

The Indonesian BIOMEDICAL JOURNAL



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Jl. Kramat Raya No.150,
Jakarta 10430, Indonesia
Tel.: +62-21-3144182
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E-mail: Secretariat@InaBJ.org
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Secretariat of The Indonesian Biomedical Journal
Prodia Tower 9th Floor
Jl. Kramat Raya No.150, Jakarta 10430, Indonesia
Tel.: +62-21-3144182, ext. 3872
Fax.: +62-21-3144181
WhatsApp No.: +62 877-3616-3117
E-mail: Secretariat@InaBJ.org
Website: www.InaBJ.org

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RESEARCH ARTICLE

High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients

Nuri Dyah Indrasari^{1,2}, Marcellus Simadibrata³, Primariadewi Rustamadji⁴,
Yusra², Aria Kekalih⁵, Suhendro³, Alida Roswita Harahap², Heri Wibowo⁶,
Ida Parwati⁷, Ferry Sandra^{8,*}

¹Doctoral Program in Medical Sciences, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia

²Department of Clinical Pathology, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Central General Hospital, Jl. Diponegoro No.71, Jakarta 10430, Indonesia

³Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Central General Hospital, Jl. Diponegoro No.71, Jakarta 10430, Indonesia

⁴Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Central General Hospital, Jl. Diponegoro No.71, Jakarta 10430, Indonesia

⁵Community Medicine Department, Faculty of Medicine, Universitas Indonesia, Jl. Pegangsaan Timur No.16, Jakarta 10430, Indonesia

⁶Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia

⁷Department of Clinical Pathology, Faculty of Medicine, Universitas Padjadjaran, Dr. Hasan Sadikin General Hospital Bandung, Jl. Pasteur No. 38, Bandung 40132, Indonesia

⁸Department of Biochemistry and Molecular Biology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No. 260, Jakarta 11440, Indonesia

*Corresponding author. Email: ferry@trisakti.ac.id

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Abstract

BACKGROUND: Currently, laboratory diagnosis of intestinal tuberculosis (ITB) is limited based on clinical manifestations, providing opportunities for alternative laboratory tests to diagnose ITB. At the present time, the role of serum adenosine deaminase (ADA) and hematological tests in ITB patients are not widely known. The objective of this study was to determine the role of ADA and hematological tests in patients suspected with ITB.

METHODS: Subjects that were suspected of ITB were classified as ITB group, while subjects with inflammatory bowel disease, hemorrhoid, and intestinal malignancy were classified as non-ITB group. Colonoscopy, histopathological examinations, and hematological test were performed. ADA measurement was also performed with clinical chemistry analyzer based on enzymatic colorimetry principle.

RESULTS: Out of 143 subjects, 16 (11.2%) subjects were diagnosed with ITB and 127 (88.8%) subjects were

classified as non-ITB group. ADA level and erythrocyte sedimentation rate (ESR) of ITB group were significantly higher than the ones of non-ITB group ($p < 0.05$). Cut-off, sensitivity, and specificity of ADA level were 12.56 IU/L, 75%, and 57%, respectively. Cut-off, sensitivity, and specificity of ESR were 32.5 mm/hour, 81%, and 62%, respectively. Colonoscopy of ITB subjects displayed multiple ulcerations, edema, and hyperemic mucosa. Histopathological examination of ITB subjects exhibited granulomatous inflammation, epithelioid cells, giant cells, and lymphocyte aggregates.

CONCLUSION: ADA level and ESR were significantly higher among ITB patients compared with non-ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests could be considered as a screening test for ITB.

KEYWORDS: intestinal tuberculosis, adenosine deaminase, hematological tests

Indones Biomed J. 2023; 15(4): 362-8

Introduction

According to the Global Tuberculosis Report 2019 from the World Health Organization (WHO), Indonesia was ranked third among the countries with highest burden of tuberculosis (TB).(1,2) The global prevalence of extrapulmonary TB was 14% and the prevalence in Indonesia was $\leq 9.9\%$. Intestinal tuberculosis (ITB), as a part of abdominal TB, had a prevalence of approximately 3-16%.(3-5)

Currently, ITB diagnosis was established based on clinical manifestations, Ziehl-Neelsen (ZN) acid fast bacilli (AFB) stain of the stool, fecal culture, colonoscopy, and histopathology.(3,6-10) The limitations of the respective laboratory pose difficulties to diagnose ITB, and hence, providing opportunities for alternative laboratory tests to diagnose ITB.(11)

Adenosine deaminase (ADA) is an enzyme involved in purine metabolism which catalyzes the conversion of adenosine to inosine. ADA is also involved in the proliferation and differentiation of lymphocytes and maturation of monocytes.(11,12) Elevated ADA levels in blood or body fluid during tuberculosis infection is due to the stimulation of T lymphocytes by *Mycobacterium tuberculosis* (MTB) antigen.(12,13) The activity of ADA differs significantly between patients with pulmonary TB and the normal healthy people.(14,15)

In ITB, acute or chronic inflammation is present due to infection process. Hematological tests are often requested by clinicians in patients suspected of having ITB, as several parameters can be utilized as markers of inflammation or infection. Some of them are leukocyte count, leukocyte differential count (basophil, eosinophil, neutrophil, lymphocyte, and monocyte), neutrophil to lymphocyte ratio (NLR), monocyte to lymphocyte ratio (MLR), and erythrocyte sedimentation rate (ESR).(3,6,16,17) At the present time, the role of serum ADA and hematological tests in ITB patients are not widely known. The objective of this study was to determine the role of ADA and hematological tests in patients suspected with ITB.

Methods

Study Design and Subjects

Subjects were recruited at the Gastroenterology Outpatient Clinic, Department of Internal Medicine, Gastrointestinal Endoscopy Center of Dr. Cipto Mangunkusumo Central General Hospital from December 2020 until December

2022, with the inclusion criteria as follows; for the ITB group: ≥ 18 years old and suspected ITB. Meanwhile the exclusion criteria: being treated with anti-tuberculosis drugs for >3 months, post-treatment with anti-tuberculosis drugs for <6 months.

