Memorial Lecture by Imamura Award Winner

STUDY OF TUBERCULOSIS IN PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION

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Abstract The research on tuberculosis (TB) comorbid with human immunodeficiency virus infections (HIV/TB), for which this prize was awarded, began with the author's experience with Japan's first HIV/TB case in 1992. In 1997, the clinical characteristics of six HIV/TB cases were presented in the *Japanese Journal of Thoracic Disease*s. In 2001, the author published a paper in *Kekkaku* on the anti-HIV antibody positive rate of TB patients. As part of a research team with the Japanese Ministry of Health, Labour and Welfare (2011–2013), the author surveyed the HIV/TB patients in the National Hospital Organization, and found a more or less unchanging mean 0.39% HIV-positive rate among TB patients. Among these TB cases, 2.1% were multidrug-resistant TB. In 2007, the results of QuantiFERON®-TB-2G (QFT-2G) HIV/TB analysis were reported in *Kekkaku*, showing the usefulness of QFT-2G in immunosuppression cases. Positive rates obtained with QFT-2G and QuantiFERON®-TB Gold (QFT-3G) declined when the peripheral blood lymphocyte count decreased, thought to be a result of QFT's whole-blood collection methods. The author further studied the usefulness of interferon-gamma release assays (IGRAs) in HIV/TB with another health ministry research team (2009–2011). Enzyme-linked immunospot assay and QFT-3G were compared, which yielded better sensitivity and fewer indeterminate cases with the former. Periodic IGRAs were performed in IGRA-positive patients. Ten such cases (2 received isoniazid) were observed for more than 3 years, but none developed TB; however, IGRA values fluctuated during the observation period. It seems highly likely that immune function recovery through antiretroviral therapy lowered the risk of developing active TB. The author further examined the therapeutic interaction of rifampicin with anti-HIV drugs, confirming the feasibility of combining efavirenz and raltegravir. These results were presented at the annual meeting of the Japanese Society for Tuberculosis in Tokyo in 2012. The author intends to continue research with the hope of reducing HIV/TB incidence and improving prognosis.

Key words: Human immunodeficiency virus, Acquired immune deficiency syndrome, Tuberculosis, Interferon-gamma release assay, Multidrug-resistant tuberculosis, Efavirenz, Raltegravir

Introduction

The risk of developing active tuberculosis (TB) increases in many immunodeficient states but is highest in cases of human immunodeficiency (HIV) infection, which involve a marked decline in cellular immunity.

Japan's TB prevalence has declined to 16.1 cases per 100,000 people (2013); however, among other Western nations, prevalence is no more than five cases per 100,000 people, meaning TB is still moderately prevalent in Japan. The number of HIV/AIDS patients in Japan increased until 2008, eventually exceeding 1,500 new cases per year. Growth plateaued in 2009 but rose again in 2013 to the second-highest number of reported cases ever. In this environment, the number of comorbid HIV/TB cases appears unlikely to decrease.

The author treated Japan's first case of HIV/TB at Tokyo National Hospital in 1992[1]. By 1997, the author's team reported a summary of six cases[2], and, to date, the author has experienced 85 HIV/TB cases. Since that initial case, the author has engaged in a variety of research on HIV/TB. The main findings are described below.

1. Surveying changes in HIV-positive rates among TB patients in Japan

The number of HIV/TB patients seen at Tokyo National Hospital has increased yearly since 1992. In most cases, an HIV test is performed if the TB is miliary or nonspecific, but some cases of classical pulmonary TB are found to be HIV-positive purely by chance. Thus, to examine the true extent of HIV-positivity among TB patients, we performed HIV tests on

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all consenting TB patients for 2 years starting in January 1998[3]. In all, HIV tests were performed on 164 TB patients (4 HIV-positive) in 1998 and on 149 patients (6 HIV-positive) in 1999, amounting to a 3.2% HIV-positive rate for the 2 years. Typically not suspected of being HIV-positive, only 1.0% of classical pulmonary TB cases were positive for HIV, but miliary TB cases exhibited a high HIV-positive rate of 28.6%. As TB patients in the Tokyo area may have a high rate of HIV infection, it is important to test TB patients for HIV so infections can be discovered and treatment initiated earlier.

