Article

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Interferon-Gamma Release Assay Combined with Renal Indicators to Reduce the False-Negativity of Latent Tuberculosis Infection in End-Stage Renal Disease with Hemodialysis Patients

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Background: Tuberculosis (TB) is still the main cause of mortality due to a single transfectant, *Mycobacterium tuberculosis* (MTB). Latent tuberculosis infection (LTBI) is a condition characterized by the presence of tuberculosis (TB) that is not clinically apparent but nonetheless shows a sustained response to MTB. Presently, tuberculin skin test (TST) and interferon gamma (IFN-γ) release assays (IGRAs) are mainly used to detect LTBI via cell-mediated immunity of T-cells. For people with end-stage renal disease (ESRD), the diagnosis of patients infected with MTB is difficult because of T-cell dysfunction. To get more accurate diagnosis results of LTBI, it must compensate for the deficiency of IGRA tests.

Methods: Sixty-seven hemodialysis (HD) patients and 96 non-HD patients were enrolled in this study and the study population is continuously included. IFN-γ levels were measured by the QuantiFERON-TB Gold In-Tube (QFT-GIT) test. Kidney function indicators, blood urea nitrogen (BUN), serum creatinine (Cr), and estimated glomerular filtration rate (eGFR) were used to compensate for the declined IFN-γ levels in the IGRA test.

Results: In individuals who were previously undetected, the results of compensation with serum Cr increased by 10.81%, allowing for about 28% more detection, and compensation with eGFR increased by 5.41%, allowing for approximately 14% more detectable potential among them and employing both of them could enhance the prior shortcomings of IGRA tests. when both are used, the maximum compensation results show a sensitivity increase rate of 8.81%, and approximately 23% of patients who were previously undetectable may be found.

Conclusion: Therefore, the renal function markers which are routine tests for HD patients to compensate for the deficiency of IGRA tests could increase the accuracy of LTBI diagnosis.

Keywords: *Mycobacterium tuberculosis* (MTB); latent tuberculosis infection (LTBI); hemodialysis (HD); blood urea nitrogen (BUN); serum creatinine (Cr); estimated glomerular filtration rate (eGFR)

Introduction

Tuberculosis (TB) is caused by a single transfectant bacillus *Mycobacterium tuberculosis* (MTB), it represents a worldwide health issue despite the improvements in medical science and technologies, as it still has high infection and mortality rates [1]. Latent tuberculosis infection (LTBI) is characterized as a persistent immune response to MTB antigens without any clinical testimony of active TB [2]. Nevertheless, LTBI may remain feasible and intransitive at any time and then develop into active TB (ATB) [3].

TB might become the primary cause of death from a single transfectant, substituting the coronavirus infectious disease 2019 (COVID-19) in the future [2]. The COVID-19

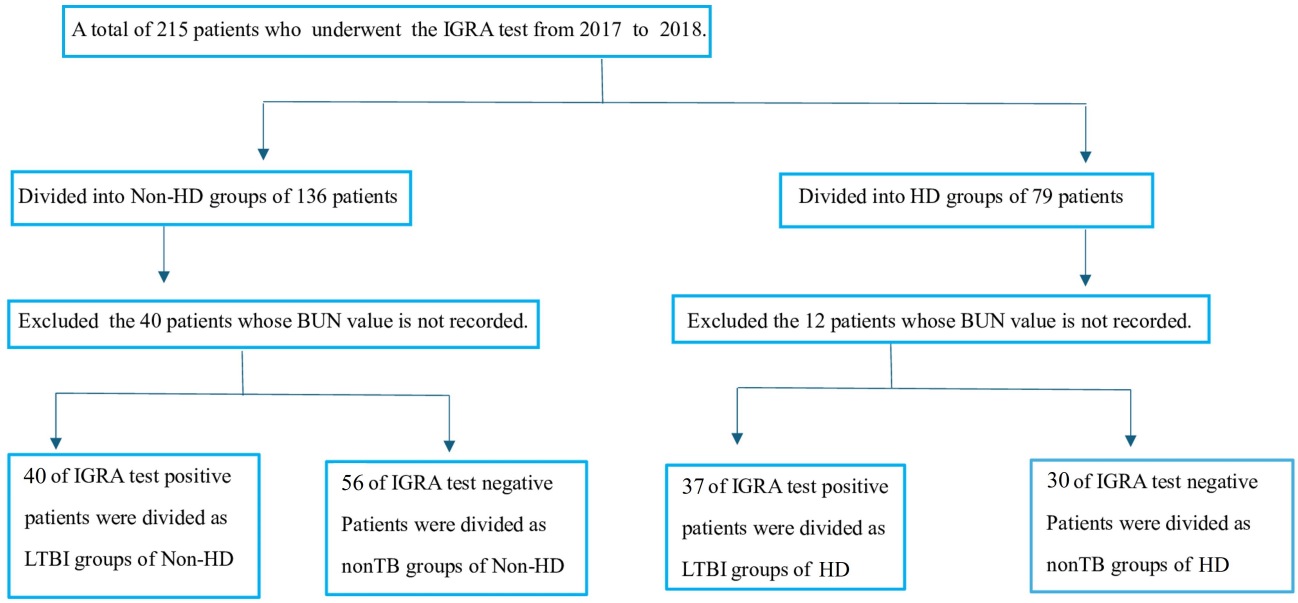

Fig. 1. Flowchart of this study. IGRA, interferon gamma release assay; HD, hemodialysis; BUN, blood urea nitrogen; TB, tuberculosis; LTBI, latent tuberculosis infection.

pandemic continues to have negative effects on accurate TB diagnosis and delays the prompt treatment for TB, in comparison to 2019, there has been a considerable decrease in the number of newly identified cases of TB in 2020. After large raises between 2017 and 2019, there was a decrease of 18% between 2019 and 2020, from 7.1 million to 5.8 million. It recovered to 6.4 million in 2021 [2]. Abatement of the announced population of novel diagnosed with TB in 2020 and 2021 indicated the increased risk of TB transmission [2].

