Elevation of matricellular proteins in dengue virus infection. 口頭	Chagan-Yasutan H, Hattori T	The 10th China-Japan international conference of VIROLOGY. 長春	25-28th August 2014	国外
The Levels of Matricellular Proteins in Plasma of Active Tuberculosis and Latent Tuberculosis in the Setting of Helicobacter Pylori Co-infection 口頭	Hasibuan FM, Senoputra MA, Shiratori B, Chagan-Yasutan H, Cundarani R, Apriani L, Alisjahbana B, Hattori T	4th Annual Conference of Japan Association for Human Security Studies 仙台	2014年9月6日--2014年9月7日	国内
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Workshop on disaster management	服部　俊夫	仙台・東北大学医学部主催	2014年6月27日	国内

Antibody responses against multiple antigens of Mycobacterium tuberculosis to differentiate active tuberculosis from latent form	Toshio Hattori, Beata Shiratori, Muhammad Andrian Senoputra, Fakhrial Mirwan Hasibuan, Cundarani Raspati, Apriani Lika, Bachti Alisjahbana, Makoto Matsumoto, and Yasuhiko Suzuki	National Tuberculosis Research Parade (The 4th Indonesia TB Research Parade). Jakarta, Indonesia.	11-12th March 2015.	国外
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Different expression of galectin-9, osteopontin and tenascin-C in granulomatous diseases. ポスター	Beata Shiratori, Fakhrial Mirwan Hasibuan, Muhammad Andrian Senoputra, Haorile Chagan-Yasutan, Cundarani Raspati, Apriani Lika, Yayoi Takahashi, Toshiro Niki, Bachti Alisjahbana, Toshio	NIH-Japan-JSPS Joint Symposium. Washington DC, USA.	23-24th October 2014.	国外

Diversity of matricellular protein secretion in active and latent tuberculosis infection. 口頭	Beata Shiratori, Haorile Chagan-Yasutan, Toshio Hattori	The 63th Annual Meeting of Japanese Association for Infectious Diseases. Tokyo, Japan.	2014年10月29日 -- 2014年10月31日	国内
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Expression of matricellular proteins in latent and active tuberculosis ポスター	Shiratori Beata, Haorile Chagan-Yasutan, Hattori Toshio	The 43rd Annual Meeting of The Japanese Society for Immunology 京都	2014年12月10日 -- 2014年12月12日	国内
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Construction of a virtual Mycobacterium tuberculosis consensus genome and its application to data from next generation sequencers.	Okumura K, Kato M, Kirikae T, Kayano M, Miyoshi-Akiyama T	American society of microbiology 114th General meeting, USA	2014年7月	国内

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An in vitro model of sick building syndrome using human bronchial epithelial cells	Matsushita I, Hijikata M, Ito H, Keicho N	第19回アジア太平洋呼吸器学会総会	2014.11	国際
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２．学会誌・雑誌等における論文掲載

掲載した論文（発表題目）	発表者氏名	発表した場所 （学会誌・雑誌等名）	発表した時期	国内・外の別
Elevated levels of full-length and cleaved osteopontin during acute dengue virus infection.	Chagan-Yasutan, H., Lacuseta, T-L., Ndholvu, L. C., Pguma, S., Leano, P.S.A., Telan, E.F.O., Kubo, T., Morita, K., Uede, T., Dimaano, E.M., and Hattori T.	Thrombosis Research PMID: 24861695 Thrombosis research 2014, 134(2)449-454.	2014	国外

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呼吸器治療薬の副作用とその対策　抗結核薬	永井英明	呼吸	2014年7月	国内
疾患解説　感染症の基礎知識 肺結核	永井英明	感染症道場	2014年9月	国内
Study of tuberculosis in patients with human immunodeficiency virus infection	Nagai H	Kekkaku	2015年1月	国内

（注１）発表者氏名は、連名による発表の場合には、筆頭者を先頭にして全員を記載すること。
（注２）本様式はexcel形式にて作成し、甲が求める場合は別途電子データを納入すること。

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Regular Article

Elevated levels of full-length and thrombin-cleaved osteopontin during acute dengue virus infection are associated with coagulation abnormalities

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ABSTRACT

Introduction: Dengue virus (DENV) is transmitted by the mosquito vector, and causes a wide range of symptoms that lead to dengue fever (DF) or life-threatening dengue hemorrhagic fever (DHF). The host and viral correlates that contribute to DF and DHF are complex and poorly understood, but appear to be linked to inflammation and impaired coagulation. Full-length osteopontin (FL-OPN), a glycoprotein, and its activated thrombin-cleaved product, trOPN, integrate multiple immunological signals through the induction of pro-inflammatory cytokines.
Materials and Method: To understand the role of OPN in DENV-infection, we assessed circulating levels of FL-OPN, trOPN, and several coagulation markers (D-dimer, thrombin-antithrombin complex [TAT], thrombomodulin [TM], and ferritin in blood obtained from 65 DENV infected patients in the critical and recovery phases of DF and DHF during a dengue virus epidemic in the Philippines in 2010.
Results: Levels of FL-OPN, trOPN, D-dimer, TAT, and TM were significantly elevated in the critical phase in both the DF and DHF groups, as compared with healthy controls. During the recovery phase, FL-OPN levels declined while trOPN levels increased dramatically in both the DF and DHF groups. FL-OPN levels were directly correlated with D-dimer and ferritin levels, while the generation of trOPN was associated with TAT levels, platelet counts, and viral RNA load.
Conclusion: Our study demonstrated the marked elevation of plasma levels of FL-OPN and thrombin-cleaved OPN product, trOPN, in DENV-infection for the first time. Further studies on the biological functions of these matricellular proteins in DENV-infection would clarify its pathogenesis.

Introduction

Dengue is an acute febrile disease that is caused by the dengue virus (DENV), which is transmitted to the host through the bite of blood-feeding mosquitos [1]. The number of countries reporting dengue cases has been increasing annually. An estimated 50 million dengue infections occur annually worldwide, and approximately 2.5 billion people live in countries where dengue is endemic [2]. In the majority of cases, infection with any of the 4 DENV serotypes is asymptomatic. However, a wide spectrum of clinical symptoms are associated with cases of symptomatic infection. These symptoms range from dengue fever (DF), which is a mild flu-like syndrome, to the more severe dengue hemorrhagic fever (DHF), which is characterized by coagulopathy and increased vascular fragility and permeability. DHF may even progress to hypovolemic shock (dengue shock syndrome) and death [3].