We also conducted fact-finding surveys of National Hospital Organization hospitals nationwide regarding HIV/TB and multidrug-resistant TB (MDR-TB) from 2007 to 2012[4]. Little variation was seen in HIV-positive rates among TB patients, ranging from 0.29% to 0.46% (mean 0.39%) (Table). There were 96 cases of HIV/TB in total, but 82 (85.4%) of these were concentrated in the major urban centers of Tokyo, Osaka, and Nagoya. These regions combined had a 0.91% HIV-positive rate, higher than other, more rural regions. Accordingly, we recommended promoting HIV screening of TB patients in major urban areas. The male-female ratio of the HIV/TB patients was 18:1, and patients' median age was 43 years. In 56% of cases, HIV positivity was discovered owing to TB onset. The mean CD4 count was 156/μL, with many cases of reduced immune function. There were 48 cases of pulmonary TB, and 39 cases of extrapulmonary TB (25 of which were miliary TB). Side effects due to antitubercular agents were common, occurring in 53 of 83 cases (63.9%). While being treated for TB, 42 patients began antiretroviral therapy (ART). Immune reconstitution inflammatory syndrome was observed in 16 of the 26 patients (62%) who began ART within 8 weeks of starting TB therapy. All seven patients who began ART within 4 weeks of starting TB therapy developed the syndrome. There were two cases (2.1%) of MDR-TB, of which one patient was a foreign national. No cases of MDR-TB have been found since 2009, so there appears to be no increasing trend.

2. The usefulness of interferon-gamma release assays (IGRAs) in HIV/TB

The risk of TB infection is extremely high in cases of HIV infection. In recent years, IGRAs have been more frequently used for diagnosing TB infection than the tuberculin skin test (TST). Forms of IGRAs include QuantiFERON®-TB-2G (QFT-2G), QuantiFERON®-TB Gold (QFT-3G), and T-SPOT®TB (T-SPOT); the latter two are currently in use. In 2006, the QFT-2G was the first to be put to use. It has superior sensitivity and specificity, but in cases of HIV infection with markedly reduced cellular immunity, we expected sensitivity to decrease and the number of indeterminate cases to increase. To examine this assumption, we studied the usefulness of QFT-2G in cases of HIV infection[5]. We examined the QFT-2G results, CD4 count, and the TST results in known cases of HIV/TB. In 13 HIV/TB cases, QFT-2G's sensitivity was 76.9%, significantly higher than that of the TST (erythema 38.5%, induration 15.4%). The one indeterminate case had the lowest CD4 count (16/μL). Thus, we considered QFT-2G highly sensitive and sufficiently useful in HIV/TB, although HIV cases with very low CD4 count may return indeterminate results.

Next, QFT-2G and enzyme-linked immunospot assay (ELISPOT; this study used T-SPOT) were performed simultaneously on 230 pulmonary TB patients with positive tubercle bacilli cultures[6]. Sensitivity was compared based on the lymphocyte count. The overall positive rates were 74% for QFT-2G and 92% for ELISPOT, showing better sensitivity in the latter. In the group with a lymphocyte count of 1,000/μL or more, QFT-2G's positive rate was 88% and ELISPOT's was 97%. With a lymphocyte count of 500/μL or less, the positive rate with QFT-2G was 39% and 81% with ELISPOT, a marked decline in QFT-2G's accurate, conclusive positive rate (Fig. 1). A comparison of QFT-3G and ELISPOT produced similar results. That is, when lymphocyte count declined, the positive rate of QFT-3G declined, and the number of indeterminate cases increased (Fig. 2).

We used periodic, simultaneous tests to compare the positive rates of QFT-3G and ELISPOT in HIV-infected patients[7], performing QFT-3G as many as 50 times in 35 HIV-infected patients. Cases that were indeterminate or equivocal using QFT were not found with ELISPOT. There were 13 positive cases diagnosed with QFT versus 22 positive cases with ELISPOT. Patients were grouped per 100/μL of CD4 to compare QFT-3G and ELISPOT (Fig. 3). In all count groups, the ELISPOT positive rate was the same or higher. In the CD4 200/μL or less group, three cases were indeterminate by using QFT-3G. No cases were indeterminate by using ELISPOT, which found two negative cases and one positive case.