Patients were suspected of ITB if 3 out of 4 main clinical findings were present, and 1 out of 3 additional history criteria for ITB was met. The main clinical findings of ITB include weight loss, non-specific abdominal pain, fever of unknown origin, and chronic diarrhea or constipation. The additional history includes history of pulmonary TB, active pulmonary TB with or without anti-tuberculosis drugs treatment (<3 months of treatment), and positive contact history with TB patients. Patients with inflammatory bowel disease (Crohn's disease or ulcerative colitis), hemorrhoid, and intestinal malignancy were classified as non-ITB group.

Prior to the recruitment, all subjects were explained and asked for their consents of participation by signing an informed consent form. The research protocol was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia and Dr. Cipto Mangunkusumo Central General Hospital (No. KET-1498/UM2.F1/ETIK/PPM.00.02/2020).

Colonoscopy Examination

After being given laxatives, sedatives and analgesics, subjects lied in a supine position for a colonoscope insertion. If abnormalities were found, biopsies were taken for histopathological examination. Various features could be identified during colonoscopy of patient with suspected ITB, such as ulceration, nodules, polyps, narrowing of the lumen, and irregular multiple fibrous bands. ITB may affect extensive parts of the intestine, in the form of continuous lesions or single or skip lesions.(16)

Histopathological Examination

Samples for examination were harvested from at least 5 granulomas originating from a single segment. After tissue fixation, grossing, embedding and slicing, tissue sections were stained with hematoxylin (Eprexia, Kalamazoo, MI, USA) and eosin (Merck, Darmstadt, Germany), and mounted. Prepared tissue slides will be read by an anatomic pathologist.

ADA Test

Nine mL of venous blood was drawn from the cubital vein. Three mL were collected in an EDTA tube for complete hematological test, while 6 mL in non-anticoagulated tube for ADA test. Blood in non-anticoagulated tubes were

allowed to stand at room temperature for about 30 minutes, until blood clot formed. Immediate centrifugation of 3,000 revolutions per minute (rpm) for 15 minutes was performed to separate the serum from the rest of the remaining sample. Quantitative test of blood ADA was performed by BS-200 automated clinical chemistry analyzer (Mindray, Shenzhen, China) with enzymatic colorimetry principle test used. The measurement was determined by the increase of photometric absorbance at a wavelength of 546 nm.

Hematological Test

Hematology test was performed by XN-2000 hematology analyzer (Sysmex, Kobe, Hyogo, Japan) with semiconductor laser flowcytometry for measurement of leukocyte, basophil, eosinophil, neutrophil, lymphocyte, monocyte, NLR, and MLR. K3EDTA blood was diluted by the reagent that lysed erythrocytes and perforated the cytoplasmic membrane of all nucleated cells. This process allowed fluorescent dyes to enter the cells and bind to the nucleic acids of the cells. ESR measurement was performed using Starsed RS automated ESR analyzer (Sysmex).

Statistical Analysis

Data were processed using SPSS software version 20 (IBM Corporation, Armonk, NY, USA) and Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA). Data distribution was checked using Kolmogorov-Smirnov test. Bivariate analysis was performed to investigate the

relationship between two measured variables. Analysis of the difference between the two groups in the abnormal data distribution was done by using the Mann-Whitney U Test. The level of significance used was $p < 0.05$.

The calculation of cut-off and area under the curve (AUC) was obtained from the receiver operating characteristic (ROC) curve. Sensitivity and specificity of the examination compared to the gold standard (combined results of histopathology, colonoscopy, and therapeutic response) were then calculated.

Results

A total of 143 subjects, 16 (11.2%) of them were diagnosed with ITB, and 127 (88.8%) subjects were non-ITB (Table 1). In percentage, the ITB group had higher weight loss, loss of appetite, chronic diarrhea, and constipation.

ADA and ESR of ITB and non-ITB groups

From the results of laboratory test, ADA level and ESR of ITB groups were significantly higher than the ones of non-ITB group (Table 2). Meanwhile, all other hematological parameters of ITB and non-ITB groups were almost similar.

The median of ADA level in blood of ITB and non-ITB groups were 16.51 (11.82-35.61) IU/L, and 12.11 (8.89-15.99) IU/L, respectively (Table 2). In the ROC curve analysis, AUC of 0.695 (95% CI: 0.542-0.849) was obtained

Table 1. Characteristics of ITB and non-ITB groups.

Characteristics	ITB (n=16)	Non-ITB (n=127)
Age (years), mean±SD	42.19±18.28	43.53 ±16.65
Gender, n (%)		
Male	10 (62.5%)	40 (31.5%)
Female	6 (37.5%)	87 (68.5%)
Clinical manifestation, n (%)		
Weight loss	13 (81.3%)	69 (54.3%)
Night sweat	1 (6.3%)	5 (3.9%)
Cough	2 (12.5%)	5 (3.9%)
Fever	5 (31.3%)	16 (12.6%)
Loss of appetite	9 (56.3%)	41 (32.3%)
Non-specific abdominal pain	12 (75.0%)	111 (87.4%)
Chronic diarrhea	13 (81.3%)	73 (57.5%)
Constipation	13 (81.3%)	82 (64.6%)
Blood in stool	8 (50.0%)	69 (54.3%)
Mucus in stool	8 (50.0%)	70 (55.1%)
Blood and mucus in stool	8 (50.0%)	68 (53.5%)
TB history, n (%)		
History of contact with TB patient	1 (6.3%)	1 (0.8%)
History of pulmonary and non-pulmonary TB	2 (12.5%)	10 (7.9%)

Table 2. Laboratory results of ITB and non-ITB groups.

