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"EVALUATION OF THE PERFORMANCE OF IP-10 ASSAY FOR THE DIAGNOSIS OF LATENT TUBERCULOSIS INFECTION"

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THESIS APPROVAL

YÜKSEK LİSANS TEZİ ONAYI

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DECLARATION

I declare that this study is an individual work in which there was no unethical behavior during all the stages from planning the thesis until its writing, and that all the information in this thesis was obtained according to academic and ethical rules. I declare that I have referenced all the information and interpretations not obtained in this study and that these sources are listed in the list of sources and that there is no violation of patents or copyrights during the study and the writing of the thesis.

ASMA SALEH ALI SALEM

DEDICATION

I dedicate this thesis to my family and many friends for their great support during this period.

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SYMBOLS / LIST OF ABBREVIATIONS

AIDS: Acquired Immune Deficiency Syndrome

APCs: Antigen Presenting Cells

AUC: Area Under Curve

BCG: Bacillus Calmette-Guérin

CFP-10: Culture Filtrate Protein-10

DMSO: Dimethyl Sulfoxide

ELISA: Enzyme-Linked Immunosorbent assay

ESAT-6: Early Secretory Antigenic Target-6

HIV: Human Immune Deficiency Virus

IFN- γ: Interferon Gamma

IGRAs: Interferon-Gamma Release Assays

IL: Interleukin

IP-10: Interferon-Gamma Inducible Protein 10

IPRA: IP-10 Release Assay

Kda: Kilodalton

LTBI: Latent Tuberculosis Infection

NTM: Nontuberculous Mycobacteria

PCR: Polymerase Chain reaction

PPD: Purified Protein Derivative

QFT-GIT: QuantiFERON-TB Gold-In Tube

RD1: Region of Difference 1

ROC: Receiver Operating Curve

TB: Tuberculosis

TNF: Tumor Necrosis Factor

TH: T Helper

T- Reg: Regulatory T Cell

TST: Tuberculin Skin Test

WBC: White Blood Cell Count

WHO: World Health Organization

ÖZET

SALEM. A.S (2018). Latent Tüberküloz Enfeksiyonu Tanısında IP-10 Testinin Performansının Değerlendirilmesi. İstanbul Üniversitesi,Deneysel Tıp Araştırma Enstitüsü, İmmünoloji Anabilim Dalı.Yüksek Lisans Tezi, İstanbul.

Anahtar Kelimeler: Tüberküloz, LTBE, IP-10, IGST, TDT.

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Interferon gamma salınım testleri (IGST) hücresel bağışıklık bazlı testler olup, *Mycobacterium tuberculosis*'e spesifik antijenlerine karşı oluşan IFN-γ yanıtını ölçerler. IGST'ler tüberkülin deri testi ile karşılaştırıldığında özgüllük açısından farklı şekilde ileride olmasına karşın, duyarlılık açısından üstün olduklarını söylemek zordur.
İnterferon-γ ile indüklenebilir protein (IP)-10, isminden de anlaşıldığı gibi, her enflamatuvar reaksiyonda IFN-γ'ya paralel olarak artar, bu da onu IFN-γ'ya potansiyel olarak alternatif bir kemokin yapar. Bu çalışmada, IP-10 salinim testlerinin (IPST) latent tüberküloz tanısı icin duyarlık ve özgüllüğünü saptanması hedeflendi. Bu amaçla, 84 adet mikrobiyolojik olarak onaylı, yeni aktif tüberküloz hastası ve 57 adet tüberküloz dışı solonum yolları hastalığı olan hastadan oluşan bir çalışma popülasyonu oluşturuldu. Bütün hastaların 17 yaşından büyük ve immunokompetan olmalarına dikkat edildi.

IP-10 yanıtını ölçmeden önce antijen ve mitojenle stimüle edilen kan tüplerini ve negatif kontrol kan tüpünü tıpkı tüberkülin deri testinde olduğu gibi 48 saat enkübe ettik. Plasma medyan IP-10 seviyeleri aktif tüberküloz ve non-tüberküloz hasta grupları için, sırasıyla, (3269.6pg/ml) and (18.1pg/ml) olarak saptandı. Receiver-operating curve analizinde IPST'nin teşhis edebilme doğruluğu yüksek bulundu, eğrinin altındaki alan neredeyse 1'e yakındı, AUC=0.962 ($p < 0.001$). Maksimum Youden indeksine göre (duyarlık+özgüllük-1) testin cut-off'u 439pg/ml olarak tespit edildi. Bu cut-off kullanıldığında duyarlık ve özgüllük sırasıyla %89.3 ve %98.3 olarak saptandı, sonuçların önceki çalışmaları destekler nitelikte olduğu ve latent tüberküloz enfeksiyonlarında IPST'nin tek başına kullanılabilecek bir tanı marker olduğunu gösterdi.

ABSTRACT

SALEM.A.S (2018).Evaluation of The Performance of IP-10 Assay For The Diagnosis of Latent Tuberculosis Infection. İstanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology. Master Thesis. İstanbul.

Key Words: Tuberculosis. LTBI. IGRAs. IP-10 .TST.

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Interferon gamma release assays (IGRAs) are cell-mediated immunity based tests that measure IFN-γ responses specific to *Mycobacterium tuberculosis* antigens. Though IGRAs far surpass tuberculin skin test in specificity, they hardly are superior to skin test in terms of sensitivity.

The interferon-γ inducible protein (IP)-10, as its name implies, increase in line with IFN-γ in every inflammatory reaction, which makes it a potential alternative chemokine to IFN-γ. In this study, we aimed to assess the sensitivity and specificity of the IP-10 release assay (IPRA) for the diagnosis of latent tuberculosis infection. For this purpose, 84 newly diagnosed, microbiologically confirmed active tuberculosis patients and 57 patients with various respiratory conditions other than tuberculosis constituted the study population. All patients were immunocompetent and older than 17 years of age. Similar to tuberculin skin test, we incubated antigen and mitogen stimulated blood tubes, and the nil tubes, for 48 hours before measuring the IP-10 responses. The median IP-10 levels in plasma were (3269.6pg/ml) and (18.1pg/ml) in the active tuberculosis and the non-tuberculosis patients, respectively. Analysis of the results by receiver-operating-curve analysis showed that the IP-10 assay had a high diagnostic accuracy, with an AUC of 0.962 ($p < 0.001$). The cut-off for our IPRA was found 439pg/ml at maximum Youden Index (sensitivity + specificity - 1). At this cut-off, the sensitivity and specificity was found 89.3% with 98.2 %, respectively. This study supports previous findings and shows that the IPRAs can be used as stand-alone diagnostic biomarker for the diagnosis of latent tuberculosis infection.

1. INTRODUCTION AND AIM

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is a serious infectious disease that affects people worldwide. World Health Organization (WHO) has reported in Global Tuberculosis Report (2017) that TB is the number one killer as a single infectious agent. According to the report, approximately one out of three individuals has estimated to have the TB infection. An estimate of 10.4 million people has been infected with TB in 2016, 10 % of whom have been living with HIV.(Organization 2017)

LTBI patients are asymptomatic, but they exhibit an adaptive immune response to the mycobacterial fragments.(Barry, et al. 2009; Chan and Flynn 2004)

It is common knowledge that around 10-15% of latently infected individuals advance to active TB throughout their lifetimes, half of which do so within the first five years of exposure to the bacillus.(Getahun, et al. 2015) A recent study has reported that the risk accrued in a relatively shorter time after infection; half of the infected individuals progressed to active TB within the first two years, while for the 56% of the children aged <5 years, the risk came off within 5 months after infection (Trauer, et al. 2016).

Since the recently infected individuals are undoubtedly comprising the high-risk group for progression to active TB disease, new diagnostic tools and biomarkers for detecting the recently acquired LTBI are of ever increasing importance to control the disease.(Halliday, et al. 2017; Wallis, et al. 2010) The LTBI diagnosis, today, relies substantially on a hundred-years-old skin test which contains tuberculin antigen and abbreviated as TST. For a relatively smaller part of LTBI diagnosis a new alternative test, which developed nearly two decades ago, called interferon-γ release assays (IGRAs) is used. These two tests, however, are not able to distinguish active disease from LTBI and more importantly remote from recent infection. Therefore, TST and IGRAs per se, are not good predictors of TB disease(Huebner, et al. 1993; Mack, et al. 2009). This is the rationale why targeted testing of only selected subpopulations, i.e. patients with high risk factors, is recommended.(LoBue and Mermin 2017)

The TST is an easy-to-do, affordable test that has been used for almost a century.(Huebner, et al. 1993) However, the test has low specificity (false positive results) in patients with nontuberculous mycobacteria and patients who were given BCG vaccine , and requires two visits to read the induration. The antigen used in this test is called purified protein derivative(PPD) and in contrast to its name it is not a purified form of proteins only, contains nearly every mycobacterial cell wall component. It elicits a "delayed type hypersensitivity reaction".(Mack, et al. 2009) The so-called PPD, contains mycobacterial cell wall components; glycolipids, fatty acids and proteins, which are common to *Mycobacterium* genus and not specific to *M. tuberculosis*.

By contrast, the IGRAs use two *M. tuberculosis* specific antigens. One of them is 6 kDA protein and called early secretory antigenic target 6 (ESAT-6). The second TB specific antigen is the culture filtrate protein (CFP-10) antigens. These strong T-cell antigens are encoded inside the region of difference 1 (RD1) of the *M. tuberculosis* strains. *M.*bovis BCG strains and most of the nontuberculous mycobacteria (NTM) do not contain this region. This is the reason why IGRAs have no positivity in BCG vaccinated and almost no positivity in patients infected with NTM. Nevertheless, RD1 genes are present in some NTM such as *M. kansasii*, *M. marinum* ,*M. riyadhense*, *M. szulgai,* and *M. smegmatis* and may cause false positivity.(van Ingen, et al. 2009a; van Ingen, et al. 2009b) The elicited immune response thereof is more specific than the reaction elicited by TST.

However, IGRAs have a sensitivity rate around 80% (Sester, et al. 2011), which is comparable to TST and even lower, in immunocompromised patients(Syed Ahamed Kabeer, et al. 2011). The suboptimal sensitivity of IGRAs might be related to the usage of IFN-γ as a biomarker, which can be produced at low levels within the lymphocytes.(Ruhwald, et al. 2011) To overcome this particular pitfall of IGRAs, alternative biomarkers expressed at high levels are needed.

One of the most potential candidates is interferon-gamma inducible protein, also known as CXCL10, is encoded by the CXCL10 gene .Therefore it is called IP-10 and secreted at very high levels by antigen presenting cells (APCs).(Ruhwald, et al. 2007)

The IP-10 release assay (IPRA) measure IP-10 response in a blood sample upon stimulation with TB specific antigens; exactly the same ones that are used in IGRAs. IP-10 is a promising diagnostic marker for TB because of its favorable characteristics. Its

advantages originates from its high level of expression compared to the current biomarker; IP-10 (7.4 kDa) is 2.5 times smaller than IFN-γ (18.8kDa) as amolecule, and therefore can be stored in relatively larger amounts within the APCs .(Ruhwald, et al. 2012)

Like IGRAs , IPRA's are principally cell-mediated immune assays based on the determination of the T-cell responses to peptide antigens specific toTB.

The length of antigenic stimulation is an important variable in both IGRAs and IPRA, and the use of a longer incubation period might be one way to improve the IPRA. (Butera, et al. 2009; Sutherland, et al. 2010)

In our study, we aimed to use IPRA test as an alternative to IGRAs and to determine the performance characteristics of this home-made test such as sensitivity and specificity.