The results of the previous studies ultimately show that when the CD4 count declines, the sensitivity of QFT-3G decreases, and the number of indeterminate cases increases. The ELISPOT test was not influenced by CD4 count, indicating the effectiveness of this test in cases of reduced immune function.

Table Cases of TB patients with HIV infection in National Hospital Organization hospitals

Year	No. of TB patients	No. of HIV-positive TB patients	No. of HIV-positive MDR-TB patients
2007	4388	15 (0.34%)	1
2008	4165	19 (0.46)	1
2009	4129	18 (0.44)	0
2010	4122	16 (0.39)	0
2011	4091	18 (0.44)	0
2012	3502	10 (0.29)	0
Total	24397	96 (0.39)	2

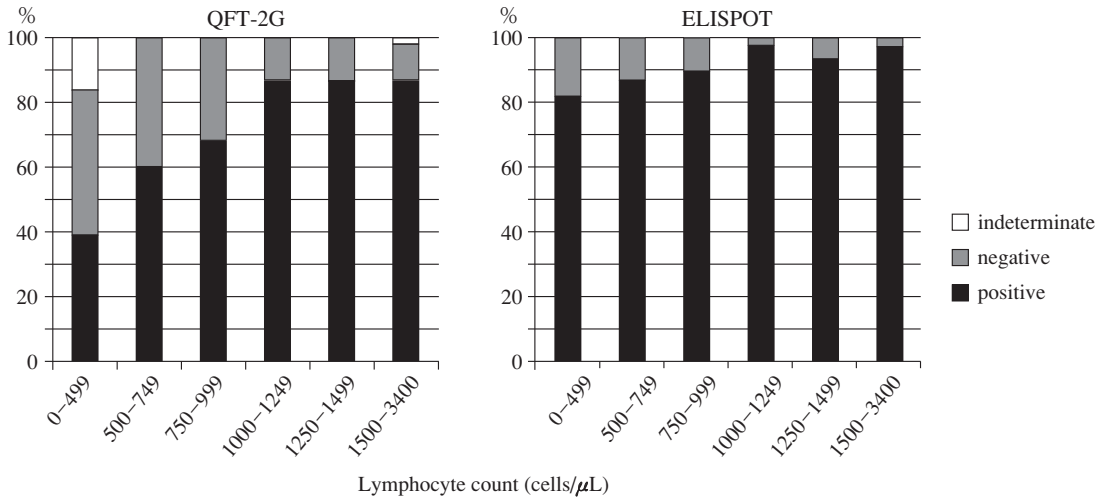

Fig. 1 Influence of lymphocyte count on QFT-2G and ELISPOT performance in pulmonary TB patients

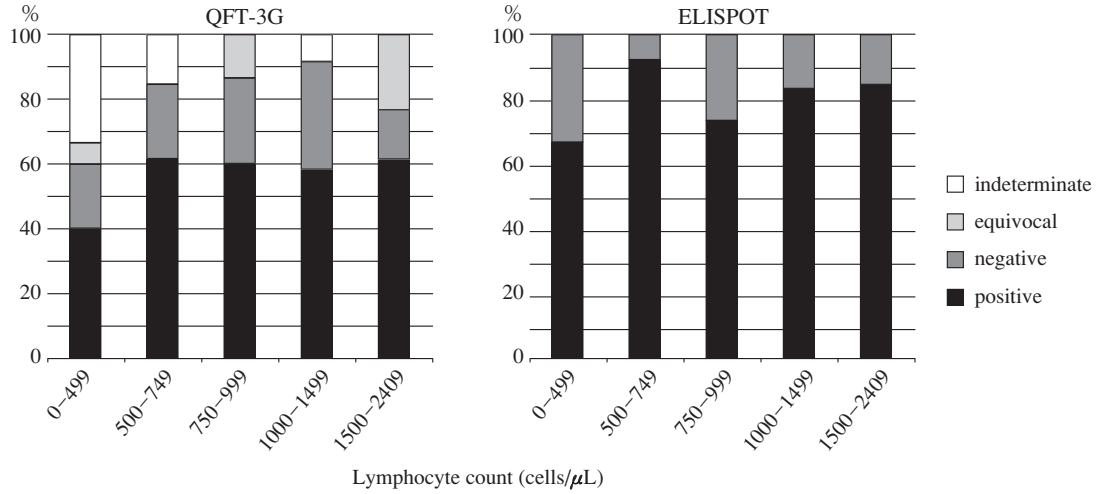

Fig. 2 Influence of lymphocyte count on QFT-3G and ELISPOT performance in pulmonary TB patients

