MBL is encoded by *MBL2*, which is located on chromosome 10, and six *MBL2* single nucleotide polymorphisms (SNPs) are associated with serum levels and/or functions of MBL. Three nonsynonymous nucleotide substitutions in exon 1 change the wild *A* allele to the three variant alleles (*A/B*, *A/C*, and *A/D*), which disrupt the collagenous structure and the formation of functional oligomers. The other alleles, *H/L*, *X/Y*, and *P/Q*, are distinguished by the SNPs in the promoter and 5′-untranslated regions, and the *X* allele shows the lowest transcriptional activity among them [5]. Because of strong linkage disequilibrium, seven haplotypes are commonly observed and often classified into three groups of higher producing (*HYPA*, *LYPA*, and *LYQA*), lower producing (*LXPA*), and nonfunctional (*LYPB*, *LYQC*, and *HYPD*) haplotypes.

These genetic variations that result in MBL deficiency are associated with a wide variety of diseases, including respiratory tract infections, presumably because of the leak of circulating MBL into inflamed airways [6]. However, *MBL2* polymorphisms show conflicting results and confer either resistance or susceptibility toward pulmonary TB [2]. According to some studies, MBL deficiency is associated with protection against TB disease, raising the hypothesis that the uptake of microorganisms by phagocytes is enhanced by MBL binding, which results in the promotion of infection by intracellular pathogens [7,8]. In contrast, other investigators have suggested that high MBL levels have a protective effect against TB [9,10].

Immune responses control *Mtb* infection in the latent phase, but *Mtb* is reactivated from an immunological equilibrium to develop TB disease [11]. The persistence of the latency period in adult patients with TB varies greatly among individuals, and this may reflect the duration of successful *Mtb* containment. During this process, it is believed that pathogenic factors, including different genotype strains, may also play a role [12]. We found a protective role of the interferon gamma receptor 2 gene (*IFNGR2*) polymorphism against TB; furthermore, we found that the resistant alleles tended to be less frequent in younger patients at diagnosis when we investigated polymorphisms in the Th1-immune response genes in Vietnamese patients with TB [13]. Grant et al. also found an age-dependent association of thymocyte selection-associated high mobility group box gene (*TOX*) variants in Morocco and Madagascar and highlighted the importance of age at TB diagnosis, which is correlated with the duration of the latency period in endemic areas [14]. The inconsistent association between *MBL2* and TB in different studies may be attributable to the different stages of *Mtb* infection from latent TB infection (LTBI) to TB disease; therefore, in the present study, we explored whether *MBL2* polymorphisms or MBL levels are associated with the development of active TB in apparently immunocompetent patients of various ages or the stage of LTBI in Viet Nam, a country with high TB prevalence.

2. Materials and methods

2.1. Study population

The patients and controls were recruited from Hanoi, Viet Nam [13,15,16]. In total, 832 patients (age, 41 ± 14.4 years; 77.6% males) without a previous TB episode were recruited immediately after the diagnosis of new smear-positive pulmonary TB was made. Pulmonary physicians treated them with anti-TB drugs according to the guidelines of the national TB program. Fifty-three HIV-positive patients with TB, four with no information about HIV status, and nine with missing age data were excluded from further analysis. *Mtb* genotyping method was described elsewhere [16]. Beijing genotype of *Mtb* isolates was distinguished from non-Beijing genotype in 429 TB patients with no HIV infection.

The control group for this genetic association study consisted of 556 healthy volunteers (age, 36 ± 10.3 years; 48.6% males) who had the same ethnicity and were residents in the same area of Hanoi city. Information of their LTBI status was not available, but 109 disease-free healthcare workers (HCWs; age, 34 ± 10.1 years; 23.9% males) were also recruited and their LTBI status was assessed by an enzyme-linked immunosorbent assay (ELISA)-based interferon gamma release assay (IGRA; QuantiFERON-TB Gold In-Tube™, Cellestis, Victoria, Australia) [17]. All were unrelated Vietnamese. Informed consent was obtained from all participants. The study protocol was approved by the ethics committees of the Ministry of Health, Viet Nam (4481/QD-BYT, 2529/QD-SYT), the National Center for Global Health and Medicine (NCGM-A-000185-00, 63), and the Research Institute of Tuberculosis (RIT/IRB25-1, 25-2), Japan.

2.2. Haplotype analysis of MBL2 SNPs in the Vietnamese HCWs and patients with TB

Genomic DNA samples from the 109 HCWs and 156 patients with TB were randomly subjected to polymerase chain reaction (PCR) amplification of the *MBL2* promoter and exon 1 regions with the primers 5′-GACCTATGGGGCTAGGCTGCTGAG-3′ and 5′-CCCC AGGCAGTTTCCTCTGGAAGG-3′ using TaKaRa LA Taq (TaKaRa, Shiga, Japan). The amplified products (1112 bp) were purified and sequenced with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) using the 3130xl Genetic Analyzer (Applied Biosystems). The synonymous SNP in exon 4 (rs930507) was amplified by PCR with the primers 5′-CTTTG TACCAGTCTGTCTGTTCAC-3′ and 5′-GGCCTGGAACTTGACACACAAG GC-3′ and genotyped using the restriction fragment length polymorphism method with *Ban* II (TaKaRa).

2.3. Plasma MBL level assay

Plasma MBL levels in samples were assayed by ELISA (Human MBL Quantikine ELISA Kit; R & D Systems, Minneapolis, MN, USA), which can specifically detect oligomeric forms of natural human MBL in serum, heparinized plasma, and EDTA plasma samples. Whole blood was divided into an EDTA tube and a negative control tube for the IGRA (nil tube). Plasma was separated immediately from the EDTA tube, whereas the nil tube was incubated at 37 °C for 16–24 h and centrifuged to separate the plasma. MBL levels in the plasma from the two procedures were compared for 31 individuals, and the coefficient of variation was calculated as 13.2%. Because incubation with heparin did not affect the results considerably, MBL levels were assayed in plasma supernatants from the control tubes for 109 HCWs and 65 patients with TB before the initiation of anti-TB treatment (0 month), after the initial phase of treatment (2 months), and at the end of treatment (7 months). These subjects were selected randomly from the abovementioned 156 patients.

2.4. MBL2 X/Y and A/B genotyping

X/Y (rs7096206) and *A/B* (rs1800450) polymorphisms were amplified in one DNA fragment by PCR using primers 5′-ACCTGG GTTTCCACTCATTCTCAT-3′ and 5′-CCCCAGGCAGTTTCCTCTGGAA GG-3′. An amplified product of 623 bp was digested with *Btg* I (New England Biolabs, Ipswich, MA, USA) to genotype *X/Y* or with *Ban* I (New England Biolabs) to genotype *A/B*, and they were electrophoresed on 2% agarose gels with ethidium bromide. Genotypes were determined by the length of the digested PCR products (*Y* allele with 540 bp after *Btg* I digestion and *A* allele with 536 bp after *Ban* I digestion).

2.5. Statistical analysis

Frequencies of haplotypes containing multiple polymorphic sites were estimated by Haploview ver. 4.2 [18]. Each individual's haplotypes were estimated by the PHASE program v.2.1 [19]. The association between the *MBL2* polymorphisms and plasma MBL levels after logarithmic transformation was analyzed using a multiple regression model. Differences in levels among the *MBL2* diplotype-based groups were further assessed by one-way analysis of variance (ANOVA) with the Tukey–Kramer method. Hardy–Weinberg exact tests were conducted to examine whether the genotype frequencies in the populations were compatible with Hardy–Weinberg equilibrium. TB development associated with a particular *MBL2* diplotype was assessed by odds ratios (ORs) and 95% confidence intervals (CIs) using a logistic regression model in which the interaction term between a particular MBL diplotype and age was also considered. The age-dependent trend for the presence of the *MBL2* diplotype in the patient population and their subgroups divided by *Mtb* genotypes was assessed by the change in odds at the 10-year interval in another logistic model.

Plasma MBL levels between groups with and without LTBI, as estimated by the IGRA results, were compared by analysis of covariance after adjusting for age and gender. The overall effects of *MBL2* diplotype, age, and gender on plasma MBL levels throughout the anti-TB treatment period were assessed using a random intercept model. All statistical analyses were performed using JMP ver. 9.0.0 (SAS Institute, Cary, NC, USA). Stata ver. 11 (StataCorp, College Station, TX, USA) was also used for the random intercept model analysis. *P*-values of <0.05 were considered statistically significant.

3. Results

3.1. Distribution of MBL2 haplotypes in the Vietnamese population

The *HYPA*, *LYPA*, *LYQA*, *LXPA*, and *LYPB* haplotypes in the Vietnamese HCWs and TB patients were estimated by the PHASE program (Table 1). The *LYQC* and *HYPD* haplotypes were not found. Thus the five haplotypes were observed in the Vietnamese population and they were identical to those directly determined by long-range PCR from other Asian populations [20,21]. Promoter −503 A/G SNP (rs7100749) was also polymorphic in the Vietnamese population; the −503 A allele was strongly associated with the *LYPA* haplotype. No novel mutation was found in the 156 patients with TB, including the randomly selected 65 individuals whose plasma MBL levels were measured afterwards in this study. Exon 4 synonymous C/G SNP (rs930507) is known to affect the MBL level of *LXPA* [22], but the *LXPA-rs930507 G* haplotype was rarely found; the estimated frequency was 0.02 in the Vietnamese population. Therefore, rs930507 was excluded from further analysis.

