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Original Article

Diagnostic role of multiplex cytokine assay for osteoarticular tuberculosis

Xiao-Hong Jiang*, Hui Wang*, Xu Fang, Xiao-Qiang Zhuang, Sheng-Lin Lu, Yu Bai, Ya-Dong Zheng

*Department of Orthopedic Surgery, Affiliated Guangxi Minzu Hospital, Guangxi Medical University, Nanning, People's Republic of China. *Equal contributors.*

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Abstract: Objective: This study aims to investigate the diagnostic value of multiplex cytokine (CK) assay inosteoarticular Tuberculosis (TB). Methods: A total of 144 patients with suspected osteoarticular TB during hospitalization from March 2014 to June 2015 were finally enrolled. CKs secreted by PBMCs were detected by using Milliplex human cytokine kits. The area under the receiver operating characteristic (ROC) curve was used to evaluated the performance of different CKs in diagnosis of osteoarticular TB. Results: The mean age of the patients was 56.6 years, of which 47.2% patients (n=68) were male. 60.4% patients (n=87) were diagnosed as osteoarticular TB. IL-8, IL-15 and IP-10, showed statistical differences between the confirmed osteoarticular TB patients and not having osteoarticular TB patients (P<0.01). When compared with individual CK, IL-8 & IL-15 & IP-10 scores howed a better performance of auROC, 0.97 (95% CI: 0.92-0.99), in ROC analysis. Conclusion: IL-8, IL-15 and IP-10 are useful biomarker for diagnosis of osteoarticular TB and incorporation of these biomarkers significantly improved the diagnostic accuracy in osteoarticular TB.

Keywords: IP-10, IL-8, IL-15, multiplex cytokine assay, osteoarticular tuberculosis

Introduction

Osteoarticular tuberculosis (TB) is observed in about 5% of all TB patients [1]. Among all patients with osteoarticular TB, spinal TB is most common, followed by joint TB and extraspinal TB [2]. As early diagnosis and proper treatment can reduce the rates of disability and functional impairment for osteoarticular TB, it is very important to make the right diagnosis and differentiate TB from bacterial, fungal, and neoplastic diseases of bones and joints [3, 4]. However, it still remains a challenge for diagnosing of osteoarticular TB because it is invasive to obtain specimens of spine or joint and many clinical samples got from relatively inaccessible sites may bepaucibacillary, which would result in low sensitivity of current diagnostic tests [5-8].

By using the Mycobacterium tuberculosis (Mtb)-specific T-cell interferon γ (INF γ) release assays (IGRAs), the sensitivity and specificity for diagnosis of TB had been improved a lot compared with the traditional tuberculin skin test (TST) [9, 10]. What's more, recent studies using Mtb-specific antigens encoded by genes in the RD1 region to stimulate peripheral blood mononuclear cells (PBMCs) demonstrated that measuring multiplex cytokines other than INF γ like IL-2, IFN-a, IFN-b, IL-27, IL-17, IL-23, TNF-α and IL-1b could not only improve the diagnosis of pulmonary TB, but also discriminate latent TB infection (LTBI) from active TB infection [11, 12]. However, it remains unknown whether this PBMCs immune-based assay have any clinical role in the diagnosis of osteoarticular TB. Therefore, we conduct this prospective, blinded, observational study to exam the diagnostic usefulness of this PBMCs immune-based assay and further explore whether measuring multiplex cytokines could improve the sensitivity and specificity for diagnosis of osteoarticular TB.