To effectively control TB contagion, early diagnosis, and prompt treatment of LTBI are required [4]. Detection for LTBI can fundamentally decrease the potential for developing into ATB. Presently, the traditional tuberculin skin test (TST) and interferon gamma (IFN-γ) release assays (IGRAs) are optional uses for diagnostic methods [5]. The TST used the Mantoux technique to induce an immunological reaction to MTB and then measured the response of the skin after 2 to 3 days of the infection. A major limitation of TST is the false-positive result for Bacillus Calmette-Guérin (BCG)-vaccinated individuals, and nontuberculous mycobacteria (NTM) infections [6]. Contrary to *in vivo* tests TST, IGRAs are *ex vivo* whole blood tests of cell-mediated immune response that are laboratory-based [5]. IGRAs measure IFN-γ that are released by T-cells based on stimulation via specific antigens to the MTB complex, such as culture filtrate protein 10 (CFP-10) and early secreted antigenic target 6 (ESAT-6) [7]. However, IGRAs might have a cross-reaction with other environmental mycobacterial species such as *M. marinum* and *M. szulga*. They are also much more expensive than TST due to the use of reaction reagents and laboratory equipment [8].

Nevertheless, the gold standard for LTBI diagnosis still has some limitations, both TST and IGRAs are appropriate however defective methods [9]. They are circumstantial methods that, according to the host's sensitivity to MTB antigens, assess the existence of persistent MTB-specific T-cell responses [10]. Therefore, MTB is more dangerous among immunocompromised patients, who are on dialysis and are recipients of organ transplants [11]. End-stage renal disease (ESRD) is defined as the irreversible failure of renal function that requires another kidney replacement therapy such as dialysis and transplantation [12].

The diagnosis of TB disease in ESRD is usually difficult due to the T-cell dysfunction of HD patients [13]. This causes irregular IFN-γ levels, which would affect the cell-mediated tests TST and IGRAs and lead to false-negative results in the diagnosis of LTBI [14]. To estimate renal function, we generally used easily gained plasma markers containing blood urea nitrogen (BUN) and serum creatinine (Cr) [15]. Both BUN and Cr are produced by the process of metabolism of nitrogen compounds [16]. The most commonly used marker of renal function is serum Cr, which supplies short-term ATP in muscle and other tissues. It is cleared by glomerular filtration rate (GFR) and expelled in the urine [17]. For patients who were both suffering from LTBI and renal insufficiency, their IFN-γ levels might be lower than the individuals who only suffered from LTBI because of their immune suppression due to HD. Therefore, the IGRA test may lead to a false-negative result in individuals that were suffering from both LTBI and renal insufficiency.

Therefore, in the present study, for a more accurate diagnosis of LTBI with ERSD who receive hemodialysis (HD), three renal function indicators, BUN, serum Cr [1],

Table 1. Characteristics of study participants.

Characteristics		Non-HD subjects, n (%) (median = 59 SD ± 14, range = 23–86)	HD patients, n (%) (median = 60 SD ± 11.84, range = 32–90)
Age	20 s	4 (4.2)	0 (0.0)
	30–40 s	20 (20.8)	11 (16.4)
	50–60 s	55 (57.3)	40 (59.7)
	≥70 s	17(17.7)	16 (23.9)
Sex	Male	56 (58.3)	30 (44.8)
	Female	40 (41.7)	37 (55.2)
Current smoker		8 (8.3)	1 (1.5)
BCG scar or vaccination		96 (100.0)	37 (55.2)
Contact TB history		3 (3.1)	0 (0.0)
Abnormal chest X-ray lesions		0 (0.0)	0 (0.0)
Underlying diseases			
Diabetes mellitus		29 (30.2)	32 (47.8)
Autoimmune disease		17 (17.7)	3 (4.5)
Total		96 (100.0)	67 (100.0)

BCG, Bacillus Calmette-Guérin; SD, Standard Deviation.

and estimated glomerular filtration rate (eGFR), are used to compensate for the whole blood IFN-γ level of IGRA tests. We focus on the ESRD patients who received hemodialysis treatment, and those patients are in the TB high-risk group due to immune deficiency. However, the situation that ESRD patients infected with LTBI and the gold standard for LTBI diagnosis rely on indirect immune response [13]. ESRD patients frequently receive hospital treatment or are hospitalized, this poses a significant risk for immunocompromised patients who have a weakened immune system. To detect more patients who were potentially infected with LTBI which easily develops into ATB such as ESRD patients, we compensate the original IGRA results with the renal function. The flow chart for classifying the data is shown in Fig. 1. A total of 215 patients who underwent the IGRA test between 2017 and 2018 were included in the study. Patients with missing BUN values were excluded. The remaining patients were then separated into two groups: Non-HD group and HD group. As a result, 67 ESRD-HD patients and 96 non-HD patients were registered in the present study, and their IFN-γ levels were measured by the QuantiFERON-TB Gold In-Tube (QFT-GIT) test (Qiagen, Hilden, Germany). The compensated whole blood IFN-γ level with BUN, serum Cr, and eGFR levels could overcome the false-negativity of the QFT-GIT test (Qiagen) results in the HD patient group to the diagnosis of LTBI. In addition to the high risk caused by the immune suppression for ESRD-HD patients, it is necessary to compensate for the cut-off value for the diagnosis of LTBI in ESRD-HD patients who might be infected with LTBI.