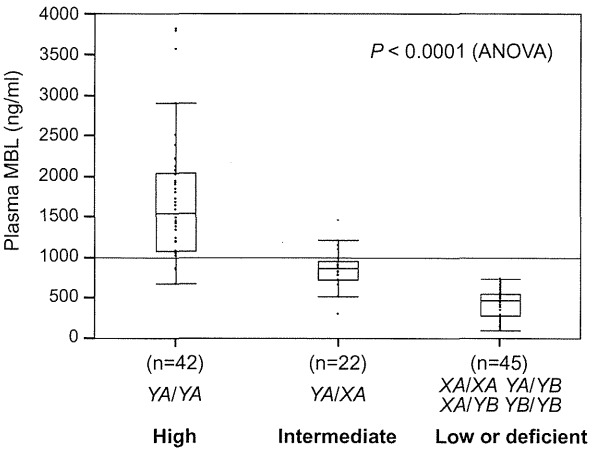

Fig. 1. Mannose-binding lectin *(MBL)2* diplotypes and plasma MBL levels in healthcare workers. Box-and-whisker plots of plasma MBL levels according to *MBL2* diplotypes of *X/Y* and *A/B* polymorphisms in healthcare workers. Each dot represents an individual. Differences in levels among *MBL2* diplotype-based groups were assessed by one-way analysis of variance.

3.2. MBL2 haplotypes associated with plasma MBL levels in the population

In agreement with previous studies [3], *X/Y* and *A/B* polymorphisms were strong determinants of plasma MBL levels in the multiple regression model ($P < 0.0001$ and $P < 0.0001$). *H/L* was only weakly associated with MBL in the same model ($P = 0.0378$). Neither *P/Q* nor age was associated with MBL levels in these 109 Vietnamese HCWs ($P = 0.1566$ and $P = 0.2484$). On the basis of these findings, the *MBL2* diplotypes were divided into three groups according to MBL levels, i.e., high (*YA/YA*), intermediate (*XA/YA*), and low or deficient (*XA/XA*, *YA/YB*, *XA/YB*, and *YB/YB*; Fig. 1). MBL levels were actually different among the three groups ($P < 0.0001$ by ANOVA), and the difference was significant between any two of the three groups (data not shown). The plasma level that defines MBL deficiency has not been clearly determined, but a recent study with a large sample size ($n = 1037$) using the same ELISA kit adopted ⩽1000 ng/ml as the category for partial and severe MBL deficiency [23]. Our classification almost matched with this definition, as MBL levels were >1000 ng/ml in 38/42 (90.5%) of the *YA/YA*-carrying individuals and ⩽1000 ng/ml in 45/45 (100.0%) of the *XA/XA*- or *B*-carrying individuals.

3.3. YA/YA diplotype associated with protection against TB in younger patients

Because plasma MBL levels largely depended on the combinations *X/Y* and *A/B*, frequencies of these variants were compared between all patients with TB and the controls. The genotype

Table 1
Estimated haplotypes and their frequencies in the Vietnamese healthcare workers ($n = 109$) and TB patients ($n = 156$).

	Promoter								Exon1		HCWs		TB patients	
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	*n*	Frequency	*n*	Frequency
	−550						−221		+4	G54D				
Haplotype	*H/L*						*X/Y*		*P/Q*	*A/B*				
HYPA	G	G	A	A	A	ins	G	C	C	G	97	0.445	136	0.436
LYPA	C	A	A	A	A	ins	G	C	C	G	16	0.064	24	0.077
LYQA	C	G	C	G	G	del	G	T	T	G	21	0.096	26	0.083
LXPA	C	G	A	A	A	ins	C	C	C	G	46	0.211	74	0.237
LYPB	C	G	A	A	A	ins	G	C	C	A	38	0.174	52	0.167

(1) rs11003125, (2) rs7100749, (3) rs11003124, (4) rs7084554, (5) rs36014597, (6) rs10556764, (7) rs7096206, (8) rs11003123, (9) rs7095891, (10) rs1800451.
Abbreviations: ins, insertion; del, deletion.
Statistically significant difference between the haplotype frequencies of 156 TB patients and those of 109 HCWs was not observed.

Table 2
MBL2 diplotypes in controls and patients with TB.

MBL levels	Diplotype	Full population					≤45 years					>45 years				
		Controls (n = 556)		TB (n = 765)		OR (95% CI)	Controls (n = 436)		TB (n = 457)		OR (95% CI)	Controls (n = 120)		TB (n = 308)		OR (95% CI)
		No.	(%)	No.	(%)	[P value]	No.	(%)	No.	(%)	[P value]	No.	(%)	No.	(%)	[P value]
High	*YA/YA*	224	(40.3)	262	(34.2)	**0.77 (0.62–0.97) [P = 0.028]**	178	(40.8)	135	(29.5)	**0.61 (0.46–0.80) [P = 0.0004]**	46	(38.3)	127	(41.2)	1.13 (0.73–1.74) [P = 0.66]
Intermediate	*YA/XA*	148	(26.6)	231	(30.2)		122	(28.0)	154	(33.7)		26	(21.7)	77	(25.0)	
Low	*XA/XA*	35	(6.3)	50	(6.5)		23	(5.3)	30	(6.6)		12	(10.0)	20	(6.5)	
	YA/YB	98	(17.6)	142	(18.6)		76	(17.4)	91	(19.9)		22	(18.3)	51	(16.6)	
	XA/YB	33	(5.9)	56	(7.3)		24	(5.5)	34	(7.4)		9	(7.5)	22	(7.1)	
	YB/YB	18	(3.2)	24	(3.1)		13	(3.0)	13	(2.8)		5	(4.2)	11	(3.6)	

TB development associated with *YA/YA* was assessed by odds ratios (OR) with non-*YA/YA* as a reference. *P* values and ORs with confidence intervals (CIs) shown in bold type are statistically significant.

Table 3
Tendency for the presence of high or low MBL level diplotypes in the order of age strata: subgroup analysis by *Mtb* strains.

Age (years)	TB (n = 765)	(%)	Beijing (n = 250)	(%)	Non-Beijing (n = 179)	(%)
High MBL level (*YA/YA*) diplotype (n/N)						
Total	262/765	(34.2)	87/250	(34.8)	69/179	(38.5)
16–25	34/122	(27.9)	10/39	(25.6)	9/28	(32.1)
26–35	51/168	(30.4)	19/68	(27.9)	7/32	(21.9)
36–45	50/167	(29.9)	19/53	(35.8)	9/29	(31.0)
46–55	73/180	(40.6)	27/64	(42.2)	26/47	(55.3)
56–65	33/81	(40.7)	7/19	(36.8)	10/24	(41.7)
66-	21/47	(44.7)	5/7	(71.4)	8/19	(42.1)
OR per 10-year change (95% CI)	**0.85 (0.76–0.94)**		**0.77 (0.63–0.94)**		**0.82 (0.67–0.99)**	
Low MBL levels (XA/XA and YB-positive) diplotypes (n/N)						
Total	272/765	(35.6)	85/250	(34.0)	67/179	(37.4)
16–25	50/122	(41.0)	19/39	(48.7)	12/28	(42.9)
26–35	58/168	(34.5)	27/68	(39.7)	9/32	(28.1)
36–45	60/167	(35.9)	16/53	(30.2)	13/29	(44.8)
46–55	59/180	(32.8)	21/64	(32.8)	14/47	(29.8)
56–65	27/81	(33.3)	2/19	(10.5)	12/24	(50.0)
66-	18/47	(38.3)	0/7	(0.0)	7/19	(36.8)
OR per 10-year change (95% CI)	1.05 (0.94–1.16)		**1.43 (1.16–1.78)**		0.98 (0.81–1.19)	

The trend for the presence of the corresponding diplotype was calculated as an odds ratio in a logistic model when the patients were 10 years younger at the time of diagnosis. The corresponding *P*-values are also shown in the Section 3. The bold font denotes statistically significant odds ratio (OR); CI, confidence interval. The frequencies of diplotypes in TB patients with Beijing genotype and with non-Beijing genotype are shown in Supplementary Table.

distribution of the *X/Y* and *A/B* polymorphisms did not deviate from the Hardy–Weinberg equilibrium. We found that *YA/YA* was significantly associated with protection against TB (P = 0.028, OR, 0.77; 95% CI, 0.62–0.97; Table 2), even after adjustment for gender (data not shown). When *YA/YA* was further analyzed together with age (≤45 years or >45 years), the genotype–age interaction was significant (P for interaction = 0.018). Therefore, the patients with TB and the corresponding controls were divided into two subgroups. A significant negative association was observed between TB and *YA/YA* only in the subgroup (mean age = 32) equal to or younger than 45 years old (P = 0.0004, OR, 0.61; 95% CI, 0.46–0.80; Table 2).

Considering the importance of the genotype–age interaction in TB pathogenesis, we investigated the age-dependence of the *YA/YA* frequencies in the patient group. When the patients were 10 years younger at the time of diagnosis, the trend of carrying *YA/YA* was significantly lower (P = 0.0021; Table 3), whereas such age-dependency was not observed in the control subjects (data not shown). The frequencies of *B*-allele-positive or *XA/XA* diplotypes resulting in low or deficient MBL levels were not significantly different between patients and controls, regardless of age stratification (Table 3).

Genetic information about the pathogen was available for 429 patients with TB among the HIV-negative cases. Beijing genotype *Mtb* strains are known to infect Asians and are spreading worldwide [24]. These strains were isolated from 250 patients in our Vietnamese study, and non-Beijing strains were found in 179 patients [16]. Regardless of *Mtb* genotype, the *YA/YA*-resistant diplotype was significantly less frequent when patients were 10 years younger at the time of diagnosis (P = 0.02 in the Beijing strain group and P = 0.04 in the non-Beijing strain group; Table 3), whereas low-level or deficient *MBL2* diplotypes containing the *B*-allele or *XA/XA* were more frequent in the younger age group only among patients with TB of the Beijing genotype strains (P = 0.0014; Table 3). When clinical information from the 506 patients with TB was analyzed, *MBL2* variations were not associated with TB severity, as estimated by the area of lung infiltration or recurrence of TB after therapy (data not shown).