2. GENERAL INFORMATION

2.1. Tuberculosis and Its Epidemiology

Tuberculosis (TB) caused by *"Mycobacterium tuberculosis"* which is an intracellular mycobacterial pathogen.(LoBue, et al. 2010)It is considered as one of the most ancient and common infectious diseases of the human history, that is shown by morphologically and also genetically in the extensive studies.(Gagneux 2018; Galagan 2014; Sandhu 2011) It remains its position in the world as a major health problem currently because it is one of the most causes of death. Around 10 million new cases occur in all over the world each year. Moreover, it causes even more deaths than HIV\AIDS, which is one of the diseases caused by a single infectious agent like TB for last five years. (Gagneux 2018; Organization 2017)

TB can affect different parts of the body other than the respiratory system, which is called 'extra pulmonary TB ; if it involves the respiratory system only, we talk about 'pulmonary "TB".(Organization 2017) Airborne contagion is the major route for transmitting the disease (Sandhu 2011)Of course, there are other factors for the transmission of the disease from one individual to others including the environment nature where transmission occurs and infection level of the infected patient. Also, a person who has pulmonary TB expels and spreads the disease by coughing into the air. (Walzl, et al. 2018)

World Health Organization WHO reported that approximately 10.4 million people has been infected with TB in 2016, and about 10-15% of that population will develop TB during their lifetime. Some factors such as having HIV infection, or being undernutrition, smoking, consuming alcohol, and having diabetes increase the probability of the disease progression. (Organization 2017). The most number of cases with TB took place in the South East Asia Region with the approximately half percent of the all total cases in the world in 2016. The incident case was followed by African Region with 25%, Western Pacific Region with 17%, Eastern Mediterranean Region with 7%, European Region with 3%, and America Region with 3%. India is the country where the most cases occur. It is followed by Indonesia, China, Philippines, and Pakistan. (Organization 2017; Organization 2018; Sandhu 2011)

In spite of the fact that TB is possessed of the chance to be cured for most of the people, as long as it is diagnosed early enough and treated well. On the other hand, the incidence of mortality for TB is in rise unless accurate and enough treatment is not applied. Together with this, the mortality rate is directly proportional to the correct and timely diagnosis. It is the fact that drug sensitive and drug-resistant TB diagnosis also important in order to prevent death caused by TB all around the world. (LoBue, et al. 2010; Walzl, et al. 2018)

The biggest global problem about TB comes with the lack of correct diagnosis and the low rate of reporting of the estimated incidence. WHO reported that approximately 40% of the possible cases were not reported in 2016. Particular regions where health facilities are beyond adequate have struggled to diagnose TB because of the economic and social inadequacy of the countries. Determination of the disease which is based on the indications reported by the patients who are with TB to the health services, and efficient treatment is mostly delayed in the countries with a low socio-economic state. This unfavorable situation leads to the transmission of TB by time and maintenance of the epidemic process in the population. (Walzl, et al. 2018)

Another problem about the detection of TB comes with its specific forms such as extra pulmonary TB. By contrast , detection of TB in children and testing drug resistance of the possible patients has a great deal of difficulties. This kind of failures in a diagnostic manner close the ways for the treatment of the disease and increase the possibility of TB to spread more and more. (Simmons, et al. 2018; Walzl, et al. 2018)

The rate of death caused by TB is elevated in the absence of treatment. Investigations showed that death took place in the lack of treatment involved approximately 70% of patient with smear-positive pulmonary tuberculosis in 10 years after diagnosis. Especially, in the countries, where health care is relatively limited and the population becomes a huge burden economically, hardly apply appropriate treatment in terms of economic and overpopulation situations. (Griffiths, et al. 2014; Simmons, et al. 2018)

Because of the fact that tuberculosis has the potential for global health risk, diagnosis of TB and its types followed by an applicable treatment become a crucial case in all over the world. Understanding bacteriology of the disease better and developing

diagnostic tests and treatments maintains its importance among other infectious diseases such as HIV. (Griffiths, et al. 2014)

2.2.The Life Cycle of *Mycobacterium tuberculosis*

Even though technology and science has been proceeding in time, scientific researchers have been failed controlling of evolving of bacterial strains which have resistance to drugs. Furthermore, recent knowledge of biological characteristics of *"M. tuberculosis "* still restrains the understanding of pathogenicity of *M. tuberculosis*. The lifecycle assessment of "*M. tuberculosis*" remains critical in order to develop new strategic approaches for the places where TB is counted as a high burden. (Russell, et al. 2010)

In order to find out the life cycle of *M. tuberculosis* to understand the disease better, clinical epidemiological and immunological studies have been proceeding with various animal models to observe the infectious disease pattern in the biological system. Moreover, observing human tuberculosis has been leading up a great deal of understanding of the life cycle of the species. (Ernst 2012; Russell, et al. 2010)

M. tuberculosis is pathogenic intracellular bacteria that live in the host which is the causative agent of "TB". When bacterial infection occurs, immune system of the human body generates the main response against the infection in order to eliminate it. However, the immune response becomes relatively efficient against to *M. tuberculosis* by driving the pathogens into a latent period, but it mostly fails to eliminate all the infection completely. Nonetheless, the latent stage of *M. tuberculosis* is not irreversible, and the disease goes to reactivation stage at some point of the infection. This is counting as one of the biggest reason for the transmission of "TB". (Ernst 2012; Galagan 2014; Russell, et al. 2010)

Although immunological life cycles of the pathogen in the host have been resolving, recent studies in humans and other animal models have not answered yet all the questions of the failure of immune system to eradicate the infection completely. Of course, scientific findings help to understand the mechanism of infection and the stages of TB in humans, but there are a lot of parts in the life cycle of *"M. tuberculosis*" in a host, which is still not understood completely. Life cycle of *M. tuberculosis* includes four stages in general. (Ehrt, et al. 2018; Ernst 2012; Nunes-Alves, et al. 2014)

M. tuberculosis is transmitted via aerosol between human. Aerosol droplets which are inhaled by the host contain the bacteria. This is the initial stage of the infection where inflammatory cells are gathered as an innate immune cells to the lungs. Even though, *M. tuberculosis* infects different types of cells, macrophages are the favorable place for the pathogen. Other immune cells including neutrophils, dendritic cells, and monocytes are inhabited by *M. tuberculosis* during the first stage of infection, when the immune cells "are recruited" to eliminate pathogens. Then, intracellular replication of *M. tuberculosis* continues when infected immune cells migrate to "lymph nodes". Expected restriction or elimination response by phagocytic cells in this stage is manipulated by the pathogens for their own advantage as supplying new cellular niches for the growth of their bacterial population. It indicates that *M. tuberculosis* is dominant during the innate immune stage rather than the innate immune cells. (Ehrt, et al. 2018; Ernst 2012; Nunes-Alves, et al. 2014; Simmons, et al. 2018)

Adaptive immune response occurs after reaching of *M. tuberculosis* to the draining lymph nodes and presenting bacterial antigens by dendritic cells to "naïve""CD4+ T cells". Adaptive immune stage is followed by "priming of T cells" and expansion of antigen specific T cells. After differentiating of "naïve T cells" to effector "T cells" in the lymph nodes, "effector T cells" are transferred to the lungs where the infection is occurred. Then, recruitment of monocytes, lymphocytes, infected macrophages, effector T cells and B cells with fibroblasts form a structure called granuloma in the lung which is known as the pathological hallmark of "TB". (Ehrt, et al. 2018; Ernst 2012; Nunes-Alves, et al. 2014)

At this point of infection, most of the cases enter a latent stage where there is only a slow or arrested replication of pathogen. Patients which develop latent stage of TB show almost no clinical symptoms and may last for decades because of maintaining of *"M. tuberculosis "* within the granulomas. Nevertheless, granulomas can expose the pathogen to the environment and eventually bacteria are released into the airways. Infectious droplets are generated and easily transmitted when the patient with active TB cough. Even though, immune system tries to eradicate the infection, a complete sterilization of pathogen does not occur. The latent stage which is estimated that one third of the world population possess this stage of infection, forms a major reserve for the potential infection in the world. Studies show that almost 10% of that population

with latent TB will develop active TB at some point of their lifetime. (Ehrt, et al. 2018; Nunes-Alves, et al. 2014; von Both, et al. 2018)

Reactivation stage of TB starts whenever pathogens pass through the latent stage of infection. It is the symptomatic stage of the disease where the immune system of the infected individual weakens. Although, there is couple of possible mechanisms why immune system fails such as T cell exhaustion, altered antigen expression of the pathogen, and altered cell trafficking, the main reason behind the "failure of immune system" and reactivation of TB is poorly understood. (Ehrt, et al. 2018; Ernst 2012; Nunes-Alves, et al. 2014)

The last stage of TB is transmission of the disease to a new host. The main route is known as airborne transmission occurs when the infected individual who has active disease coughs and expels *M. tuberculosis* via aerosol droplets. (Ehrt, et al. 2018; Ernst 2012)

2.3. Immunological Biomarkers and Diagnostics of Tuberculosis

Estimated number of people who live with latent TB is approximately two billion in all over the world, which is counting as one of the biggest potential sources for future disease. The major factor for prevalence of TB and hardly management of the disease is the lack of rapid, effective, and accurate diagnostic tests. In terms of controlling TB in global level, development of diagnostic tests and effective vaccines followed by a novel effective therapy are necessary. (Kaufmann, et al. 2017; Singh, et al. 2017; Yerlikaya, et al. 2017)

It is known that biomarkers used for detection of TB differ from latent stage of infection to active stage of infection. Thus, biomarkers are essential for not only the detection of TB but also prediction of the stage of infection and the disease progression which is important to know in order to eradicate TB and understand the possible treatment failure and relapse of the disease. It is also crucial for monitoring of the patient, and providing accurate predictions for clinical trials of new TB drugs and vaccines.(Walzl, et al. 2018) However, there are only few numbers of biomarkers which have been approved for specific usage as new diagnostic biomarkers despite all scientific research, high amount of investment, and findings of new biomarkers. (Ottenhoff, et al. 2012; Yerlikaya, et al. 2017)

Even though detection of intact *"M. tuberculosis "* by microscopy or culture is not the case for the point of care of the disease, it possible to identify the characteristics of the bacilli, macromolecules such as components of the cell wall, proteins, or nucleic acids. Bacterial metabolites or host metabolites caused by the infection may be evaluated in terms of evidence of the disease, but their detection or discovery requires different types of approaches. Host and pathogen-derived biomarkers are needed for the diagnosis of active TB. Host-derived biomarkers refer the biomarkers that are resulted from the immune system of the host against to *M. tuberculosis* infection. A great number of studies have been focused on host-derived biomarkers in terms of diagnosis. In the other respects, there are tests currently using that detect pathogen in clinical samples by microscopy, bacterial culture, or specific nucleic acid amplification. (McNerney and Daley 2011)

It is an advantage that host response differs at each stage of infection in terms of determining the individual biomarkers or bio signatures, which refer marker combinations, because they have a potential for diagnosis or prognosis of the infection. (Walzl, et al. 2011)

In order to control "*M. tuberculosis*", T helper (TH) cell balance is required, which consists of pro-inflammatory TH1-type responses and "TH17-type responses". There are also responses that limit immunopathology such as TH2-type responses which are associated with IL-4 production and regulatory T (T-Reg) cell phenotypes.(Walzl, et al. 2011; Weiner and Kaufmann 2017)

Replicating stage of the infection is symptomatic where *M. tuberculosis* have achieved to escape the immune control, granulomas are spoilt, acute stage of infection is activated, and pro-inflammatory marker levels increase. Immunosuppression at this stage refers that balance of TH cells is disturbed. Also, changes in memory T cell populations and antibody production may have seen. (Walzl, et al. 2011; Weiner and Kaufmann 2017)