Materials and Methods

Study Participants

A total of 67 ESRD-HD patients and 96 non-HD subjects were enrolled in this study, and their whole blood samples were gathered from March 2017 to June 2018 at the Kosin University Gospel Hospital, Busan, the Republic of Korea. These clinical samples were obtained from the Institutional Ethics Committee of Catholic University of Pusan and Kosin University Gospel Hospital approved the study (approval numbers CUPIRB-2017-036 and KUGH 2017-11-042). All demographic clinical data were gathered from an electronic medical chart review which contains sex, age, TB contact history, prior TB, Bacillus Calmette-Guérin (BCG) scar or vaccination history, co-morbidity diabetes mellitus (DM) and ischemic heart disease (IHD), smoking history, chest X-ray (CXR), and LTBI treatment history if available. The criteria of abnormal CXR were fibrotic infiltrates with pleural thickening or calcified nodules over the upper lung fields or other fibrotic lesions documented from previous TB.

The characteristics of the participants registered in the present study are described in Table 1. The statistic for comparison of characteristics of the participants was implemented by an unpaired *t*-test using GraphPad Prism v. 8.00 (GraphPad Software, San Diego, CA, USA). All enrolled participants were more than 19 years old; the median age of ESRD-HD patients was 60 years old, with a range of 32 to 90 years old, and the male-to-female ratio was 30:37 (44.8%:55.2%). HD groups of ESRD patients received more than three months of HD therapy. Those who are co-infected with human immunodeficiency virus (HIV) infection, liver cirrhosis of Child-Pugh class C, cancer or autoimmune disease, and patients who received chemother-

Table 2. Formula of conversion factors used in this study.

Conversion factor	
BUN	A = BUN value of HD patient/(BUN mean value of non-HD patients) BUN index = (A value) of each HD patients/the lowest (A value) among All HD patients
Cr	B = Serum Cr value of HD patient/(Serum Cr mean value of non-HD patients) Serum Cr index = (B value) of each HD patients/the lowest (B value) among All HD patients
eGFR	C = eGFR mean value of non-HD patients/eGFR value of HD patients eGFR index = (C value) of each HD patients/the lowest (C value) among All HD patients
Mitogen-nil	D = 10/Mitogen-nil value of HD patients Mitogen-nil index = (D value) of each HD patients/the lowest (D value) among All HD patients

Cr, creatinine; eGFR, estimated glomerular filtration rate.

Table 3. Formula of revisions used in this study.

Formula of revisions	
BUN revision	BUN conversion factor × HD patients IFN-γ value (TB-nil)
Cr revision	Cr conversion factor × HD patients IFN-γ value (TB-nil)
eGFR revision	eGFR conversion factor × HD patients IFN-γ value (TB-nil)
Mitogen-nil revision	Mitogen-nil conversion factor × HD patients IFN-γ value (TB-nil)
Cr + eGFR	Cr revision value × eGFR revision value × HD patients IFN-γ value (TB-nil)

IFN-γ, interferon gamma.

apy within the last three months, besides those who had any active TB treatment history, were excluded. The QFT-GIT test was performed for screening of LTBI for all participants. LTBI was diagnosed depending on positive results of the QFT-GIT test, with no symptoms or signs of active TB and no history of previous TB. HCs all displayed no symptoms or signs of active TB and no signs of active TB on chest radiographs. Those eligible subjects were separated into HD and non-HD groups depending on their medical chart results.

Blood Collection and Serum Preparation

Whole blood samples were gathered, and serum was separated using VACUETTE® Plain tube (Greiner Bio-One, Frickenhausen, Australia). As recommended by the instructions, A VACUETTE® Plain tube (Greiner Bio-One) was kept at room temperature (RT) for 10 min, and centrifuged the whole blood samples at 4000 ×g for 15 min for separated serum samples and transferred to a 1.5 mL Eppendorf tube and stored at –20 °C until use. ESRD-HD patients' whole blood samples were performed before hemodialysis, and those blood samples were performed in the IGRAs in advance, and then conducted the biochemical tests.

Measurement of Blood Urea Nitrogen (BUN)

BUN was measured by an Atellica Ch 930 Analyzer (Siemens Healthineers AG, Erlangen, Germany) and Atellica® CH Urea Nitrogen (Siemens Healthineers AG). Urea creates ammonia and carbon dioxide in the situation with water and urease. The ammonia reacts with 2-oxoglutarate when glutamate dehydrogenase (GDH) and reduced nicotinamide adenine dinucleotide (NAD)+hydrogen (H) (NADH) exist. It is measured at 340/410 nm for the inverse rate reaction of the oxidation of NADH to oxidized nicotinamide adenine dinucleotide (NAD). Automated analysis was performed according to the manufacturer's instructions.