3.4. Plasma MBL levels and diplotype frequencies not associated with the status of LTBI

Among the 109 HCWs, 68 (age, 33 ± 9.5 years; 22.1% males) were IGRA-negative while 41 (age, 35 ± 11.1 years, 26.8% males) were IGRA-positive. Plasma MBL levels and *MBL2* diplotype frequencies were not significantly different between IGRA-positive and -negative HCWs (Fig. 2), even after adjusting for age and gender (data not shown).

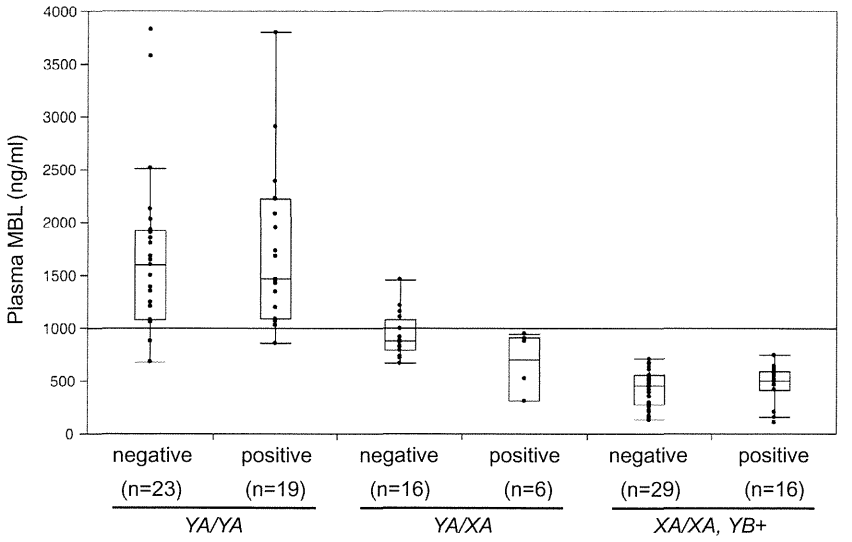

Fig. 2. Plasma mannose-binding lectin (MBL) levels in healthcare workers with and without latent tuberculosis infection (LTBI). Box-and-whisker plots of plasma MBL levels according to interferon gamma release assay (IGRA)-positive and -negative healthcare workers. Each dot represents an individual. Plasma MBL levels between groups were compared after adjusting for age and gender by analysis of covariance.

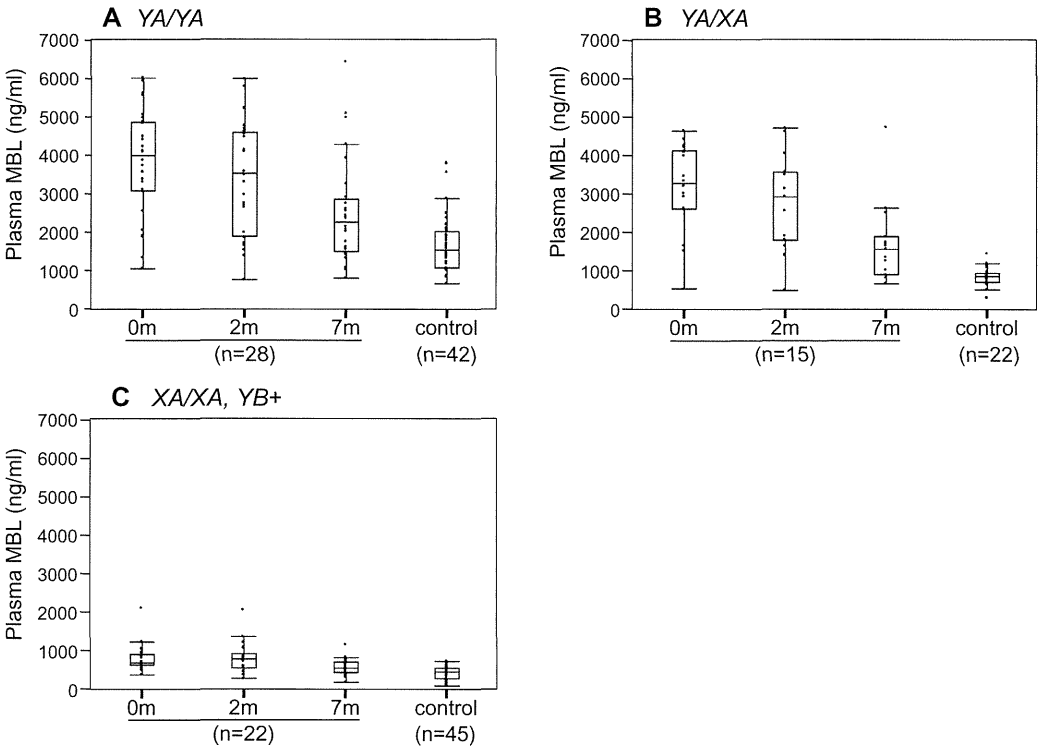

Fig. 3. Time-dependent changes in plasma mannose-binding lectin (MBL) levels in patients with tuberculosis (TB). Box-and-whisker plots of plasma MBL levels from the patients with TB [before (0 month), during (2 months), and at the end of (7 months) treatment] and from healthcare workers (control) with the *YA/YA* diplotype and high MBL levels (A), *YA/XA* diplotype with intermediate MBL levels (B), and *XA/XA* and YB-positive diplotypes with low or absent MBL levels (C). Each dot represents an individual. The overall effects of *MBL2* diplotype, age, and gender on plasma MBL levels throughout the anti-TB treatment period were assessed by using a random intercept model.

3.5. MBL diplotype-dependent plasma MBL levels and their changes during anti-TB treatment

MBL is an acute-phase reactant protein [25,26], and its plasma level is elevated in patients with TB [9]. We assayed MBL levels in serial plasma samples from 65 patients with TB [before (0 month), during (2 months), and at the end of (7 months) anti-TB treatment]. Plasma MBL levels were the highest before treatment and decreased during treatment (Fig. 3). MBL levels were significantly affected by *MBL2* diplotype throughout the treatment course ($P < 0.0001$), whereas age and gender did not affect the MBL levels ($P = 0.452$ and $P = 0.866$). The MBL concentrations at 7 months were still significantly higher than those of controls in any diplotype groups.

4. Discussion

We found that *YA/YA* associated with high plasma MBL levels was associated with protection against the development of new pulmonary TB. This result is consistent with that of a previous study on European descendants, which reported that *HYA/HYA* subjects are protected against TB [10]. Interestingly, the association was found only in younger patients when TB patients were stratified by age in our study. Nongenetic factors that may directly affect MBL levels include inflammatory condition and age, and MBL levels are higher in children than in adults [3]. In contrast, no association has been found between age and MBL levels among adults in some studies [27,28], whereas others reported that serum and plasma MBL levels in healthy adults decrease with age [29,30], although the levels were not stratified by *MBL2* genotypes in those studies. We did not find a significant age-dependent difference in MBL levels in either adult patients with TB or the control group, whereas we clearly demonstrated *YA/YA* diplotype-dependent effects on MBL levels even in the disease state during anti-TB treatment.

The protective role played by high MBL levels only in the younger patients with TB may be related to the complicated immune balance involved in TB development, which occurs within a shorter duration after *Mtb* infection in younger patients. On the other hand, older patients may have maintained LTBI status and *Mtb* in granulomas for a longer time. Because previous studies have suggested that the interaction between MBL and pathogens enhances proinflammatory cytokine production [31–33], high MBL levels may contribute to the maintenance of *Mtb* containment. Moreover, MBL affects monocyte and dendritic cell function at high levels without forming a complex with pathogens [34–37]. Although high MBL levels may limit *Mtb* infection into LTBI status during the younger days of a patient, the decline in the immune response with aging may result in the development of TB disease. The interaction between MBL and host cells and the subsequent immunological response should be elucidated in both the young and the elderly. The effects of associated SNPs in other genetic studies of TB are also restricted in young patients with TB [13,14,38]. Although the mechanism of age-dependent association may be different among genes, studies on the association between host genetic polymorphisms and TB should carefully consider the effects of age.

It has been speculated that the lack of MBL as an opsonin may suppress *Mtb* uptake by macrophages and prevent infection. However, we did not find significantly different plasma MBL levels and the frequencies of *MBL2* genotypes between HCWs with and without LTBI. Therefore, high MBL levels may not promote the establishment of LTBI, but *YA/YA* may confer protection against the development of TB. This is a new concept because the relationship between the *MBL2* genotype and TB infection, as assessed by IGRA or the tuberculin skin test, has not been clearly investigated so far. A limitation of the present study is that the number of asymptomatic individuals according to the IGRA results was relatively small; therefore, larger-scale studies are necessary to elucidate the role of MBL in various stages of TB.

In addition, differences in bacterial strains should be considered as some studies have reported associations between human polymorphisms in patients with TB and particular *Mtb* strains [39]. Furthermore, the binding of MBL to *Mtb* can be different among *Mtb* strains. A nonfunctional haplotype of a population in Ghana (*LYQC*) is associated with protection against TB caused by *Mycobacterium africanum*, which was identified in 30% of their isolates [8]. The *Mtb* Euro-American lineage accounted for 65% of their isolates, and they did not find any association between *MBL2* genotype and TB caused by *Mtb*. The distribution of *Mtb* lineages is different between Africa and Asia [24], and the Beijing genotype, which was <4% in the Ghanaian study, was identified in 58% of our Vietnamese isolates [16]. In our study, *YA/YA* was less frequently found in both younger patients with TB caused by the Beijing genotype strain and those with TB caused by the non-Beijing genotype strain, whereas diplotypes with low or deficient MBL levels tended to be more frequently found only in younger patients with the Beijing genotype strain. Although we should be cautious in interpreting the results of subgroup analysis, we speculate that human *MBL2* nonfunctional or low-producing alleles (*B*, *C*, *D*, and *X*) originally evolved to dampen phagocyte-mediated spreading of intracellular pathogens, including the ancestral type of *Mtb* such as *M. africanum*, that may have exploited binding to MBL [40]. However, the recently expanding modern *Mtb* strains, such as the Beijing genotype strains, may have further evolved to be unaffected by MBL deficiency or to take advantage of low MBL levels. It is postulated that 'modern' *Mtb* such as Beijing strain is more pathogenic than other 'ancient' lineages, and that infection with modern *Mtb* progresses faster to active disease presumably because it elicits weaker innate immunity and poorer defense mechanism [41]. In this context, lower MBL levels may facilitate the early progression to active disease caused by the 'modern' *Mtb*. To explain the controversial results from studies on the association between *MBL2* and TB, a study design collecting both host and bacterial information is required because the outcome of TB infection and disease depends on interactions between host and pathogen genotypes [12].