Parameter	ITB (n=16)	Non-ITB (n=127)	p- value
ADA level (IU/L)	16.51 (11.82-35.61)	12.11 (8.89-15.99)	0.011*
Leukocyte (10 ³ /μL)	7.63 (4.85-10.96)	6.98 (5.58-8.45)	0.656
Basophil (%)	0.50 (0.30-0.70)	0.50 (0.40-0.80)	0.358
Eosinophil (%)	1.80 (0.80-4.15)	1.90 (1.00-3.70)	0.941
Neutrophil (%)	63.85 (49.97-74.22)	61.0 (54.10-71.00)	0.522
Lymphocyte (%)	25.70 (16.65-36.82)	29.10 (21.70-34.90)	0.520
Monocyte (%)	7.20 (4.62-8.32)	6.60 (5.40-7.90)	0.670
NLR	2.48 (1.44-4.49)	2.12 (1.56-3.31)	0.495
MLR	0.29 (0.15-0.44)	0.22 (0.19-0.29)	0.401
ESR (mm/hour)	65 (33.50-92.50)	26 (13.00-45.00)	0.002*

Tested with Mann-Whitney U test, *significant if $p < 0.05$. Data were presented in median (min-max).

with a cut-off of 12.56 IU/L. Sensitivity of ADA were 75%, and specificity of 57% (Table 3).

The median of ESR of ITB group and non-ITB group were 65 (33.50-92.50) mm/hour and 26 (13.00-45.00) mm/hour, respectively (Table 2). In the ROC curve analysis, AUC of 0.741 (95% CI: 0.623-0.860) was obtained with a cut-off of 32.5 mm/hour. Sensitivity of ESR was 81%, and specificity of 62% (Table 4).

ROC analyses were conducted for the combination of ADA and ESR testing. For the utilization of ADA and ESR in diagnosing ITB, the cut-off was 12.56 IU/L and 32.5 mm/hour, respectively. Subsequently, ROC curve analysis was performed and AUC of 0.768 (95% CI: 0.656-0.880), sensitivity of 81.3%, and specificity of 62.2 % were obtained (Supplementary 1).

Colonoscopic and Biopsy Histopathological Appearances of ITB Subjects

In this study, colonoscopy of subjects with ITB displayed multiple ulcerations, edema, and hyperemic mucosa

in various areas, such as descending colon, transverse colon, ascendent colon, caecum, and terminal ileum (Figure 1). Meanwhile, histopathological examination of patients with confirmed ITB exhibited granulomatous inflammation, epitheloid cells, giant cells (Datia Langhans), and lymphocyte aggregates (Figure 2). Some of the other subjects showed incomplete and varied overview.

Discussion

There were 16 ITB subjects among all 143 recruited subjects from the Gastroenterology Outpatient Clinic, Department of Internal Medicine, Gastrointestinal Endoscopy Center of Dr. Cipto Mangunkusumo Central General Hospital. The subject population of 16/143 (11.2%) was in accordance with the previous reported ITB prevalence (3-16%).(1,18)

ADA level was significantly higher in ITB group compared with the one of non-ITB group. The sensitivity

Table 3. ADA results of ITB and non-ITB groups with cut-off=12.56 IU/L.

ADA Result	ITB	Non-ITB	Total
Positive	12	54	66
Negative	4	73	77
Total	16	127	143
Sensitivity	= 75%		
Specificity	= 57%		
Positive Predictive Value (PPV)	= 18%		
Negative Predictive Value (NPV)	= 95%		

Table 4. ESR results of ITB and non-ITB groups with cut-off=32.5 mm/hour.

ESR Result	ITB	Non-ITB	Total
Positive	13	48	61
Negative	3	79	82
Total	16	127	143
Sensitivity	= 81%		
Specificity	= 62%		
Positive Predictive Value (PPV)	= 21%		
Negative Predictive Value (NPV)	= 96%		

and specificity of ADA test were 75% and 57%, respectively, with a cut-off of 12.56 IU/L. These data could be useful for further investigation since in the present time there is not any available data of ITB patients' ADA level. Most studies reported ADA results of patients with pulmonary tuberculosis and abdominal tuberculosis.(15,19) The cut-off value of ADA level in this study was lower than the one in Serbian study on extrapulmonary tuberculosis (24 U/L) (19) and Nepalese study on pulmonary/extrapulmonary tuberculosis (25 U/L) (20).

There was no significant difference in leukocyte count and leukocyte differential count between ITB and non-ITB groups in this study. These results are in accordance with the ones in Ethiopian study, reporting that the counts between the two groups were not significantly different.(21)

Although NLR may indicate an early TB infection, there was not any significant difference between NLR of ITB and non-ITB groups in this study. This result was similar to the findings reported by previous study.(22)

However, contradictory findings were reported in a study in Bandung, Indonesia, whereas pulmonary TB subjects exhibited significantly higher NLR.(23)

There was no significant difference in MLR between ITB and non-ITB groups in this study. Both similar (22) and contradictive findings (24) in the MLR of patients with TB, have been reported which might be influenced by the study site, existence of any comorbidities (HIV, diabetes mellitus), age, and TB treatment.

Compared with non-ITB group (26 (13.00-45.00) mm/hour), the ITB group had significant higher ESR (65 (33.50-92.50) mm/hour). Similarly, a previous study reported that ESR was significantly higher in patients with pulmonary TB compared with those without TB.(25) In this study, high sensitivity of ESR (81%) was calculated, which could be promoted as a screening test for ITB. ESR test has been used for a long time to assess acute phase proteins in chronic diseases, including TB. Pro-inflammatory cytokines produced in ITB will stimulate the liver to produce acute phase proteins, such as fibrinogen which may lead to an increase in ESR. ESR measurement has limitations, such as being affected by anemic conditions and hypoalbuminemia, which cause a false-high ESR.(25-27)

The combination of ADA and ESR test results with cut-offs of 12.56 IU/L and 32.5 mm/hour, respectively, did not provide a significant increase in sensitivity and specificity compared to individual ADA or ESR test result. Therefore, each of ADA or ESR test can be used independently to assist the diagnose of ITB.