Measurement of Serum Creatinine (Cr) and Estimated Glomerular Filtration Rate (eGFR)

Serum Cr was measured using a Chemistry Analyzer AU5800 (Beckman Coulter, Brea, CA, USA) and an AU Creatinine reagent (Beckman Coulter). The kinetic modification of the Jaffe procedure was used, which forms a yellow-orange complex through creatinine having a reaction with picric acid at pH 11.5. The rate of change in absorbance at 520/800 nm depends on the creatinine concentration of the sample. The eGFR was calculated using the following formulas [18], for males eGFR = 175 × (serum Cr)$^{-1.154}$ × (age)$^{-0.203}$, for females eGFR = 175 × (serum Cr)$^{-1.154}$ × (age)$^{-0.203}$ × 0.742.

QuantiFERON-TB Gold In-Tube (QFT-GIT) Test

All enrolled participants were administered the QFT-GIT test according to the manufacturer's instructions. The patients' whole blood samples were gathered into a negative control nil tube, TB antigen tube, and positive control T-cell mitogen stimulation tube. All these tubes required incubation for 16 to 24 hours at 37 °C. The quantity of IFN-γ (IU/mL) was detected by ELISA, and test results were analyzed by QFT-GIT ELISA software (version no. 2.43; Cellestis Ltd., Victoria, Australia). The response to IFN-γ with ESAT-6/CFP-10/TB7.7 mixture $\geq$0.35 IU/mL above

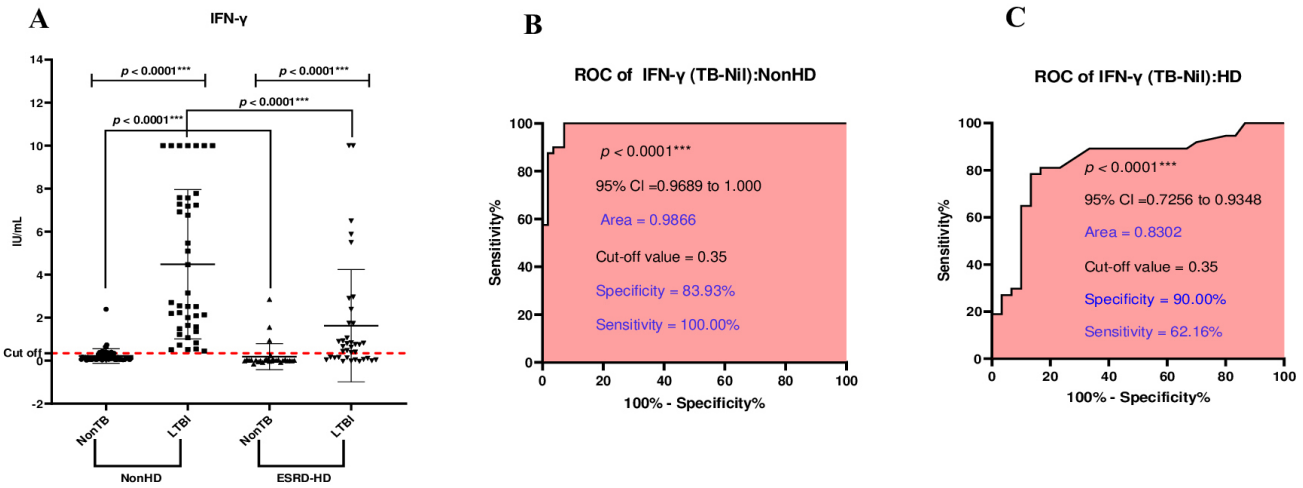

Fig. 2. Comparison of IFN-γ levels between the non-HD and end-stage renal disease (ESRD)-HD patients and ROC curve analysis in non-HD and HD groups. (A) The comparison results of IFN-γ levels in LTBI and non-TB patients between the non-HD and HD patients. Note: The IFN-γ levels were measured by QuantiFERON-TB Gold In-Tube (QFT-GIT), which were stimulated by *Mycobacterium tuberculosis* (MTB)-specific antigens for 24 h. The red line is cut-off value that the kit recommended. (B) ROC curve analysis based on results of IFN-γ levels in non-HD patients. (C) ROC curve analysis based on results of IFN-γ levels in HD patients. Note: Receiver operating characteristic (ROC) curves compare sensitivity versus specificity. The comparison of specificity and sensitivity based on ROC curve in non-HD and ESRD-HD patients. Non-HD Patients (n = 96), ESRD-HD Patients (n = 67); *p* value was measured by ROC curve, *** $p < 0.0001$.