In conclusion, *MBL2 YA/YA*, involved with high levels of plasma MBL, played a protective role against the development of TB in younger (mean age = 32) patients in Viet Nam. Further studies are required to fully elucidate the role of MBL in *Mtb* infection and TB development.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.humimm.2014.06.006.

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Association between tuberculosis recurrence and interferon-γ response during treatment

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Summary *Objectives:* We investigated the relationship between tuberculosis recurrence and *Mycobacterium tuberculosis* antigen-stimulated interferon-gamma (IFN-γ) responses during

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Recurrence;
Interferon-γ release assay;
Cellular response

treatment.
Methods: Plasma IFN-γ levels in active pulmonary tuberculosis patients ($n = 407$) were analyzed using QuantiFERON-TB Gold In-Tube™ (QFT-IT) at 0, 2, and 7 months of the 8-month treatment received from 2007 to 2009 and the patients were followed up for another 16 months after treatment. Risk factors for recurrence were assessed using the log-rank test and Cox proportional hazard models. Random coefficient models were used to compare longitudinal patterns of IFN-γ levels between groups.
Results: QFT-IT showed positive results in 95.6%, 86.2%, and 83.5% at 0, 2, and 7 months, respectively. The antigen-stimulated IFN-γ responses varied significantly during the treatment course ($P < 0.0001$). Unexpectedly, positive-to-negative conversion of QFT-IT results between 0 and 2 months was significantly associated with earlier recurrence (adjusted hazard ratio, 5.57; 95% confidence interval, 2.28–13.57). Time-dependent changes in IFN-γ levels were significantly different between the recurrence and nonrecurrence groups ($P < 0.0001$).
Conclusions: Although the IGRA response varies individually, early response during the treatment course may provide an insight into host immune responses underlying tuberculosis recurrence.

Introduction

Tuberculosis (TB) remains a major global health problem, resulting in 8.7 million new cases and 1.4 million deaths annually, and multidrug-resistant TB occurs in approximately 3.7% new cases and 20% previously treated cases.[1] Recurrence is thus a major risk factor for multidrug-resistant TB cases[2] and increases the TB burden.[3–5] TB recurrence is defined as a second episode of active disease as a result of relapse (endogenous reactivation) or exogenous reinfection after completion of previous treatment.[6] Biomarkers are necessary for the assessment of treatment effectiveness including early recurrence.[7]

The interferon-gamma (IFN-γ) release assay (IGRA) is an immunological diagnostic test designed to detect TB infection. In this assay, IFN-γ levels produced by primed blood lymphocytes after stimulation with *Mycobacterium tuberculosis* (MTB)-specific antigens in vitro are measured. According to the assay's principle and research findings obtained from animal models, the IGRA response may be attenuated proportional to decreased bacterial antigen load as a result of successful anti-TB treatment.[8,9] However, clinical researchers argue that little correlation exists between the commercial IGRA response and bacillary burden,[10] on the basis of various tests including grade of sputum smear or presence of cavities on chest X-rays (CXRs).[11]

Although many studies have demonstrated a decrease in IFN-γ values during treatment,[12–16] others have shown inconsistent changes and increases have also been reported occasionally.[17–20] Thus, most clinicians believe that monitoring changes in the IGRA response during anti-TB treatment may have limited use in evaluating the effectiveness of treatment[21]; however studies on the relationship of the IGRA response to subsequent episodes of TB recurrence are lacking. In this study, we investigated whether longitudinal patterns of the IGRA response during the treatment period are associated with TB recurrence.

Materials and methods

Ethics statement

A written consent was obtained from each participant. In the case of minors, the parents provided the written consent. The study was approved by the ethical committees of the Ministry of Health, Vietnam and National Center for Global Health and Medicine, Japan.

Study population

In total, 506 unrelated patients aged ≥16 years with smear- and culture-positive pulmonary TB and without history of TB treatment, were consecutively recruited from July 2007 to March 2009 in Hanoi, Vietnam. The MTB culture test was performed using Löwenstein–Jensen media. MTB isolates were subjected to niacin and drug susceptibility tests for streptomycin (SM), isoniazid (INH), ethambutol, and rifampicin. Peripheral blood samples were obtained at diagnosis before initiation of anti-TB treatment (0 months, baseline) for analyzing total blood count, human immunodeficiency virus (HIV) status, and IGRA. IGRA test was repeated at 2 months immediately after the intensive treatment period and at 7 months at the final stage of the maintenance treatment period of the standard 8-month regimen of 2SHRZ/6HE, which was commonly administered during the study period in Vietnam. CXRs were obtained at the baseline and results were interpreted by two unbiased readers blinded to the IGRA results. In the present analysis, patients with multidrug-resistant TB as well as HIV coinfection were excluded.

Follow-up and definitions

During treatment, culture tests were repeated when smear tests were confirmed positive at 2, 5 or 7 months. During the 16-month post-treatment follow-up, sputum smear and culture tests were performed at 2, 4, 7, 10, and 16 months for all accessible cases.

Treatment failure was defined based on the WHO Global Tuberculosis Report in 2012,[1] when the smear and culture were positive at ≥5 months or when the smear was positive but culture was not performed, clinical and/or CXR findings indicated failure, and category switched to category II of anti-TB treatment.

Recurrence was defined when patients were cured after treatment, and then suffered from the second TB episode. The second episode was bacteriologically confirmed if the sputum culture was positive at the time of recurrence or the smear was positive or the culture revealed <5 colonies, clinical and/or CXR findings indicated recurrence, and category switched to category II anti-TB treatment.

Interferon-gamma release assay

An enzyme-linked immunosorbent assay-based IGRA kit, QuantiFERON-TB Gold In-Tube™ (QFT-IT; Cellestis, Victoria, Australia), was used for analysis. The guidelines for algorithm and software (QuantiFERON-TB Gold Analysis Software, version 2.50; Cellestis) provided by the manufacturer were followed for the interpretation of results. The testing procedure was carefully monitored as described earlier,[22] and test quality control was performed during each run according to the manufacturer's instructions. When IFN-γ values of negative control "Nil" and positive control "Mitogen-Nil" fell within the appropriate range, the QFT result was assessed as positive when IFN-γ value of "TBAg-Nil" was above the cutoff value (0.35 IU/ml) and negative when the value was below the cutoff value. A positive-to-negative change of QFT results was designated as "negative conversion" in this study.

Measurement of cytokines and chemokines in QuantiFERON-TB Gold In-Tube™ samples

Cytokines and chemokines released in QFT-IT plasma supernatants were collected before treatment and at 2 months and 7 months after the initiation of treatment, from 10 randomly selected recurrence patients and 10 age- and sex-matched nonrecurrence patients were measured using Bio-Plex multiplex system with a 27-plex cytokine-bead kit (Bio-Plex Pro Human Cytokine 27-plex Assay; Bio-Rad Laboratories Hercules, CA). Only values within the asymptotic range were calculated using the standard curve for statistical analysis.

Statistical analysis

Chi square or log-rank tests were used to compare the incidence of recurrence (events) between groups. Influence of time course on the proportion of IGRA-positive results was assessed using generalized estimating equations. Wilcoxon's rank-sum test was used to compare nonparametric distributions between groups. A logistic regression model was used to investigate risk factors involved in treatment failure. The log-rank test of equality across strata and Cox models after testing the proportional hazard assumption were used to assess risk factors for recurrence. The random coefficient model was used to assess influence of time course on the IGRA response and the post-estimation Wald test was used to compare the longitudinal patterns of the response between recurrence and nonrecurrence groups. Bonferroni's correction was applied to correct multiple comparisons. When the IFN-γ value was greater than 10.00 IU/ml, statistical analysis was performed in both two conditions, using a truncated value (10.00 IU/ml) or a value based on extrapolation. Truncated values are presented in parenthesis along with those based on extrapolation when appropriate. The statistical results confirmed that all significant differences found here were demonstrated in both conditions. *P* values of <0.05 were considered statistically significant unless otherwise specified. Statistical analysis was performed using Stata version 11 (StataCorp, College Station, TX).