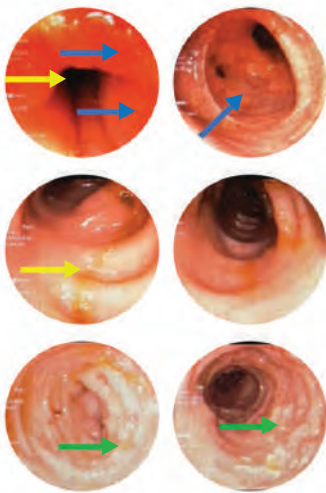


Figure 1. Colonoscopic results of ITB-suspected-subjects with ileocolitis. Blue arrow: hyperemic intestinal mucosa; Yellow arrow: edema; Green arrow: ulceration.

Conclusion

ADA level and ESR were significantly higher among ITB patients compared with non-ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests could be considered as a screening test for ITB.

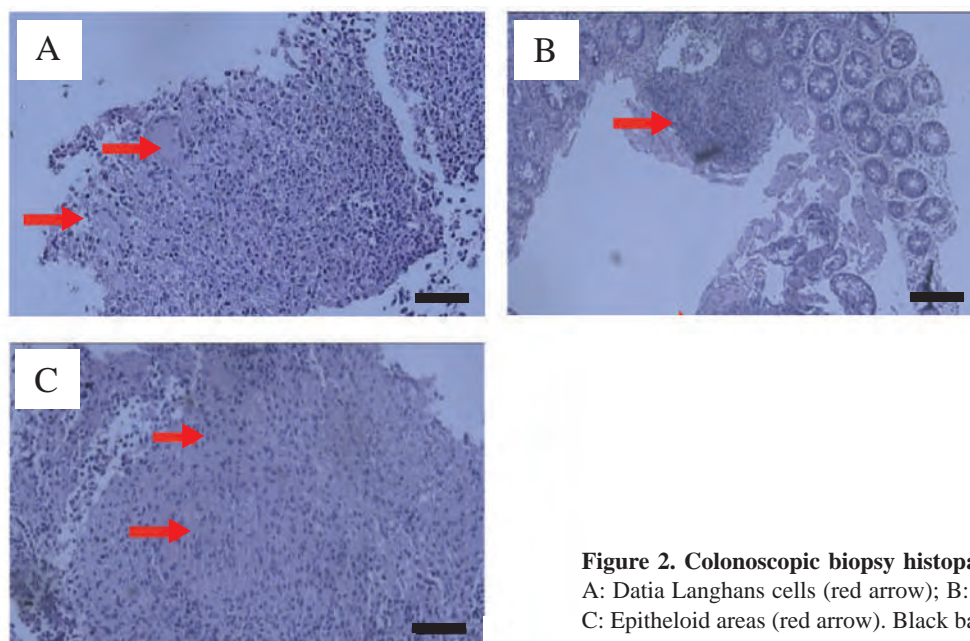


Figure 2. Colonoscopic biopsy histopathological results of ITB group.
 A: Datia Langhans cells (red arrow); B: Lymphoid aggregates (red arrow);
 C: Epitheloid areas (red arrow). Black bar: 50 µm.

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Authors Contribution

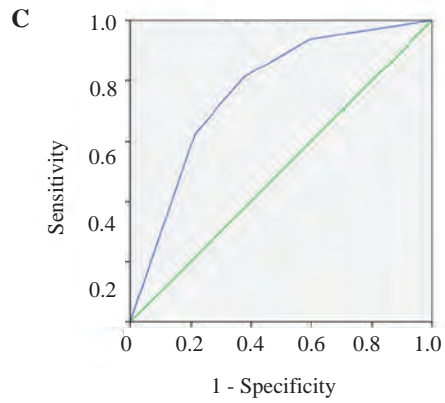
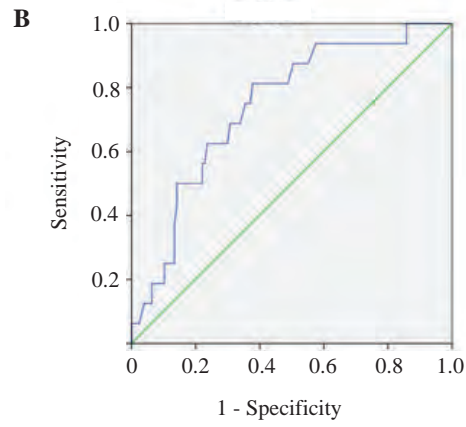
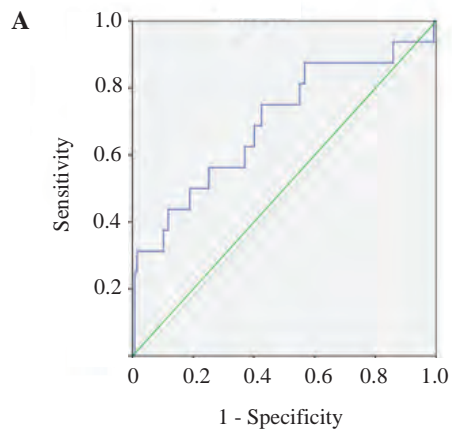
NDI, MS, PR, Y, AK, S, ARH, HW, and IP were involved in concepting and planning the research. NDI, MS, PR, AK, ARH, HW, and IP performed the data acquisition/collection. NDI, MS, PR, Y, AK, and S calculated the experimental data and performed the analysis. NDI, MS, PR, Y, AK, ARH, HW, IP, and FS drafted the manuscript. NDI, MS, AK and S designed the figures and tables. NDI, MS, PR, Y, AK, S, ARH, HW, IP, and FS aided in interpreting the results. NDI, MS, PR, Y, AK, and FS took parts in giving critical revision of the manuscript.

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Supplementary 1.



ROC curve for (A) ADA test, (B) ESR test, and (C) ADA+ ESR tests.