Material and methods

Patients

The research was conducted in strict accordance with the protocol approved by the Ethics

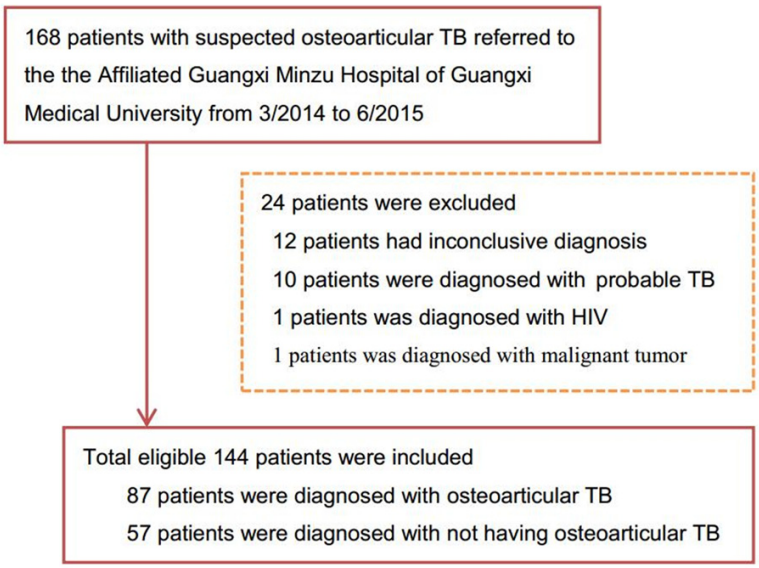

Figure 1. A flow diagram of study participants.

Committee of the Affiliated Guangxi Minzu Hospital of Guangxi Medical University. After obtaining written informed consent, 168 patients with suspected osteoarticular TB were prospectively enrolled from March 2014 to June 2015. Medical records were reviewed and data were collected on age, sex, underlying diseases, pathology and microbiological results, and follow-up observations. Diagnosis of osteoarticular tuberculosis was clinically confirmed by clinical specimens positive for M. tuberculosis on culture or by a PCR assay. Probable TB was diagnosed if they responded to anti-TB therapy and had radiographic findings consistent with osteoarticular TB. Not having TB was diagnosed if another diagnosis was made or if there was clinical improvement without anti-TB therapy [13]. Individuals with malignant tumor, HIV infection or in-take immunosuppressive agents were excluded.

Purification of peripheral blood mononuclear cells from blood

Anti-coagulated peripheral blood was obtained from each participant. Peripheral blood mononuclear cells (PBMCs) were isolated with density gradient centrifugation by using FicollePaque (GE Biosciences, USA). The PBMCs were washed twice with DMEM (GIBCO, New York, USA) supplemented with 2 mM L-glutamine, 10% heat inactivated human AB serum and 100 U/ml penicillin, 100 mg/mlstreptomycin, and re-suspended in DMEM to a concentration of 2×10^6 cells/ml. Then, about 4×10^5 cells were added to 96 well tissue culture plate (Corning, New York, USA).

Antigen stimulation

PBMCs from each participant were stimulated with M. tuberculosis ESAT-6 and CFP-10 peptide pools composed of 15-mers overlapping by 10 amino acids [2]. The ESAT-6 and CFP-10 peptides were HPLC purified (>80% purity) (SBS Genetech, Beijing, China) and dissolved in phosphate-buffered saline with 5% dimethyl sulfoxide as a stock solution of 2 mg/ml. Following incubation at 37°C for 24 hours, supernatants were harvested and stored at -80°C for further analysis.

Detection of CKs

Detection of IL-8, IL-15, IL-4, IL-6, IL-9, IL-12, IL-13, IL-14, IL-2R, sIL-2Ra, IP-10, MIP-1a, M-CSF, PF-4, ENA-78, PDGF, EGF, TNF-β1, TNF-β2, BMP, IGF-1, NGF and OSM was performed in duplicate by using Milliplex human cytokine kits (Millipore Corp., Billerica, MA) according to the manufacturer's instruction, with analyses conductedon a Luminex 200 analyzer (Luminex Corp., Austin, TX).

Statistical analysis

Continuous variables were summarized as mean ± standard deviation (SD), and categorical variables were displayed as counts or percentages. Student-t test was used for continuous variables and χ^2-test for categorical variables.

To assess differences in diagnostic efficiency between the CKs, area under the receiver operating characteristic curve (auROC), which is a measure of discrimination, was calculated. Furthermore, the standard index of validity, such as Youden index, sensitivity, specificity was calculated according to the ROC results.

The value of $P<0.05$ was considered to be statistically significant.

