RESEARCH

Differences of Plasma Interleukin-6 and Tumor Necrosis Factor-A Levels in Healthy People, Rifampicin Resistant and Sensitive Pulmonary Tuberculosis Patients
Wahyu Setiani Wibowo, Jusak Nugraha, Soedarsono ................................................................. 129 - 134

Association between Specific Enolase Serum Levels and Outcome Acute Ischemic Stroke One Month After Onset
Yuri Haiga, Darwin Amir, Yullarni Syafrita .................................................................................. 135 - 139

Analysis of Hemoglobin Levels And Leukocyte Count in Neonates with Hyperbilirubinemia
Dewi Suharti, Sulina Yanti Wiraba, Muthmainnah ....................................................................... 140 - 144

Diagnostic Value of Ca-125 in Patients with Epithelial Ovarian Cancer at the Dr. Soetomo General Hospital Surabaya in 2016
Kintan P. R. Kania, Betty A. Tambunan, Willy Sandhika ................................................................. 145 - 149

Analysis of Vitamin D in Patients with Type 2 Diabetes Mellitus
Arfandhy Sanda, Uleng Bahrun, Ruland DN. Pakasi, Andi Makbul Aman ........................................ 150 - 154

Proportion of Rhesus Blood Phenotypes at the Blood Donor Unit in Bandung City
Ivana Dewi, Nadjwa Zamalek Dalimoenthe, Anna Tjandrawati, Nida Suraya ................................. 155 - 160

Correlation of Total Lymphocyte Count with CD4 Count in HIV/TB Coinfected Patients
Herniaty Rampo, Uleng Bahrun, Mansyur Arif ............................................................................. 161 - 164

Using Six Sigma to Evaluate Analytical Performance of Hematology Analyzer
Robiul Fuadi .................................................................................................................................. 165 - 169

Correlation of AA Index with Degree of Liver Fibrosis in Chronic Hepatitis B Patients
Rika Andriany, Ibrahim Abdul Samad, Mansyur Arif ................................................................... 170 - 173

Difference in HbA1c Level between Boronate Affinity and Ion Exchange-High Performance Liquid Chromatography Method in Diabetic Patient
Tuti Asryani, Ellyza Nasrul, Rikarni, Tutty Prihandani ................................................................... 174 - 179

Diagnostic Value of Neutrophil Lymphocyte Ratio to Differentiate Ischemic and Hemorrhagic Stroke
Martina Rentauli Sihombing, Liong Boy Kurniawan, Darwati Muhadi ........................................... 180 - 183

D-Dimer and Fibrinogen in Patients Underwent Surgery in Malignant and Benign Ovarian Tumor
Ismail Aswin, Herman Hariman, Fauzie Sahil .................................................................................. 184 - 190
Relationship between Specific Gravity of Cupric Sulfate and Saturation of Blood Droplets During Donor's Hemoglobin Screening

Resna Hermawati, Solichul Hadi .......................................................................................................................... 191 - 193

Vancomycin-Resistant Staphylococcus aureus at the Dr. Wahidin Sudirohusodo Hospital Makassar

Fatmawaty Ahmad, Nurhayana Sennang, Benny Rusli .......................................................................................... 194 - 198

The Levels of Interleucin-6 (IL-6) and Tumor Necrosis Factor Alpha (TNF-ALFA) in Preeclampsia Patient and Normal Pregnancy

Mawardi, Ratna Akbari Ganie, Sarma N. Lumbanraja ............................................................................................. 199 - 201

Analysis of Platelet Volume Mean, Platelet Distribution Width, and Platelet Count in Hemorrhagic and Non-Hemorrhagic Stroke

Gita Medita Sunusi, Darwati Muhadi, Mansyur Arif ............................................................................................... 202 - 206

High Fluorescent Lymphocyte Count Examination in Dengue Hemorrhagic Patients with Sysmex Xn-1000 Hematology Analyzer

Budiono Raharjo, Solichul Hadi ............................................................................................................................ 207 - 210