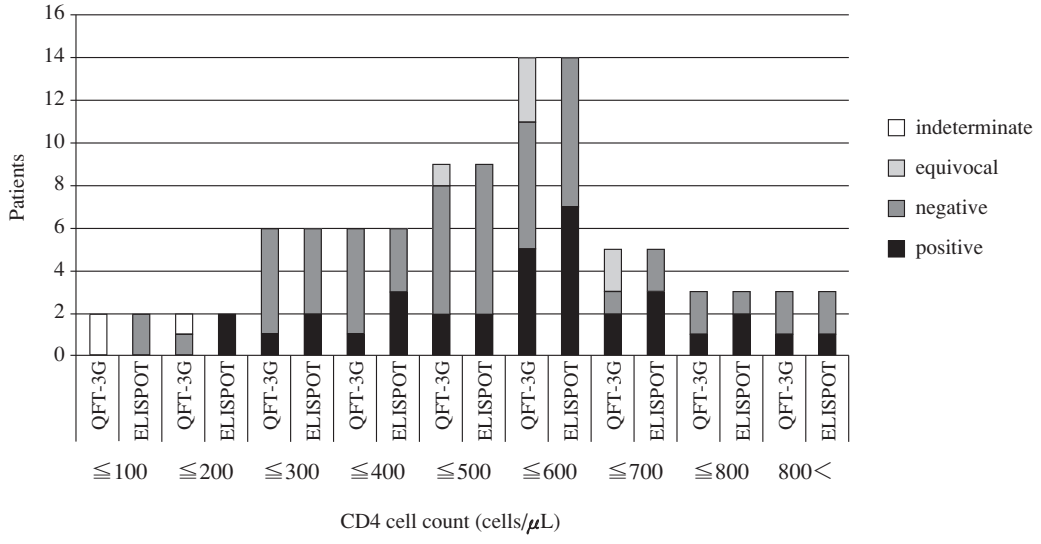

Fig. 3 Influence of lymphocyte count on QFT-3G and ELISPOT performance in HIV/TB patients

3. Research on the early discovery and treatment of latent tuberculosis infection comorbid with HIV infection

As IGRAs can become positive in HIV-infected patients undergoing ART, we sought to determine if TB would unavoidably occur in the future, or if isoniazid (INH) administration could suppress developing active TB in such patients.

We observed six patients with no TB history with positive IGRA results for 3 to 5 years, but none developed TB. In addition, both total conversion to negative results and fluctuations between results were observed.

Of the patients cases with no history of TB that became IGRA-positive during observation, two patients received prophylactic treatment with INH and later became IGRA-negative. The other two patients, observed with no treatment, also became IGRA-negative. For the 3 to 4 years following conversion to IGRA positivity, TB failed to appear in any of these four patients.

Of the 10 aforementioned IGRA-positive cases with no TB history, active TB did not occur over at least 3 years of observation in the eight patients who did not receive INH. It has been reported that repeated IGRA testing can produce variable results regardless of the presence or absence of infection risk, and that this can occur over relatively short time periods[8]. Our hospital confirmed this phenomenon with case fluctuations from positive to negative. The reasons for these fluctuations are unclear, though factors such as instability of test methods, variable interpretations of results, and immune system changes unrelated to infection are thought to play a role. Owing to these IGRA variations, uniformly administering INH to IGRA-positive patients in periodic testing may be premature. In the above cases, it is highly likely that immune function recovery (CD4 $\geqq 200\,\mu$/L) due to ART lowered the risk of developing active TB.

Among ELISPOT-positive cases with TB history at our hospital, three patients continued to test positive for 7 to 13 years, and four patients tested negative after 8 to 15 years. Clearly, it takes substantial time for ELISPOT to become negative in HIV/TB patients.