Current studies on proteomics for detection of new protein biomarkers of "*M. tuberculosis*" showed that immune response of the host to proteome subsets is much more dominant for transmembrane and secreted proteins. ESAT-6and CFP-10 are immune dominant *M. tuberculosis* antigens which are identified as diagnostic biomarkers and secreted by *M. tuberculosis* during the infection. Broad range of

scientific studies have displayed that there are a wide range of proteins identified as biomarkers for detection of *M. tuberculosis* and evaluated for their value in diagnostic manner alone or in combination. Therefore, new scientific approaches and further studies are needed to investigate more effective diagnostic biomarkers in terms of detection of TB. (Walzl, et al. 2011; Zhou, et al. 2015a)

A comprehensive list of immunological biomarkers that vary according to the different stages of tuberculosis is shown in Table 2-1

Table 2-1: Varying biomarkers for tuberculosis

Biomarkers	Diagnosis	Correlate of risk or of protection*	Treatment outcome‡
Cytokines and chemokines			
IFN γ	Latent or active "TB"	Vaccine efficacy or disease progression	Treatment response
CXCL10, IL-10	Latent or Active "TB"	Increased after "BCG"	Under evaluation
IL-6	Active "TB"	Increased after "BCG"	Treatment response
IL-4	Active "TB"	Progression	Under evaluation
CXCL8, CCL8 and IL-12	Active "TB"	ND	Under evaluation
IL-4δ2/IL-4 ratio	Extent of disease	ND	Treatment response
IFN γ/IL-4 ratio	Latent or active "TB"	ND	Treatment response
IL-17 and TNF	Latent or active "TB"	Increased after "BCG"	Under evaluation
Receptors and soluble receptors			
Soluble urokinase PAR	Extent of disease	ND	Treatment response
Soluble ICAM1	Extent of disease	ND	Treatment response
Soluble E-selectin receptor	"M. tuberculosis " infection status	ND	ND
Soluble IL-2R, soluble TNFR1, soluble TNFR2	Extent of disease	ND	Treatment response
CD11c	Extent of disease	ND	Treatment response
LAG3	Extent of disease	ND	Treatment response
CXCR4, CCR5	Extent of disease	ND	ND
Other inflammation markers			
Neopterin	Extent of disease	ND	Treatment response relapse
Procalcitonin	Extent of disease	ND	ND
CRP	Extent of disease	ND	Treatment response or treatment failure
Granzyme B	Extent of disease	ND	Month 2 sputum conversion
Adenosine deaminase	Extent of disease	ND	Treatment response

Biomarkers	Diagnosis	Correlate of risk or of protection*	Treatment outcome‡
Immune cells and their markers			
Poly functional T cells	"M. tuberculosis " infection status	Vaccine efficacy (inconsistent data)	Treatment response
Single-positive TNF-expressing CD4+ T cells	"M. tuberculosis " infection status	ND	ND
MHC class I and II tetramer-specific T cells	Under evaluation	ND	ND
CD3lowCD56+ NKT cells	"M. tuberculosis " infection status	ND	Treatment response
FOXP3, CXCL8, IL-12β	"M. tuberculosis " infection status	ND	ND
TNF/TNFR1 ratio	Extent of disease	ND	ND
Total white blood cell and monocyte or neutrophil numbers	Extent of disease	ND	Treatment response
Antibodies to M. tuberculosis antigens and autoantibodies			
Antibodies specific for 38 kDa antigen, ESAT6 and LAM	M. tuberculosis infection status, extent of disease	ND	Treatment response
Antibodies specific for Rv3369 and CFP10	M. tuberculosis infection status	ND	ND
Antibodies specific for 38 kDa antigen, MPT64, TRXC and HSPX	Extent of disease	ND	ND
Antibodies specific for alanine dehydrogenase and malate synthetase	No differences between TB and control	ND	Treatment failure
BPI-specific ANCA	ND	ND	Treatment response
Differential gene or protein expression profiles			
CIS, SOCS3, IL-2RA, JAK3, PIM1	Diagnosis active "TB", latent "TB"	ND	ND
Lacto transferrin, CD64 and RAB33A	"M. tuberculosis " infection status, extent of disease	ND	ND
Fcγ RIB	"M. tuberculosis " infection status, extent of disease	ND	ND
RIN3, LY6G6D, TEX264, MP68, SOCS3, KIAA2013, ASNA1, ATP5G1, NOLA3	"M. tuberculosis " infection status	ND	Treatment response
SAA, transthyretin, neoptertin, CRP	"M. tuberculosis " infection status	ND	Treatment response
Neutrophil driven transcript signature of IFN-γ and type I IFN signaling	"M. tuberculosis " infection status, extent of disease	ND	Treatment response

ANCA, anti-neutrophil cytoplasmic autoantibodies; ASNA1, arsenite-stimulated ATPase; ATP5G1, mitochondrial ATP synthase lipid-binding protein; BCG, Mycobacterium bovisbacillus Calmette–Guérin; BPI, bactericidal permeability-increasing protein; CCL, CC chemokine ligand; CCR, CC chemokine receptor; CFP10, 10 kDa culture filtrate antigen; CIS, cytokine-inducible SH2-containing protein; CRP, C reactive protein; CXCL, CXC-chemokine

ligand; CXCR, CXC-chemokine receptor; ESAT6, 6 kDa early secretory antigenic target; FcγRIB, high-affinity IgG Fc receptor IB; FOXP3, forkhead box P3; HSPX, heat shock protein X, ICAM1, intercellular adhesion molecule 1; IFN, interferon; IL, interleukin; IL 2RA, IL 2 receptor antagonist; JAK3, Janus kinase 3; LAG3, lymphocyte activation gene 3; LAM, lipoarabinomannan; MP68, 6.8 kDa mitochondrial proteolipid (also known as C14orf2); M. tuberculosis , Mycobacterium tuberculosis; ND, not determined; NKT, natural killer T; NOLA3, nucleolar protein family A, member 3; PAR, plasminogen activator receptor; PIM1, proto-oncogene serine/ threonine-protein kinase PIM1; RIN3, RAS and RAB interactor 3; SAA, serum amyloid A protein; SOCS3, suppressor of cytokine signalling 3; TB, tuberculosis; TEX264, testis-expressed gene 264; TNF, tumour necrosis factor; TNFR, TNF receptor; TRXC, thioredoxin. *Correlates of risk represents the markers which are detectable at the lower possibility of developing the disease or unable to detect markers at the increased possibility of developing the disease. On the other hand, correlates of protection represents the reliable estimation of the protection level generated by a vaccine when compare vaccinated and unvaccinated subjects for immunological indications. ‡Treatment outcome contains initial impact of the treatment which is detected by turning positive to negative of sputum smear or culture tests, later on treatment to accomplish or fail to treat, and recurrence after primary treatment. 40. (Walzl, et al. 2011)

Lack of suitable diagnostic test for TB to detect the bacteria or its products is one of the reasons for searching for new tuberculosis biomarkers. Sputum smear test is most commonly used test to detect TB based on microscopic detection of the acid-fast bacilli. Sputum smear test has approximately 34-80% of sensitivity. However, percentage of sensitivity reduces in children and immunocompromised individuals. Also, sputum culture test is another widely used test and more sensitive than sputum smear test, but it may take up to 6-8 weeks to get results. It is laboratory-based test and requires a setup laboratory which is hard to find in the countries where TB is in high incidence. In other respects ,there is also common test for LTBI diagnosis which is tuberculin skin test (TST) that provides "screening for" only latent infection of *"M. tuberculosis*" and has low specificity. It exhibits high cross reactivity with other "mycobacteria species". Thus, the test is not able to differentiate *"M. tuberculosis*" from other *mycobacterium* infections. (Arinaminpathy and Dowdy 2015; Walzl, et al. 2011; Zhou, et al. 2015a)

Rapid culture techniques and T-cell-based interferon-γ release tests are brand new developments in terms of diagnosis of TB. "*M. tuberculosis*" gene amplification test (GeneXpertM. TUBERCULOSİS / RIF) has been also developed and it has a capacity to detect antibiotic resistance for several antibiotics used in the treatment of TB such as rifampicin. The test has an excellent sensitivity, allows automatized sample processing and gives results in two hours. However, it is unable to detect latent disease. It serves for the detection of active pulmonary tuberculosis. (Arinaminpathy and Dowdy 2015; Jenum, et al. 2016; Ottenhoff, et al. 2012; Walzl, et al. 2011; Zhou, et al. 2015a)

IFN-γ release assays (IGRAs) which are for measuring production of IFN-γ in response to specific TB antigens which are, early secretory antigenic target (ESAT6) and culture filtrate protein (CFP-10) are cell-mediated immunity based assays .These strong T-cell antigens are encoded inside "the region of difference 1 (RD1) of the *M. tuberculosis* strains. *M* .bovis BCG strains and most of the nontuberculous mycobacteria (NTM) do not contain this region. This is the reason why IGRAs have no positivity in BCG vaccinated and almost no positivity in patients infected with "NTM". However , RD1 genes" are present in some NTM such as *M. kansasii*, *M. marinum*,*M. riyadhense* and *M. szulgai*. IGRAs are considered much more accurate in vaccinated patients. In other respects , IGRA is not specialized test for latent tuberculosis and not suitable for large scale of screening. For this reason, tests which have capacity for rapid, economic, and accurate diagnosis of active TB with low cost are required. (Arinaminpathy and Dowdy 2015; Jenum, et al. 2016; Liu, et al. 2016; Ottenhoff, et al. 2012; Walzl, et al. 2011; Zhou, et al. 2015a)

2.4. IP-10 Tests for Diagnosis of TB

Tuberculin skin test (TST) has preserved its position as the primary immune diagnostic test for LTBI for almost a century. About 10 years ago, the IFN-γ release assays were introduced as an alternative test for the diagnosis of LTBI. The biggest issue of TST comes with its low specificity, and IGRAs were designed to solve this problem especially. They provide excellent specificity rate. In other respects, the performance of IGRA is slightly better only in this manner. "IGRAs" have a sensitivity rate around 80%, which is comparable to TST and even lower, in immunocompromised patients. The suboptimal sensitivity of IGRAs might be related to the usage of IFN-γ as a biomarker, which can be produced at low levels within the lymphocytes. To overcome this particular pitfall of IGRAs, alternative biomarkers expressed at high levels are needed . Multiple cytokines and chemokines were measured as substitutional biomarkers to IFN-γ. (Chegou, et al. 2014; Zhang, et al. 2017)

IGRAs measure T-cell response which is stimulated overnight with specific *M .tuberculosis* antigens. They are *in vitro* assays. The well-known IGRAs worldwide are QuantiFERON- TB Gold In-Tube (QFT-GIT) assay and the T-SPOT assay are the most widely used IGRAs. (Banaei, et al. 2016; Du, et al. 2018; Liu, et al. 2016; Moses, et al. 2016; Zhou, et al. 2015b)

In terms of diagnosis of LTBI, IFN-γ is one of the potential biomarkers in order to detect antigen specific response. By contrast , interferon gamma inducible protein 10 kDa (IP-10) is an alternative biomarker candidate to IFN-γ. Also, it is expressed at 100-fold higher level compared to IFN-γ, and increases the sensitivity of IGRAs. This can be count as an advantage in terms of people with "high risk factors" ,i.e. recently infected people .