Prevalence and Characteristics of Multidrug-Resistant Acinetobacter baumannii Cases at the Dr. Wahidin Sudirohusodo General Hospital in Makassar

Dewi Kartika Tungadi, Nurhayana Sennang, Benny Rusli ........................................................................................... 211 - 217

The Correlation of Anemia and Hepcidin Serum Levels in Regular Hemodialysis Patients with Chronic Hepatitis C

Wingsar Indrawanto, Adi Koesoema Aman, Alwi Thamrin ................................................................................... 218 - 223

The Comparison between HbA1c and Glycated Albumin Level Patient with Type II Diabetes Mellitus with or without CKD

M. Rusli, Zulfikar, Santi Syafril ............................................................................................................................... 224 - 227

Differentiation of Tgd Lymphocyte Cells Expressing Interleukin-17 on Healthy Persons and Adult Acute Myeloid Leukemia Patients

Elvan Dwi Widyadi, Yetti Hernaningsih, Endang Retnowati, Ugroseno, Ryzky Widi Atmaja ........................................ 228 - 232

LITERATURE REVIEW

Hormone Examination in Menopause

Ferdy Royland Marpaung, Trieva Verawaty Butarbutar, Sidarti Soehita ........................................................................ 233 - 239

CASE REPORT

Chronic Myelogeneous Leukemia Transformation into Acute Lymphoblastic Leukemia

Endah Indriastuti, Arifoel Hajat .................................................................................................................................. 240 - 245

Rapid Progression of Clavicular Solitary Plasmacytoma to Multiple Myeloma

Hantoro Gunawan, Paulus Budiono Notopuro ........................................................................................................ 246 - 249
DIFFERENCES OF PLASMA INTERLEUKIN-6 AND TUMOR NECROSIS FACTOR-α LEVELS IN HEALTHY PEOPLE, RIFAMPICIN RESISTANT AND SENSITIVE PULMONARY TUBERCULOSIS PATIENTS

Wahyu Setiani Wibowo1, Jusak Nugraha1, Soedarsono2

1 Department of Clinical Pathology, Faculty of Medicine, Airlangga University/Dr. Soetomo Hospital Surabaya, Indonesia. E-mail: 
2 Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Airlangga University/Dr. Soetomo Hospital Surabaya,

ABSTRACT

Increased tuberculosis in the world is caused by increased HIV-infected and antituberculous drugs (rifampicin) resistant individuals. IL-6 and TNF-α play an essential role in explaining the different degrees of inflammation in Rifampicin Resistant (RR) and Rifampicin Sensitive (RS) pulmonary tuberculosis patients, and healthy people. The research aimed to analyze the differences in plasma IL-6 and TNF-α levels in healthy people, Rifampicin Resistant (RR), and Rifampicin Sensitive (RS) pulmonary tuberculosis patients. A cross-sectional study was conducted from July-September 2017. Thirty-nine subjects were classified into RR pulmonary tuberculosis (n=15), RS pulmonary tuberculosis (n=12) based on GeneXpert examination and treated by antituberculous drugs for ≤1 month, and healthy people (n=12) based on AFB results, Thorax X-ray, and tuberculin tests. IL-6 and TNF-α were done in all subjects using ELISA U-CyTech® (Biosciences, Inc.). ANOVA analyzed differences of IL-6 and TNF-α levels between groups. The mean IL-6 levels (pg/mL) in RR and RS pulmonary tuberculosis patients, and healthy people were 54.56±59.13, 27.05±37.04, 4.42±2.83, respectively. The mean TNF-α levels (pg/mL) in RR and RS pulmonary tuberculosis patients, and healthy people were 263.54±327.58, 250.25±314.20, 9.04±5.89, respectively. The mean differences between IL-6 and TNF-α levels (pg/mL) between RR pulmonary tuberculosis patients and healthy people were 50.14±15.29 (p<0.05) and 254.59±8460 (p<0.05). Significant differences of mean IL-6 and TNF-α levels were found between RR pulmonary tuberculosis patients and healthy people.