4. Examination of efavirenz and raltegravir plasma concentrations during rifampicin (RFP) administration

Rifampicin (RFP) induces cytochrome P450 in the liver, which accelerates the metabolism of a variety of drugs and reduces their plasma concentration, including that of key anti-HIV drugs. This phenomenon can make anti-HIV drug selection difficult. The nonnucleoside reverse transcriptase inhibitor efavirenz (EFV) or the integrase inhibitor raltegravir (RAL) are often selected, as these key drugs are not easily affected by RFP. An effect is still present, however, as the plasma concentration of both drugs were shown to decline when combined with RFP. Thus, it was thought the doses of both drugs need to be increased when combined with RFP, but the pharmacodynamics in Japanese patients have not yet been clarified.

To help clarify this phenomenon, we examined the achievement of optimal doses in patients who received EFV or RAL combined with RFP by measuring EFV and RAL plasma concentration[9]. Plasma concentrations of EFV and RAL were measured in 15 patients with HIV/TB. Patients received RFP combined with EFV or RAL at our hospital from 2001 to 2011. There were 14 men and one woman (14 received EFV and one received RAL) who received 600 to 800 mg/day of EFV. Blood samples were taken, on average, 13.7 hours after EFV administration. The median plasma concentration of EFV was 1,696 ng/mL (900−12,685 ng/mL), which was

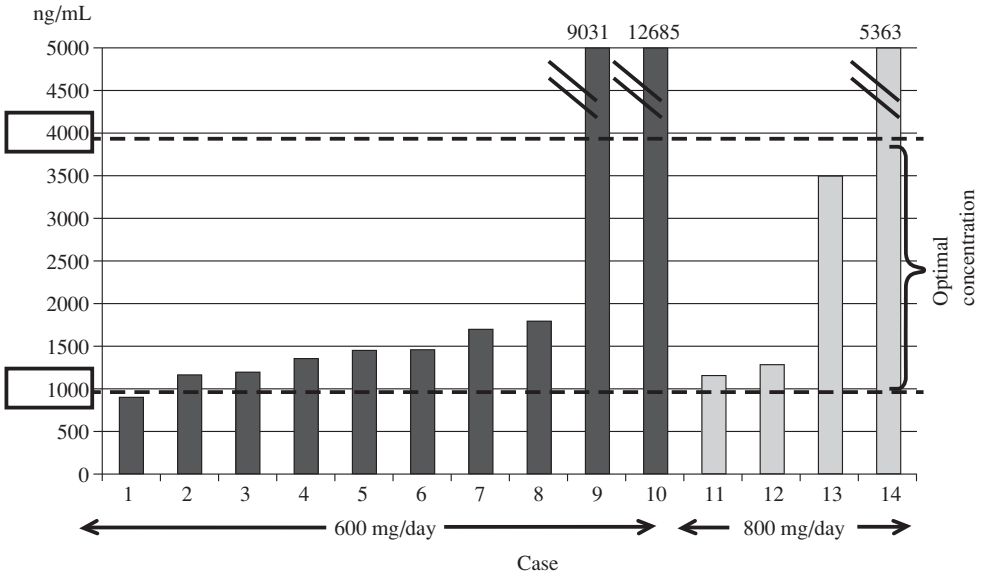

Fig. 4 Plasma concentration (trough) of efavirenz

above the target concentration of 1,000 ng/mL at 14 hours after administration (Fig. 4). Several patients exhibited extremely high plasma EFV concentration, and the dose was actually lowered to reach the optimal concentration. The patient who received RAL (1,600 mg/day) exhibited a trough plasma concentration of 26.6 ng/mL, well above RAL's IC95 of 14.5 ng/mL.

In general, RFP lowers the plasma concentration of anti-HIV drugs by inducing CYP3A4, but there are differences in this reaction across individuals. While EFV is mainly metabolized in the liver by CYP2B6, the gene has three genotypes (G/G, G/T, T/T). The T/T genotype is known to metabolize EFV slowly, which increases serum EFV concentration. However, none of the patients at our hospital had the T/T genotype.

Moreover, the neuropsychiatric symptoms that appear as a side effect of EFV are known to be dependent on plasma concentration. Thus, plasma concentration levels must be monitored to maintain the continuous administration that sustains the anti-HIV effect and suppresses side effects.

Conclusion

The author has researched HIV/TB since 1992. While HIV/TB cases are concentrated in large cities, it is doubtful that Japan's situation will lead to an immediate decline in the number of cases, prompting continued research to reduce HIV/TB incidence and improve prognosis.

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