IP-10 is a chemokine and also known as CXCL10 (C-X-C motif chemokine). It is expressed by antigen presenting cells (APCs) after several cytokines activation ; especially, IFN-γ, TNF-α , IL-2 and IL-17. IP-10 is expressed by viruses and bacteria infected cells and initiation of the expression related to the recognition of specific peptides presented on the APCs by T cells. (Chegou, et al. 2014; Ruhwald, et al. 2012; Ruhwald, et al. 2011)

Antigen specific T cells directly interact with monocytes and other related cells responding to the T-cell derived cytokines and secretion of IP-10 occurs in IP-10 release with IFN-γ, but 100-fold higher than IFN-γ at levels. Several researches displayed that IP-10 might be used with IFN-γ, to improve sensitivity for LTBI diagnosis (2–11% increase) without decrease of the specificity rate.. (Chegou, et al. 2014; Ruhwald, et al. 2011)

IPRA is a cell mediated immunity based assay and induction occurs through the response of adaptive immunity. Firstly, T cells recognize "*M. tuberculosis*" specific antigens on the MHC molecules on the APC surface. By this interaction, activation of T cells and APCs occurs. This activation initiates secretion of several pro inflammatory cytokines from both T cells and APCs. With the further activation of the APCs by the released cytokines, IP-10 is secreted from APCs as a host biomarker molecule. (Ruhwald, et al. 2012; Ruhwald, et al. 2011; Walzl, et al. 2011)

IP-10 release assays measure IP-10 response in blood sample when the sample is stimulated with *M. tuberculosis* antigens such as ESAT-6 and CFP-10. IP-10 is considerable as an alternative and promising diagnostic marker for LTBI diagnosis because of its favorable characteristics. Its advantages come with its high level of expression compared to the current biomarker, IFN-γ, and IP-10 (7.4 kDa) is 2.5 times smaller than IFN-γ (18.8 kDa) as a molecule. Furthermore, consistent expression of IP-10 in response to *M. tuberculosis* antigens makes it even more preferable marker rather

than IFN-γ in a diagnostic manner. (Ruhwald, et al. 2012; Ruhwald, et al. 2011; Walzl, et al. 2011)

IP-10 as an alternative biomarker expressed in high amount promises to enable a more sensitive test in terms of detection. Furthermore, high-level secretion of IP-10 allows for using small amount of blood volume, simple and cheaper sample preparation.(Banaei, et al. 2016; Ruhwald, et al. 2012; Ruhwald, et al. 2011; Walzl, et al. 2018; Walzl, et al. 2011)

3. MATERIALS AND METHODS

3.1. Study population and setting

The study included two groups of patients and for defining the two groups no prior TST or IGRA or IP10 test was used.

The active TB patients group: Blood samples of active TB patients were collected from two centers; Süreyapaşa Chest Diseases Hospital, Saglik University and Taksim Central Laboratory of Istanbul Union Against Tuberculosis. To yield this, following inclusion criteria were used for active patient group; i) patients should be >18 years of age and immunocompetent, ii) cases should be newly-diagnosed and maximally within the first four weeks of TB therapy, iii) all active TB cases should be smear and/or culture positive or PCR(+).

The exclusion criteria for this group were defined as follows; i) patients with bleeding diathesis, ii) patients under 18 years of age, iii) patients with immunosuppression for any reason (immunodeficiency, HIV/AIDS infection, cancer, immunosuppressive treatment)

The non-TB patients as control group: Patients who were known not to have tuberculosis infection were selected. The inclusion criteria of this group were defined as follows; i) a definite clinical diagnosis of any pulmonary disease other than tuberculosis, ii) patients that were not fallen into high risk group for TB.

The exclusion criteria were; i)patients with bleeding diathesis, ii) patients under 18 years of age, iii) patients with immunosuppression for any reason (immunodeficiency, HIV/AIDS infection, cancer, immunosuppressive treatment) iv) patients who were carrying more than two high risk factors asked in the "Volunteer Data Form".

In the Volunteer Data Form following questions were asked:

1. Is TB diagnosed and/or treated previously?

2. Is LTBI diagnosed /or treated previously?

3. Any known contact with someone diagnosed as TB?

4. Did the volunteer currently or previously

a. work in a hospital for more than 1 year?

b. stay in correctional facility, nursing home, drug rehabilitation center, or alike for more than 1 year?

c. live in a homeless shelter for more than 1 year?

5. Is the volunteer suffering from diabetes, chronic renal failure, cancer, HIV/AIDS, organ transplant or IV drug abuse?

6. Has the volunteer used any immunosuppressive agent such as steroids, TNF-α blockers, etc. within the last 6 months?

7. Is the volunteer coughing up blood within the last two months?

8. Has the volunteer complaints of coughing, fever and sweating within the last 3 weeks?

9. Is the volunteer losing weight for some unknown reasons within the last 2 months?

10. Has the volunteer stayed in a foreign country with a TB prevalence of >20/100000 for 3 months or more?

If the volunteer replied "Yes" to any three of the above questions, he/she was considered as high risk individual and excluded from the study.

This study was conducted between May 2017 and September 2018 at the Institute of Experimental Medicine, Department of Immunology, Istanbul University. The study was approved by Istanbul University's Ethics Committee (approval No. 2017/740) and informed written consent was obtained from all participants before blood samples were taken. The main objective of this research was to evaluate the sensitivity and specificity as intrinsic performance characteristics of IP-10 release assay for the detection of LTBI.

3.2. Blood sampling

Preparation of the ESAT-6/CFP-10 antigen cocktail

The 101 amino acid (AA) long ESAT-6 protein was synthesized in peptide fragments, which were 15 AA long. Every fragment's last 11 AA's constituted the first 11 AA's of the ensuing fragment. Thus the entire ESAT-6 protein was covered through 23 peptide fragments. Similarly the 96 AA long CFP10 protein was covered through 21 fragments. The purity of the synthesis was adjusted to >70% (JPT Peptide Tech., Berlin, Germany).

Peptide fragments were diluted in 100% DMSO, mixed and final concentration of each peptide fragment in the solution was adjusted to 5 µg/ml.

Blood samples were taken from both patient groups in 9-ml heparinized tubes and 950 µl of blood was transferred aseptically to each of the following tubes. These tubes are provided with the following contents;

1. 50 µl of glucose solution (40mg/ml) (negative control tube),
2. 5µg/ml phytohemagglutinin in 50 µl of glucose solution (40mg/ml)(positive control tube),
3. 2.5µl of ESAT-6 and CFP-10 antigen-cocktail (44 peptides in total, each with a concentration of 5 µg/ml) + 47.5µl glucose solution (40mg/ml)

The incubation of tubes was completed at 37°Cfor 48 hours to stimulate TB specific antigenic response. After the incubation, 10 µl of supernatant was obtained from each tube and transferred to an identical new tube containing 320 µl of Diluent A (33×dilution), and the tubes were stored at 4°C.

3.3 IP-10 Release assay

Ray Bio® Human IP-10 ELISA Kit (Ray Biotech, USA) was used according to the manufacturer's instructions. All assay reagents, standards and patient samples for the experiment were brought to room temperature at least 30 min before starting the assay procedure.

Table3.1 Reagents

Item	Component	Size / Description
A	IP-10 microplate	96-well plate coated with anti-human IP-10
B	Wash buffer 20x	25 ml of 20x solution
C	Standard protein	2 vials of human IP-10. 1 vial is enough to run each standard in duplicate.
F	Detection antibody for IP-10	2 vials of biotinylated anti-human IP-10. Each vial is enough to assay half the microplate.
G	HRP-streptavidin concentrate	200 "µl" of 500x HRP-conjugated streptavidin solution
H	TMB One-Step substrate reagent	12 ml of 3,3,5,5-tetramethybenzidine (TMB) in buffer solution
I	Stop Solution	8 ml of 0.2 M sulfuric acid
D	Assay diluent A	30 ml of diluent buffer with 0.09% sodium azide as preservative
E	Assay diluent B	15 ml of 5x buffer

3.3.1 Preparation of standard

Item C vial was centrifuged for 10 seconds, 400 µl of 1×Assay Diluent A was added to Item C vial in order to prepare a 50 ng/ml stock standard dissolved by gentle mixing and 80 µl of this IP-10 stock standard was transferred to a tube containing 586.7 µl of Assay Diluent A to prepare a 6000 pg/ml standard solution, which was used to prepare a three-fold dilution series by serially transferring 200 µl of the well-mixed last dilution to 400 µl of Assay Diluent A. The dilutions prepared ranged from 6000 pg/ml to 8.23 pg/ml, with the diluent alone serving as the zero dilution.

Table3.2 Standard preparation

		Three-fold serial dilution series							
		1	2	3	4	5	6	7	Zero
Diluent volume	Item C 400 µl	586.7 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl
Concentration	50 ng\ml	6000 pg/ml	2000 pg/ml	666.7 pg/ml	222.2 pg/ml	74.07 pg/ml	24.69 pg/ml	8.23 pg/ml	0 pg/ml

Volumes of 100 µl of each standard and samples were added into the appropriate wells and the incubation of the plate was completed at room temperature for 2.5 h by shaking gently on a shaker. After incubation, the supernatant was decanted and washing was done with 1x wash solution eight times by using an auto washer. Then, 1x biotinylated antibody was prepared and the antibody incubation was performed with 100 µl of antibody per well at 22^{o}Cfor one hour with vortexing at low speed. The supernatant was decanted followed by washing for four times. Next, plate was incubated 22^{o}C after adding 100 µl of the streptavidin solution to each well for 45 min on a shaker. After a second washing, 100 µl of tetramethylbenzidine substrate was poured to each well and the plate was incubated at 22^{o}C with for 10-30 min in the dark on a shaker. Finally, reaction halted using 50 µl of Stop Solution and optical density at 450 nm was evaluated just after the reading.

3.4. Interpretation of the results and Data analysis

The mean absorbance values were calculated for standards and samples and the zero standard optical density was subtracted .A standard curve was plotted by using "Sigma plot software". The results were assessed as was given in the manufacturer's instructions.

The values obtained from each well of the plate were calculated. The stimulated TB antigen value was obtained by subtracting the nil tube value from the value obtained from the antigen tube . Mitogen induced result was obtained by subtracting the nil tube value from the IP10 value of the mitogen tube.

The cut-off value for a positive test, based on receiver operating characteristics curve analysis using "MedCalc statistical software".(2018) A cut-off value of 439pg\ml was chosen by the software.

If the mitogen induced value is less than 1000pg/ml and the stimulated antigen tube considered negative, the result of the test was considered indeterminate. If the stimulated TB antigen response was higher or lower than the cut-off, the result was considered positive and negative, respectively.

Table3.3 Results interpretation

Antigen-Nil	IP-10 assay result	Interpretation
< 439	Negative	*"MTB*" infection unlikely
≥ 439	Positive	"*MTB*" infection likely

4. RESULTS

4.1 Demographic and General Features Of The Study Population

The population consisted of two groups: 84 active TB patients and 57 non-TB patients. The active TB patients were younger (mean age 44.5 years vs. 57.7years) and mostly males (69% vs. 47%) with a higher percentage of immigrants (11.9% vs. 1.8%) (Table 4.1).

Table 4.1: Features of active TB and non-TB patients.

	Active-TB	**Non-TB**
N	84	57
Median age in years (range)	46(17-84)	56.5(30-92)
Ave. age in years (mean ± SD)	44.5 ± 18.1	57.7 ± 18.90
Immigrants n(%)	10 (11.9%)	1 (1.8%)
Gender	58 ♂, 26 ♀	27 ♂, 30 ♀
TB diagnostic tests, n(%)		
smear (-) culture (+)	21(25%)	
culture and smear (+)	57 (67.8%)	
smear(+),culture(-)	4(4.7%)	
"PCR", n(%)*	2(2.3%)	
Non-TB patients		Total=57
Sleep apnea		17 (29.8%)
Sarcoidosis		9 (15.7%)
Pneumonia		9 (15.7%)
Chronic obstructive *pulmonary disease*		9 (15.7%)
Pulmonary hypertension		6 (10.5%)
Pulmonary embolism		3
Bronchiectasis		1
Vasculitis		1
Pleuritic		1
Type 2 dyspnea		1

Actually, we collected blood samples from four other patients who were smear (+), atypical mycobacteria were grown in cultures and excluded from the study.

Out of the 84 of the active TB patients,57 were smear and culture positive,21 patients were only culture positive, and four were smear positive" but their cultures remained negative, Of the culture positive patients two were also subjected to PCR and their PCR tests gave positive results. Most of the specimens were sputum, four were biopsies, and two were pleural fluid.