Results

Characteristics of the study population

The characteristics of 506 patients recruited have been reported elsewhere.[23] In the present study, we analyzed 407 patients who were enrolled in the directly observed treatment, short-course (DOTS) program at various study sites and did not have multidrug-resistant TB or HIV coinfection at the time of initial diagnosis. Adherence to anti-TB therapy was supervised by the healthcare staff, in cooperation with the patients' family members under the DOTS strategy of the national TB control program. Out of these

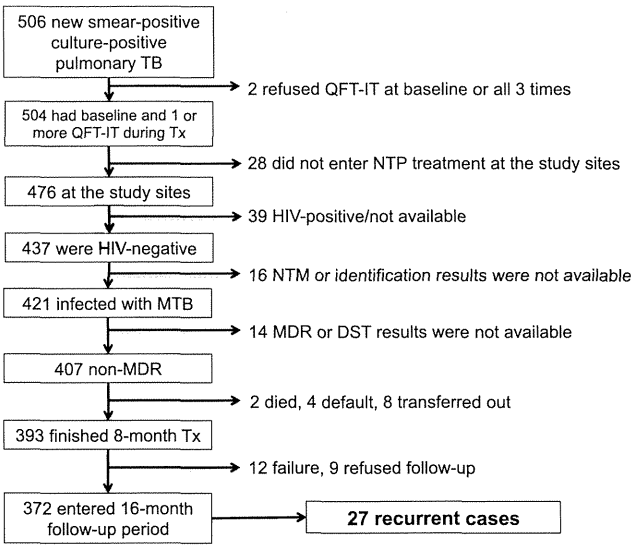

Figure 1 Study flow. TB: tuberculosis; QFT-IT: QuantiFERON-TB Gold In-Tube; NTP: National tuberculosis program; NTM: nontuberculous mycobacterium; MDR: multi-drug resistance; DST: drug sensitivity test; MTB: *Mycobacterium tuberculosis*; Tx: treatment.

Table 1 Patterns of qualitative QFT-IT results during the treatment course (n = 407).

QFT-IT pattern[a]		n	% (95% CI)
Positive-to-Positive-to-Positive	(PPP)	265	65.1 (60.3–69.7)
Positive-to-Positive-to-Negative	(PPN)	27	6.6 (4.4–9.5)
Positive-to-Positive-to-others	(PP_)	40	9.8 (7.1–13.1)
Positive-to-Negative-to-Positive	(PNP)	14	3.4 (1.9–5.7)
Positive-to-Negative-to-Negative	(PNN)	12	2.9 (1.5–5.1)
Positive-to-Negative-to-others	(PN_)	5	1.2 (0.4–2.8)
Negative-to-Negative-to-Negative	(NNN)	8	1.9 (0.9–3.8)
Others		36	8.8 (6.3–12.0)

QFT-IT: QuantiFERON TB-Gold In-tube; TB: tuberculosis; 95% CI: 95% confidence interval.
[a] QFT-IT was performed three times: before treatment, two months, and seven months after starting anti-tuberculosis treatment.

407 patients, 393 completed the 8-month standard treatment course; 381 (97.0%) were cured and 12 (3.0%) did not show treatment response (12/393). Among the cured patients, 372 (97.6%) entered the 16-month post-treatment follow-up (Fig. 1).

The median age of the 372 follow-up patients was 39.7 years (Interquartile range or IQR, 29.0–50.1); 77.7% (289/372) were male, and 238 (64.0%) were current or ex-smokers. Of the MTB isolates tested, 22.8% (85/372) displayed INH resistance with or without SM resistance, and 66.7% (248/372) were sensitive to all 4 major anti-TB drugs tested (data not shown). During the follow-up period, 27 patients (7.3%) showed recurrence.

QuantiFERON-TB Gold In-Tube™ results during treatment period

QFT-IT results were positive in 95.6% (389/407), 86.2% (337/391), and 83.5% (294/352) of the patients tested at 0, 2, and 7 months, respectively, after treatment onset. The proportion of positive IGRA responses varied significantly during the treatment course ($P < 0.0001$). The proportion of negative conversion (positive-to-negative; PN) between 0 and 2 months, 0 and 7 months, and 2 and 7 months were 7.9% (31/391), 12.2% (43/352), and 8.4% (29/347), respectively. The patterns of QFT-IT results as measured at the three time points during the course of the treatment period are shown in Table 1.

QuantiFERON-TB Gold In-Tube™ interferon-gamma values during treatment

The median values of IFN-γ, "TBAg-Nil" at 0, 2, and 7 months were 7.33 [IQR 2.53–14.53 (10.00)], 3.22 (1.03–9.54), and 2.54 (0.77–7.80) IU/ml, respectively. IFN-γ values significantly varied during the treatment course ($P < 0.0001$).

QuantiFERON-TB Gold In-Tube™ results and recurrence

The overall proportion of recurrence was significantly higher in the PN (between 0 and 2 months) group than in the positive-to-positive (PP) group [7/27 (25.9%) vs. 18/311 (5.8%), $P = 0.0001$] (Table 2). The 1-year recurrence rate was also significantly higher in the PN (between 0 and 2 months) group than in the PP group {25.9% [95% confidence interval (CI), 13.3–46.8] vs. 5.5% [95% CI, 3.4–8.7]}. The log-rank test

Table 2 Proportion of treatment failure and recurrence in TB patients showing positive-to-positive and positive-to-negative patterns of QFT-IT results.

Patterns of QFT-IT results	Treatment failure n/N (%)	P value[a]	Recurrence n/N (%)	P value[a]
Between month 0 and month 2				
Positive-to-Positive	8/325 (2.5)	0.203	18/311 (5.8)	0.0001
Positive-to-Negative	2/30 (6.7)		7/27 (25.9)	
Between month 0 and month 7				
Positive-to-Positive			19/285 (6.7)	0.222
Positive-to-Negative			5/43 (11.6)	
Between month 2 and month 7				
Positive-to-Positive			15/264 (5.7)	>0.999
Positive-to-Negative			1/29 (3.5)	

TB: tuberculosis; QFT-IT: QuantiFERON-TB Gold In-Tube.
[a] By Chi square or Fisher's exact test; comparisons were made between the two groups with different patterns of QFT-IT results; Positive-to-Positive and Positive-to-Negative.

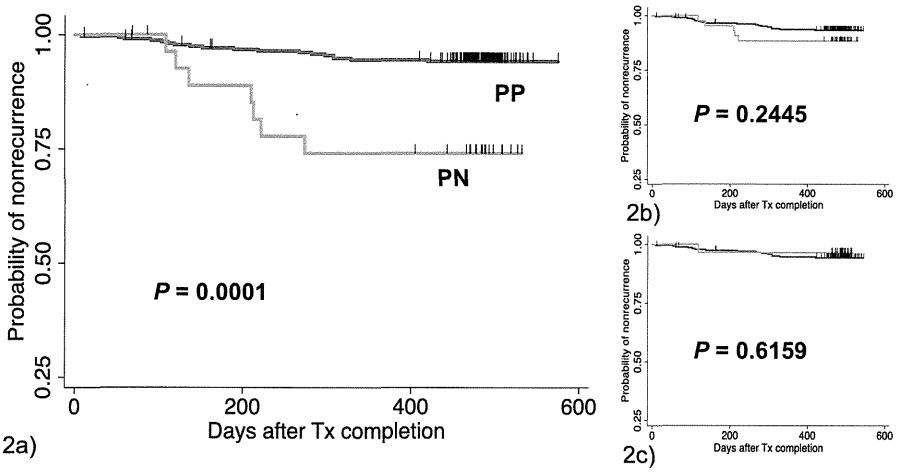

Figure 2 Kaplan–Meier plots stratified by the conversion of QFT-IT results between 0 and 2 months (2a), 0 and 7 months (2b), and 2 and 7 months (2c). QFT-IT: QuantiFERON-TB Gold In-Tube; Tx: treatment; Blue line: positive-to-positive (PP) QFT-IT results. Red line: positive-to-negative (PN) QFT-IT results. The *P* values were obtained by the log-rank test.

confirmed the difference between the two groups ($P = 0.0001$; Fig. 2a), whereas the conversion of QFT-IT results between 0 and 7 months and between 2 and 7 months did not affect recurrence ($P = 0.2445$ and $P = 0.6159$, Fig. 2b and c, respectively). Among the 27 recurrence cases, MTB isolates were sensitive to all drugs tested in 14 cases (51.9%). INH resistance with or without SM resistance was seen in 8 cases (29.6%). This percentage was slightly higher than that of the nonrecurrence group (77/345 or 22.3%), but the difference was not statistically significant ($P = 0.189$, data not shown). The proportion of this drug resistance was also not different between groups with and without the negative conversion (7/31 or 22.6% vs. 83/332 or 25.0%, $P = 0.919$) (data not shown). Using the Cox proportional hazard model, the association between recurrence and the negative conversion of QFT-IT results between 0 and 2 months remained significant (hazard ratio, 5.57; 95% CI, 2.28–13.57) after adjusting for BMI at baseline, smear results at 2 months, drug resistance, and smoking status in the final model (Table 3).

QuantiFERON-TB Gold In-Tube™ interferon-gamma values and recurrence

We further assessed possible changes in the actual IFN-γ values using a random coefficient model with log-transformed IFN-γ values of "TBAg-Nil" set as an outcome variable and time of testing, recurrence status, and the

Table 3 Multivariate analysis using Cox proportional hazard model[a] to assess risk factors for recurrence ($n = 372$).

	Proportion (%)	Hazard ratio	95% CI
QFT-IT status at baseline to 2 months after starting treatment			
Positive-to-Positive	18/311 (5.8)	Reference	–
Positive-to-Negative	7/27 (25.9)	5.57	2.28–13.57
BMI		0.86	0.71–1.04
Result of sputum smear at 2 months after starting treatment			
Negative	22/326 (6.8)	Reference	–
Positive	5/46 (10.9)	2.28	0.84–6.16
Drug resistance profile			
Sensitive to all 4 drugs tested[b]	14/248 (5.7)	Reference	–
INH resistance (± SM resistance)	8/85 (9.4)	1.66	0.65–4.19
Other resistant patterns	5/39 (12.8)	2.77	0.96–8.06
Smoking status			
No	8/134 (6.0)	Reference	–
Yes[c]	19/238 (8.0)	1.48	0.61–3.61

95% CI: 95% confidence interval; QFT-IT: QuantiFERON-TB Gold In-Tube; BMI: body mass index; INH: isoniazid; SM: streptomycin.