The mechanisms that lead to severe forms of dengue illness are complex, but undoubtedly relate to increased coagulation and fibrinolysis activity during DENV-infection [4,5]. The activation of coagulation pathways and fibrinolysis have also been reported in DENV-infection,

Abbreviations: DENV, dengue virus; DF, dengue fever; DHF, dengue hemorrhagic fever; TAT, thrombin-antithrombin complex; TM, thrombomodulin; NS1, nonstructural protein-1; FL, full-length; tr, thrombin-cleaved; OPN, osteopontin; RGD, arginine-glycine-aspartic acid; TAFIa, thrombin-activatable fibrinolysis inhibitor; HC, healthy control; MMP, matrix metalloproteinase.

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as reflected by increased thrombin-antithrombin complex (TAT), D-dimer (fibrin degradation product), tissue plasminogen activator and prothrombin fragment [6,7]. In addition, it has been reported that thrombomodulin (TM) is induced in endothelial cells after infection with DENV *in vitro*, and may contribute to anticoagulation pathways in cells during DENV-infection [8]. (TM is an integral membrane protein that is expressed on the surface of endothelial cells, and serves as a cofactor for protein C activation by thrombin.) Nonstructural protein-1 (NS1) is a 43 kDa glycoprotein of DENV and is expressed on cell surface or secreted as a soluble hexamer after infection [9,10]. It is of note that this protein was reported to bind prothrombin and inhibits its activation into thrombin and it has also been shown that antithrombin antibodies recognize NS1 protein in the sera of patients with dengue [11,12]. Hemophagocytic syndrome is a final common form of a cytokine storm, which is induced by the uncontrolled proliferation and activation of macrophages, and results in systemic inflammatory responses and multi-organ dysfunction. Elevated ferritin, a marker of hemophagocytic syndrome, has also been reported in patients with dengue [13,14]. The precise mechanism of DENV activity in the disturbance of capillary permeability is unclear. However, this mechanism is generally thought to be related to the dysregulation of immune and inflammatory factors. There is a need for soluble biomarkers that reflect both inflammation and coagulopathy during DENV-infection. Sosothikul et al. demonstrated that von Willebrand factor (vWF) was the best indicator of the hemorrhagic form of dengue fever. Increased levels of soluble TM, vWF antigen, tissue factor and plasminogen activator were reported during the acute phase and were associated with disease severity. In contrast, the levels of ADAMTS-13 were lower in DHF patients compared to DF patients [15,16]. Here, we studied inflammatory molecule of osteopontin, which have potential cleavage site of thrombin, in DENV-infection.

Full-length osteopontin (FL-OPN) is a highly phosphorylated and glycosylated matricellular protein. Although FL-OPN is secreted into the extracellular environment or matrix, intracellular form of OPN was also reported [17]. Rather, FL-OPN modulate cell function by interacting with cell-surface receptors, proteases, hormones, other bioeffector molecules, and structural matrix proteins, such as collagens [18]. FL-OPN is an acidic protein that consists of approximately 300 amino acids and is widely expressed in immune cells (for example, macrophages, T cells, and B cells) that are involved in bone resorption, wound repair, immune function, angiogenesis, cell survival, and cancer biology [19–24]. FL-OPN contains the arginine-glycine-aspartic acid (RGD) sequence, a classic cell-binding motif that is recognized by cell surface RGD-recognizing integrins such as $\alpha v\beta 1$, $\alpha v\beta 3$, and $\alpha 5\beta 1$ [25,26]. In addition to the RGD motif, FL-OPN also contains 2 heparin-binding sites, 1 thrombin cleavage site, and 1 putative calcium-binding site [27]. Proteolytic cleavage of FL-OPN by thrombin (between Arg^{168} and Ser^{169}) generates a functional fragment of N-terminal thrombin-cleaved OPN (trOPN, also known as OPN-R [28]), which contains a cryptic binding site for integrin $\alpha 9\beta 1$ and $\alpha 4\beta 1$ that enhances the attachment of trOPN to integrins [26,29]. Elevation of trOPN levels has been reported in plasma and tissue of patients with atherosclerotic status, and also in the synovial fluid from knee osteoarthritis [29–31].

A previous study demonstrated that DENV-infection induces *OPN* gene expression in human macrophages [32]. Given the importance of coagulation and inflammation abnormalities in DENV-infection, we designed a prospective clinical study to investigate FL-OPN and trOPN as candidate biomarkers in patient cohorts from Manila, the Philippines, during a dengue epidemic.

Materials and Methods

Subjects and Study Design

During 2010, a study on dengue was conducted at San Lazaro Hospital in Manila, the Philippines. A total of 65 patients with clinical diagnoses of DF (n = 53) or DHF (n = 12) were enrolled in the study. DF and DHF were defined in accordance with the World Health Organization guidelines. Medical histories, physical examination results, and laboratory examination results were obtained from each of the enrolled patients. For each of the patients infected with DENV and 30 healthy controls (HC), plasma and serum samples were collected during the critical phase (day 4 or 5 of illness) and recovery phase (day 7 or 8 of illness), as described previously [33]. In brief, blood was collected in tubes with or without the anti-coagulant EDTA. EDTA plasma was obtained by centrifugation at 3,000 rpm for 10 min at 4 °C. Serum was centrifuged and collected after clot formation at room temperature. Samples were aliquoted and stored at −80 °C until use. Multiple thawing was avoided.

Ethics Statement

The study was conducted in accordance with the Declaration of Helsinki (Seoul, 2008) and was approved by the Ethics Committees of San Lazaro Hospital, Manila, the Philippines (2009-003), and Tohoku University Hospital, Sendai, Japan (2009-425). Written informed consent was obtained from all study participants.

RNA Extraction and DENV Quantification

Dengue viral RNA was quantified as previously reported [33]. In brief, genomic viral RNA was extracted from 140 μl of patient serum (critical phase only, n = 65) using the QIAamp viral RNA mini kit (QIAGEN, Hilden, Germany). The extracted RNA was stored at −80 °C until use. The DENV copy number was measured by a TaqMan® real-time reverse transcription polymerase chain reaction assay (7500 Real-Time PCR System, Applied Biosystems, Foster City, CA, USA) using an *in vitro* transcribed quantitative RNA standard, as described previously [34].

Inflammatory and Coagulation Marker Quantification

OPN levels in plasma were quantified by 2 different commercially available ELISA kits (IBL, Gunma, Japan; R&D Systems, Minneapolis, MN, USA) [35]. In the first kit, polyclonal rabbit antibody (O-17) specific to the N-terminus of OPN (Ile17-Gln31, accession #NP_000573.1) was used as a capture antibody, and a mouse monoclonal antibody (10A16) raised against synthetic peptides corresponding to the internal sequence of human OPN (Lys166-Glu187) was used as a detector antibody. The system does not allow us to detect trOPN. The standard range of this kit is 5-320 ng/ml or 76.9 ~ 4920 pmol/L. Here, the result was expressed as pmol/L. In the second ELISA kit, the proprietary capture monoclonal antibody and the detection polyclonal antibodies were both raised against recombinant human OPN (NS0-derived, amino acids Ile17-Asn300). The standard range of this kit is 62.5 ~ 4000 pg/ml. Final result was obtained based on dilution factor of 50-200 and expressed as ng/ml.