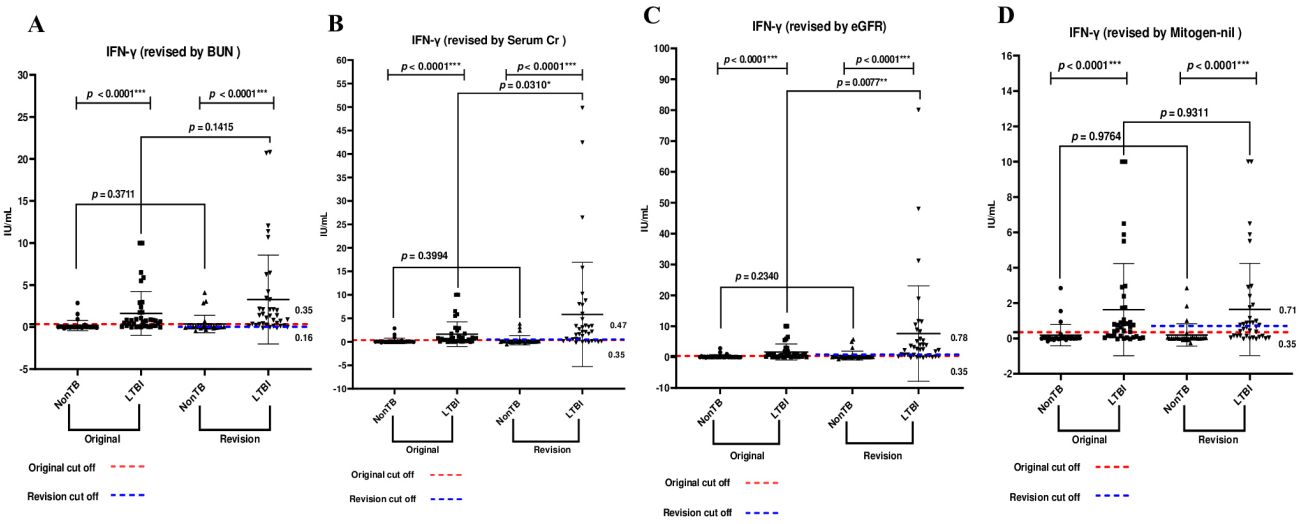

Fig. 3. Comparison of the original IFN-γ levels and the IFN-γ levels revised by diagnostic indicator in ESRD-HD patients. (A) Comparison of the original IFN-γ levels and the IFN-γ levels revised by BUN in ESRD-HD patients. (B) Comparison of the original IFN-γ levels and the IFN-γ levels revised by Serum Cr in HD patients. (C) Comparison of the original IFN-γ levels and the IFN-γ levels revised by eGFR in ESRD-HD patients. (D) Comparison of the original IFN-γ levels and the IFN-γ levels revised by Mitogen-nil in ESRD-HD patients. Note: The red line is cut-off value that the kit recommended. The blue line is cut-off line for the revision results. *p* value were measured by ROC curve, *** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$.

the nil control value was recognized as a positive result. In the case of the IFN-γ level <0.35 IU/mL and mitogen control ≥0.5 IU/mL, the test was recognized as a negative result. In the case of the IFN-γ level <0.35 IU/mL and mitogen control <0.5 IU/mL, the test result was recognized as indeterminate.

Formula of IFN-γ Revisions

The IFN-γ revision formulae for all of the biomarkers calculates the conversion factor at first, and the formulae of revisions are shown in Tables 2,3.

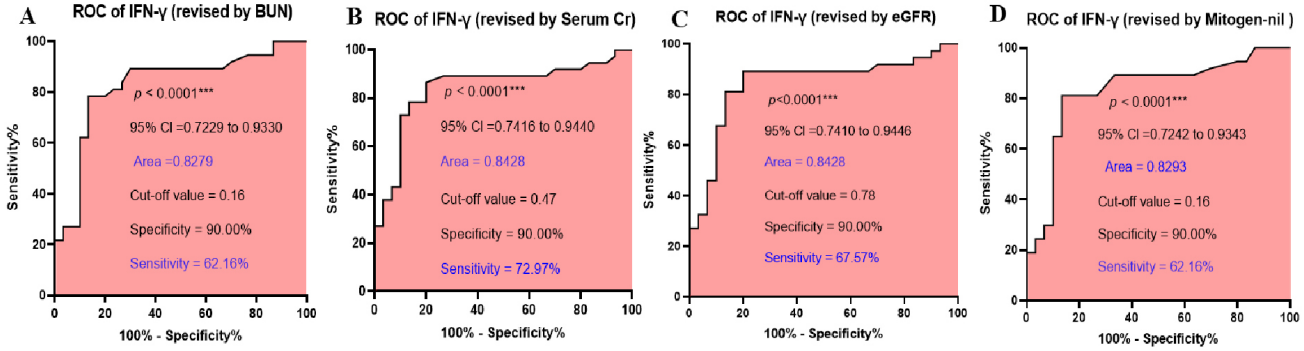

Fig. 4. ROC curve based on the IFN-γ levels revised by diagnostic indicator. (A) ROC curve analysis based on results of IFN-γ levels revised by BUN in ESRD-HD patients. (B) ROC curve analysis based on results of IFN-γ levels revised by Serum Cr in ESRD-HD patients. (C) ROC curve analysis based on results of IFN-γ levels revised by eGFR in ESRD-HD patients. (D) ROC curve analysis based on results of IFN-γ levels revised by Mitogen-nil in ESRD-HD patients. Note: Receiver operating characteristic (ROC) curves compare sensitivity versus specificity. *p* value were measured by ROC curve, *** $p < 0.0001$.

Statistical Analysis

The statistical analysis was performed using GraphPad Prism v. 8.00 (GraphPad Software, San Diego, CA, USA). In order to compare the ESRD-HD patients' and non-HD patients' IFN-γ release level, and in addition to compare the IFN-γ revision results with the original IFN-γ release level of ESRD-HD patients, the Receiver operator characteristic (ROC) curve analysis was performed on the ESRD-HD group and the non-HD groups, by additional ROC curve analysis to obtain *p* values and area under curve (AUC). *p* values < 0.05 were considered statistically significant. Venkatraman test which compares the difference between two AUCs was performed using RStudio Team 2023 (RStudio: Integrated Development for R. RStudio, PBC, Boston, MA, USA). To compare the IFN-γ revision ROC results with the original IFN-γ release ROC of ESRD- HD patients, the Venkaraman_test function was used.