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RESEARCH ARTICLE

High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients

Nuri Dyah Indrasari^{1,2}, Marcellus Simadibrata³, Primariadevi Rustamadji⁴, Yusra⁵, Aria Kekalih⁶, Subendro⁷, Alida Roswita Harahap⁸, Heri Wibowo⁹, Ida Parwati¹, Ferry Sandra^{1*}

¹Doctoral Program in Medical Sciences, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
²Department of Clinical Pathology, Faculty of Medicine, Universitas Indonesia Dr. Cipto Mangunkusumo Central General Hospital, Jl. Diponegoro No.71, Jakarta 10430, Indonesia
³Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia Dr. Cipto Mangunkusumo Central General Hospital, Jl. Diponegoro No.71, Jakarta 10430, Indonesia
⁴Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia Dr. Cipto Mangunkusumo Central General Hospital, Jl. Diponegoro No.71, Jakarta 10430, Indonesia
⁵Community Medicine Department, Faculty of Medicine, Universitas Indonesia, Jl. Pajajaran Timur No.16, Jakarta 10430, Indonesia
⁶Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
⁷Department of Clinical Pathology, Faculty of Medicine, Universitas Padjadjaran, Dr. Hasan Sadikin General Hospital Bandung, Jl. Pasteur No. 38, Bandung 40132, Indonesia
⁸Department of Biochemistry and Molecular Biology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No. 206, Jakarta 11440, Indonesia

*Corresponding author. Email: ferry@trisakti.ac.id
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Abstract

BACKGROUND: Currently, laboratory diagnosis of intestinal tuberculosis (ITB) is limited based on clinical manifestations, providing opportunities for alternative laboratory tests to diagnose ITB. At the present time, the role of serum adenosine deaminase (ADA) and hematological tests in ITB patients are not widely known. The objective of this study was to determine the role of ADA and hematological tests in patients suspected with ITB.

METHODS: Subjects that were suspected of ITB were classified as ITB group, while subjects with inflammatory bowel disease, hemorrhoid, and intestinal malignancy were classified as non-ITB group. Colonoscopy, histopathological examinations, and hematological tests were performed. ADA measurement was also performed with clinical chemistry analyzer based on enzymatic colorimetry principle.

RESULTS: Out of 143 subjects, 16 (11.2%) subjects were diagnosed with ITB and 127 (88.8%) subjects were

classified as non-ITB group. ADA level and erythrocyte sedimentation rate (ESR) of ITB group were significantly higher than the ones of non-ITB group ($p < 0.05$). Cut-off, sensitivity, and specificity of ADA level were 12.56 IU/L, 75%, and 57%, respectively. Cut-off, sensitivity, and specificity of ESR were 32.5 mm/hour, 81%, and 62%, respectively. Colonoscopy of ITB subjects displayed multiple ulcerations, edema, and hyperemic mucosa. Histopathological examination of ITB subjects exhibited granulomatous inflammation, epithelioid cells, giant cells, and lymphocyte aggregates.

CONCLUSION: ADA level and ESR were significantly higher among ITB patients compared with non-ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests could be considered as a screening test for ITB.

KEYWORDS: intestinal tuberculosis, adenosine deaminase, hematological tests

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Ida Parwati⁷, Ferry Sandra^{8,*}

¹Doctoral Program in Medical Sciences, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia

²Department of Clinical Pathology, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Central General Hospital, Jl. Diponegoro No.71, Jakarta 10430, Indonesia

³Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Central General Hospital, Jl. Diponegoro No.71, Jakarta 10430, Indonesia

⁴Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Central General Hospital, Jl. Diponegoro No.71, Jakarta 10430, Indonesia

⁵Community Medicine Department, Faculty of Medicine, Universitas Indonesia, Jl. Pegangsaan Timur No.16, Jakarta 10430, Indonesia

⁶Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia

⁷Department of Clinical Pathology, Faculty of Medicine, Universitas Padjadjaran, Dr. Hasan Sadikin General Hospital Bandung, Jl. Pasteur No. 38, Bandung 40132, Indonesia

⁸Department of Biochemistry and Molecular Biology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No. 260, Jakarta 11440, Indonesia

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KEYWORDS: intestinal tuberculosis, adenosine deaminase, hematological tests

Indones Biomed J. 2023; 15(4): 362-8



Introduction

According to the Global Tuberculosis Report 2019 from the World Health Organization (WHO), Indonesia was ranked third among the countries with highest burden of tuberculosis (TB).(1,2) The global prevalence of extrapulmonary TB was 14% and the prevalence in Indonesia was $\leq 9.9\%$. Intestinal tuberculosis (ITB), as a part of abdominal TB, had a prevalence of approximately 3-16%.(3-5)

Currently, ITB diagnosis was established based on clinical manifestations, Ziehl-Neelsen (ZN) acid fast bacilli (AFB) stain of the stool, fecal culture, colonoscopy, and histopathology.(3,6-10) The limitations of the respective laboratory pose difficulties to diagnose ITB, and hence, providing opportunities for alternative laboratory tests to diagnose ITB.(11)

Adenosine deaminase (ADA) is an enzyme involved in purine metabolism which catalyzes the conversion of adenosine to inosine. ADA is also involved in the proliferation and differentiation of lymphocytes and maturation of monocytes.(11,12) Elevated ADA levels in blood or body fluid during tuberculosis infection is due to the stimulation of T lymphocytes by *Mycobacterium tuberculosis* (MTB) antigen.(12,13) The activity of ADA differs significantly between patients with pulmonary TB and the normal healthy people.(14,15)

In ITB, acute or chronic inflammation is present due to infection process. Hematological tests are often requested by clinicians in patients suspected of having ITB, as several parameters can be utilized as markers of inflammation or infection. Some of them are leukocyte count, leukocyte differential count (basophil, eosinophil, neutrophil, lymphocyte, and monocyte), neutrophil to lymphocyte ratio (NLR), monocyte to lymphocyte ratio (MLR), and erythrocyte sedimentation rate (ESR).(3,6,16,17) At the present time, the role of serum ADA and hematological tests in ITB patients are not widely known. The objective of this study was to determine the role of ADA and hematological tests in patients suspected with ITB.

Methods

Study Design and Subjects

Subjects were recruited at the Gastroenterology Outpatient Clinic, Department of Internal Medicine, Gastrointestinal Endoscopy Center of Dr. Cipto Mangunkusumo Central General Hospital from December 2020 until December

2022, with the inclusion criteria as follows; for the ITB group: ≥ 18 years old and suspected ITB. Meanwhile the exclusion criteria: being treated with anti-tuberculosis drugs for >3 months, post-treatment with anti-tuberculosis drugs for <6 months.