Key words: Pulmonary tuberculosis, rifampicin resistant, rifampicin sensitive, healthy people, interleukin-6, tumor necrosis factor-α

INTRODUCTION

Tuberculosis is a disease that has long been known and is still a health problem of people in the world.1,2 Mycobacterium tuberculosis as a bacterium that causes tuberculosis has been discovered by Robert Koch in 1882.3 The spread of tuberculosis infection occurs through droplet nuclei with the airway as the port of entry of Mycobacterium tuberculosis (95%).4 Increased tuberculosis infection is caused by increased HIV-infected and anti tuberculous drugs (rifampicin) resistant individuals.5

The incidence rate of pulmonary tuberculosis in the world in 2015 was 580,000 or 7.9 people per 100,000 population. New cases of RR tuberculosis in the world in 2015 were 3.9%, and cases of RR tuberculosis with previous anti tuberculous drugs in the world in 2015 were 21%. The incidence rate of pulmonary tuberculosis in Indonesia in 2015 was 32,000 or 12 people per 100,000 population. New cases of RR tuberculosis in Indonesia in 2015 were 2.8%, and cases of RR tuberculosis with previous anti tuberculous drugs in Indonesia in 2015 were 16%.6 As many as 12.5% of RS pulmonary tuberculosis patients became RR pulmonary tuberculosis patients in TB DOTS/MDR Wards, Department of Pulmonology and Respiratory Medicine, Dr. Soetomo Hospital Surabaya in October 2016-July 2017. This is likely influenced by IL-6 and TNF-α. Besides tuberculosis, other infectious diseases, trauma, diseases which cause inflammation such as diabetes mellitus, liver disease, kidney disease, and others can influence the levels of IL-6, and TNF-α.

The role of IL-6 and TNF-α in the pathogenesis of Mycobacterium tuberculosis resistance to rifampicin remains unclear. IL-6 and TNF-α may be factors that increase the occurrence of pathological processes in RR pulmonary tuberculosis.7 IL-6 dan TNF-α in RR and RS pulmonary tuberculosis have an essential role because it can explain the pattern of different inflammatory reactions.7

Ladel et al. suggested that IL-6 has a protective role against tuberculosis. In-vitro, CD4 T cells from M. tuberculosis-infected mice produce high levels of...
IL-6 during the early immune response, and IL-6 induces mycobacterial growth inhibition in macrophages. Consistent with the functional effects of IL-6 in murine tuberculosis, analyses of human pulmonary tuberculosis and levels of IL-6 in human plasma point to a role of IL-6 in infection with M. tuberculosis. However, other studies showed that IL-6 was involved in the inability of the cellular immune response to eradicate tuberculosis.²

TNF-α was needed to control tuberculosis. TNF-α was also involved in the host pathological response to tuberculosis and often cited as a significant factor in lung tissue damage.⁹ Increased TNF-α was positively correlated with clinical deterioration in tuberculosis patients.¹⁰

Ieremenchuk et al. and Correia et al. conducted researches on plasma IL-6 levels in RR and RS pulmonary tuberculosis and obtained increased IL-6 levels in RR pulmonary tuberculosis higher than RS pulmonary tuberculosis.⁷,⁸ Eum et al. obtained TNF-α level in PBMC or whole blood of RS pulmonary tuberculosis higher than RR pulmonary tuberculosis.¹¹

Research on plasma IL-6 and TNF-α levels in RR and RS pulmonary tuberculosis patients has not been done in Indonesia and also in the Dr. Soetomo Hospital Surabaya at this time. From this idea, this research should be done to know and compare plasma IL-6 and TNF-α levels in pulmonary tuberculosis patients with different sensitivity to rifampicin.

METHODS

The study was conducted in July-September 2017 using a cross-sectional design, and samples were taken consecutively. Subjects consisted of RR and RS pulmonary tuberculosis patients (anti tuberculosis drugs ≤ one month) in the TB DOTS/MDR Wards, Department of Pulmonology and Respiratory Medicine, Dr. Soetomo Hospital Surabaya, and healthy people. The GeneXpert examination was performed in RR and RS pulmonary tuberculosis patients. Tuberculin tests were performed in healthy people. AFB results and thorax X-ray were performed in all subjects.