Results

Population Characteristics

As for non-HD patients, the median age was 59 years old, with a range of 23 to 86 years old, the male-to-female ratio was 56:40 (58.3%:41.7%), and the group contained eight smokers. The mean ages of the ESRD-HD group and the non-HD group were approximately the same. In contrast to the ESRD-HD group, almost 37 participants had received the BCG vaccination, and the non-HD group had only two participants who received the BCG vaccination. As for TB contact history, none of the ESRD-HD patients had any contact, while two non-HD patients previously had TB. None of the participants received any radiological lesions.

IFN-γ Level Analysis Results between ESRD-HD and Non-HD Groups

The IFN-γ level of IGRA tests for the ESRD-HD group and the non-HD group were significantly different, as described in Fig. 2. For the ESRD-HD groups, when applied, the recommended cut-off value from the kit was 0.35, specificity was 90.00%, sensitivity was 62.16%, *p* value $<$ 0.0001, and ROC curve was 0.8302. As for non-HD groups, most of the data were much higher than the HD groups. Cut–off value is 0.35, specificity was 83.93%, sensitivity was 100%, *p* value was $<$ 0.0001, and the AUC was 0.9866. As a result, it is indicated that ESRD-HD patients' IGRA test results are less accurate than non-HD patients.

Comparing IFN-γ Revised by BUN, Serum Cr, eGFR and Mitogen-nil in the HD Groups

In order to compare the available biomarker for IGRAs revision, three renal function biomarkers were selected, including BUN, serum Cr, and eGFR. The comparison figure and ROC curve are shown in Figs. 3,4. For the BUN revisions of the IFN-γ, the results were lower than the original data of the results, the cut-off value was 0.16, specificity was 90.00%, sensitivity was 62.16%, *p* value $<$ 0.0001, and AUC was 0.8279. As for the serum Cr revision of IFN-γ, most of the data were much higher than the original IFN-γ level. The cut-off value was 0.47, specificity was 90.00%, sensitivity was 72.97%, the *p* value was $<$0.0001, and the AUC was up to 0.8428. Compared with the original data of ESRD-HD patients' IGRAs results, the sensitivity was increased by 10.81%, allowing about 28.00% more detection among previously undetectable patients. Similar to the eGFR revision of IFN-γ, the data were mostly much higher than the original IFN-γ level. The cut-off value was 0.78, specificity was 90.00%, sensitivity was 67.57%, the *p* value was $<$0.0001, and the AUC was up to 0.8428. Compared with original data of ESRD-HD patients' IGRAs results, the sensitivity was increased by

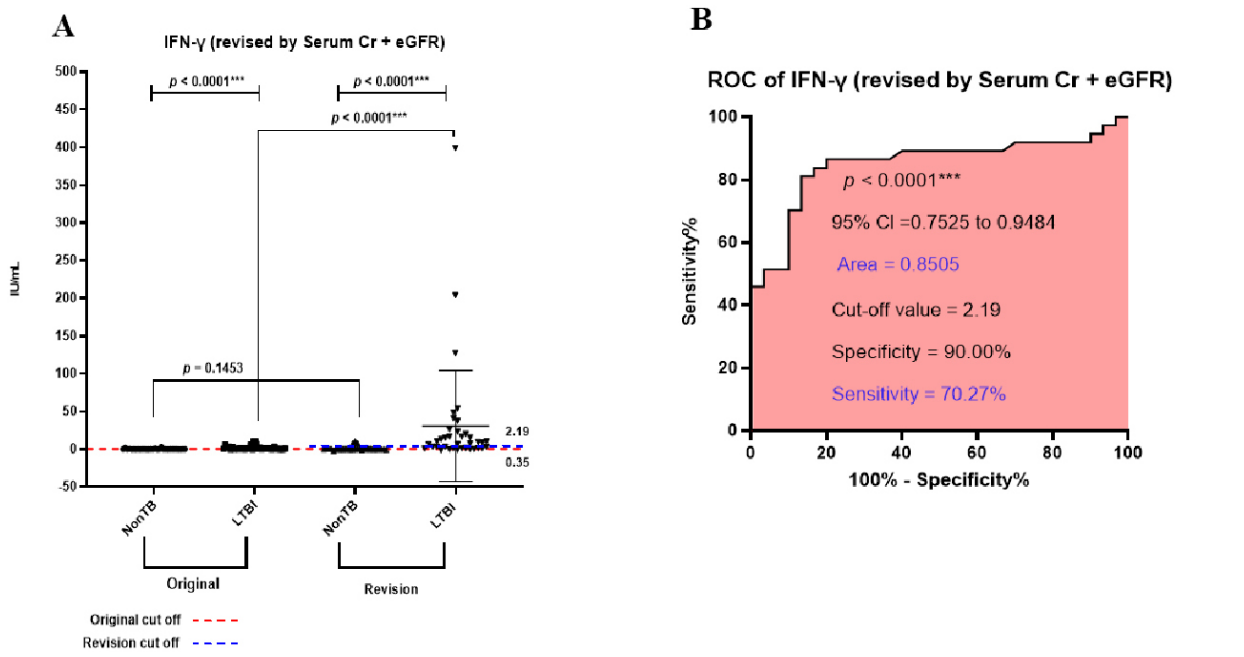

Fig. 5. Comparison of the original IFN-γ levels and the IFN-γ levels revised by serum Cr and eGFR and ROC curve analysis based on results of IFN-γ levels revised by serum Cr and eGFR in ESRD-HD patients. (A) The comparison results of the original IFN-γ levels and the IFN-γ levels revised by serum Cr and eGFR. Note: The red line is cut-off value that the kit recommended. (B) ROC curve analysis based on results of IFN-γ levels revised by Serum Cr and eGFR. Note: The red line is cut-off value that the kit recommended. The blue line is cut-off line for the revision results. *p* value were measured by ROC curve, *** $p < 0.0001$.