We also collected blood samples from 85 non-TB patients, but 28 were excluded when it became clear that they had taken immune-suppressive medications. For the remaining 57 patients who were included" in the study, 17 had sleep apnea, nine each had pneumonia, chronic obstructive pulmonary disease or sarcoidosis, and the others various other conditions.

Furthermore, 16 out of the total 85 non-TB patients had replied one yes to the our ten exclusion criteria.

The above-mentioned data for the TB group and the non-TB group were obtained from the medical records.

White blood cell and lymphocyte counts were provided by the healthcare institutions (Table4.2). The mean lymphocyte counts in active TB patients($20.7 \pm 8.73 \backslash mm^3$) were higher than in non-TB patients ($16.66 \pm 8.85 \backslash mm^3$), and the same was true for the medians.

Table 4.2: WBC and lymphocyte counts in active TB patients and non-TB patients

Group		WBC /µl	Lymphocytes/mm^3
Active TB group n = 84	Mean ± SD	9021±3014 SD	20.7 ±8.73
	Median (range)	8600 (4300 – 16,600)	19.5 (5.5-42.9)
Non-TB group n =35*	Mean ± SD	9552.64±3461.3	16.66± 8.85
	Median (range)	9100 (4200 – 15,900)	15.75 (2.6-35.4)

* No cell counts are available for 22 of the 57 patients.

4.2."IPRA" Biomarker Levels

Most of blood samples were analyzed within an hour of blood collection and the others within max. 4hours. The time between blood collection and analysis and "the length of the incubation period" are shown in Table4.3.

Table4.3: The time between blood collection and analysis and the incubation time during analysis.

Group	Statistic	Time between blood collection and analysis (min)	Incubation time (h)
Active TB	Median (range)	12 (2-237 min)	48 (45-50)
Non TB	Median (range)	26.5 (3-95 min)	48.5 (45-53)

- As expected, antigen induced IP-10 level (median) was higher for active TB patients (3269.6pg/ml) compared to the non-TB group (18.1pg/ml). Mitogen-induced "IP-10" level was higher for active TB patients (8700.1pg/ml) than non-TB patients (5916.1pg/ml). To have a general view how far the active TB cases' IP-10 levels dispersed from the background, antigen induced values of active TB were divided to nil values. The difference was found to be 17.5-fold. The same ratio was 1.2 for non-TB patients.

Table 4.4:Median levels (range) of IP-10 (pg|ml)) for active TB patients and non-TB patients.

IP-10 result	Active TB n =84	Non-TB n =57
Nil tube	198.7 (0-7308)	80.4 (0-6433)
Mitogen tube	8898.8 (21.7-57850)	5996.5 (749.6-52612)
Ag tube	3468.3 (21.7-54252)	98.5 (0-4664)
Mitogen - Nil	8700.1 (-910-57209)	5916.1 (660.2 – 51824)
Ag*- Nil	3269.6 (-108.6-52776)	18.1 (-1858-554)

Ag*: *M. tuberculosis* -specific antigensESAT-6 and CFP-10.

4.3. Intrinsic Performance Characteristics Of The IPRA Test

The IPRA results from active TB patients and non-TB patients were used to assess its diagnostic performance on the basis of receiver operating curve analysis (ROC) by using statistical software .(2018) The area under curve was calculated as 0.962 ($p < 0.001$), which pointed out to a high diagnostic performance of the test. Again by using the ROC analysis, a cut-off value of 439pg/ml was determined at maximum Youden Index. Accordingly, the sensitivity of the test was 89.3% and specificity was 98.2 %. As shown in the Table 4.5, if we choose different cut off values, the sensitivity and specificity will be changed. For example, if we use(290 pg\ml) as a cut-off value ,the sensitivity will be 90.4% with 94.7% specificity. Also with a higher cut-off value ,such as (554pg\ml), the sensitivity will be decreased to 83.3% with 100% specificity.

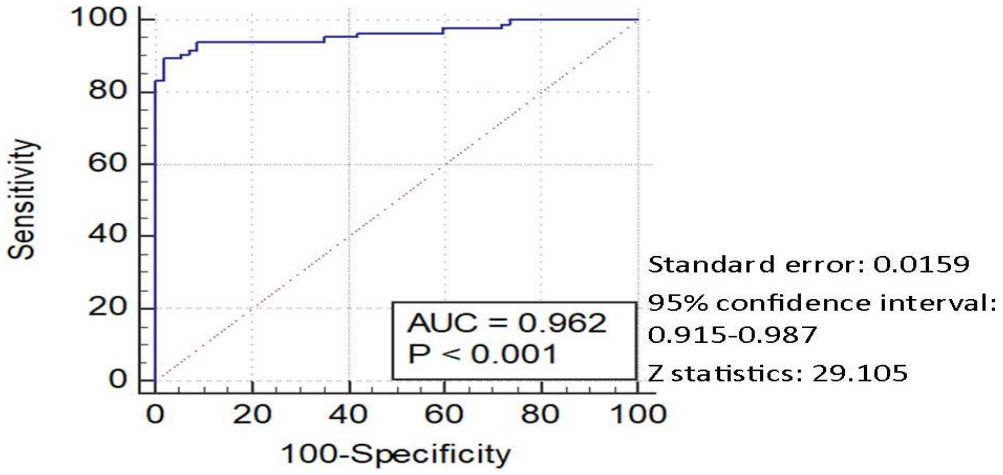

Figure 4.1.AUC plot of specificity and sensitivity of IP-10 test for 84 active TB patients and 57 non-TB patients.

Variable	IP10_Result
Classification variable	Diagnosis

Sample size	141
Positive group [a]	84(59.57%)
Negative group [b]	57(40.43%)

[a]Diagnosis = 1

[b]Diagnosis = 0

Disease prevalence (%)	unknown

Area under the" ROC curve" (AUC)

Area under the ROC curve (AUC)	0.962
Standard Error[a]	0.0159
95% Confidence interval[b]	0.915 to 0.987

z statistic	29.105
Significance level P (Area=0.5)	<0.0001

[a] DeLong et al., 1988

[b] Binomial exact

Youden index

Youden index J	0.8753
Associated criterion	>439.1
Sensitivity	89.29
Specificity	98.25

Table **4.5"**

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR
≥-1857.64	100.00	95.7 - 100.0	0.00	0.0 - 6.3	1.00	
>-123.97	100.00	95.7 - 100.0	26.32	15.5 - 39.7	1.36	0.00
>-108.64	98.81	93.5 - 100.0	26.32	15.5 - 39.7	1.34	0.045
>-78.49	98.81	93.5 - 100.0	28.07	17.0 - 41.5	1.37	0.042
>-65.2	97.62	91.7 - 99.7	28.07	17.0 - 41.5	1.36	0.085
>-17.88	97.62	91.7 - 99.7	40.35	27.6 - 54.2	1.64	0.059
>-16.3	96.43	89.9 - 99.3	40.35	27.6 - 54.2	1.62	0.089
>11.2	96.43	89.9 - 99.3	57.89	44.1 - 70.9	2.29	0.062
>16.254	95.24	88.3 - 98.7	57.89	44.1 - 70.9	2.26	0.082
>31.93	95.24	88.3 - 98.7	64.91	51.1 - 77.1	2.71	0.073
>32.6	94.05	86.7 - 98.0	64.91	51.1 - 77.1	2.68	0.092
>125.556	94.05	86.7 - 98.0	91.23	80.7 - 97.1	10.72	0.065
>168.08	91.67	83.6 - 96.6	91.23	80.7 - 97.1	10.45	0.091
>206.7	91.67	83.6 - 96.6	92.98	83.0 - 98.1	13.06	0.090
>217.3	90.48	82.1 - 95.8	92.98	83.0 - 98.1	12.89	0.10
>290.13	90.48	82.1 - 95.8	94.74	85.4 - 98.9	17.19	0.10
>367.46	89.29	80.6 - 95.0	94.74	85.4 - 98.9	16.96	0.11
>439.1	89.29	80.6 - 95.0	98.25	90.6 - 100.0	50.89	0.11
>525.95	83.33	73.6 - 90.6	98.25	90.6 - 100.0	47.50	0.17
>554	83.33	73.6 - 90.6	100.00	93.7 - 100.0		0.17
>52776.209	0.00	0.0 - 4.3	100.00	93.7 - 100.0		1.00

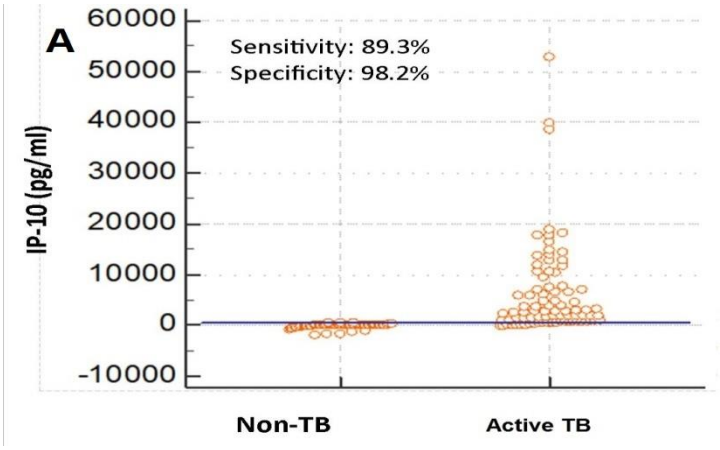

Figure 4.2. A*.Distribution of IP-10 levels in 84 active TB patients and 57 non-TB patients. The horizontal blue line represents the cutoff value of >439.1 pg/ml for diagnosis of LTBI.

*In the figure 4.2.A : The distribution of IP-10 values were shown without logarithmic transformation .

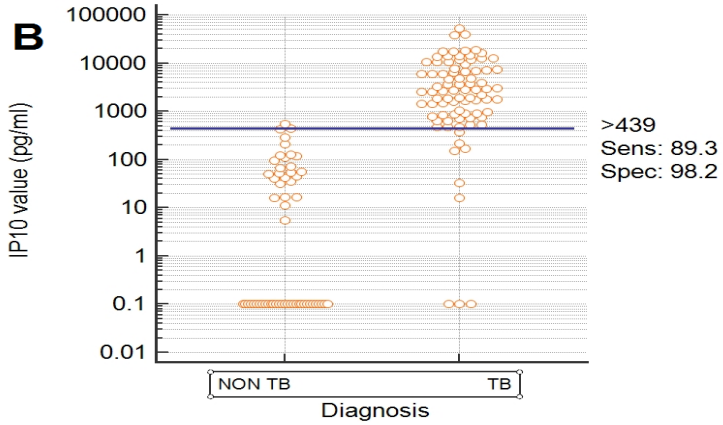

Figure 4.2.B . shows the same data in the figure 4.2.A with logarithmic transformation For the logarithmic figure all values less than zero were considered as (0.1pg\ml) .

5. DISCUSSION

This study was designed to assess the performance of our home-made IPRA for the diagnosis of LTBI by using the intrinsic parameters of the test such as sensitivity and specificity.

As we know no laboratory gold standard for LTBI diagnosis, we could not include latently infected patients to test the accuracy, but instead, included those patients who were definitely known to have the disease to determine the sensitivity, and those who were definitely known to not have the disease to calculate the specificity of the home-made IPRA. For this purpose, 84 newly diagnosed and microbiologically confirmed TB patients who fell into the active TB group to calculate the sensitivity and 57 patients with respiratory diseases other than tuberculosis comprised the non-TB group to determine the specificity of the assay.