Patients were suspected of ITB if 3 out of 4 main clinical findings were present, and 1 out of 3 additional history criteria for ITB was met. The main clinical findings of ITB include weight loss, non-specific abdominal pain, fever of unknown origin, and chronic diarrhea or constipation. The additional history includes history of pulmonary TB, active pulmonary TB with or without anti-tuberculosis drugs treatment (<3 months of treatment), and positive contact history with TB patients. Patients with inflammatory bowel disease (Crohn's disease or ulcerative colitis), hemorrhoid, and intestinal malignancy were classified as non-ITB group.

Prior to the recruitment, all subjects were explained and asked for their consents of participation by signing an informed consent form. The research protocol was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia and Dr. Cipto Mangunkusumo Central General Hospital (No. KET-1498/UM2.F1/ETIK/PPM.00.02/2020).

Colonoscopy Examination

After being given laxatives, sedatives and analgesics, subjects lied in a supine position for a colonoscope insertion. If abnormalities were found, biopsies were taken for histopathological examination. Various features could be identified during colonoscopy of patient with suspected ITB, such as ulceration, nodules, polyps, narrowing of the lumen, and irregular multiple fibrous bands. ITB may affect extensive parts of the intestine, in the form of continuous lesions or single or skip lesions.(16)

Histopathological Examination

Samples for examination were harvested from at least 5 granulomas originating from a single segment. After tissue fixation, grossing, embedding and slicing, tissue sections were stained with hematoxylin (Eprexia, Kalamazoo, MI, USA) and eosin (Merck, Darmstadt, Germany), and mounted. Prepared tissue slides will be read by an anatomic pathologist.

ADA Test

Nine mL of venous blood was drawn from the cubital vein. Three mL were collected in an EDTA tube for complete hematological test, while 6 mL in non-anticoagulated tube for ADA test. Blood in non-anticoagulated tubes were

allowed to stand at room temperature for about 30 minutes, until blood clot formed. Immediate centrifugation of 3,000 revolutions per minute (rpm) for 15 minutes was performed to separate the serum from the rest of the remaining sample. Quantitative test of blood ADA was performed by BS-200 automated clinical chemistry analyzer (Mindray, Shenzhen, China) with enzymatic colorimetry principle test used. The measurement was determined by the increase of photometric absorbance at a wavelength of 546 nm.

Hematological Test

Hematology test was performed by XN-2000 hematology analyzer (Sysmex, Kobe, Hyogo, Japan) with semiconductor laser flow cytometry for measurement of leukocyte, basophil, eosinophil, neutrophil, lymphocyte, monocyte, NLR, and MLR. K3EDTA blood was diluted by the reagent that lysed erythrocytes and perforated the cytoplasmic membrane of all nucleated cells. This process allowed fluorescent dyes to enter the cells and bind to the nucleic acids of the cells. ESR measurement was performed using Starrsed RS automated ESR analyzer (Sysmex).

Statistical Analysis

Data were processed using SPSS software version 20 (IBM Corporation, Armonk, NY, USA) and Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA). Data distribution was checked using Kolmogorov-Smirnov test. Bivariate analysis was performed to investigate the

relationship between two measured variables. Analysis of the difference between the two groups in the abnormal data distribution was done by using the Mann-Whitney U Test. The level of significance used was $p < 0.05$.⁶

The calculation of cut-off and area under the curve (AUC) was obtained from the receiver operating characteristic (ROC) curve. Sensitivity and specificity of the examination compared to the gold standard (combined results of histopathology, colonoscopy, and therapeutic response) were then calculated.

Results

A total of 143 subjects, 16 (11.2%) of them were diagnosed with ITB, and 127 (88.8%) subjects were non-ITB (Table 1). In percentage, the ITB group had higher weight loss, loss of appetite, chronic diarrhea, and constipation.

ADA and ESR of ITB and non-ITB groups

From the results of laboratory test, ADA level and ESR of ITB groups were significantly higher than the ones of non-ITB group (Table 2). Meanwhile, all other hematological parameters of ITB and non-ITB groups were almost similar.

The median of ADA level in blood of ITB and non-ITB groups were 16,51 (11.82-35.61) IU/L, and 12,11 (8.89-15.99) IU/L, respectively (Table 2). In the ROC curve analysis, AUC of 0.695 (95% CI: 0.542-0.849) was obtained

Table 1. Characteristics of ITB and non-ITB groups.

Characteristics	ITB (n=16)	Non-ITB (n=127)
Age (years), mean±SD	42.19±18.28	43.53 ± 16.65
Gender, n (%)		
Male	10 (62.5%)	40 (31.5%)
Female	6 (37.5%)	87 (68.5%)
Clinical manifestation, n (%)		
Weight loss	13 (81.3%)	69 (54.3%)
Night sweat	1 (6.3%)	5 (3.9%)
Cough	2 (12.5%)	5 (3.9%)
Fever	5 (31.3%)	16 (12.6%)
Loss of appetite	9 (56.3%)	41 (32.3%)
Non-specific abdominal pain	12 (75.0%)	111 (87.4%)
Chronic diarrhea	13 (81.3%)	73 (57.5%)
Constipation	13 (81.3%)	82 (64.6%)
Blood in stool	8 (50.0%)	69 (54.3%)
Mucus in stool	8 (50.0%)	70 (55.1%)
Blood and mucus in stool	8 (50.0%)	68 (53.5%)
TB history, n (%)		
History of contact with TB patient	1 (6.3%)	1 (0.8%)
History of pulmonary and non-pulmonary TB	2 (12.5%)	10 (7.9%)

Table 2. Laboratory results of ITB and non-ITB groups.