To detect N-terminal trOPN, a commercially available ELISA kit was used (IBL, Gunma, Japan). The standard range of this kit is 6.25 ~ 400 pmol/L. ELISA assay was performed using an anti-trOPN monoclonal antibody (34E3) as the capture antibody, and the O-17 antibody as the detection antibody. This capture antibody specifically reacts to the epitope Ser162–Arg168 exposed by thrombin and dose not reacts to matrix metalloproteinase-3, 7 (MMP-3, 7) cleaved N-terminal trOPN [36]. It is also known that thrombin-activatable fibrinolysis inhibitort (TAFIa) treated trOPN reduce its adherent capacity on Jurkat cells [37], but the treated form was not confirmed to bind the monoclonal antibody [31,38].

ELISA kits to detect TAT (Abcam, Cambridge, MA, USA), D-dimer (Hyphen BioMed, Neuville-Sur-Oise, France), TM (R&D Systems, Minneapolis, MN, USA) and ferritin (Bio-vendor, Brno, Czech Republic) were used according to the manufacturer's instructions.

Statistical Analysis

Data were expressed as medians because the distributions were non-Gaussian. The Kruskal–Wallis test was used to assess differences in the plasma FL-OPN and trOPN levels among the HC, DF, and DHF groups. When a significant difference was found among these groups, Dunn's multiple comparison test was used to assess between-group differences for each pair of groups. Differences between the critical and recovery phases were assessed using the Wilcoxon signed-rank test. Relationships between parameters were assessed using Spearman's rank correlation coefficients. Two-tailed tests were used in all appropriate instances, and values of $P < 0.05$ were considered statistically significant. All statistical analyses were performed using GraphPad Prism software, version 6 (GraphPad Software Inc., San Diego, CA, USA).

Results

Elevated Levels of Plasma FL-OPN in the Critical Phase of DENV-Infection Decline During the Recovery Phase

Plasma levels of FL-OPN were measured in patients with DF and DHF during both the critical and recovery phases. Two different ELISA kits (IBL and R&D Systems) were used to determine FL-OPN levels because it has previously been demonstrated that these ELISA systems can have discordant results [35,39,40]. Analysis with the IBL kit showed that the levels of OPN were markedly elevated in both patients with DF (median, 25,951 pmol/L; 9.2-fold increase) and patients with DHF (median, 27,550 pmol/L; 9.7-fold increase), as compared with the HCs (2,814 pmol/L; Fig. 1A). The R&D Systems kit also demonstrated elevated levels in patients with DF (median, 540 ng/ml; 7.9-fold increase) and

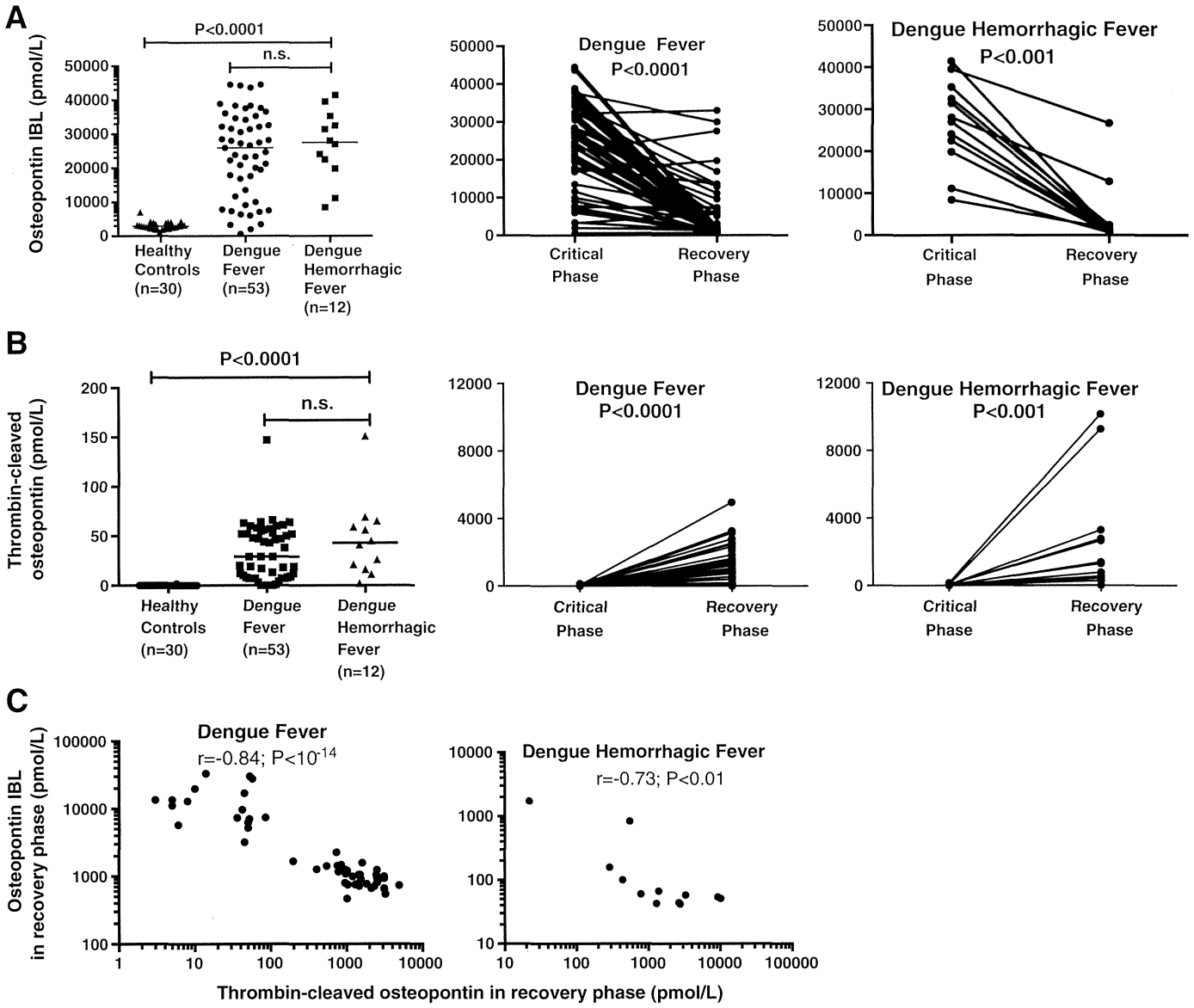

Fig. 1. Elevated plasma full-length osteopontin (FL-OPN) and thrombin-cleaved OPN (trOPN) levels in patients infected with dengue virus. (A & B) The levels of FL-OPN (measured by the IBL ELISA kit) and trOPN differed significantly between patients with dengue fever, patients with dengue hemorrhagic fever, and healthy controls during the critical phase of dengue virus infection. The levels of FL-OPN declined in the recovery phase, while those of trOPN increased. (C) A significant inverse correlation was observed between the recovery phase OPN (measured by the IBL ELISA kit) and trOPN levels.