Table 1. Baseline characteristics of patients

	Total	Non-TB (n=57)	Definite TB (n=87)	*P*-value
Male	68 (47.2%)	28 (49.1%)	40 (46%)	0.712
Age	56.6±6.8	59.9±6.2	53.9±8.6	<0.001
History of:				
Diabetes	22 (15.3%)	14 (24.6%)	8 (9.2%)	0.012
Transplantation	4 (2.8%)	3 (5.3%)	1 (1.1%)	0.142
Liver cirrhosis	4 (2.8%)	1 (1.8%)	3 (3.4%)	0.545
Chronic renal failure	2 (1.4%)	0	2 (2.3%)	0.249
Rheumatologic disease	2 (1.4%)	0	2 (2.3%)	0.249
No underlying illness	112 (77.8%)	39 (68.4%)	73 (83.8%)	0.029
History of TB	73 (50.7%)	21 (36.8%)	52 (59.8%)	0.007
Old TB lesion on chest radiograph	26 (18.1%)	3 (5.3%)	18 (20.7%)	0.01
Suspected sites of infection				
Spine	74 (51.4%)	21 (36.9%)	53 (60.9%)	0.005
Joint	35 (24.3%)	19 (33.3%)	16 (18.4%)	0.041
Prosthetic joint	16 (11.1%)	5 (8.8%)	11 (12.6%)	0.470
Femur	4 (2.8%)	2 (3.5%)	2 (2.3%)	0.666
Tibia	2 (1.4%)	2 (3.5%)	0	0.079
Pelvis	5 (3.5%)	0	5 (5.7%)	0.065
Combined pulmonary TB	13 (9.0%)	2 (3.5%)	11 (12.6%)	0.061

Note: TB: tuberculosis.

Results

Study population

A total of 158 patients with suspected osteoarticular TB were prospectively enrolled from March 2014 to June 2015. Of these, 12 patients (7.6%) were excluded owing to "inconclusive" final diagnoses. 10 patients (6.3%) with probable TB were also excluded from the final analysis. 1 patients (0.6%) excluded for HIV and 1 patients (0.6%) excluded for malignant tumor (**Figure 1**). Of the 144 finally included patients, 87 patients (60.4%) were classified as confirmed osteoarticular TB and 57 patients (39.6%) were classified as not having osteoarticular TB (**Table 1**). The 87 patients with confirmed osteoarticular TB included 53 (60.9%) with TB spondylitis, 16 (18.4%) with TB arthritis, 11 (12.6%) with prosthetic joint infection, and 7 (8.0%) with extra-spinal TB (2 femur TB, 5 pelvic TB). The baseline clinical characteristics of the patients finally included in this study are shown in **Table 1**.

Detection of CKs by microbead-based assay

23CKs including IL-8, IL-15, IL-4, IL-6, IL-9, IL-12, IL-13, IL-14, IL-2R, sIL-2Ra, IP-10, MIP-1a, M-CSF, PF-4, ENA-78, PDGF, EGF, TNF-β1, TNF-β2, BMP, IGF-1, NGF and OSM were detected by using culture supernatant of PBMCs after stimulation with ESAT-6 and CFP-10 peptides pool. Three cytokines, IL-8, IL-15 and IP-10, showed statistical differences between the confirmed osteoarticular TB and not having osteoarticular TB (**Table 2**).

ROC analysis of CKs for diagnosis of osteoarticular TB

IL-8, IL-15 and IP-10 were selected for further analysis. The diagnostic performance of IL-8, IL-15 and IP-10 were analyzed by ROC analysis and the cut-off values were determined based on maximum Youden's index. The auROC of IL-8, IL-15 and IP-10was 0.87 (95% CI: 0.80-0.92), 0.87 (95% CI: 0.80-0.92), 0.91 (95% CI: 0.86-0.96) respectively (**Figure 2**). When using a best cut-off value of 2.95 for IL-8, the sensitivity was 87.4%, the specificity was 75.4%; a cut-off value of 3.68 for IL-15, the sensitivity was 65.5%, the specificity was 98.3%; a cut-off value of 8765.7 for IP-10, the sensitivity was 82.7%, the specificity was 96.5%. The diagnostic performance of these three CKs had no statistically difference (**Table 3**).