In the present study we have determined a cut- off for a positive IPRA assay on the basis of receiver operating characteristics curve (ROC) analysis using MedCal statistical software.(2018) A cut-off value of 439pg\ml was defined at the maximum Youden Index. Based on this cut-off value, the area under the IP10 - (100-specificity) curve' covered nearly the entire area; it was found 0.962, with sensitivity and specificity values of 89.3% and 98.2 %, respectively. A drastic difference obtained in the median Ag - Nil values between the active TB and non-TB patients (3269.1 vs. 18.1pg/ml, respectively) evidenced that IPRA test gave a confident discrimination between the two groups as expected.

Similar to previous studies done with IFN-γ(Auguste, et al. 2017; Diel, et al. 2011; Ruhwald, et al. 2011; Sester, et al. 2011) and IP10 cytokines (Guo, et al. 2014; Ruhwald, et al. 2012; Santos, et al. 2018), a very high specificity rate of 98.3% was obtained.

A meta-analysis published in 2018 compared the diagnostic assessment of "IPRA" for diagnosing LTBI in four studies published between 2012 and 2015 reported that the pooled sensitivity was 85% and specificity was 63%. Our 89.3% sensitivity and 98.2% specificity are higher than the reported pooled sensitivity and specificity.(Santos, et al. 2018)

In another study published in 2011, IPRA and IGRAs were compared. They found that IPRA had an AUC of 0.924 and IFN-γ had an AUC coverage of 0.937. While IPRA showed 97% specificity and 81% sensitivity, IFN-γ had 100% specificity and 84%sensitivity.(Ruhwald, et al. 2011)

A previous study displayed that IPRA sensitivity was 83% with 100% specificity rate.(Ruhwald, et al. 2008) On the other hand, another study demonstrated that the "IPRA" test shows more promising results with 91.8% and 93.5% sensitivity and specificity, respectively. However, that study had used a cut off value of (300pg\ml), which is less than our cut off value (439pg\ml). According to our ROC curve data sheet, if we had used the same "cut off" value our results would have been on par with that study.(Kabeer, et al. 2010)

A great difference was observed between active TB and non-TB patients based on IP-10 levels(3269.1 vs 18.1 pg\ml), which supported the usefulness of IP-10 for detecting LTBI as an alternative to IFN-γ used in standard IGRAs .

Therefore, "IP-10" has a potential for improving the sensitivity of IGRA with its considerably high level of expression in terms of "LTBI" diagnosis. Even though IFN-γ measurements were not included in the study, it has been shown that TB patients produce antigen-induced "IFN-γ" in smaller amounts compared to IP-10.(Chegou, et al. 2014; Ruhwald, et al. 2012; Ruhwald, et al. 2011). Thus, the lower amount of produced IFN- γ decreases the sensitivity of "IGRAs" and gives false negative results.

Even though "IGRAs" overcome the drawback of the relatively lower specificity of tuberculin skin test (TST), the sensitivity of "IGRAs" seems hardly to be superior to "TST". The potential reasons of suboptimal sensitivity of "IGRAs" might be two-pronged; first reason might be the relatively lower secretion rate of "IFN-γ" when compared with IP-10 secretion. A second reason could be related to the incubation time(Sutherland, et al. 2010). Commercially available IGRAs are suggesting overnight incubation before measuring the TB specific IFN-γ responses, which in fact, is contrasting with the conventional TST. As is known, the incubation period of TST is minimally 48 hours; if the measurement at the 48th hour is below the threshold, the antigenic stimulation will be extended for another day. Therefore we preferred an incubation period of 48 for the IPRA as to increase the sensitivity of the assay. Of the 84 active TB patients, 9 (10.7%) turned out negative. The sensitivity of 89.3% is higher than obtained for IFN-γ, This suggests that IPRA with 48-hour incubation might be

superior to commercial IGRAs, which are suggesting overnight incubation in their test protocols.

One of the limitations of this study is the absence of a proper comparison of overnight incubation with 48-hour incubation for the both patient groups. The results of two sets of incubation times would clearly give robust data whether to extend incubation period for another day and change working protocols.

In conclusion, IP-10 release assay showed very high sensitivity and specificity in our setting, and may be a robust stand-alone test for detecting LTBI.

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FORMS

E-postalayın Yazdır

Çalışma Başlığı: IP10 stimulanı olarak Phytohemagglutinin, Concavalin A ve Poly I:C maddelerinin immunokompetan ve immunodefektif hastalarda karşılaştırılması

Değerli araştırmacı,
Lütfen formu doldurup sol üstteki "E-postalayın" düğmesini tıklayarak gönderiniz.
Bu formu doldurabilmeniz için en az Adobe Acrobat version 7.0 gereklidir.

Araştırmacı Merkez

Genel hasta bilgileri

Hasta Kimliği [1] Göçmen

Doğum tarihi Evet ise, ne zamandan beri

Cinsiyet Doğum yeri (ülke)

Kan örneğine ilişkin bilgiler

Hasta grubu

1 Klinisyen gönüllünün ismini gizli tutmak isterse, hasta kimliğini kendi isim ve soyadının ilk harflerini takiben verilmiş hasta numarası, örneğin Dr Zeki Kılıçaslan ilk hastasını ZK-1, ikinci hastasını ZK-2 tarzında bildirebilir.

(Aşağıda yer alan IP10 testinin yapılması ve sonuçları ile ilgili veriler lab. tarafından doldurulacaktır)

Hasta ve kontrol gönüllülerine ait bilgiler

IP10 ELISA assay

Kan örneğinin alındığı gün ve saat:dak

Kan alınmasından analiz başlangıcına kadar geçen süre (saat:dak)

Kan örneğinin enkübasyon süresi (saat:dak)

(Lütfen heparinli tüpün etiketine de kanın alındığı gün, saat ve dakikayı yazınız)

IP10 konsantrasyonları (pg/µl)	1 gün enkübasyon	2 gün enkübasyon
Nil		
TB Ag		
Mitojen$_1$ (PHA)		
Mitojen$_2$ (ConA)		
Mitojen$_3$ (Poly I:C)		

Güncel kan sayımı

Lökosit sayısı /µl

Lenfosit yüzdesi

TB şüphesi veya aktif hastalık

Şu anda TB şüphesi var mı?

Eğer evet ise, nasıl belirlendi?

Şu anda aktif TB var mı?

Eğer evet ise, nasıl belirlendi?

Klinik forma

Kaynak

Özgeçmiş ve TB için risk faktörleri

Önceki yıllarda TB tanısı var mı?		Eğer evet ise, tanı yılı (yyyy)	
TB tedavisi görmüş mü?		Eğer evet ise, tedavi tarihi (aa/yyyy)	
Latent TB durumu biliniyor mu?		Eğer evet ise, tanı konulduğu tarih	
Koruyucu tedavi almış mı?		Eğer evet ise, koruyucu ted. tarihi	
BCG aşısı olmuş mu?		Eğer evet ise aşılanma tarihi	
Maruziyet hikayesi var mı?		Varsa maruz kalma tarihi	
IV ilaç kullanımı			
Alkol kullanımı			
Diabetes mellitus tanısı almış mı?			
Sigara kullanımı		Eğer evet ise kaç paketyıl[1]	
Akciğerde sekel lezyonlar var mı?			

1... bir paket yıl, bir yıl boyunca hergün bir paket sigara tüketimine karşılık gelir

Immunokompetan spesifisite kontrol grubu için risk değerlendirmesi

Aşağıdaki sorular sadece testin spesifisitenin ölçülmesi için çalışmaya dahil edilen gönüllü grubuna sorulacaktır. Bu grup, TB dışı bir hastalığı olduğu kesinleşmiş immunokompetan gönüllülerden oluşur. Aşağıdaki sorular düşük riskli gönüllüleri risk faktörüne sahip gönüllülerden ayırmak için düzenlenmiştir

Tanı			
Önceki yıllarda TB tanısı var mı?		Evet ise, tanı tarihi (yyyy)	
TB tedavisi görmüş mü?		Evet ise, tedavi tarihi (aa/yyyy)	
Latent TB durumu biliniyor mu?		Evet ise, tanı tarihi (yyyy)	
Koruyucu tedavi almış mı?		Evet ise, tarihi (aa/yyyy)	
TB tanısı almış bir hastayla teması olmuş mu?			

Gönüllü geçmişte veya halen 1 yıldan fazla süreyle;

- Hastanede çalışmış mı?
- Hapishane, bakım evi, ilaç rehabilitasyon merkezi ve benzeri kurumda kalmış mı?
- Evsiz olarak yaşamış mı?

Gönüllünün mevcut diabet, böbrek yetmezliği, silikoz, kanser, HIV/AIDS, organ nakli ameliyatı, damar içi ilaç kullanımı hikayesi var mı?

Gönüllü son 6 ay içinde steroid, TNF antagonisti vb immunosupressan ilaç kullanmış mı?

Gönüllü hamile mi?

Son 2 ay içinde öksürükle beraber kan geldi mi?

Gönüllü son üç haftadır süren öksürük, ateş ve terleme şikayetleri var mı?

Gönüllü son 2 ay içinde sebebi bilinmeyen bir şekilde 5kg'dan fazla kilo kaybetti mi?

Gönüllü ≥3 ay TB prevalansı >20/100.000 olan bir ülkede bulundu mu?

Gönüllü düşük riskli bir kontrol olarak kabul edilebilir mi?

Gönüllü yukarıdaki bütün sorulara "Hayır" yanıtı veriyorsa düşük riskli kontrol olarak kabul edilir.

Gönüllü risk faktörlü bir kontrol müdür?

Gönüllü yukarıdaki sorulardan en az birine "Evet" yanıtı verdiyse risk faktörlü kabul edilir.

RAW DATA

Appendix(1)

	Study Group	Time btw BC and analysis	Incubation period	N 2d	Ag 2d	M 2d	AG-NIL	WBCs	% Lymph	Source	Diagnosis	Age	Gender	Immigrant	Country of birth
001ga	ACTIVE TB	22min	48:49:00	180.5	651.8	4277	471.3	10200	16.3	sputum	Smear &cul (+)	17	F		
002ma	ACTIVE TB	10min	47:38:00	144.07	7858	2841	7714	6000	14.8	sputum	Smear &cul (+)	46	F		
003yg	ACTIVE TB	10min	47:35:00	2356.2	3065	17612	708.4	13100	23.3	sputum	Smear &cul (+)	25	F		
004ha	ACTIVE TB	20min	47:13:00	253.3	6166	13898	5913	12700	9	sputum	Smear &cul (+)	24	M		
006Hk	ACTIVE TB	3min	47:28:00	1475.3	54252	15173	52776	7100	39.3	sputum	Smear &cul (+)	58	M		
007mb	ACTIVE TB	6min	47:26:00	2312.1	5348	19300	3035	11000	25.8	sputum	Smear &cul (+)	46	M		
008mk	ACTIVE TB	6min	47:15:00	1321.2	14048	7209	12727	6700	24.4	sputum	Smear &cul (+)	19	M		
010mr	ACTIVE TB	10min	47:18:00	1299.2	2717	22231	1418	15700	19.9	sputum	Culture (+)	57	M	YES,IN 2009	TÜRKMENSTAN
011hd	ACTIVE TB	3min	49:13:00	689.4	18850	4975	18160	5400	14.9	pleural fluid	Culture (+)	81	F		
018aÕ	ACTIVE TB	6min	48:17:00	946.8	4181	35132	3234	12700	14.4	sputum	Smear &cul (+)	51	M		
019gk	ACTIVE TB	8min	48:20:00	22.3	1916	1365	1893	5300	13.7	sputum	Culture (+)	84	F		
021mc	ACTIVE TB	5min	49:58:00	7307.7	45786	48723	38478	6200	26.5	sputum	Smear &cul (+)	17	M		
022yu	ACTIVE TB	3min	49:16:00	924.8	2668	2817	1743	4500	23.9	sputum	Smear &cul (+)	80	F		
026dy	ACTIVE TB	16min	49:12:00	1145	7708	12097	6563	5600	19.1	sputum	PCR &cul (+)	25	F		
027mk	ACTIVE TB	28min	47:59:00	67.07	7408	5496	7341	11400	18.4	sputum	Smear &cul (+)	60	M		
028oe	ACTIVE TB	23min	48:09:00	1321.2	13223	39336	11901	10400	20.4	sputum	Smear &cul (+)	48	M		
029ay	ACTIVE TB	44min	48:43:00	253.3	1740	56958	1486	6600	16.9	sputum	Smear &cul (+)	52	M		
035bk	ACTIVE TB	6min	49:51:00	1761.6	2668	18100	906	10100	22.4	sputum	Smear &cul (+)	28	F		
036ag	ACTIVE TB	12min	49:45:00	1673.5	3114	27357	1441	4800	34.9	sputum	Smear &cul (+)	45	M		
037ag	ACTIVE TB	50min	49:55:00	3643.2	6586	4646	2943	4300	13.7	Pleural fluid	Culture (+)	42	M		