5.41%, allowing about 14.00% more detection among previously undetectable patients. For the Mitogen-nil revision of the IFN-γ, the results were lower than the original data of the results, the cut-off value was 0.71, specificity was 90.00%, sensitivity was 62.16%, *p* value was <0.0001, and AUC was 0.8293. As a result, it is indicated that the IFN-γ revised by serum Cr and the results revised by eGFR are both improved.

Comparing IFN-γ Revised by Serum Cr and eGFR in the ESRD-HD Group

Maximizing the benefit of the revision and, utilizing the two increasing biomarkers which are serum Cr and eGFR, results in better data. The comparison figure and ROC curve are shown in Fig. 5. The cut-off value was 2.19, specificity was 90%, sensitivity was 70.27%, the *p* value was <0.0001, and AUC was up to 0.8505. Compared with original data of HD patients' IGRA results, the sensitivity was increased by 8.81%, allowing about 23% more detection among previously undetectable patients. As a result, it is indicated that the IFN-γ revised by serum Cr and eGFR is improved.

Comparison of the AUC of IFN-γ Levels in HD Patients and Revised Data

To compare the AUC after the revision, the Venkataraman tests were carried out. The comparison data are shown in Table 4. The *p*-value of the AUC comparison value is increased when using the recommended diagnostic markers in this study. Meanwhile, the value for the test statistic E in the Venkatraman test represents the sum of squared differences in sensitivity values between the two paired ROC curves at each threshold considered. E is used to evaluate the degree of difference between the two ROC curves [19].

Table 4. Venkatraman test results between the AUC of IFN-γ levels in HD patients and revised data.

Venkatraman test objects	E	Boot.n	*p*-value
IFN-γ (ESRD-HD) & BUN revision	578	2000	0.9605
IFN-γ (ESRD-HD) & Cr revision	716	2000	0.8685
IFN-γ (ESRD-HD) & eGFR revision	716	2000	0.8685
IFN-γ (ESRD-HD) & Cr & eGFR revision	1852	2000	0.126

Discussion

TB still maintains high mortality rate worldwide as a single transfectant, despite being an ancient contagious disease [1]. Latent tuberculosis infection (LTBI) is regarded as the primary obstacle in achieving the objectives of the End TB Strategy, as declared by the World Health Organization (WHO).

Accurate diagnosis is key for controlling LTBI and precautions for developing active TB. Therefore, the WHO recommends TB testing for high-risk groups such as HIV-positive individuals, patients who undergo dialysis, individ-

uals with TB history, and patients who have initiated immunotherapy. However, because of the limitation of the present diagnosis for LTBI, it may delay the treatment of TB; without treatment, the mortality rate is approximately 50% [2].

Patients who are under immune suppressive situations such as ESRD and receive HD with LTBI faced an increased risk of developing active TB [14]. HD causes variations in T-cell activation and leads to T-cell dysfunction, which can affect IFN-γ release level [20]. IGRAs measure IFN-γ level through T-cells that stimulate specific MTB complex antigens [3]. Therefore, for patients who received HD with LTBI, the IGRA tests result in false-negatives due to their lower IFN-γ release levels. Many laboratory parameters have been used to evaluate renal function. The most universal parameters include BUN and serum Cr concentrations [21].

In order to overcome this deficiency and more accurately diagnose LTBI, in the present study, three renal function markers BUN, serum Cr, and eGFR were selected to compensate for whole blood IFN-γ level of IGRA tests. Assessments of the levels of BUN, Cr, and eGFR are generally used as renal function biomarkers. Serum Cr has been considered a universal marker of kidney function for decades, and the eGFR measured by plasma levels is based on it [15,22]. As a result, eGFR is generally original from Cr-based equations which through age, sex, weight, and race to calculate in clinical. BUN is considered a biomarker for renal function that penetrates via the glomerulus, and at tubules, urea absorbs once more. Consequently, BUN relies on tubular function and eGFR simultaneously [15]. Therefore, these three kidney function markers were selected to compensate IFN-γ release levels for HD patients with LTBI due to their ability to be processed with little or no renal regulation on glomerular filtration or the reduction of renal function.

In order to usher in significant achievements in TB elimination and end the global TB epidemic by 2035, it is important to continue innovating accurate diagnosis methods and medical knowledge [2]. Nevertheless, it is time-consuming to develop novel techniques, launch them, and then create corresponding commercial products with clinical applications. For example, the latest generation of IGRAs, QFT-Plus test, is an upgraded version of QFT-GIT test launched in 2015 [23]. Contrary to the original version, QFT-Plus test adds another MTB antigen tube for a total of four reaction tubes. QFT-GIT test only has one TB antigen tube containing ESAT-6, CFP-10, and TB 7.7 to mainly stimulate $CD4^+$ T-cells. However, in addition to $CD4^+$ T-cells, $CD8^+$ T-cells can also release IFN-γ when stimulated by MTB antigens. Therefore, as the latest generation of IGRAs, the second added TB antigen tube could both stimulate $CD4^+$ and $CD8^+$ T-cells based on the original version [24]. However, evidence of recent research results indicates that compared with QFT-GIT test, the newest generation QFT-Plus test has not reflected any meaningful advancement in TB patients, including high- and low-risk groups [5].