[a] Initial model included BMI, sex, age, status of QFT-IT results at baseline to 2 months after starting treatment, presence of cavity or extension of infiltrate on chest radiograph, the results of smear testing at 2 months, patterns of drug resistance, and smoking status. Variables showing $P > 0.2$ were removed from the final model.

[b] The drugs INH, SM, rifampicin, and ethambutol were tested.

[c] Current or ex-smokers.

Table 4 Analysis of time-dependent change of interferon-γ values during treatment period using random coefficient model.

	Coefficient	*P* value	95% CI
TBAg−Nil (log-transformed values) as outcome variable[a]			
Month 2	−0.64	<0.001	−0.74 to −0.54
Month 7	−0.96	<0.001	−1.09 to −0.82
Recurrence	−0.17	0.535	−0.70 to 0.36
Interaction term between 2 months and recurrence	−0.83	<0.001	−1.20 to −0.46
Interaction term between 7 months and recurrence	−0.17	0.513	−0.67 to 0.33
Constant	1.73	<0.001	1.58 to 1.87
Mitogen−Nil (log-transformed values) as outcome variable[a]			
Month 2	0.43	<0.001	0.26 to 0.59
Month 7	1.05	<0.001	0.88 to 1.22
Recurrence	−0.04	0.902	−0.61 to 0.54
Interaction term between 2 months and recurrence	0.22	0.465	−0.37 to 0.81
Interaction term between 7 months and recurrence	−0.04	0.904	−0.64 to 0.57
Constant	1.12	<0.001	0.96 to 1.28

95% CI: 95% confident interval; TBAg−Nil: tuberculosis-specific antigen values minus Nil values; Mitogen−Nil: mitogen values minus Nil values.

[a] 0 month and nonrecurrence group are reference categories.

interaction between the two as independent variables. IFN-γ levels differed significantly with time between the recurrence and nonrecurrence groups according to the post-estimation Wald test ($P < 0.0001$) (Table 4). Fig. 3 shows the linear prediction lines of nonrecurrence and recurrence groups, being overlaid on the basis of individual changes in IFN-γ values.

Statistical significance in the overall difference in QFT-IT IFN-γ values between the two groups prompted us to characterize further estimates. Among the three time points measured, IFN-γ values at 2 months were significantly lower in the recurrence group than in nonrecurrence group {1.36 [IQR, 0.25−3.15] vs. 3.82 [1.12−10.51 (10.00)] IU/ml, $P = 0.003$}, whereas IFN-γ values at 0 months were not significantly different between the recurrence and nonrecurrence groups ($P = 0.1467$). In addition, magnitudes of IFN-γ level changes measured at two consecutive points of time were divided equally into three levels for comparing recurrence time. This indicated that increase of IFN-γ values between 2 and 7 months was significantly associated

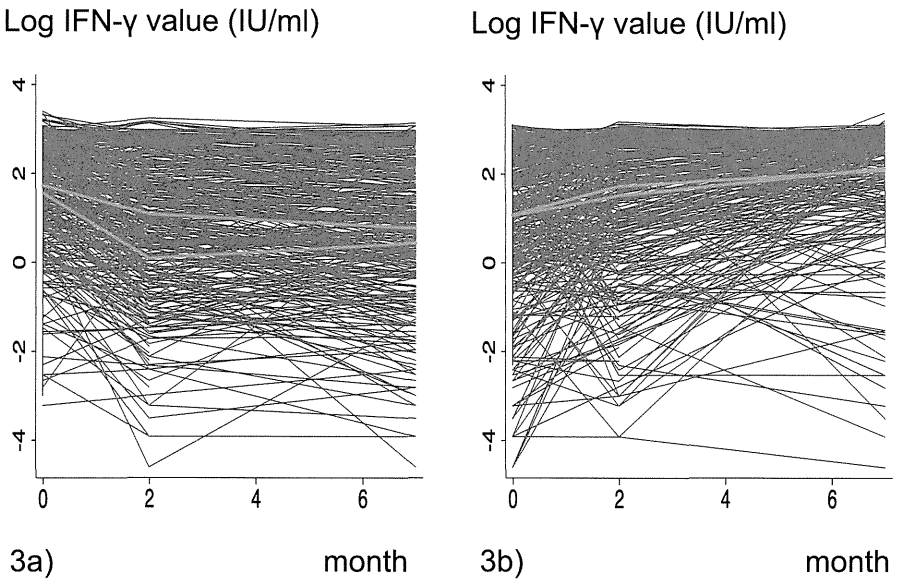

Figure 3 Linear prediction of transition patterns of interferon-γ that responded to TB-specific antigens (3a) and to mitogen (3b) among recurrence and nonrecurrence cases. TB: tuberculosis; IFN-γ: interferon-γ; Blue line: individual IFN-γ pattern; Upper red line: linear prediction line of nonrecurrence group; Lower red line: linear prediction line of recurrence group.

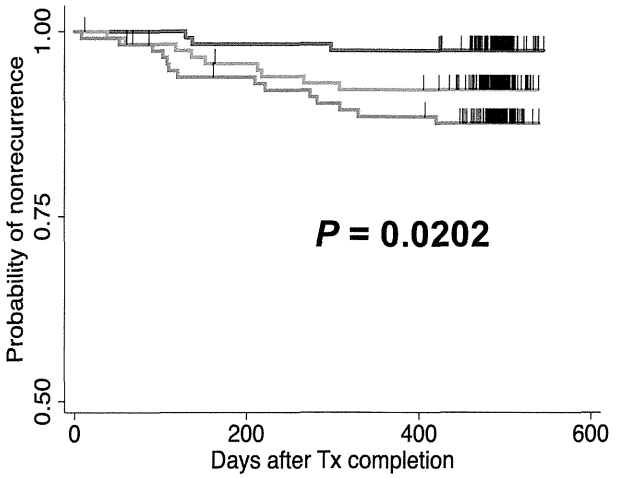

Figure 4 Kaplan–Meier plots stratified by the magnitude of increase in interferon-γ values that responded to TB-specific antigens. The magnitude of increase in interferon-γ values between 2 and 7 months was divided equally into three levels: small (blue line), medium (red line) and large (green line); TB: tuberculosis; Tx: treatment; The *P* value was obtained by the log-rank test.

with TB recurrence ($P = 0.0202$). Kaplan–Meier plots are shown in Fig. 4.

QuantiFERON-TB Gold In-Tube™ and treatment failure

The proportion of failure was slightly higher in the PN group (between 0 and 2 months) than in the PP group (Table 2), although no significant association was found even after we ran the logistic regression model with treatment failure as an outcome variable, and the status of negative conversion and result of smear testing at 2 months both as independent variables (data not shown). However, similar to the results observed between IFN-γ patterns and recurrence, the IFN-γ values of "TBAg-Nil" at 2 months were significantly lower in the failure group than in the cure group [median = 1.04, (IQR = 0.21–3.44) vs. 3.46 (1.03–9.82) IU/ml, $P = 0.0285$].

Cytokines and chemokines in QuantiFERON-TB Gold In-Tube™ plasma supernatants after stimulation with tuberculosis-specific antigens

Among the 27 cytokines and chemokines tested, IL-2, IL-1RA, IP-10, and IFN-γ levels were increased after TB-specific antigen stimulation, and the levels were significantly different compared with unstimulated control levels (data not shown). IL-2, IP-10, and IFN-γ levels tended to be lower in the recurrence group than in the nonrecurrence group (Table 5). Difference in IP-10 and IFN-γ levels at 2 months remained significant even after Bonferroni's correction. IL-10 levels were not different between the conditions (Table 5).

Discussion

In our study, >80% patients had a positive IGRA response until treatment completion, although TB-antigen-stimulated IFN-γ values gradually decreased with time. Interestingly, negative-conversion of the IGRA response after 2 months of treatment was significantly associated with early TB recurrence, and longitudinal patterns of the IGRA response during the treatment course were different between the recurrence and nonrecurrence groups.

According to most of the previous studies, the proportions showing positive IGRA responses before, during, and after anti-TB treatment tend to decrease in a time-dependent manner, but are largely variable.[12–15] High proportions of positivity before and during the treatment period in our study may have resulted from strong TB-antigen-specific IFN-γ response before treatment (median IFN-γ levels = 7.33 IU/ml), presumably because of high bacillary burden in immunocompetent individuals. IFN-γ levels are known to not easily decrease below the cutoff level in such cases.[24,25] Frequent exposure to MTB is one of the reasons for relatively high IFN-γ values during treatment course.[26]

Nevertheless, our study showed that IFN-γ values varied and gradually decreased with time. This finding is consistent with earlier reports,[12–15] but varies from other results in which IFN-γ values did not change remarkably[17] or increased.[19] In the Indian study cohort,[17] a large proportion of the subjects were hospitalized as compared with our study (80.0% vs. 22.1%), and the patients may have suffered from more severe disease than those in the present study. In such cases, recovery or elevation of IFN-γ levels may be observed after starting the effective treatment, although the bacterial antigen load may decrease. Such a paradoxical response may be often observed after several days of blood-cell incubation, to allow proliferation of IFN-γ-producing cells, as reported previously.[27]

Negative conversion of the IGRA response after 2 months of treatment was significantly associated with early recurrence in our cohort, even after adjustment for possible confounding factors. This was different from our expectation; no studies have attempted a possible association between actual recurrence and the IGRA response during treatment. TB antigen-specific IFN-γ levels observed at 2 months were also significantly lower in the recurrence group than in the nonrecurrence group. Pretreatment IFN-γ levels were slightly lower in the recurrence group (median = 6.03 IU/ml) than in the nonrecurrence group (median = 7.89), but were considerably higher than the cutoff value. Therefore, frequent negative conversion at 2 months in the recurrence group cannot be attributed to a simple fluctuation of IFN-γ levels around the cutoff value. As a possible confounder, the proportion of INH-resistance was not significantly associated with recurrence or negative conversion in our study, indicating that 2SHRZ/6HE, a previous standard treatment regimen during the study period in this area, did not affect our main findings.