Table 2. The expression of 23 kinds of CKs in the two group (x±s, pg/ml)

	Non-TB (n=57)	Definite TB (n=87)	F	P
IL-8	2.62±0.69	3.93±1.17	56.05	<0.001
IL-15	2.28±1.09	4.37±1.70	138.02	<0.001
IP-10	5990.12±1502.11	10793.53±3121.60	74.35	<0.001
IL-2R	122.83±24.96	141.73±33.87	3.41	0.441
sIL-2Ra	25.53±5.41	32.62±15.04	1.45	0.701
MIP-1a	1006.38±268.13	1252.46±369.32	1.845	0.643
IL-4	345.23±23.45	363.91±49.53	4.21	0.4223
IL-6	37.43±5.35	41.32±8.43	3.44	0.5103
IL-9	2.43±0.43	3.21±0.33	2.67	0.5511
IL-12	123.52±10.43	142.65±16.22	1.93	0.5911
IL-13	24.72±2.98	19.43±3.44	5.92	0.1329
IL-14	8.34±0.52	10.34±0.44	3.23	0.5317
M-CSF	0.92±0.02	1.03±0.03	2.09	0.5821
PF-4	435.46±23.24	452.33±33.78	5.89	0.1109
ENA-78	83.74±8.27	82.98±6.45	6.32	0.1081
PDGF	91.32±4.27	95.34±6.74	1.45	0.6412
EGF	35.53±2.46	41.43±3.84	2.53	0.6017
TNF-β1	5.32±0.02	4.99±0.04	1.55	0.6218
TNF-β2	6.87±0.06	7.88±0.07	4.99	0.2145
BMP	122.54±10.75	133.54±12.44	3.42	0.5243
IGF-1	453.76±69.23	542.88±55.89	4.78	0.3992
NGF	4.91±0.43	5.12±0.66	2.33	0.5263
OSM	12.24±0.99	14.22±1.01	4.11	0.4017

Improved diagnostic performance of combined detection of IL-8, IL-15 and IP-10 for diagnosis of osteoarticular TB

Then, we combinedIL-8, IL-15 and IP-10 to make an IL-8 & IL-15 & IP-10 score (IL-8 & IL-15 & IP-10 score =-11.6945+1.151* IL-8+1.194* IL-15+0.00058*IP-10), of which the coefficients were calculated by the multivariate logistic regression with just IL-8, IL-15 and IP-10 included. The score had an auROC of 0.97 (95% CI: 0.92-0.99), significantly higher than that of individual IL-8 (P<0.001) or IL-15 (P<0.001) or IP-10 (P<0.001) along (**Figure 3**). When we had a cut-off value of 0.435 for this score, the sensitivity was 89.7%, the specificity was 91.2% (**Table 3**). These results demonstrated that combined detection of IL-8, IL-15 and IP-10 resulted in significantly improved diagnostic sensitivity and specificity for detection of osteoarticular TB, as compared with individual CK.

Discussion

As clinicalor radiographic findings are often inconclusive, accurate diagnosis of osteoarticular TB is difficult for orthopedist [14, 15]. Besides, the clinical value of conventional tests for the diagnosis of osteoarticular TB is limited because the clinical samples obtained may not contain bacilli [16]. In addition, the cultures of mycobacterial at least take several weeks, which often result in delaying diagnosis and the initiation of therapy [17-19]. In order to improve the sensitivity and specificity for rapid diagnosis of osteoarticular TB, several new tests had been developed these years like nucleicacid amplification tests and TST [20]. Nucleicacid amplification tests have shown sensitivities ranging from 57% to 83% for the diagnosis of osteoarticular TB, but, their role in this clinical setting has not been fully defined [2, 4, 21]. In immunocompetent patients with osteoarticular TB, TST showed high positivity which almost reached 90%. However, in immunosuppressed patients, this test is much less sensitive. Soit has little practical value in regions of high TB prevalence and widespread BCG vaccination like China [22].