Rifampicin resistant pulmonary tuberculosis patients were determined based on the clinical examination and GeneXpert examination which showed positive DNA TB and resistant to rifampicin. Rifampicin sensitive pulmonary tuberculosis patients were identified based on the clinical study and GeneXpert examination which showed positive DNA TB and sensitive to rifampicin. Rifampicin resistant pulmonary tuberculosis patients and RS pulmonary tuberculosis patients infected with HIV, with autoimmune disease (lupus), trauma, sepsis, liver disease, kidney disease, hepatitis B, hepatitis C, diabetes mellitus, and was receiving immunosuppressant therapy or corticosteroids were excluded.

Healthy people were determined based on the normal results of thorax X-ray, negative AFB result, and tuberculin test <10 mm. Healthy people who had been diagnosed as pulmonary tuberculosis, with a history of liver disease, kidney disease, diabetes mellitus, HIV, autoimmune disease (lupus), were suffering from a respiratory infection or other infections, and was receiving immunosuppressant therapy or corticosteroids were excluded.

Laboratory examinations were performed in the Clinical Pathology Laboratory, Dr. Soetomo Hospital Surabaya. Plasma IL-6 and TNF-α levels were performed using Enzyme-Linked Immunosorbent Assay (ELISA) U-CyTech® (Biosciences, Inc.).

Statistical analysis was performed using SPSS ver 16.0. Differences of IL-6 and TNF-α levels between groups were analyzed by Anova if the data of each group was normally distributed or analyzed by Kruskal Wallis if the data of each group was not normally distributed. P-value <0.05 was considered as statistically significant, with a 95% confidence interval.

RESULTS AND DISCUSSION

The results of this study revealed that 27 subjects were diagnosed as pulmonary tuberculosis. The GeneXpert examination which positive DNA TB and resistant to rifampicin were detected in 15 subjects, while GeneXpert examination which positive DNA TB and sensitive to rifampicin were detected in 12 subjects. Healthy people who were willing to participate in this research and meet the inclusion criteria obtained by 12 subjects.

Based on the median age of patients, RR and RS pulmonary tuberculosis patients in this research tend to occur in productive age. The majority of subjects in RS pulmonary tuberculosis in this research were males. The results of this research were similar to Correia’s research in Brazil.⁷ The ratio of male to female in RR pulmonary tuberculosis patients in this research was almost the same. The results of this research were similar to the study of the incidence of RR pulmonary tuberculosis at the Bahtera Mas Regional Hospital of Southeast Sulawesi Province in 2016 and Correia’s research. Characteristics of subjects could be seen in Table 1.
The highest level of IL-6 in RR pulmonary tuberculosis patients was 145.53 pg/mL, while the lowest level of IL-6 in RR pulmonary tuberculosis patients was 0 pg/mL. The highest level of IL-6 in RS pulmonary tuberculosis patients was 102.01 pg/mL, while the lowest level of IL-6 in RS pulmonary tuberculosis patients was 0 pg/ml. Healthy people have IL-6 levels ranging from 0-7.67 pg/mL. The distribution of IL-6 in three groups could be seen in Figure 1.

Means, standard deviations, and p-values of IL-6 in three groups can be seen in Table 2.

Mean IL-6 levels between RR pulmonary tuberculosis patients, RS pulmonary tuberculosis patients, and healthy people showed a significant difference (p < 0.05). Mean IL-6 of RR pulmonary tuberculosis patients was higher than mean IL-6 of RS pulmonary tuberculosis patients and healthy people. Mean IL-6 of RS pulmonary tuberculosis patients was higher than mean IL-6 of healthy people. This finding was due to the increased inflammatory response in RR pulmonary tuberculosis patients from RS pulmonary tuberculosis patients and healthy people, and increased inflammatory response in RS pulmonary tuberculosis patients from healthy people. The results of this research were consistent with Correia’s research and Ieremenchuk’s research.