Appendix(2)

	Study Group	Time btw BC and analysis	Incubation period	N 2d	Ag 2d	M 2d	AG-NIL	WBCs	% Lymph	Source	Diagnosis	Age	Gender	Immigrant	Country of birth
038de	ACTIVE TB	64min	48-49	125.8	912.5	27520	786.7	8000	9.9	sputum	Smear &cul(+)	58	M		
039my	ACTIVE TB	3min	49:45:00	65.578	543.2	4254	477.6	9900	19.5	sputum	Smear &cul (+)	66	F		
040ok	ACTIVE TB	2min	49:42:00	5.418	21.67	2989	16.2	8300	16.5	sputum	Culture (+)	37	M		
041em	ACTIVE TB	3min	49:04:00	733.293	2924	1991	2191	7400	29.4	sputum	Smear&cul (+)	22	F		
048ok	ACTIVE TB	5min	49:32:00	41.725	1665	21.67	1623	10400	13.8	sputum	Smear&cul (+)	47	M		
050mw	ACTIVE TB	5min	47:42:00	77.504	597.5	17507	520	15500	5.5	sputum	Smear&cul (+)	22	M	Yes, in 2016	PAKISTAN
051ms	ACTIVE TB	15min	47:55:00	349.187	4990	5728	4641	6500	12.2	sputum	Smear&cul (+)	24	F		
052sy	ACTIVE TB	10 min	48:00:00	2303.48	4178	1393	1875	13800	6.5	sputum	Smear&cul (+)	48	M		
053mk	ACTIVE TB	20min	46:47:00	216.5	5008	9162	4792	8900	27.4	SPUTUM	Culture (+)	71	M		
058ma	ACTIVE TB	10min	46:30:00	84.78	7167	7148	7082	11600	5.5	sputum	Smear&cul (+)	49	M		
065co	ACTIVE TB	35min	45:00:00	73.17	2905	4547	2832	8800	16	sputum	Culture (+)	68	M		
066ek	ACTIVE TB	6min	48:17:00	50.3	1822	1410	1771	8500	18.7	sputum	Smear&cul (+)	40	M		
067 Çg	ACTIVE TB	11min	48:30:00	33.5	39889	814.7	39855	6300	33.5	Biopsy	Culture (+)	52	F		
072 Şy	ACTIVE TB	5 min	46:25:00	67.07	1822	101.5	1755	15000	17.9	sputum	Smear&cul (+)	53	M		
073ok	ACTIVE TB	13min	46:20:00	198.7	1848	1252	1649	9000	9.3	sputum	Smear&cul (+)	18	M		
074f Ş	ACTIVE TB	18min	46:15:00	444.4	1080	11077	635.3	10900	15.8	sputum	Smear&cul (+)	53	M		
075mg	ACTIVE TB	12min	46:10:00	186.5	666.6	2812	480.1	15500	7.4	sputum	Smear &cul (+)	46	M		
076ko	ACTIVE TB	17min	46:05:00	271.5	15075	4735	14804	8400	22.7	sputum	Smear &cul (+)	67	M		
077f Ş	ACTIVE TB	4min	49:44:00	296.2	1133	1570	836.5	8900	26.2	SPUTUM	Culture (+)	20	F		
085am	ACTIVE TB	12min	47:35:00	144.07	1190	4815	1045	7800	16.2	sputum	Culture (+)	58	M		
086dk	ACTIVE TB	12min	47:30:00	488.8	18187	2249	17698	5300	42.9	Biopsy	Culture (+) P/XP	67	F		

Appendix(3)

	Study Group	Time btw BC and analysis	Incubation period	N 2d	Ag 2d	M 2d	AG-NIL	WBCs	% Lymph	Source	Diagnosis	Age	Gender	Immigrant	Country of birth
087ha	ACTIVE TB	17min	47:25:00	23.8	6477	16960	6453	6400	29.7	sputum	Culture (+)	74	M		
088kc	ACTIVE TB	9min	47:20:00	53.6	4840	10000	4786	9700	18.8	sputum	Smear&cul (+)	54	M		
089oz	ACTIVE TB	3min	47:35:00	1059.2	2933	8506	1874	10100	24.4	sputum	Culture (+)	39	M		
091ha	ACTIVE TB	7min	46:35:00	17.8	235.1	10720	217.3	11500	20.6	sputum	Smear(+)cul(-)	66	F		
092mo	ACTIVE TB	3min	45:34:00	47.6	10693	7813	10645	8500	16.1	sputum	Culture (+)	63	M		
093oa	ACTIVE TB	184min	48:59:00	63.8	977.7	162.3	913.9	9500	15.4	sputum	Smear &cul(+)	38	f		
094ka	ACTIVE TB	3min	47:43:00	29.7	3837	4302	3808	8800	11.4	sputum	Smear &cul(+)	25	F		
095yg	ACTIVE TB	2min	47:28:00	35.7	760.4	12213	724.7	9700	39.4	sputum	Culture (+)	56	M		
096ak	ACTIVE TB	30min	47-48 hours	125.8	10800	7839	10674	16580	10.7	sputum	Smear &cul(+)	58	M		
097ak	ACTIVE TB	30min	47-48 hours	198.7	14507	13307	14308	8260	32.6	sputum	Smear& cul(-)	41	M		
098as	ACTIVE TB	30min	47-48 hours	398.3	4052	22001	3654	11020	11.3	sputum	Smear & cul(+)	37	M		
099mt	ACTIVE TB	30min	47-48 hours	3161.2	22001	22001	18840	6730	33.7	sputum	Smear &cul(-)	49	M		
100ad	ACTIVE TB	52min	48:00:00	271.5	9650	22001	9379	11510	19.8	sputum	Smear &cul (+)	46	M		
102Nb	ACTIVE TB	13min	47:45:00	0	2654	8977	2654			sputum	Smear &cul (+)	30	F		
104ag	ACTIVE TB	12min	47:36:00	35.7	12837	20222	12802	10800	19.4	sputum	Smear &cul (+)	38	F		
105nk	ACTIVE TB	12min	47:33:00	787.6	7735	15221	6948	5400	20.6	sputum	Smear &cul (+)	50	F		
106Hg	ACTIVE TB	19min	47:28:00	426.7	18101	20007	17675	7000	29.7	sputum	Smear &cul (+)	80	F		
107ad	ACTIVE TB	20min	47:23:00	1098.9	3847	11875	2748	8000	26.2	sputum	Smear &cul (+)	20	M		
108ny	ACTIVE TB	219min	48-49	1450.2	1434	13656	-16.3	8500	31.5	sputum	PCR& cul(+)	69	F		
109hn	ACTIVE TB	17min	47:10:00	235.1	6242	4450	6007	7100	31.1	sputum	Culture (+)	24	M	Yes, in 2012	SYRIA
124fa	ACTIVE TB	2min	47:40:00	84.9	235.1	9039	150.2	5200	26.1	sputum	Smear &cul (+)	26	F		
125c Ş	ACTIVE TB	2min	46:40:00	67.	235.2	11077	168.1	8500	17.8	sputum	Culture (+)	21	M	Yes, in 2012	SYRIA

Appendix(4)

	Study Group	Time btw BC and analysis	Incubation period	N 2d	Ag 2d	M 2d	AG-NIL	WBCs	% Lymph	Source	Diagnosis	Age	Gender	Immigrant	Country of birth
141n Ç	ACTIVE TB	12min	50:00:00	35.7	1004	4256	968.6	9600	18.1	sputum	Smear &cul (+)	56	M		
142hk	ACTIVE TB	12min	49:55:00	1800	3710	11565	1910	4300	27	Biopsy Xpulm.	Smear &cul (+)	78	F		
143mi	ACTIVE TB	2min	49:42:00	89.4	3769	3749	3679	6000	22.5	SPUTUM	Culture (+)	41	M	Yes in 2016	SYRIA
153ay	ACTIVE TB	19min	46:24:00	189.6	4152	31163	3962	9400	25.8	sputum	Smear(+)& cul(+)	54	M		
154rs	ACTIVE TB	30min	48-49	162.2	1027	21643	864.3	7700	12.7	sputum	Smear(+)& cul(+)	36	M		
155sh	ACTIVE TB	2MIN	48:00:00	369.3	401.9	16964	32.6	9300	28.5	sputum	Smear &cul (+)	18	M	Yes, in 2016	PAKISTAN
156sr	ACTIVE TB	7MIN	47:50:00	488.8	423.6	5859	-65.2	12400	9.6	sputum	Smear &cul (+)	27	M	Yes, in 2016	PAKISTAN
157at	ACTIVE TB	10MIN	46:47:00	180.5	803.9	4954	623.4	6800	35	sputum	Culture (+)	63	M		
164es	ACTIVE TB			640.9	14510	57850	13869	8870	20.9	sputum	Smear &cul (+)	24	M		
167hb	ACTIVE TB	2MIN	47:25:00	186.5	554	8820	367.5	16600	7	sputum	Smear &cul (+)	23	M	Yes, in 2015	SYRIA
169tb	ACTIVE TB	237min	49:06:00	63.8	2640	651.8	2576	8700	20.9	sputum	Smear &cul (+)	23	m		
171ea	ACTIVE TB	2MIN	46:50:00	380.2	3226	4693	2846	5900	34.2	sputum	Smear &cul (+)	24	F		
172mm	ACTIVE TB	4MIN	46:45:00	380.2	10778	10021	10398	6900	21.6	BIOBSY	Smear(+)cul(-)	30	M	Yes, in 2014	ÖZBEKISTAN
179rb	ACTIVE TB	3h40min	49:32:00	488.8	3055	3495	2566	6100	17.1	sputum	Smear &cul (+)	29	F		
180bk	ACTIVE TB	03:40	49:33:00	107.6	13722	12418	13614	7400	36	sputum	Smear &cul (+)	38	M		
181 Şk	ACTIVE TB	03:39	49:30:00	125.8	651.8	1157	526	15100	12	sputum	Smear &cul (+)	58	M		
182kd	ACTIVE TB	03:43	49:27:00	488.8	380.2	1923	-108.6	10400	12.1	sputum	Smear &cul (+)	28	M	Yes, in 2017	PAKISTAN
183ak	ACTIVE TB	01:28	49:29:00	0	760.5	2835	760.5	6200	34	sputum	Smear &cul (+)	57	f		

Appendix(5)