IGRAs have been showed as a promising test for the diagnosis of TB infection, and have already been used to assist diagnosis of active TB by measuring multiplex cytokines secreted by PBMCs other than INFγ [23, 24]. We hypothesize that this PBMCs immune-based assay might also have diagnostic value for osteoarticular TB and multiplex biomarker detection might improve diagnostic accuracy than singly using one cytokine. In this study, 23 kinds of cytokines secreted by PBMCs from 87 osteoarticular TB patients and 57 noneosteoarticular TB patients were measured. We found that IL-8, IL-15 and IP-10 differed significantly between individuals infected with osteoarticular TB and those with no evidence of TB infection. These data indicated the potential diagnostic role of IL-8, IL-15 and IP-10 for osteoarticular TB and prompted us to further focus on these three CKs in the following study. We demonstrated the high diagnostic value of IL-8, IL-15 and IP-10 with ROC analysis. What's more, our results

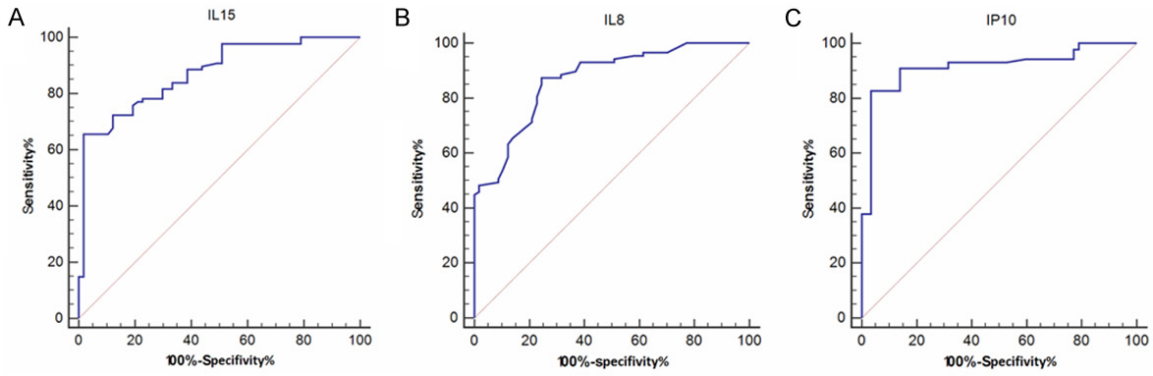

Figure 2. ROC analysis of IL-8, IL-15 and IP-10 on diagnosis of osteoarticular TB. A: ROC analysis of IL-15 on diagnosis of osteoarticular TB. B: ROC analysis of IL-8 on diagnosis of osteoarticular TB. C: ROC analysis of IP-10 on diagnosis of osteoarticular TB.

Table 3. ROC analysis of CKs and combined detection of CKs on the diagnosis of osteoarticular TB

Values	auROC	95% CI	P*	Youden	Cut-off	Sensitivity	Specificity
IL-8	0.87	0.80-0.92	<0.001	0.63	2.95	87.4%	75.4%
IL-15	0.87	0.80-0.92	<0.001	0.64	3.68	65.5%	98.3%
IP-10	0.91	0.86-0.96	<0.001	0.79	8765.67	82.9%	96.5%
IL-8 & IL-15 & IP-10	0.97	0.92-0.99	<0.001	0.81	0.44	89.7	91.2

Note: Value of (IL-8 & IL-15 & IP-10)=-11.6945+1.151* IL-8+1.194* IL-15+0.00058*IP-10.