The process of phagocytosis rifampicin sensitive Mycobacterium tuberculosis by macrophages results in rifampicin sensitive Mycobacterium tuberculosis destroyed in the phagosome of macrophages. This process results in increased differentiation of M2 macrophages and decreased M1 macrophages. Rifampicin resistant Mycobacterium tuberculosis is phagocytosed by macrophages and is not destroyed in macrophage phagosomes resulting in increased differentiation of M1 macrophages and decreased M2 macrophages. Increased M1 macrophages produce higher levels of IL-6.

Post Hoc analysis results showed that statistically significant differences were obtained in RR pulmonary tuberculosis patients with healthy people (p < 0.05), while RS pulmonary tuberculosis patients with healthy people and RR pulmonary tuberculosis patients with RS pulmonary tuberculosis patients showed no statistically significant differences. The mean differences of IL-6 between groups could be seen in Table 3.
Table 3. The mean differences of IL-6 (pg/mL) between RR pulmonary tuberculosis, RS pulmonary tuberculosis, and healthy people

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of samples</th>
<th>Mean differences</th>
<th>Standard deviations</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR pulmonary tuberculosis</td>
<td>15</td>
<td>27.51</td>
<td>18.64</td>
<td>0.320</td>
</tr>
<tr>
<td>RS pulmonary tuberculosis</td>
<td>12</td>
<td>50.14</td>
<td>15.29</td>
<td>0.014</td>
</tr>
<tr>
<td>Healthy people</td>
<td>12</td>
<td>22.63</td>
<td>10.72</td>
<td>0.133</td>
</tr>
</tbody>
</table>

Table 4. Means (pg/mL), standard deviations, p-values of TNF-α

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of samples</th>
<th>Means</th>
<th>Standard deviations</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR pulmonary tuberculosis</td>
<td>15</td>
<td>263.54</td>
<td>327.58</td>
<td></td>
</tr>
<tr>
<td>RS pulmonary tuberculosis</td>
<td>12</td>
<td>250.25</td>
<td>314.20</td>
<td>0.038</td>
</tr>
<tr>
<td>Healthy people</td>
<td>12</td>
<td>9.04</td>
<td>5.89</td>
<td></td>
</tr>
</tbody>
</table>

The mean differences of IL-6 that not significant between RS pulmonary tuberculosis patients and healthy people in this research were probably caused by *Mycobacterium tuberculosis* destroyed in RS pulmonary tuberculosis patients, so that the inflammatory response decreased. The mean differences of IL-6 that not significant between RR pulmonary tuberculosis patients and RS pulmonary tuberculosis patients were probably caused by data variations were too vast. Other intervening variables that could not be controlled or measured were probably as the cause of wide data variations.

The role of IL-6 in RR and RS pulmonary tuberculosis patients is crucial because it will glue information about the different degrees of inflammation. IL-6 is a proinflammatory cytokine group that initiates an inflammatory response. Ieremenchuk stated that increased IL-6 showed a high inflammatory response activity and was shown maximally in RR pulmonary tuberculosis patients compared with RS pulmonary tuberculosis patients. This inflammatory response was critical in detaining *Mycobacterium tuberculosis*.

The highest level of TNF-α in RR pulmonary tuberculosis patients was 882.53 pg/mL, while the lowest level of TNF-α in RR pulmonary tuberculosis patients was 0 pg/mL. The highest level of TNF-α in RS pulmonary tuberculosis patients was 835.58 pg/mL, while the lowest level of TNF-α in RS pulmonary tuberculosis patients was 0 pg/mL. Healthy people have TNF-α levels ranging from 0-17.66 pg/mL. The distribution of TNF-α in three groups could be seen in Figure 2.

Figure 2. The distribution of TNF-α (pg/mL)

Means, standard deviations, and p-values of TNF-α in three groups could be seen in Table 4.