patients with DHF (median, 692 ng/ml; 10.1-fold increase), as compared with the HCs (68 ng/ml; Suppl. Fig. 1A). FL-OPN levels significantly differed among the 3 groups ($P < 0.0001$), and multiple-comparison corrected assessments indicated that the FL-OPN levels differed between the patients with DF and the HCs ($P < 0.001$), as well as between the patients with DHF and the HCs ($P < 0.001$), based on measurements from both the IBL and R&D Systems kits. However no significant differences in FL-OPN levels were found between patients with DF and patients with DHF (Fig. 1A; Suppl. Fig. 1A).

FL-OPN levels were significantly lower during the recovery phase than they were during the critical phase for patients with DF (median:1,199 pmol/L; $P < 0.00001$), as well as for patients with DHF (median: 907 pmol/L; $P < 0.001$) (IBL kit). The FL-OPN levels measured by the R&D Systems kit were also significantly decreased in patients with DF (121 ng/ml; $P < 0.0001$) and patients with DHF (285 ng/ml; $P < 0.01$). Interestingly, the levels were significantly lower than those of HCs, 0.57 fold and 0.68 fold reduction in DF and DHF by using IBL kit, respectively ($P < 0.05$; Fig. 1A). However, using the R&D Systems kit, FL-OPN levels remained greater in DENV-infected subjects than they were in HCs during the recovery phase (1.7-fold increase in DF, not significant; 4.2-fold increase in DHF, $P < 0001$; Suppl. Fig. 1A). A Spearman rank correlation coefficient revealed a significant correlation between IBL and R&D Systems assessments of the DENV-infected patients' FL-OPN levels during the critical phase. However, no correlation was evident during the recovery phase (Suppl. Fig. 1B), indicating that the IBL ELISA kit only measured the FL-OPN, and specifically did not measure the cleaved form, whereas the R&D Systems kit measured both forms, but could not differentiate between them.

Elevated Plasma Levels of TrOPN Persist and Iincrease During the Recovery Phase of DENV-Infection

The levels of trOPN were elevated during the critical phase, both in patients with DF (median: 38 pmol/L) and patients with DHF (median: 43 pmol/L), as compared with the HCs (1 pmol/L). As assessed using the Kruskal–Wallis test, the trOPN levels of patients with DF and patients with DHF were significantly different from those of the HCs ($P < 0.0001$ and $P < 0.0001$, respectively; Fig. 1B). Interestingly, the levels of trOPN during the recovery phase were significantly higher than those during the critical phase in both the DF and DHF groups (median: 979 and 1348 pmol/L; $P < 0.0001$ and $P < 0.01$, respectively; Fig. 1B).

Elevated Levels of TrOPN are Inversely Associated with FL-OPN Levels During the Recovery Phase of DENV-Infection

We found no correlation between FL-OPN and trOPN during the critical phase (data not shown). During the recovery phase, however, a strong inverse correlation was observed between the trOPN levels and IBL FL-OPN levels in both the DF group ($r = -0.84$, $P < 0.0001$) and the DHF group ($r = -0.73$, $P < 0.05$) (Fig. 1C). We did not observe a similar correlation for the R&D FL-OPN levels in recovery phase (Suppl. Fig. 1C).

Coagulation Marker Levels in DENV-Infected Patients

The levels of TAT, D-dimer, and TM were significantly higher in -infected patients than they were in HCs based on the results of a Mann-Whitney test. Ferritin levels appear to be elevated in DENV- infected patents (the reference range was 25-283 ng/ml in HC according to manufacturer). Furthermore, a Wilcoxon signed-rank test indicated that the levels of each of these markers had declined significantly between the critical and recovery phases (Fig. 2).

Plasma Levels of OPN Correlated with Hematological and Coagulation Biomarkers Throughout the Course of DENV-Infection

To study whether plasma FL-OPN levels were correlate with clinical and laboratory markers during DENV-infection, we examined potential correlations using Spearman's rank correlation coefficient from all DENV-infected patients, because the levels of these markers did not differ significantly between patients with DF and patients with DHF. In the critical phase, FL-OPN levels were positively correlated with elevated hematocrit, D-dimer, and ferritin levels ($r = 0.37$, 0.26, and 0.25, respectively) and negatively correlated with platelet count ($r = -0.44$). In the recovery phase, an even stronger correlation was observed between FL-OPN and D-dimer levels ($r = 0.42$), a moderate correlation was observed with TAT ($r = 0.42$), and a negative correlation was observed with lymphocyte levels ($r = -0.29$; Table 1).

TrOPN Levels were Associated with Virological, Hematological, and Coagulopathy Markers in DENV-Infection

The Spearman rank correlation coefficient was used to determine the extent to which laboratory findings and coagulation markers were correlated with trOPN in the DENV-infected patients. During the critical phase, monocytes, DENV viral load, and TAT levels were associated with trOPN ($r = -0.26$, 0.46, and −0.37, respectively). During the recovery phase, levels of lymphocyte and ferritin were positively correlated with those of trOPN ($r = 0.28$ and 0.33, respectively) and, additionally, TAT levels and platelet counts were observed to be inversely correlated with trOPN levels ($r = -0.34$ and −0.32, respectively; Table 1).

Discussion

To the best of our knowledge, this is the first study to provide evidence that the plasma levels of matricellular protein FL-OPN and trOPN are elevated in patients with DF and DHF during the critical phase of illness, as compared with healthy subjects. During the recovery phase, FL-OPN levels declined; however, the levels of the thrombin cleaved byproduct trOPN continued to increase significantly.

The magnitudes of the increases in these proteins were much greater in this study than in previous reports on other diseases. Although trOPN has been detected in joint and ocular fluids in local inflammatory diseases, detection of trOPN in plasma (>100 pmol/L) in a disease-specific manner has not been demonstrated [30,31]. DENV infects a plethora of cell types, including endothelial cells, fibroblasts, and

Table 1
Correlation of full-length and thrombin-cleaved OPN with laboratory and coagulation markers.

