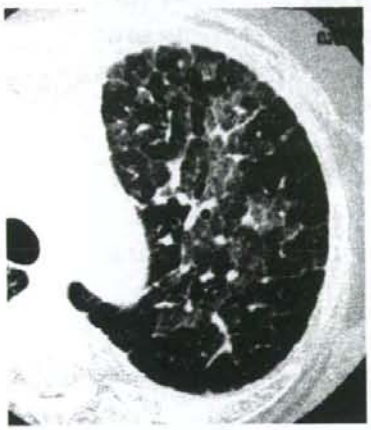

Figure 6A. GGO seen in a RA-PCP patient. A 71-year-old woman had received MTX therapy for 6 years. PCP was diagnosed based on elevated β-D-glucan and positive PCR for *P. jirovecii* in bronchoalveolar lavage fluid. CT shows type A GGO.

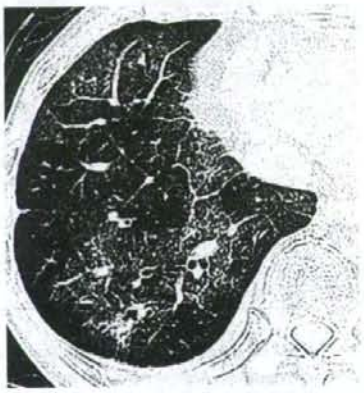

Figure 6B. GGO seen in a RA-PCP patient. A 64-year-old man had received MTX therapy for 5 years. *P. jirovecii* was identified with Grocott staining with marked elevation of serum β-D-glucan. CT shows type B GGO, nonhomogeneous pattern without lobule to lobule demarcation.

while the other 10 cases showed type B (Fig. 2B), suggesting type B to be the typical image pattern of this disease. Among the RA-PCP group, 6 cases showed type A pattern (Fig. 6A), 5 cases showed type B (Fig. 6B), and three cases type C, showing the complex nature of this disorder. The occurrence of type A GGO in RA-PCP did not differ significantly from that of MTX-P. We analyzed the relationship of these image patterns in CT and clinical features, but failed to find any relevance (data not shown).

Discussion

MTX is now widely used for the treatment of RA, because of its efficacy and low toxicity. In association with the increased use of MTX, serious and life-threatening lung complications have been increasingly reported (1-4, 10-12). One is PCP and another is MTX-P. Both diseases develop acutely and may sometimes result in serious consequences. PCP is an infectious disease in an immunosuppressed condition and should be treated with antimicrobial agents. MTX-P is a hypersensitivity reaction and should be treated by withdrawal of MTX, often followed by corticosteroids. To distinguish between these two conditions, RA-PCP and MTX-P, is therefore very important in the clinical context of acute onset lung injury during the treatment for RA with MTX.

The distinction, however, is often very difficult to make because of their similar clinical presentations. Imaging features are also so similar that no definitive difference has been reported between the two. Above all, the detection of *P. jirovecii*, which is mandatory for the diagnosis of PCP, is often very difficult in RA-PCP patients. In PCP patients without AIDS such as those of connective tissue disorders (CTD) receiving immunomosuppresive therapy (13), it is well documented that the organism numbers of *P. jirovecii* are significantly fewer in respiratory specimens (14-17). In