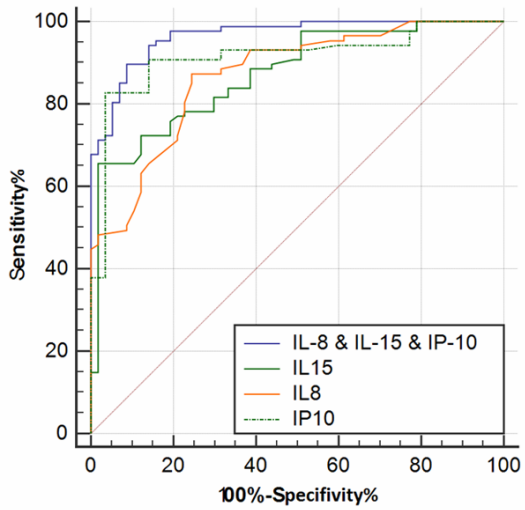

Figure 3. ROC analysis of IL-8 & IL-15 & IP-10 score on diagnosis of osteoarticular TB.

showed that high levels of diagnostic accuracy can be achieved by combining these three biomarkers.

In agreement with our findings, the diagnostic potential of IL-8, IL-15 and IP-10 has previously been highlighted in a number of studies in the field of pulmonary TB. Wang et al. revealed that patients with TB and cancer comorbidity produced higher levels of the TB-specific IP-10 release compared to normal control and as a novel biomarker for TB in China, IP-10 showed high sensitivity and specificity [25]. In addition, Wergel and et al. demonstrated that IP-10 distinguished active TB from LTBI with high accuracy and they suggested that plasma IP-10 might serve as a diagnostic biomarker for discriminating the stages of TB infection and monitoring therapy efficacy [26]. Besides, Djoba et al. suggested IL-8 and IL-15 as potential indicators of anti-tuberculosis treatment response [27], and Du et al. showed the value of IL-8 for distinguish active TB from LTBI [28].

Combination of CKs multiplex detection had better diagnostic performance than individual CK assay have already been proved by a lot of studies. Wang et al. demonstrated that combination of IFNγ, IP-10, MIG had higher diagnostic value than single cytokine/chemokine assays and could beclinically useful for the diagnosis of active pulmonary TB [17]. Our results also showed that combination detection and analyzing of IL-8, IL-15 and IP-10 significantly improved the diagnostic accuracy in osteoarticular TB than detection individual CK. With the quick

development of technology and equipment, it's not difficult to detect several different soluble proteins simultaneously from the same sample by using multiplexmicrobead-based assay. So, detecting multiplex cytokine would be a promising test for the diagnosis of TB.

In conclusion, our study showed that a number of TB-specific cytokine responses, includingIL-8, IL-15 and IP-10 were potential biomarkers for the diagnosis of osteoarticular TB. In addition, incorporation of these biomarkers significantly improved the diagnostic accuracy in osteoarticular TB. It was a rapid, sensitive and specific diagnostic test for osteoarticular TB.

This study has certain important limitation. The sample size of participants was small and all patients were from a single-center cohort. These limited the power to conduct subgroup analyses by race, age, stage of TB or other factors.

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Disclosure of conflict of interest

None.

Address correspondence to: Xu Fang, Department of Orthopedic Surgery, Affiliated Guangxi Minzu Hospital, Guangxi Medical University, 232 Mingxiudong Road, Nanning 530001, People's Republic of China. Tel: +86-13878153325; Fax: +86-771-3900863; E-mail: fangxugxnn@sina.com

References

[1] Zhu M, Li K, Zhu Y, Zhang J and Ye X. 3D-printed hierarchical scaffold for localized isoniazid/rifampin drug delivery and osteoarticular tuberculosis therapy. Acta Biomater 2015; 16: 145-155.

[2] Cho OH, Park SJ, Park KH, Chong YP, Sung H, Kim MN, Lee SO, Choi SH, Woo JH, Kim YS and Kim SH. Diagnostic usefulness of a T-cell-based assay for osteoarticular tuberculosis. J Infect 2010; 61: 228-234.

[3] Arora S, Sabat D, Maini L, Sural S, Kumar V, Gautam VK, Gupta A and Dhal A. The results of nonoperative treatment of craniovertebral junction tuberculosis: a review of twenty-six cases. J Bone Joint Surg Am 2011; 93: 540-547.

[4] Titov AG, Vyshnevskaya EB, Mazurenko SI, Santavirta S and Konttinen YT. Use of polymerase chain reaction to diagnose tuberculous arthritis from joint tissues and synovial fluid. Arch Pathol Lab Med 2004; 128: 205-209.