Laboratory findings/ Coagulation markers	Critical Phase				Recovery Phase			
	FL-OPN (IBL)		trOPN		FL-OPN (IBL)		trOPN	
	r	P	r	P	r	P	r	P
Increase of Hct (%)	0.37	<0.01	-	n.s.	-	n.s.	-	ns.
Plt (10^3/ul)	- 0.44	<0.001	-	n.s.	-	n.s.	- 0.32	<0.05
Ly (%)	-	n.s.	-	n.s.	- 0.29	<0.05	0.28	<0.05
Mono (%)	-	n.s.	- 0.26	<0.05	-	n.s.	-	ns.
Viral RNA(copy/ml)	-	n.s.	0.46	<0.001	-		-	
TAT (ng/ml)	-	n.s.	- 0.37	<0.01	0.42	<0.001	- 0.34	<0.01
D-dimer (ng/ml)	0.26	<0.05	-	n.s.	0.42	<0.001	-	ns.
TM (pg/ml)	-	n.s.	-	n.s.	-	n.s.	-	njs.
Ferritin (ng/ml)	0.25	<0.05	-	n.s.	-	n.s.	0.33	<0.01

Abbreviation: TAT, thrombin anti-thrombin complex; TM, thrombomodulin; OPN, osteopontin; FL, full-length; tr, thrombin-cleaved.

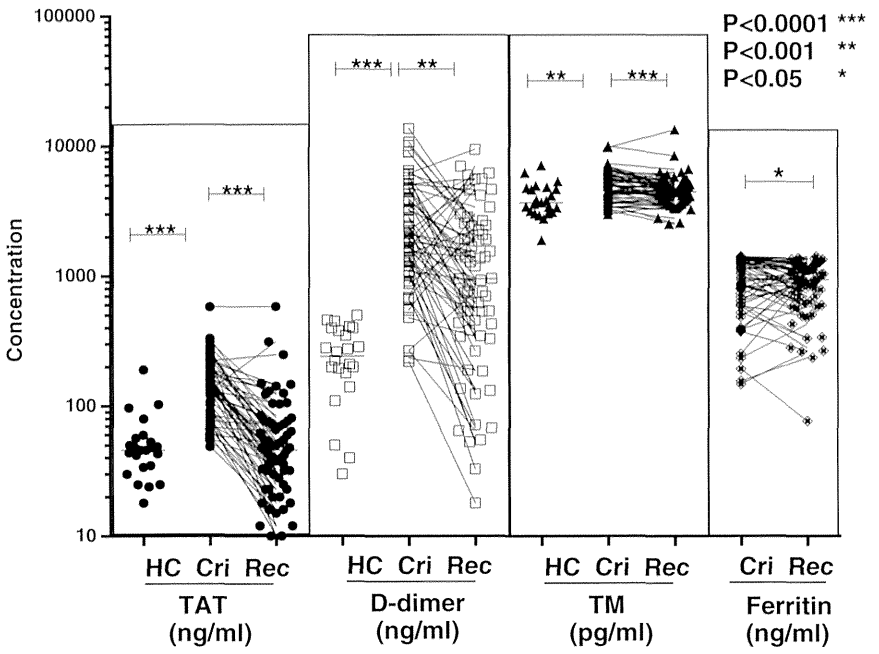

Fig. 2. Coagulation marker levels. Plasma levels of thrombin-antithrombin complexes (TAT), and D-dimer, as well as serum levels of thrombomodulin (TM) are shown for healthy controls (HCs) and dengue virus (DENV)-infected patients during the critical (cri) and recovery (rec) phases. Ferritin levels from HCs were not measured because of a sample shortage; the reference range was 25–283 ng/ml in healthy individuals.

macrophages [41–43]. Because FL-OPN is released from many of these cell types [23], DENV-infection could exacerbate the release of FL-OPN. Our study also demonstrated significant increases in the levels of D-dimer, TAT, TM and trOPN through the course of acute DENV-infection. Activation of coagulation pathways is known to be initiated by endothelial damages caused by DENV-infection [5]. We observed an inverse correlation between TAT and trOPN during the both critical and recovery phases, suggesting that the underlying mechanisms are complex. The involvement of NS1, which is known to inhibit prothrombin activation into thrombin, in delayed increase of trOPN is less likely because the levels of TAT and trOPN were inversely correlated. It is also well known that TAT is a relatively stable thrombin generation marker [44], in contrast trOPN can be substrate for several enzymes such as MMP-3, MMP-7 and TAFIa [27]. The inverse correlation may indicate the activation of these enzymes with thrombin in inflammatory critical phase. The reason of further increase of trOPN in recovery phase is unclear, but it is possible that trOPN bind to integrins in critical phase in inflammatory tissue. Furthermore, it has been proposed that TAFIa is reduced in DENV-infected patients because of consumption secondary to excessive thrombin generation [16]. More detailed kinetics of the levels of OPN and trOPN with TAFIa would clarify the underlying mechanisms of their generation.

The most unique characteristic of trOPN is the expression of a functional integrin binding site for the integrin α9. The integrin α4 can bind both full-length and trOPN via SVVYGLR^{168} [45,46]. In contrast, integrin α9 can only bind trOPN at cryptic cleaved site Arg168 [47]. Reportedly, Arg168 is required for α9 binding in addition to Val164, Tyr165, and Leu167 for cell adhesion and migration [48]. Furthermore, overexpression of FL-OPN was shown to regulate tumor metastasis and angiogenesis through the integrin αVβ3 [49]. Indeed, further cleavage of trOPN by TAFIa is believed to lose its inflammatory activities [37]. Therefore, more studies are necessary to understand the roles of OPNs in inflammation.

We have observed that FL-OPN levels are associated with both hematocrit levels and platelet counts, which suggests that FL-OPN levels may reflect the relative level of plasma leakage and thrombocytopenia during the critical phase of DENV-infection. Because FL-OPN was also positively correlated with D-dimer levels during both the critical and recovery phases (and with ferritin in the critical phase), these results suggest that plasma levels of FL-OPN may track the progression of inflammation and coagulopathy during DENV-infection. In the recovery phase, a positive correlation between trOPN and ferritin was also noted, and an inverse correlation was observed with platelet count. TrOPN is known to bind αVβ3 integrin on platelets and contributes to their migration to inflammatory sites [50]. Further, trOPN acts as a chemo-attractant for hematopoietic stem cells and possibly progenitor cells [36].

Taken together, our study demonstrated the marked elevation of plasma levels of FL-OPN and thrombin-cleaved OPN product, trOPN, in DENV-infection for the first time. Further studies on the biological functions of these matricellular proteins in DENV-infection would clarify its pathogenesis.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.thromres.2014.05.003.