Parameter	ITB (n=16)	Non-ITB (n=127)	p-value
ADA level (IU/L)	16.51 (11.82-35.61)	12.11 (8.89-15.99)	0.011*
Leukocyte ($10^3/\mu\text{L}$)	7.63 (4.85-10.96)	6.98 (5.58-8.45)	0.656
Basophil (%)	0.50 (0.30-0.70)	0.50 (0.40-0.80)	0.358
Eosinophil (%)	1.80 (0.80-4.15)	1.90 (1.00-3.70)	0.941
Neutrophil (%)	63.85 (49.97-74.22)	61.0 (54.10-71.00)	0.522
Lymphocyte (%)	25.70 (16.65-36.82)	29.10 (21.70-34.90)	0.520
Monocyte (%)	7.20 (4.62-8.32)	6.60 (5.40-7.90)	0.670
NLR	2.48 (1.44-4.49)	2.12 (1.56-3.31)	0.495
MLR	0.29 (0.15-0.44)	0.22 (0.19-0.29)	0.401
ESR (mm/hour)	65 (33.50-92.50)	26 (13.00-45.00)	0.002*

Tested with Mann-Whitney U test, *significant if $p < 0.05$. Data were presented in median (min-max).

with a cut-off of 12.56 IU/L. Sensitivity of ADA were 75%, and specificity of 57% (Table 3).

The median of ESR of ITB group and non-ITB group were 65 (33.50-92.50) mm/hour and 26 (13.00-45.00) mm/hour, respectively (Table 2). In the ROC curve analysis, AUC of 0.741 (95% CI: 0.623-0.860) was obtained with a cut-off of 32.5 mm/hour. Sensitivity of ESR was 81%, and specificity of 62% (Table 4).

ROC analyses were conducted for the combination of ADA and ESR testing. For the utilization of ADA and ESR in diagnosing ITB, the cut-off was 12.56 IU/L and 32.5 mm/hour, respectively. Subsequently, ROC curve analysis was performed and AUC of 0.768 (95% CI: 0.656-0.880), sensitivity of 81.3%, and specificity of 62.2 % were obtained (Supplementary 1).

Colonoscopic and Biopsy Histopathological Appearances of ITB Subjects

In this study, colonoscopy of subjects with ITB displayed multiple ulcerations, edema, and hyperemic mucosa

in various areas, such as descending colon, transverse colon, ascendent colon, caecum, and terminal ileum (Figure 1). Meanwhile, histopathological examination of patients with confirmed ITB exhibited granulomatous inflammation, epithelioid cells, giant cells (Datis Langhans), and lymphocyte aggregates (Figure 2). Some of the other subjects showed incomplete and varied overview.

Discussion

There were 16 ITB subjects among all 143 recruited subjects from the Gastroenterology Outpatient Clinic, Department of Internal Medicine, Gastrointestinal Endoscopy Center of Dr. Cipto Mangunkusumo Central General Hospital. The subject population of 16/143 (11.2%) was in accordance with the previous reported ITB prevalence (3-16%), (1,18)

ADA level was significantly higher in ITB group compared with the one of non-ITB group. The sensitivity

Table 3. ADA results of ITB and non-ITB groups with cut-off=12.56 IU/L.

ADA Result	ITB	Non-ITB	Total
Positive	12	54	66
Negative	4	73	77
Total	16	127	143
Sensitivity	= 75%		
Specificity	= 57%		
Positive Predictive Value (PPV)	= 18%		
Negative Predictive Value (NPV)	= 95%		

Table 4. ESR results of ITB and non-ITB groups with cut-off=32.5 mm/hour.

ESR Result	ITB	Non-ITB	Total
Positive	13	48	61
Negative	3	79	82
Total	16	127	143
Sensitivity	= 81%		
Specificity	= 62%		
Positive Predictive Value (PPV)	= 21%		
Negative Predictive Value (NPV)	= 96%		

and specificity of ADA test were 75% and 57%, respectively, with a cut-off of 12.56 IU/L. These data could be useful for further investigation since in the present time there is not any available data of ITB patients' ADA level. Most studies reported ADA results of patients with pulmonary tuberculosis and abdominal tuberculosis.(15,19) The cut-off value of ADA level in this study was lower than the one in Serbian study on extrapulmonary tuberculosis (24 U/L) (19) and Nepalese study on pulmonary/extrapulmonary tuberculosis (25 U/L) (20).

There was no significant difference in leukocyte count and leukocyte differential count between ITB and non-ITB groups in this study. These results are in accordance with the ones in Ethiopian study, reporting that the counts between the two groups were not significantly different.(21)

Although NLR may indicate an early TB infection, there was not any significant difference between NLR of ITB and non-ITB groups in this study. This result was similar to the findings reported by previous study.(22)

However, contradictory findings were reported in a study in Bandung, Indonesia, whereas pulmonary TB subjects exhibited significantly higher NLR.(23)

There was no significant difference in MLR between ITB and non-ITB groups in this study. Both similar (22) and contradictive findings (24) in the MLR of patients with TB, have been reported which might be influenced by the study site, existence of any comorbidities (HIV, diabetes mellitus), age, and TB treatment.

Compared with non-ITB group (26 (13.00-45.00) mm/hour), the ITB group had significant higher ESR (65 (33.50-92.50) mm/hour). Similarly, a previous study reported that ESR was significantly higher in patients with pulmonary TB compared with those without TB.(25) In this study, high sensitivity of ESR (81%) was calculated, which could be promoted as a screening test for ITB. ESR test has been used for a long time to assess acute phase proteins in chronic diseases, including TB. Pro-inflammatory cytokines produced in ITB will stimulate the liver to produce acute phase proteins, such as fibrinogen which may lead to an increase in ESR. ESR measurement has limitations, such as being affected by anemic conditions and hypoalbuminemia, which cause a false-high ESR.(25-27)

The combination of ADA and ESR test results with cut-offs of 12.56 IU/L and 32.5 mm/hour, respectively, did not provide a significant increase in sensitivity and specificity compared to individual ADA or ESR test result. Therefore, each of ADA or ESR test can be used independently to assist the diagnose of ITB.



Figure 1. Colonoscopic results of ITB-suspected-subjects with ileocolitis. Blue arrow: hyperemic intestinal mucosa; Yellow arrow: edema; Green arrow: ulceration.