[5] Agarwal A, Bhat MS, Kumar A, Shaharyar A, Mishra M and Yadav R. Lymphocyte/monocyte ratio in osteoarticular tuberculosis in children: a haematological biomarker revisited. Trop Doct 2016; 46: 73-77.

[6] Hu S, Guo J, Ji T, Shen G and Kuang A. Multifocal osteoarticular tuberculosis of the extremities in an immunocompetent young man without pulmonary disease: A case report. Exp Ther Med 2015; 9: 2299-2302.

[7] Magnussen A, Amirthanayagam T and Sofat R. Osteoarticular tuberculosis: The great mimicker still catches us out–a case report. Acta Orthop 2016; 87: 83-84.

[8] Sbai MA, Benzarti S, Bouzaidi K, Sbei F and Maalla R. A rare localization of tuberculosis of the wrist: The scapholunate joint. Int J Mycobacteriol 2015; 4: 161-164.

[9] Zellweger JP, Sotgiu G, Block M, Dore S, Altet N, Blunschi R, Bogyi M, Bothamley G, Bothe C, Codecasa L, Costa P, Dominguez J, Duarte R, Floe A, Fresard I, Garcia-Garcia JM, Goletti D, Halm P, Hellwig D, Henninger E, Heykes-Uden H, Horn L, Kruczak K, Latorre I, Pache G, Rath H, Ringshausen FC, Ruiz AS, Solovic I, Souza-Galvao ML, Widmer U, Witte P and Lange C. Risk Assessment of Tuberculosis in Contacts by IFN-gamma Release Assays. A Tuberculosis Network European Trials Group Study. Am J Respir Crit Care Med 2015; 191: 1176-1184.

[10] Metcalfe JZ, Cattamanchi A, McCulloch CE, Lew JD, Ha NP and Graviss EA. Test variability of the QuantiFERON-TB gold in-tube assay in clinical practice. Am J Respir Crit Care Med 2013; 187: 206-211.

[11] Dorman SE, Belknap R, Graviss EA, Reves R, Schluger N, Weinfurter P, Wang Y, Cronin W, Hirsch-Moverman Y, Teeter LD, Parker M, Garrett DO and Daley CL. Interferon-gamma release assays and tuberculin skin testing for diagnosis of latent tuberculosis infection in healthcare workers in the United States. Am J Respir Crit Care Med 2014; 189: 77-87.

[12] Arias-Guillen M, Riestra S, de Francisco R, Palacios JJ, Belda J, Escalante P, Perez-Martinez I, Molinos LM, Garcia-Clemente M, Pando-Sandoval A, Rodrigo L, Prieto A, Martinez-Camblor P, Losada A and Casan P. T-cell profiling and the immunodiagnosis of latent tuberculosis infection in patients with inflammatory bowel disease. Inflamm Bowel Dis 2014; 20: 329-338.