Mean TNF-α levels between RR pulmonary tuberculosis patients, RS pulmonary tuberculosis patients, and healthy people showed a significant difference (p <0.05). Mean TNF-α of RR pulmonary tuberculosis patients was higher than mean TNF-α of RS pulmonary tuberculosis patients and healthy people. Mean TNF-α of RS pulmonary tuberculosis patients was higher than mean TNF-α of healthy people. This results was due to the increased inflammatory response in RR pulmonary tuberculosis patients from RS pulmonary tuberculosis patients and healthy people, and increased inflammatory response in RS pulmonary tuberculosis patients from healthy people.

Eum *et al.* found that TNF-α levels in PBMC or
whole blood of RS pulmonary tuberculosis patients were higher than those in RR pulmonary tuberculosis patients. This was due to decreased production of nitric oxide in RR pulmonary tuberculosis patients that correlated with TNF-α production from monocyte cells. The results of this research were different from the results conducted by Eum. Junior stated that pulmonary tuberculosis patients with high levels of TNF-α showed a poor clinical condition.

The phagocytosis process of rifampicin resistant Mycobacterium tuberculosis by macrophages results in rifampicin resistant Mycobacterium tuberculosis destroyed in the phagosome of macrophages. This process results in increased differentiation of M2 macrophages and decreased M1 macrophages. Rifampicin resistant Mycobacterium tuberculosis is phagocytosed by macrophages and is not destroyed in macrophage phagosomes resulting in increased differentiation of M1 macrophages and decreased M2 macrophages. Increased M1 macrophages result in higher levels of TNF-α.

Post Hoc analysis results showed that statistically significant differences were obtained in RR pulmonary tuberculosis patients with healthy people (p <0.05), while RS pulmonary tuberculosis patients with healthy people and RR pulmonary tuberculosis patients with RS pulmonary tuberculosis patients showed no statistically significant differences. The mean differences of TNF-α between groups can be seen in Table 5.

The statistically significant differences in TNF-α in this research was obtained only in patients with pulmonary tuberculosis RR with healthy people (p <0.05), while the mean differences of TNF-α in RS pulmonary tuberculosis patients with healthy people and RR pulmonary tuberculosis patients with RS pulmonary tuberculosis patients showed no significant difference. The results of this research were similar to the results conducted by Eum.

The mean differences of TNF-α that not significant between RS pulmonary tuberculosis patients and healthy people in this research were probably caused by Mycobacterium tuberculosis destroyed in RS pulmonary tuberculosis patients, so that the inflammatory response decreased. The mean differences of TNF-α that not significant between RR pulmonary tuberculosis patients and RS pulmonary tuberculosis patients were probably caused by data variations were too vast. Other intervening variables that could not be controlled or measured were probably as the cause of wide data variations.

This research has a limitation, namely intervening variables that could not be controlled or measured entirely.

## CONCLUSION AND SUGGESTION

Differences of mean IL-6 and TNF-α levels were found between RR pulmonary tuberculosis, RS pulmonary tuberculosis, and healthy people, but statistically significant differences of mean IL-6 and TNF-α levels were found between RR pulmonary tuberculosis patients and healthy people. Further studies are needed using other better-differentiating markers, regarding the other intervening variables affecting IL-6 and TNF-α levels, as well as the effect of antituberculous drugs on IL-6, TNF-α, and other biomarkers in monitoring the course of tuberculosis cure.

## REFERENCES


### Table 5. The mean differences of IL-6 (pg/mL) between RR pulmonary tuberculosis, RS pulmonary tuberculosis, and healthy people

<table>
<thead>
<tr>
<th>Groups</th>
<th>Groups</th>
<th>Mean differences</th>
<th>Standard deviations</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR pulmonary tuberculosis</td>
<td>RS pulmonary tuberculosis</td>
<td>13.28</td>
<td>124.02</td>
<td>0.994</td>
</tr>
<tr>
<td>RR pulmonary tuberculosis</td>
<td>Healthy people</td>
<td>254.49</td>
<td>84.60</td>
<td>0.024</td>
</tr>
<tr>
<td>RS pulmonary tuberculosis</td>
<td>Healthy people</td>
<td>241.21</td>
<td>90.72</td>
<td>0.054</td>
</tr>
</tbody>
</table>
2006; 102-121.