In general, TB recurrence tends to occur when the initial disease course is severe and prolonged.[28] Peripheral blood cells may not sufficiently respond to TB-specific antigens in such a disease state. Notably, suppression of IFN-γ pro-

Table 5 Levels of IL-2, IL-1RA, IP-10, IFN-γ, and IL-10 in QFT-IT supernatants with and without TB-antigen stimulation in nonrecurrence and recurrence groups.

	No stimulation (Nil tubes)[a]		*P* value[b]	Stimulated with TB-antigens (TBAg tubes)[a]		*P* value[b]
	Nonrecurrence $n = 10$	Recurrence $n = 10$		Nonrecurrence $n = 10$	Recurrence $n = 10$	
IL-2						
Month 0	9.55 (4.99–10.00)	9.67 (6.45–11.96)	0.6480	119.59 (71.33–301.01)	87.65 (48.07–147.28)	0.1509
Month 2	9.42 (2.77–9.92)	9.42 (3.67–10.00)	0.7606	108.22 (67.52–199.84)	42.55 (34.23–90.57)	0.0588
Month 7	9.42 (2.59–9.92)	9.67 (2.59–10.00)	0.5656	80.86 (43.25–182.72)	40.26 (20.25–84.47)	0.1124
IL-1RA						
Month 0	383.02 (211.01–590.51)	506.14 (316.64–593.18)	0.4057	783.99 (528.86–1177.69)	920.99 (648.56–1065.19)	0.7624
Month 2	184.74 (115.41–298.36)	267.38 (205.48–469.55)	0.0821	455.26 (220.52–758.45)	406.91 (325.55–593.61)	>0.9999
Month 7	144.68 (120.22–158.43)	252.82 (197.18–306.53)	0.0156	360.04 (130.28–528.86)	328.62 (255.49–690.86)	0.4497
IP-10						
Month 0	6465.02 (3950.00–9531.21)	7291.13 (4614.94–13,068.25)	0.4497	136,362.20 (90,912.67–151,897.60)	86,495.93 (53,311.35–120,082.80)	0.0638
Month 2	5511.37 (2435.73–10,045.31)	7256.33 (5237.79–9094.84)	0.4497	128,971.10 (60,384.23–151,897.60)	38,334.16 (17,740.69–53,528.71)	**0.0072**
Month 7	3823.80 (1085.59–5388.28)	7182.96 (3012.90–14,986.52)	0.1736	106,655.30 (28,361.69–151,897.60)	32,663.32 (17,160.10–102,471.50)	0.1038
IFN-γ						
Month 0	126.01 (99.82–211.41)	124.17 (98.70–154.42)	0.9698	784.28 (387.67–1657.85)	393.75 (278.23–600.60)	0.0696
Month 2	81.33 (66.62–102.40)	102.40 (67.86–123.09)	0.2550	391.68 (217.13–1393.35)	157.00 (136.92–174.37)	**0.0041**
Month 7	86.21 (56.12–94.53)	98.34 (74.93–139.49)	0.1730	266.15 (116.70–590.04)	153.38 (113.18–316.82)	0.5453
IL-10						
Month 0	6.40 (6.12–7.97)	6.82 (6.21–12.49)	0.4267	6.12 (5.86–6.21)	7.13 (6.21–11.84)	0.0443
Month 2	6.12 (6.07–6.21)	6.21 (6.07–12.09)	0.2215	6.07 (5.90–6.12)	6.17 (6.07–6.66)	0.1457
Month 7	6.17 (6.07–6.25)	6.17 (5.86–6.59)	0.9393	6.07 (5.86–6.12)	6.21 (5.86–6.78)	0.1264

QFT-IT: QuantiFERON TB-gold In-tube; TB: tuberculosis; TbAg: tuberculosis-specific antigens; NS: non-significant.

[a] Values (pg/ml) are expressed in median (interquartile range).

[b] Compared between non-recurrent and recurrent groups. The *P* values were obtained by Wilcoxon's rank-sum tests; values in bold and underlined are those remained significant after Bonferroni's correction.

duction from CD4+ T-cells in response to certain TB-specific peptides has been observed in patients with severe pulmonary TB.[29] In such a condition, inhibitory receptors and soluble factors that induce T-cell anergy, such as CTLA-4 and IL-10,[30] contribution of regulatory T (Treg)-cells,[31–34] and compartmentalization of TB antigen-specific T-cells[35] may have played a role in low IFN-γ levels. In our study, however, the extent of pulmonary TB lesions, an indicator of TB severity (data not shown), and IL-10 production at 2 months, a marker of Treg activity, were not different between the two groups. Furthermore, according to the abovementioned mechanisms, IFN-γ levels should have been suppressed before treatment and recovered during effective treatment, contrary to the pattern observed in this study. Difference caused by nonspecific immune suppression that may occur in malnutrition or other states is also unlikely because the amount of nonspecific mitogen-induced IGRA response was not significantly different between the two groups (Fig. 3b).

Although our study provided no direct evidence, the impairment of T-cell memory function at the convalescent stage may have caused both the negative conversion and the low IFN-γ values seen in the IGRA results at 2 months in the recurrence group. Several lines of evidence have demonstrated that antigen-specific IFN-γ-only-secreting effector T cells are predominant in untreated active TB disease and also indicate that when the antigen load decreases after starting treatment, dual IFN-γ/IL-2- or single IL-2-secreting T-cells with more memory-cell characters become more predominant.[27,36–40] In the recurrence group, it is possible that dual IFN-γ/IL-2-secreting or polyfunctional T-cells have failed to expand for unknown reasons, whereas IFN-γ-only-secreting cells have continuously decreased, resulting in a significant reduction in overall IFN-γ production. Indeed, IL-2 induction in QFT-IT tended to be lower parallel with the lower IFN-γ response at 2 months in the recurrence group than in the nonrecurrence group. Further immunological studies on lymphocyte subpopulations would be necessary to elucidate the underlying mechanism.

In addition to the low IFN-γ values at 2 months, the increase in IFN-γ values between 2 and 7 months was also associated with early TB recurrence. A previous study[14] revealed a minor and insignificant increase in IFN-γ levels at 6 months after treatment completion in subjects having recurrence risk. It is well known that the IGRA response is higher in active TB than in latent TB infection despite a large overlap,[41–43] and the cytokine-producing capacity of MTB-specific CD4 and CD8 T-cells is associated with increased bacillary burden.[27,38] Collectively, a slight increase in IGRA responses between 2 and 7 months in the recurrence group may indicate significantly increased bacillary burden in the subclinical stage before recurrence.

Additionally, in the treatment failure group, the tendency for decreased IFN-γ levels at 2 months may not be explained by only change in bacillary burden, because the burden should be considerably higher in the failure group. The similarity in IFN-γ level patterns between recurrence and failure may suggest a common underlying mechanism, possibly the impairment of T-cell function, although the statistical power of our study was not strong enough to analyze this with regard to treatment failure.

IP-10 is a small chemokine expressed by antigen-presenting cells and it is induced by IFN-γ.[44] IP-10-based tests are comparable to the IGRA response[44] in different groups of TB-related subjects, including HIV-uninfected and infected subjects evaluated at the time of TB diagnosis or over time.[45,46] However, our study results are not conclusive about the better indicator among the two.

We did not distinguish reinfection from relapse in this study. However, we assumed that relapse cases are predominant because recurrence occurred during the short follow-up period (16 months). It should be emphasized that our findings cannot be used for the prediction of recurrence, because a variety of individual variations in longitudinal patterns of the IGRA response were observed. Nevertheless, our findings provide additional insights on the clinical relevance of IGRA in TB management. Negative conversion of the IGRA response after 2 months of treatment was not a good sign though it is widely believed to indicate clearance of infection.

The strength of the present study lies in the high proportion of patients completing treatment and active follow-up. However, this study has a few limitations. First, the last IGRA was performed at 7 months of treatment. The magnitude of change in IFN-γ values in the recurrence group may have been larger if the evaluation was performed at a later stage. Second, in our settings, we were unable to study the host immune response in detail so as to elucidate the underlying mechanism.[27,47] Third, diabetes, one of the possible confounding factors for TB recurrence, was not actively screened in our study protocol. However, the frequency of diabetes based on a questionnaire-based interview was relatively low (4.6%) in the study population and nonexistent (0%) in the recurrence group. Therefore, we did not include this factor in the multivariate analysis. Nevertheless, our data showed a negative association between IGRA response and recurrence, which may prompt future studies in this field.

In conclusion, this study showed that the patterns of IGRA responses to TB-specific antigens during treatment differ according to recurrence status and thus may provide insights into the immunological background prior to TB reactivation, a major question in this field.