This study is focused on ESRD patients, as for other different chronic kidney disease (CKD) stages that may get different results, we also think that the other CKD stages need to be analyzed, therefore, we will add other different CKD stage patients to compare with in further study. The BCG scar or vaccination is a significant difference between the two groups ($p < 0.0001$***), but BCG vaccination is required in South Korea. The statistical results of BCG vaccination in this study were obtained from participants' oral answers, most of them couldn't remember whether they had been vaccinated. In addition, BCG scar or vaccination has a significant influence on TST results. Unlike the TST, IGRAs are not affected by BCG vaccination in various studies for TB diagnostic methods. For underlying diseases, there are differences between the two groups.

As for ESRD patients which are characterized by proteinuria or progressive decline in the glomerular filtration rate, therefore, although TB patients have high rates underlying diabetes mellitus, morbidity of diabetes mellitus in ESRD patients may be higher than the patients who are only infected by MTB. Meanwhile, due to their lower immunity than the patients who are only infected by MTB, the morbidity of autoimmune disease may be higher than those.

In clinical research, especially for diagnostic tests it is important to evaluate the specificity and sensitivity for accurate diagnosis [23]. Initially, we computed the receiver operator characteristic (ROC) curves for both HD patients and non-HD patients. To compare the updated levels of IFN-γ using the diagnostic markers selected in this investigation from the HD patients, we conducted the Venkatraman test. The results of this test are presented in Table 4. The p-value of the AUC comparison value is increased when using the recommended diagnostic markers in this study. After careful study, it is clear that the value of E has risen. The maximum value, reaching 1852, is obtained when both Cr and eGFR are employed. This contrasts with the initial data point of 578. Therefore, it is crucial to consider all the chosen diagnostic indicators when implementing the revision formula in this study.

In conclusion, when choosing diagnostic indicators, BUN is different from serum Cr and eGFR since it can signal both liver and kidney functions. However, it is not specifically used to assess kidney function. At present, all diagnostics for latent tuberculosis infection (LTBI) rely on indirect approaches that measure cell-mediated immune responses. Consequently, individuals with weakened immune systems, such as those with end-stage renal disease on hemodialysis who are not tuberculosis insulators, are more susceptible to *Mycobacterium tuberculosis* (MTB) infection due to their significantly reduced immunity compared to healthy individuals. Thus, employing renal function markers, which are standard tests for patients with end-

stage renal disease on hemodialysis, can enhance the precision of latent tuberculosis infection diagnosis by compensating for the limitations of interferon-gamma release assay assays.

Conclusion

This study aims to revise the IGRA results of LTBI patients who received hemodialysis treatment at the end stage of renal disease. ESRD-HD patients who belong to the TB high-risk group, and those patients who have an immune deficiency that causes the LTBI gold standard diagnosis of IGRAs to get more false-negative results than usual, resulted in a higher number of undetectable patients who suffered from both LTBI and ESRD.

In this study, we used AUC which is a popular tool to assess the discriminability of different biomarkers. In addition, it is critical to select an ideal cut-off value for considering the presence or absence of disease. The groups we compared have different numbers that are not equal. Therefore, we chose an unpaired *t*-test which is a statistical procedure to compare two unrelated groups to consider whether there is a significant difference. Based on the ROC curves shown in Figs. 2,3,4,5, it is evident that there has been an improvement in sensitivity. However, the specificity has not changed significantly, particularly when compared to the IFN-γ data of patients with end-stage renal disease (ESRD). A specificity of 90% is considered high, as it increases the likelihood of finding patients who were previously undetectable and improves sensitivity. Consequently, incorporating serum Cr and eGFR into the IGRA test findings can address the limitations of the IGRA tests and enhance their effectiveness. The low sensitivity was increased and compensated with serum Cr increased by 10.81%, allowing about 28% more detection among previously undetectable patients, and eGFR increased by 5.41%, about 14% more detectable potential among them. Using both of serum Cr and eGFR, the sensitivity increase rate rises to 8.81%, and there is a possibility of detecting about 23% of previously undetectable patients.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Author Contributions

XX and HP wrote the main manuscript text and participated the conception while XX was responsible for reviewing and editing it. YJK dedicated to conducting formal analysis and acquisition of data. MJ and SohK conducted an investigation for this study and the interpretation of data. YP and EJL performed validation and analysis the data. YNK, JYP and HKC made contributions to the acquisition of data and revising it critically for intellectual content. JK were developed the theory by visualization. JL and SunK supervised and edited the manuscript, as well as the study's design. All authors contributed to significant editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

A total of 67 HD patients and 96 non-HD subjects were enrolled in this study, and their whole blood samples were gathered from March 2017 to June 2018 at the Kosin University Gospel Hospital, Busan, the Republic of Korea. These clinical samples were obtained and all of these experimental protocols for this study were approved by the Institutional Ethics Committee of Catholic University of Pusan and Kosin University Gospel Hospital (approval numbers CUPIRB-2017-036 and KUGH 2017-11-042) and all methods were carried out in accordance with relevant guidelines and regulations. Informed consent was obtained from all subjects and their legal guardian according to Declaration of Helsinki.

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Conflict of Interest

The authors declare no conflict of interest.

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