[13] Tebruegge M, Dutta B, Donath S, Ritz N, Forbes B, Camacho-Badilla K, Clifford V, Zufferey C, Robins-Browne R, Hanekom W, Graham SM, Connell T and Curtis N. Mycobacteria-Specific Cytokine Responses Detect Tuberculosis Infection and Distinguish Latent from Active Tuberculosis. Am J Respir Crit Care Med 2015; 192: 485-499.
[14] de Nijs RN. Spinal tuberculosis. Lancet 2011; 378: e18.
[15] Huang D, Li D, Wang T, Shen H, Zhao P, Liu B, You Y, Ma Y, Yang F, Wu D and Wang S. Isoniazid conjugated poly(lactide-co-glycolide): long-term controlled drug release and tissue regeneration for bone tuberculosis therapy. Biomaterials 2015; 52: 417-425.
[16] Lebowitz D, Wolter L, Zenklusen C, Chouiter A and Malinverni R. TB determined: tuberculous osteomyelitis. Am J Med 2014; 127: 198-201.
[17] Wang X, Jiang J, Cao Z, Yang B, Zhang J and Cheng X. Diagnostic performance of multiplex cytokine and chemokine assay for tuberculosis. Tuberculosis (Edinb) 2012; 92: 513-520.
[18] Ruhwald M, Petersen J, Kofoed K, Nakaoka H, Cuevas LE, Lawson L, Squire SB, Eugen-Olsen J and Ravn P. Improving T-cell assays for the diagnosis of latent TB infection: potential of a diagnostic test based on IP-10. PLoS One 2008; 3: e2858.
[19] Wang S, Diao N, Lu C, Wu J, Gao Y, Chen J, Zhou Z, Huang H, Shao L, Jin J, Weng X, Zhang Y and Zhang W. Evaluation of the diagnostic potential of IP-10 and IL-2 as biomarkers for the diagnosis of active and latent tuberculosis in a BCG-vaccinated population. PLoS One 2012; 7: e51338.
[20] Cho OH, Park KH, Kim SM, Park SJ, Moon SM, Chong YP, Sung H, Kim MN, Jeong JY, Lee SO, Choi SH, Woo JH, Kim YS and Kim SH. Diagnostic performance of T-SPOT.TB for extrapulmonary tuberculosis according to the site of infection. J Infect 2011; 63: 362-369.
[21] Lai CC, Tan CK, Liu WL, Lin SH, Huang YT, Liao CH and Hsueh PR. Diagnostic performance of an enzyme-linked immunospot assay for interferon-gamma in skeletal tuberculosis. Eur J Clin Microbiol Infect Dis 2011; 30: 767-771.
[22] Yang J, Chen J, Yue J, Liu L, Han M and Wang H. Relationship between human LTA4H polymorphisms and extra-pulmonary tuberculosis in an ethnic Han Chinese population in Eastern China. Tuberculosis (Edinb) 2014; 94: 657-663.
[23] Sester M, van Leth F, Bruchfeld J, Bumbacea D, Cirillo DM, Dilektasli AG, Dominguez J, Duarte R, Ernst M, Eyuboglu FO, Gerogianni I, Girardi E, Goletti D, Janssens JP, Julander I, Lange B, Latorre I, Losi M, Markova R, Matteelli A, Milburn H, Ravn P, Scholman T, Soccal PM, Straub M, Wagner D, Wolf T, Yalcin A and Lange C. Risk assessment of tuberculosis in immunocompromised patients. A TBNET study. Am J Respir Crit Care Med 2014; 190: 1168-1176.
[24] Rangaka MX, Wilkinson KA, Glynn JR, Ling D, Menzies D, Mwansa-Kambafwile J, Fielding K, Wilkinson RJ and Pai M. Predictive value of interferon-gamma release assays for incident active tuberculosis: a systematic review and meta-analysis. Lancet Infect Dis 2012; 12: 45-55.
[25] Wang Y, Yang Y, Li H, Liang Y, Liu J, Yu T and Wu X. Evaluation of a Whole Blood Chemiluminescent Immunoassay of Interferon-gamma Inducible Protein 10 (IP-10) for Diagnosis of Tuberculosis Patients. Clin Lab 2016; 62: 165-172.
[26] Wergeland I, Pullar N, Assmus J, Ueland T, Tonby K, Feruglio S, Kvale D, Damas JK, Aukrust P, Mollnes TE and Dyrhol-Riise AM. IP-10 differentiates between active and latent tuberculosis irrespective of HIV status and declines during therapy. J Infect 2015; 70: 381-391.
[27] Djoba Siawaya JF, Beyers N, van Helden P and Walzl G. Differential cytokine secretion and early treatment response in patients with pulmonary tuberculosis. Clin Exp Immunol 2009; 156: 69-77.
[28] du Plessis N, Loebenberg L, Kriel M, von Groote-Bidlingmaier F, Ribechini E, Loxton AG, van Helden PD, Lutz MB and Walzl G. Increased frequency of myeloid-derived suppressor cells during active tuberculosis and after recent mycobacterium tuberculosis infection suppresses T-cell function. Am J Respir Crit Care Med 2013; 188: 724-732.