	Study group	Date of BC	Time btw BC and analysis	Incubation period	Nil d2	Ag d2	M d2	AG-NIL	WBC	Lymph %	Diagnosis	Age	Gender	immigrant	country of birth
012 MÖ	NON TB	24.01 .2018	30MIN	49:07:00	162.2	271.6	3263.1	109.2	6400	15.4	Pneumonia	86	M		
013ia	NON TB	24.01 .2018	32MIN	49:03:00	1299.2	1013	14760.6	-286.2	13400	7.8	COPD	59	M		
015hk	NON TB	24.01 .2018	30MIN	48:58:00	33.5	0	946.8	-33.5	12300	24	COPD	81	M		
016gÜ	NON TB	24.01 .2018	14MIN	48:28:00	396.9	235.2	26608.3	-161.7	11900	15.8	Pneumonia	53	F		
017sb	NON TB	24.01 .2018	15MIN	48:23:00	33.5	78.2	13560.1	44.7	8900	19.6		69	F		
024zg	NON TB	29.01 .2018	40MIN	48:46:00	73.1	198.7	2942.3	125.5	15400	5.1	Pneumonia	75	F		
042yg	NON TB	06.02 .2018	25MIN	48:55:00	32.5	98.5	6742.3	66	6800	12.3	COPD	86	M		
044ed	NON TB	06.02 .2018	35MIN	48:36:00	0	16.2	1076.3	16.2	6000	7.9	Plm Emboli	88	F		
046hÜ	NON TB	06.02 .2018	35 MIN	48:28:00	56.9	98.5	6557.8	41.6	15900	9.5	Plm Emboli	80	F		
047nm	NON TB	06.02 .2018	27 MIN	48:26:00	24.3	65	6281.1	40.6	9300	30	Plm Emboli	65	F		
056km	NON TB	12.02.2018	46MIN	49:20:00	32.5	24.3	1398.1	-8.1	15800	3.1	Pneumoniae	87	F		
060ib	NON TB	19.02.2018	50MIN	45:25:00	101.5	223	1636.1	121.5	8100	15.7	Pneumoniae	92	M		
061ck	NON TB	19.02.2018	55MIN	45:23:00	11.1	16.7	8064.6	5.6	8600	9.6	Pneumoniae	69	M		
062hy	NON TB	19.02.2018	70 MIN	45:16:00	5.5	22.3	1768.6	16.7	10900	7.9	Pneumoniae	75	M		
068ka	NON TB	23.02.2018	95MIN	47:30:00	2648.8	2272	4325.9	-376.8	6700	19.1	Bronchiectasis	80	F		
069hy	NON TB	23.02.2018	60MIN	47:15:00	16.7	27.9	4612.3	11.2	13400	2.6	Pneumoniae	79	F		
070ab	NON TB	23.02.2018	60MIN	47:13:00	11.1	27.9	16848.8	16.7	13600	17.9	COPD	72	F		
080ik	NON TB	07.03.2018	58MIN	47:30:00	44.7	16.7	2311.8	-28	15000	7	Pleuresia	60	M		
101po	NON TB	30.03.2018	<1hou	47-48 hours	1319.9	1874	3921.6	554	4790	7.9	Sarcoidosis	39	F		
103ng	NON TB	03.04 . 2018	17MIN	47:40:00	1534.7	1497	11609.6	-37.8			Cirrhosis	81	F		
111dk	NON TB	04.04. 2018	32MIN	48:03:00	271.5	80.4	4033.7	-191.1			Type 2 dyspnea	52	F		
114ns	NON TB	06.04.2018	26MIN	52:44:00	17.8	0	13441.6	-17.8			Sarcoidosis	72	F		

Appendix(6)

	Study group	Date of BC	Time btw BC and analysis	Incubation period	Nil d2	Ag d2	M d2	AG-NIL	WBC	Lymph %	Diagnosis	Age	Gender	immigrant	country of birth
116eb	NON TB	06.04.2018	78MIN	51:15:00	80.4	71.5	18946.5	-8.9			Sarcoidosis	61	M		
117ze	NON TB	06.04.2018	50MIN	51:10:00	80.4	174.4	3733.8	94			Sarcoidosis	53	F		
119ta	NON TB	09.04.2018	27MIN	49:07:00	2894.1	1141	11159.1	-1753.5			Sleep apnea	47	F		
121sk	NON TB	09.04.2018	20MIN	49:58:00	198.7	488.9	33137.7	290.1			Sleep apnea	51	M		
122 öd	NON TB	09.04.2018	24MIN	48:35:00	6432.9	4664	25165.6	-1769.4			Sleep apnea	63	M		
126ma	NON TB	10.04.2018	8MIN	49:15:00	1425.8	651.8	27052.1	-774.3			Sleep apnea	37	M		
129nd	NON TB	10.04.2018	18MIN	49:00:00	787.6	186.5	52611.6	-601.1			Sleep apnea	30	M		
130ng	NON TB	11.04.2018	3MIN	49:03:00	62.6	62.6	3793.8	0			Sleep apnea	36	F		
131uk	NON TB	11.04.2018	15MIN	48:57:00	53.6	89.4	13508.8	35.8			Sleep apnea	36	M		
132ia	NON TB	11.04.2018	26MIN	48:50:00	1711	651.8	9480.8	-1059.2			Sleep apnea	36	M		
133hş	NON TB	11.04.2018	25MIN	48:45:00	1914.7	570.3	38979.9	-1344.3			Sleep apnea	33	M		
135fd	NON TB	16.04.2018	11MIN	49:55:00	5.9	0	2379.1	-5.9			Sleep apnea	37	M		
136 öu	NON TB	16.04.2018	20MIN	47:00:00	144	65.58	12239.4	-78.4			Sleep apnea	37	M		
137em	NON TB	18.04.2018	13MIN	50:40:00	564.9	216.9	7670.1	-348			Sleep apnea	38	M		
138mk	NON TB	18.04.2018	18MIN	50:30:00	1401.4	749.5	18080.8	-651.9			Sleep apnea	33	M		
140it	NON TB	18.04.2018	7MIN	50:18:00	282.4	262.4	16081.7	-20			Sleep apnea	54	M		
145ey	NON TB	18.04.2018	22MIN	49:25:00	35.7	107.6	5639.9	71.9			Sleep apnea	31	M		
146da	NON TB	18.04.2018	17MIN	49:21:00	282.4	59.6	4676.8	-222.8			Sleep apnea	34	F		
148bs	NON TB	19.04.2018	65MIN	47:17:00	0	53.6	32934.8	53.6			Sleep Apnea	73	M		
149ak	NON TB	19.04.2018	65MIN	47:14:00	125.8	564.9	4253.7	439.1	10600	11.8	COPD	74	M		
150ss	NON TB	19.04.2018	65MIN	47:25:00	216.9	423.6	1477.4	206.7	10100	18.5	COPD	62	F		
151fk	NON TB	19.04.2018	79MIN	47:08:00	0	0	1738.1	0	8100	27.3	Pneumonia	74	F	Yes, in 1957	Macedonia

Appendix(7)

	Study group	Date of BC	Time btw BC and analysis	Incubation period	Nil d2	Ag d2	M d2	AG-NIL	WBC	Lymph %	Diagnosis	Age	Gender	immigrant	country of birth
152v Ş	NON TB	19.04.2018	MIN78	47:02:00	11.9	0	1998.9	-11.9	9300	14.3	COPD	79	F		
159gi	NON TB	27.04.2018	38MIN	48:27:00	0	0	4107.7	0					F		
160n ö	NON TB	27.04.2018	40 MIN	48:22:00	0	423.6	2357.4	423.6				44	F		
161zd	NON TB	27.04.2018	22MIN	46:40:00	83.4	134.9	4603.9	51.5	9400	7.6	Pulmonary Hypertension	30	F		
162 şa	NON TB	27.04.2018	19MIN	46:45:00	57.4	174.4	1010.3	116.9	4700	35.4	Pulmonary Hypertension	79	F		
163t ö	NON TB	27.04.2018	20MIN	48:27:00	89.4	89.4	749.5	0	7200	16.1	Pulmonary alveolar protienosis	37	F		
165gt	NON TB	4.05.2018	22MIN		162.2	38.3	16546	-123.9				61	F		
173zk	NON TB	9.05 2018	9MIN	49:00:00	0	31.9	13414	31.9	9400	27.6	Hypertension	41	F		
174ah	NON TB	9.05 2018	12MIN	46:56:00	2933.1	1075	12709.3	-1857.6	5600	29.3	Hypertension	53	M		
175oy	NON TB	11.05.2018		48:42:00	6.3	57.4	5996.4	51.1			Sarcoidosis	41	M		
176hu	NON TB	15.05.2018	12MIN		69.5	49.6	2588	-19.8	7500	15	vasculitis	53	F		
177mb	NON TB	15.05.2018	21MIN		89.4	29.8	2624	-59.6	8800	31.7	pulmonary Hypertension	44	F		

ETHICAL COMMITTEE DECISION

T.C.
İSTANBUL ÜNİVERSİTESİ
İSTANBUL TIP FAKÜLTESİ
KLİNİK ARAŞTIRMALAR ETİK KURULU

Sayı : 851 **Tarih : 11.07.2017**
Konu: Doç. Dr. Orhan Kaya KÖKSALAN hk.

Sayın Doç. Dr. Orhan Kaya KÖKSALAN
Aziz Sancar Deneysel Tıp Araştırma Enstitüsü

İlgi : Aziz Sancar Deneysel Tıp Araştırma Enstitüsünün 30/05/2017 gün ve 201437 sayılı yazısı

Sorumlu araştırıcılığını üstlendiğiniz ve Dr. Asma SALEM' in yürüteceği 2017/740 dosya numaralı "IP10 Stimulanı Olarak Phytohemagglutinin, Concavalin A ve Poly I:C maddelerinin İmmunokompetan ve İmmunodefektif Hastalarda Karşılaştırılması" başlıklı çalışma kurulumuzun 23/06/2017 gün ve 12 sayılı toplantısında görüşülerek etik yönden uygun bulunmuş olup, tutanaklar ekte sunulmuştur.

Bilgilerinizi rica ederim.

Prof. Dr. A.Yağız ÜRESİN
İstanbul Tıp Fakültesi Klinik Araştırmalar
Etik Kurul Başkanı

Eki: İstanbul Tıp Fakültesi Klinik Araştırmaları Etik Kurulu Karar Formu

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FIRST PAGE OF PLAGIARISM REPORT

EVALUATION OF THE PERFORMANCE OF IP-10 ASSAY FOR THE DIAGNOSIS OF LATENT TUBERCULOSIS INFECTION

ORIJINALLIK RAPORU

%6	%3	%5	%1
BENZERLIK ENDEKSI	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCI ÖDEVLERI

BIRINCIL KAYNAKLAR

1	www.nature.com İnternet Kaynağı	<%1
2	Morten Ruhwald. "IP-10 release assays in the diagnosis of tuberculosis infection: current status and future directions", Expert Review of Molecular Diagnostics, 03/2012 Yayın	<%1
3	ddd.uab.cat İnternet Kaynağı	<%1
4	Ahmet Soysal. "T-SPOT.TB assay usage in adults and children", Expert Review of Molecular Diagnostics, 07/2011 Yayın	<%1
5	"Mycobacterial Skin Infections", Springer Nature, 2017 Yayın	<%1
6	www.tdx.cat İnternet Kaynağı	<%1

ÖZGEÇMİŞ

Kişisel Bilgiler

Adı	ASMA	**Soyadı**	SALEM
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Lise	ALMAJD SCHOOL	2001

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	Görevi	**Kurum**	**Süre (Yıl - Yıl)**
1.	general physician	my village hospital	2009-2010
2.	general physician	pediatric department at Benghazi hospital.	2011-2012
3.	Demonstrator (teaching assistant)	family and community medicine department	2012-2014

Yabancı Dilleri	**OkuduğunuAnlama***	**Konuşma***	**Yazma***	**KPDS/ÜDS Puanı**	**(Diğer) Puanı**
English	Good	Moderate	Good		

*****Çok iyi, iyi, orta, zayıf olarak değerlendirin

	Sayısal	**EşitAğırlık**	**Sözel**
LES Puanı			
(Diğer)Puanı			

Bilgisayar Bilgisi

Program	**Kullanmabecerisi**

Yayınları/Tebligleri Sertifikaları/Ödülleri