Conflict of Interest Statement

All authors declare that they have no conflicts of interest.

Acknowledgments

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REPORT FROM THE COMMITTEE OF THE JAPANESE SOCIETY FOR TUBERCULOSIS: A STUDY OF TUBERCULOSIS AMONG FOREIGNERS RESIDENT IN JAPAN, 2008

—With Particular Focus on Those Leaving Japan in the Middle of Treatment—

The International Exchanging Committee of the Japanese Society for Tuberculosis

Background

Japanese statistics of tuberculosis (TB) show a steady decline in the incidence of newly identified cases of tuberculosis. There were 19.4 new cases per 100,000 population in 2008 and it was the first year showed the rate dropped below 20 per 100,000 population. Nevertheless, it is still higher prevalence rate compared to that of other developed countries, with over 24,000 new patients developing TB disease every year, and Japan is still considered as a moderate prevalence nation[1].

The proportion of foreign residents with TB among all patients in Japan has been rising steadily and its number reached to 945 in 2008. Nearly 60% of foreigners with TB were from China, South Korea and the Philippines. Total of 70% of these cases were concentrated in the 20-39 age bracket. An analysis of therapeutic results for the cohort of new cases registered in 2007 showed many patients were transferred out withdrawing treatment, with some patients choosing to drop out or discontinue treatment in order to return home.

As the foreign population in Japan is expected to increase in the future, it is inevitable that many foreigners enter from countries with a high prevalence of TB. We conducted a national survey with the aims of elucidating the problems that compel foreign residents to return to their home country partway through treatment, and social backgrounds in foreigners with TB returning home before completion of treatment. We also analyzed demographic difference and risk factors in foreign resident patient groups who underwent successful treatment, discontinued treatment and returned to their home country.

Methodology

Questionnaires were distributed to 530 public health centers throughout Japan. Respondents were asked to fill in and return the questionnaires regarding foreign patients with TB registered between January 1 to December 31, 2008. Responses were entered into a database created using Microsoft Access 2003. Statistical analyses were performed using Minitab14.

This study was approved by the Ethics Review Committee of Nagasaki University Hospital (approval number 10022578).

Survey structure

1. Number of registered foreign TB cases — all eligible facilities nationwide
2. Patient information — for eligible facilities with registered foreign TB patients

1) Facility-specific ID of patients
2) Gender
3) Age
4) Nationality
5) Residency status
6) Occupation
7) Health insurance cover
8) Drug susceptibility of isolates
9) Treatment outcome
10) Patient returning home during treatment (yes/no)

If yes to Q11:

11) Reason(s) for returning home
12) Attempts to encourage the patient to remain in Japan for treatment
13) Adequacy of information provided to patient
14) Treatment strategy after the patient returns home
15) Tracing the treatment outcome in home country

All respondents:

16) Problems/issues in the management of foreigners with TB
17) Comments regarding the management of foreigners with TB

Privacy policy

This epidemiological study involves retrospective analysis of data on foreign residents in Japan who were diagnosed and registered as patients with TB. Personal information such as name, initials, address or date of birth — is not included in the study. Accordingly, provisions in relation to the collection of personal information are not considered applicable. The researchers have exercised due care in information collection and handling. Data held on computers is subject to ID and password protection, and is accessible only by the researchers. Data processing is performed in research rooms that can only be physically accessed by authorized personnel.

Results

Questionnaires were sent to 530 public health centers throughout Japan. Responses were received from 449 facilities, for a response rate of 84.7%. Foreign residents with TB were diagnosed in 243 facilities (54.1%). There were 834 reports between January and December, 2008. A total of 44 patients were transferred to another domestic facility and 57 patients were transferred from another domestic facility; for the purpose of simplification, however, transferred cases have been incorporated in the total and are not analyzed separately. The potential influence of transfers should be taken into account when considering the conclusions of the study.

• Nationality and age

Figs. 1 and 2 show the distribution of nationality and age of the foreign patients, respectively. Note the consistency with the study published in *Kekkaku* Vol. 84, No. 11 : 743–746 (2009). In both studies, China, the Philippines and South Korea account for around 60% of foreigners with TB in Japan, with much of the remaining 40% were also from the Asian region. Similarly, most cases (around 70%) are concentrated in the 20–39 age bracket, which is markedly younger than that of the native Japanese population.

• Residency status and health insurance

The most common residency status among foreigners with

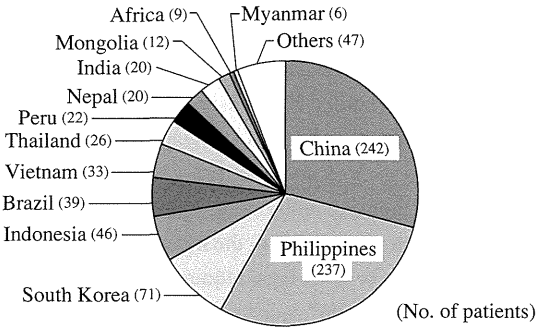

Fig. 1 Nationality distribution of foreign residents with tuberculosis in Japan

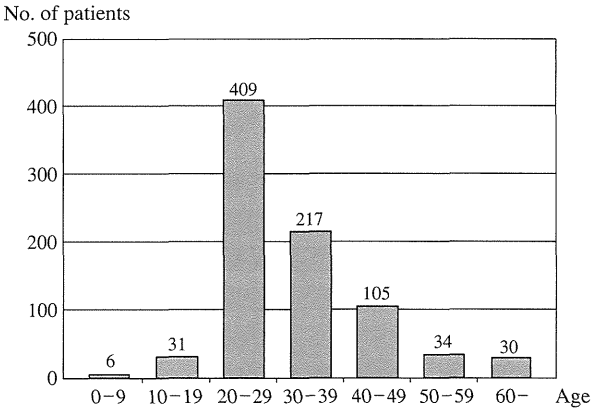

Fig. 2 Age distribution of foreign residents with tuberculosis in Japan

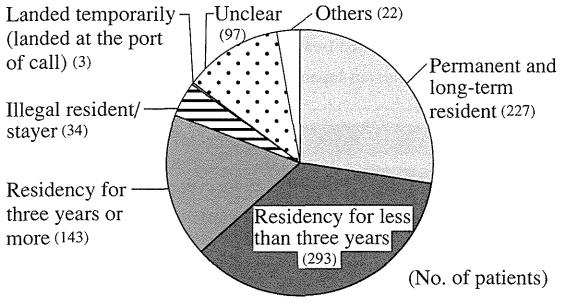

Fig. 3 Residency status of foreign residents with tuberculosis in Japan

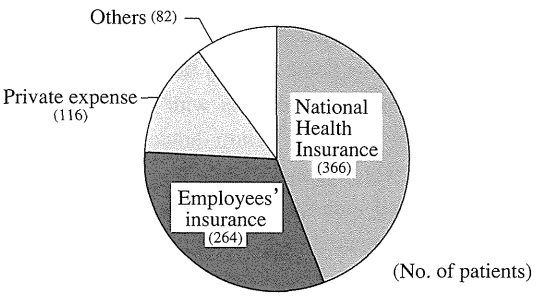

Fig. 4 Category of health insurance of foreign residents with tuberculosis in Japan