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8. 結核・非結核性抗酸菌症の現況

永井英明*

Keywords●結核，非結核性抗酸菌，*Mycobacterium avium* complex，*M. kansasii*，*M. abscessus*／tuberculosis, nontuberculous mycobacteria, *Mycobacterium avium* complex, *M. kansasii*, *M. abscessus*

要旨●抗酸菌の代表的な菌種は結核菌である。結核菌以外の抗酸菌を非結核性抗酸菌（NTM）という。日本は結核中蔓延国である（罹患率は10万対16.7）。高齢者結核が多い。NTM症は増加傾向にあり，ヒト-ヒト感染は認められないが，治療に難渋する。NTMは環境常在菌であり，土や水からの感染が確認された症例がある。

1 はじめに

抗酸菌は細胞壁に多量の脂肪を含み，加温しないと染色されにくいが，いったん染色されると酸やアルコールで脱色されにくくなる。この性質が抗酸菌と呼ばれる由縁であり，菌が酸に対して強いという意味ではない。代表的な菌種は結核菌である。結核菌以外の抗酸菌を非結核性抗酸菌（nontuberculous mycobacteria：NTM）というが，人工培地で培養可能な菌種であるという条件があり，癩菌は人工培地で培養できないのでNTMに含まれない。本稿では結核およびNTM症の代表的な菌種について述べる。

2 結　核

1）結核菌

結核菌は長さ2～5 μm，幅0.3～0.5 μmの桿菌である。細胞内寄生菌であり，酸素の豊富な環境において発育する偏性好気性菌である。発育至適温度は37℃，至適pHは6.4～7.0である。結核菌の分裂時間は13～20時間と長いので，培養検査に長時間を要し，コロニーの確認には3週間ほどかかる。培養で認められた結核菌は紐状に集まるのが特徴的であり，これをコード形成という。

結核菌はヒト以外の動物には感染症を引き起こしにくく，ヒトが最適の生存環境となっている。実験動物としてはモルモットが高い感受性を示すので結核に関する実験には用いられている。

2）結核の感染と発病

結核の感染経路は一般的には気道であり，感染者の咳，くしゃみなどによる飛沫核を吸入することにより感染する空気感染（飛沫核感染）である。結核菌を吸い込んでもただちに発病するわけではなく，気道には病原体を排除する機能があるため，感染が成立する確率は50％程度である。

感染が成立するとツベルクリン反応やインターフェロンγ遊離試験（interferon-gamma release assays：IGRAs）が陽性となるが，結核感染が成立した時点で発病するのは免疫機能が正常であれ

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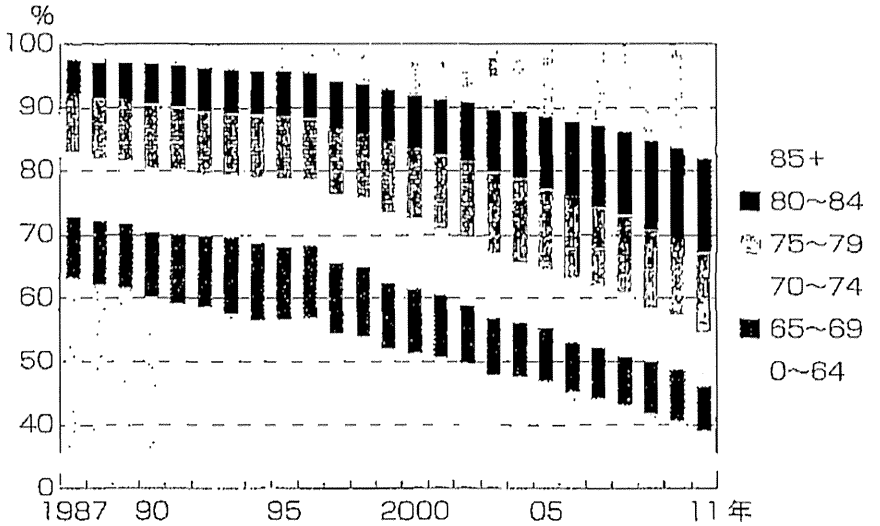

図 1　新登録結核患者内の高齢結核患者が占める割合の年齢階層別年次推移（1987～2011 年）

〔結核研究所疫学情報センター，編．結核年報 2011（2）小児結核・高齢者結核．結核 2013；88：611-6 より引用〕

ば，5～10%程度である。残りのグループの中から免疫機能が低下することにより，一生の間に5～10%の人が発病してくるといわれている。細胞性免疫機能が低下する病態では結核を発病しやすくなり，発病の危険因子としては，糖尿病，珪肺，胃切除の既往，多量喫煙，ステロイド・抗癌薬・免疫抑制薬の使用者，悪性腫瘍，人工透析，高齢，ヒト免疫不全ウイルス（human immunodeficiency virus：HIV）感染症などがある。

結核菌が体内に広がる経路には，経気道性散布，リンパ行性散布，血行性散布，経腸管性散布，局所での周囲組織への進展などがあり，結核菌は全身のあらゆる臓器に結核症を引き起こし得る。

肺結核の発病様式として，感染に引き続いてただちに発病する1次結核と，結核菌に感染して数年後に発病する2次結核があるが，明確に分けることはしばしば困難である。

結核の病理所見は多彩であり，非特異的な滲性反応が起こり，次いで細胞性免疫の成立により肉芽腫形成が見られる繁殖性反応が起こり，修復機転により周辺に線維形成が見られるようになる（増殖性反応）。その後，線維層は収縮して瘢痕組織になり治癒する（硬化反応）。肺結核における空洞形成は遅延型過敏反応によって生じるアレルギー機序によるものである。

3）結核の疫学

世界保健機関（World Health Organization：WHO）によると，2012 年には世界で 860 万人の結核患者が新しく発生し，130 万人（HIV 感染症合併例が 32 万人）が死亡したと推定されている[1]。結核はいまだに途上国を中心に社会に大きなインパクトを与えている疾患である。

日本の結核罹患率は年間 10～11%の割合で順調に減少してきたが，1977 年頃より減少率が縮小し，1997 年の結核罹患率は人口 10 万対 33.9 と 43 年ぶりに増加に転じた。その後の結核対策により 2012 年の結核罹患率は 10 万対 16.7 となり，減少のスピードもやや速まっているが，欧米先進国の結核罹患率が 3～5 前後の現状と比較すると依然として高率であり，年間 21,283 人（2012 年）の結核患者が新たに登録されている[2]。このうち喀痰塗抹陽性肺結核患者数は 8,237 人である。わが国は依然として結核の中蔓延国である。

中でも高齢者ほど結核罹患率が高い。新登録結核患者のうち 50%以上を 70 歳以上の高齢者が占めており，この割合は年々増加している（図 1）[3]。80 歳以上が結核患者の実に 1/3 を占めている。

高齢者は結核の高蔓延時代を経験しているので，年齢が高齢になるにつれ結核の推定既感染率は上昇し，この人たちの中から高齢ゆえに免疫能

が低下し内因性の再燃を起こしてくるものと思われる。したがって，高齢者で長引く咳，痰などの呼吸器症状を示す人，あるいは症状がなくても胸部X線写真で異常影を示す人については必ず結核を鑑別診断に入れなければならない。

都道府県別に罹患率をみると，大阪府（10万対27.1，2012年），東京都（21.7），沖縄県（21.2），徳島県（21.1），奈良県（20.5）の順に高く，長野県（9.5），福島県（9.9），宮城県（9.9），山形県（10.0），北海道（10.7）の順に低い。罹患率の最も高い大阪府の中でも大阪市は42.7と高値である。

受診の遅れと診断の遅れが相変わらず認められており，結核感染対策という視点に立てば改善されなければならない。有症状結核のうち症状発現から初診までの期間が2カ月以上（受診の遅れ）の割合は18.7%（2012年）であり，働き盛りの30～59歳に限ってみると33.7%と3人に1人は受診が遅れている。受診の遅れの間に多数の人々に感染を広げている可能性がある。有症状結核のうち初診から診断までの期間が1カ月以上（診断の遅れ）の割合は22.0%であり，医療機関における結核診断の遅れが依然として認められ，医療従事者の結核診断の甘さがみられる。院内感染対策上，問題である。

近年，外国出生者の新登録結核患者数は増えており，1,000人を超え，全体の5%を占めている。特に20歳代では新登録結核患者の3人に1人以上は外国出生者である。

2012年中の結核による死亡者数は2,110人で，前年に比べ56人減少し，死亡率は1.7である。死因順位は，26位である。

4）多剤耐性結核

不適切な治療や患者管理は耐性菌の排出を増加させる可能性がある。耐性結核菌は10種類ある抗結核薬のいずれかに耐性のある結核菌を指すが，最も強力な治療薬であるイソニアジド（isoniazid：INH）とリファンピシン（rifampicin：RFP）の両剤が耐性である耐性菌による結核を多剤耐性結核（multidrug-resistant tuberculosis：MDR-TB）という。この両薬剤のいずれかが欠けても十分な結核治療ができないが，さらに多剤耐性結核菌の中で，少なくとも1つの注射薬〔カナマイシン（kanamycin：KM），カプレオマイシン（capreomycin：CPM），アミカシン（amikacin：AMK）〕と1種類のフルオロキノロン薬に耐性を獲得した耐性菌による超多剤耐性結核（extensively drug-resistant tuberculosis：XDR-TB）が世界的にも増加傾向にある。

2012年のわが国のMDR-TBは60例であり，薬剤感受性検査が行われた8,347例中0.7%であり，増加傾向にない[2]。

ピラジナマイド（pyrazinamide：PZA）を含む短期化学療法で治療された患者の割合とMDR-TBの頻度との間には逆相関があると言われており，PZAの使用が浸透してきた現状では今後のMDR-TBの減少が期待される。

3 非結核性抗酸菌（NTM）症

1）NTMの種類

NTMは塵埃，土壌，水などの自然界に広く存在しており，現在，150種類以上が知られている。わが国でもその中の30種類以上の感染症例が報告されている。NTM症の中では*Mycobacterium*（*M.*）*avium* complex（MAC）症が最も多く約80%を占め，*M. kansasii*症（8%），*M. abscessus*症（3%）が続く。その他に稀少菌種によるNTM症がわずかに存在する。

2）NTMの感染と発病

NTM症では患者家族や大量排菌者との接触者からの発病例がほとんどないことから，ヒトからヒトへの感染は無視し得ると考えられている。

NTMは自然環境に広く存在するので，曝露の機会は多いが，弱毒であるため宿主側の因子が重要であると考えられてきた。後天性免疫不全症候群（aquired immune deficiency syndrome：AIDS）に合併する全身播種型のMAC症などはその典型である。興味深いことにAIDS合併全身播種型MAC症の起炎菌は97%が*M. avium*といわれている。肺局所の防御能の低下も重要な要素であ