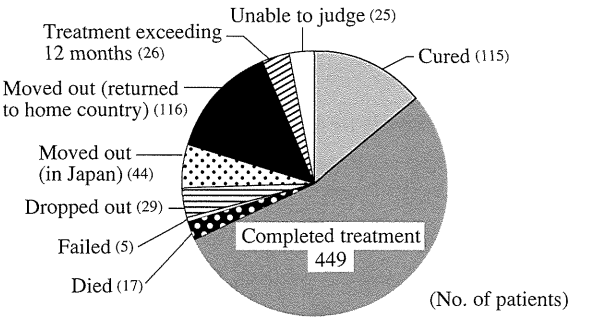

Fig. 5 Treatment outcomes of foreign residents with tuberculosis in Japan

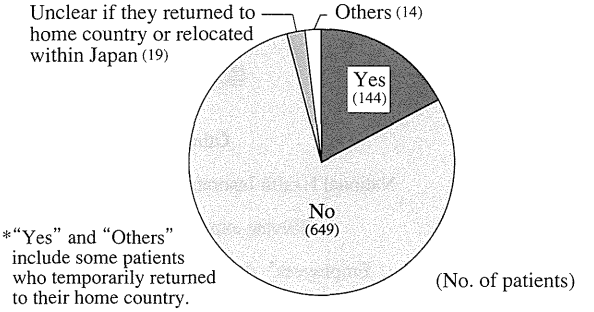

Fig. 6 Among foreigners with tuberculosis living in Japan, those who returned (or did not return) to home country midway through their treatment

TB was residency for less than three years (293 patients, 35.8 %), followed by both permanent and long-term residents (227, 27.7%), and residency for three years or more (143, 17.5%) (Fig. 3). There were 34 illegal residents (4.2%). As Fig. 4 indicates the majority of patients (630, 76.1%) had health insurance, either under the National Health or through an employee health insurance scheme, while 116 (14.0%) paid their health expenses directly.

• Treatment outcome and number of patients returning home (including partway through treatment)

As shown in Fig. 5, 116 of the 826 reported cases (14%) discontinued treatment in order to leave Japan (normally to return their origin). This number swells to 144 (17.4%) if patients who returned home temporarily during treatment are also included (see Fig. 6).

• Influence of residency status and health insurance cover on likelihood of returning home midway through treatment

Fig. 7 illustrates how foreigners with less permanency in Japan were more likely to return home during treatment. Seventy-one of 292 foreigners with residency of less than three years (24.3%) and nine of the 34 illegal residents (26.5%) returned home, compared to just seven of 141 with residency for three or more years (5.0%) and two of 223 permanent and long-term residents (1.0%). Those with health insurance were more likely to remain in Japan (Fig. 8). Only 19 of 361 patients with National Health Insurance (5.3%), and 40 of 263 patients with employee insurance (15.2%) returned home country, whereas 39 of 116 patients with no health insurance (33.6%) returned home country.

• Influence of location on likelihood of returning home midway through treatment

Figs. 9 and 10 illustrate the extent of regional variation of cases of foreigners returning home during treatment for TB, broken down by prefecture and major city. Residents of the Tokyo metropolitan area were more likely to complete treatment, whereas those in regional areas were more likely to return home country. Only 2.9% of residents in Metropolitan Tokyo returned home country during treatment (also Tokyo's 23 wards=9.9%, Yokohama=8.0%, Kawasaki=9.1% and Osaka=9.4%) compared to 50% of patients resident in Ibaraki Prefecture, 40% in Shiga Prefecture, 32% in Hyogo Prefecture and 31% in Kumamoto Prefecture.

• Reasons for returning home country

Fig. 11 shows the breakdown of reasons for leaving Japan. A significant number of respondents (36 respondents) did not provide an explanation. It is possible that some of these may have had to return home country with unexpected reasons. Where an explanation was provided, the most common reason was forced repatriation or other legal requirement (20 respondents), whereas other reasons were personal circumstances, economic necessity, cessation of employment, and the completion of studies or training (17–19 respondents for each reason).

• Encouraging patients to complete treatment in Japan/providing health advice on treatment outside of Japan

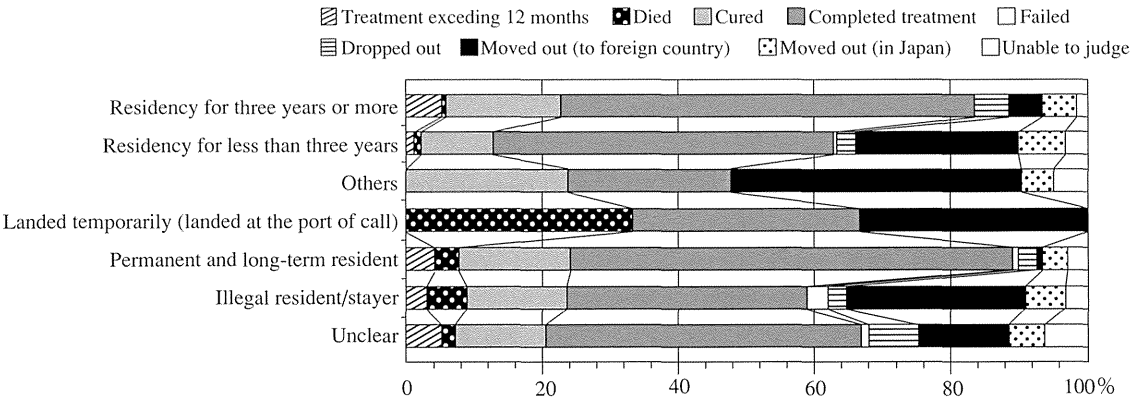

Fig. 7 Relationship between the residency status and treatment outcome of foreign residents with tuberculosis in Japan

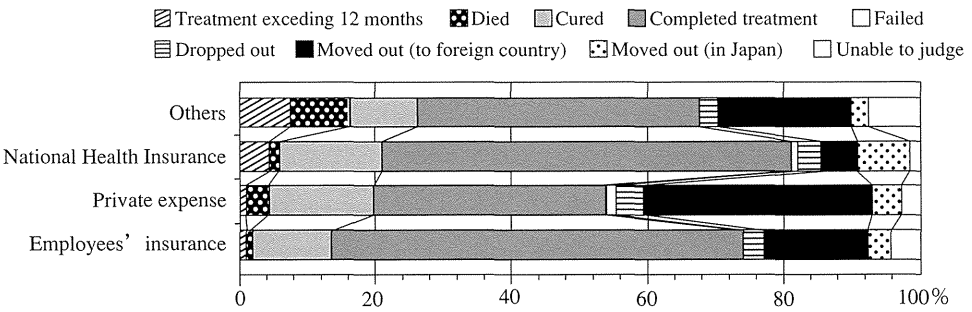

Fig. 8 Relationship between category of insurance and treatment outcomes of foreign residents with tuberculosis in Japan

In most cases, patients who returned home countries during treatment, or even before commencing treatment, had not been encouraged to remain in Japan to complete their treatment. Fig. 12 shows, only 51 facilities (34.5%) reported that they "strongly encouraged" or "encouraged" patients to complete their treatment in Japan. Meanwhile, the majority of facilities were able to provide either "considerable" or "some" health advice to patients who returned home country during treatment (see Fig. 13).

• Follow-up on patients leaving Japan

Fig. 14 shows the approach taken by the treating facilities to patients who returned home country partway through treatment. In most cases (44), patients were given a referral letter and advised to see their local doctor (the patient was responsible for locating a suitable doctor). The response of the facility is "unknown" in 24 cases. A further 22 patients were issued medication for their remaining time in Japan (considered sufficient to complete the treatment) and advised to contact

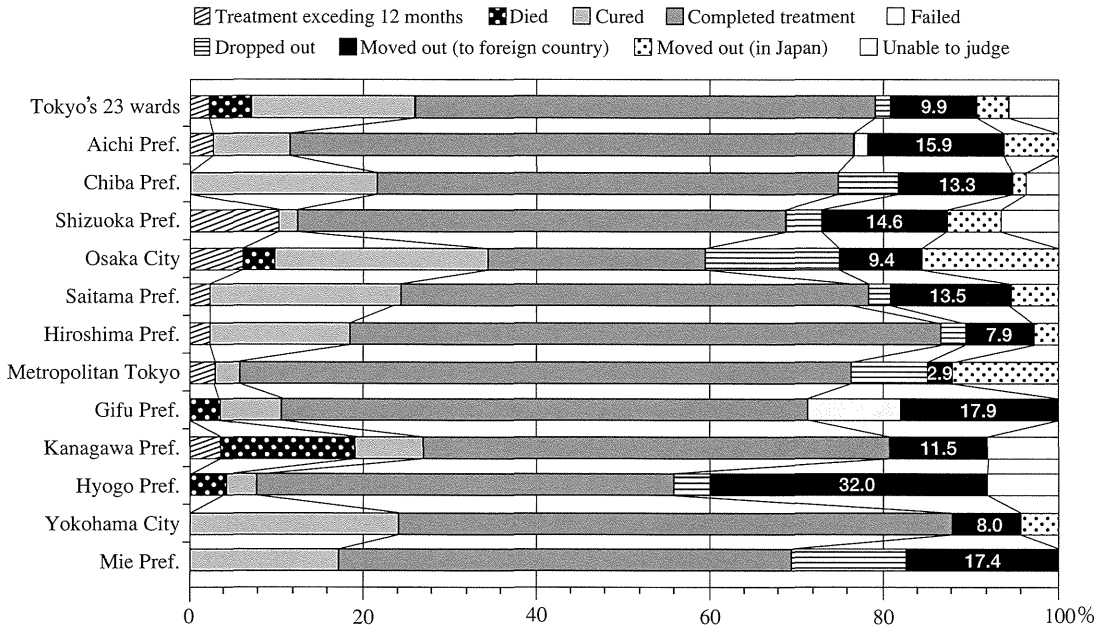

Fig. 9 Treatment outcomes of foreign residents with tuberculosis in Japan by prefecture and by ordinance-designated city (prefectures and cities that have more than 20 patients)

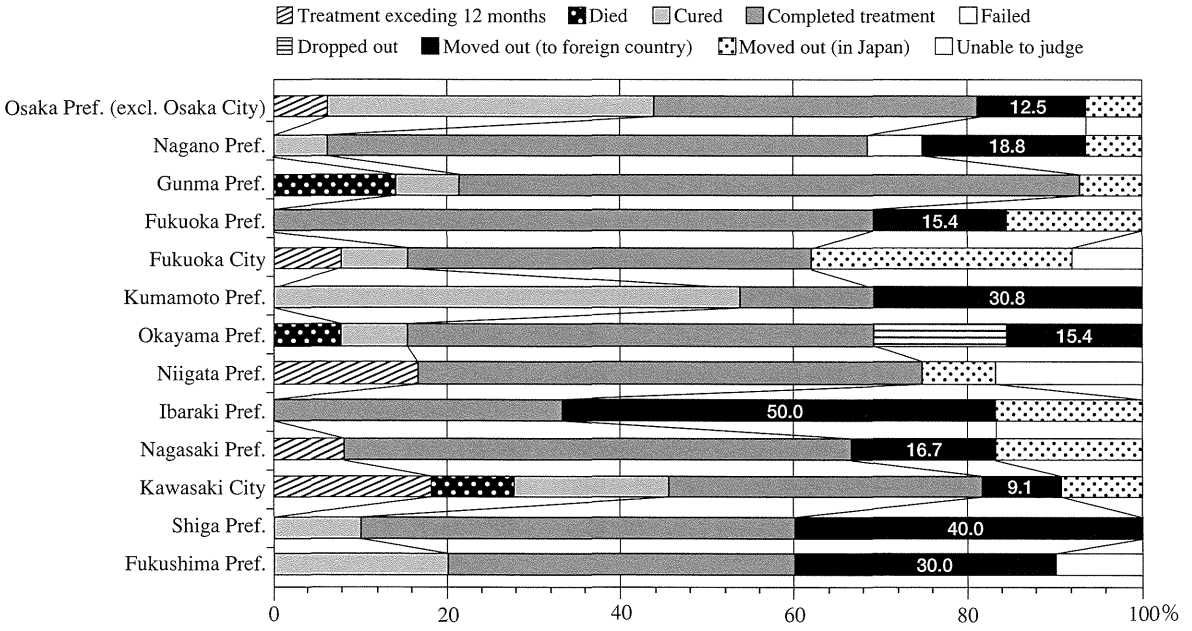

Fig. 10 Treatment outcomes of foreign residents with tuberculosis in Japan by prefecture and by ordinance-designated city (prefectures and cities that have from 10 to 19 patients)