Conclusion

ADA level and ESR were significantly higher among ITB patients compared with non-ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests could be considered as a screening test for ITB.

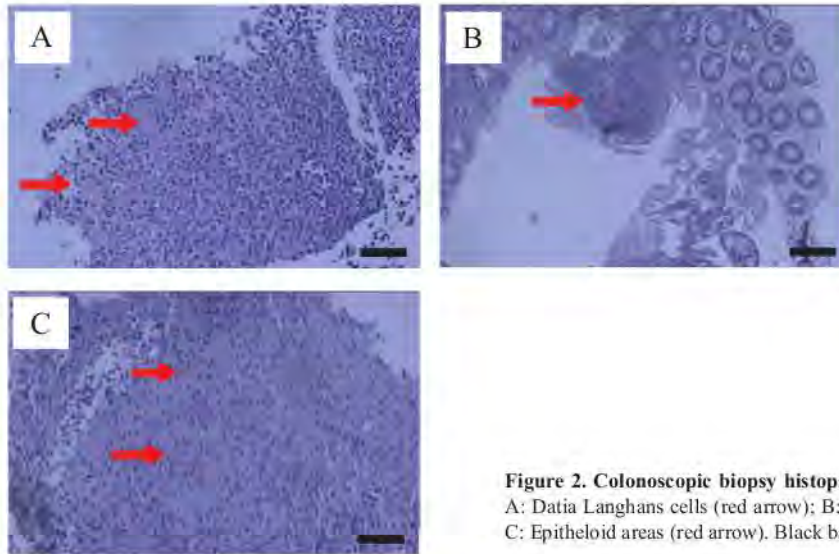


Figure 2. Colonoscopic biopsy histopathological results of ITB group.
A: Datia Langhans cells (red arrow); B: Lymphoid aggregates (red arrow); C: Epitheloid areas (red arrow). Black bar: 50 µm.

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Authors Contribution

NDI, MS, PR, Y, AK, S, ARH, HW, and IP were involved in conceiving and planning the research. NDI, MS, PR, AK, ARH, HW, and IP performed the data acquisition/collection. NDI, MS, PR, Y, AK, and S calculated the experimental data and performed the analysis. NDI, MS, PR, Y, AK, ARH, HW, IP, and FS drafted the manuscript. NDI, MS, AK and S designed the figures and tables. NDI, MS, PR, Y, AK, S, ARH, HW, IP, and FS aided in interpreting the results. NDI, MS, PR, Y, AK, and FS took parts in giving critical revision of the manuscript.

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[InaBJ] M2023128 Editor Decision Round 1 - Resubmit for Review

Secretariat of InaBJ <secretariat@inabj@gmail.com>
To: ferry@trisakti.ac.id

Mon, Jun 26, 2023 at 8:02 AM

Dear Dr. Ferry Sandra,

Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "**Blood Adenosine Deaminase and Complete Hematology Test as Potential Candidates for Laboratory Tests in Intestinal Tuberculosis**".

Our decision is: **Resubmit for Review.**

Find the file attached to see detailed comments from reviewers. Please make sure you read all the comments and revise the manuscript based on the suggestions given.

Revise this manuscript thoroughly before **July 3, 2023**. Mark/highlighted the revised part of the manuscript, so that the editor will notice the changes.

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--

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2
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4 Jha DK, Pathiyil MM, Sharma V.Indian J Gastroenterol. 2023 Feb;42(1):17-31. doi:
5 10.1007/s12664-023-01343-x.

6
7
8
9 **Blood Adenosine Deaminase and Complete Hematology Test as Potential Candidates**
10 **for Laboratory Tests in Intestinal Tuberculosis**

11
12
13 **Abstract**

14 **Background:** Currently, laboratory diagnosis of **ITB????** is limited and opens an still
15 opportunity for **other??** laboratory tests. There is not many data about adenosine deaminase
16 (ADA) and haematology tests in patients with ITB. This study aimeds to determine the role
17 of ADA and haematology tests in patients with ITB.

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18 **Method:** →PAST TENSE!

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19 The subjects are adult patients aged ≥ 18 years with suspected ITB, who come to the
20 Gastroenterology Outpatient Clinic and undergo a colonoscopy and histopathology
21 examinations. Activity of **ADA is examined by using Mindray BS-200 analyzers.**
22 Haematology tests are performed by Sysmex XN-2000 Haematology Analyzer and ESR by
23 Starrsed RS ESR.

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24 **Result:** There were 143 subjects, consist of 16 (11.2%) subjects with ITB and 127 (88.8%)
25 subjects **non-ITB →SUSPECT OF ??? CROHN???**. ADA activity of ~~all subjects~~, ITB
26 group, and non-ITB group were ~~12.15 (2.95-45.57)~~, 21.38 (± 12.79), and 12.11 (2.95-45.57)
27 IU/L. ADA activity in the ITB and non-ITB groups was higher significant ($p= 0.01$). AUC of
28 ADA was 0.695 with a cut-off of 12.56 IU/L, sensitivity of 75%, and specificity of 57%.
29 There were no significant differences in leukocyte count, differential count (basophile,
30 eosinophile, neutrophile, lymphocyte, and monocyte), NLR, and MLR between ITB and
31 non-ITB groups. ESR result **mention the mean** in the ITB and non-ITB groups was higher

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32 significant ($p=0.00$). AUC of ESR was 0.741 with a cut-off of 32.5 mm/hour, sensitivity of
33 81%, and specificity of 62%.

34 **Conclusion:** ADA activity and ESR tests can be used potential candidates for laboratory tests
35 in suspected ITB patients.

36 **Keyword:** intestinal tuberculosis, adenosine deaminase, haematology tests

37

38 **Introduction**

39 According to the Global Tuberculosis Report 2019 from the World Health Organization
40 (WHO), Indonesia is on the third rank of the countries with highest burden of tuberculosis
41 (TB).¹ The global prevalence of extrapulmonary TB is 14% and the prevalence in Indonesia
42 is $\leq 9.9\%$. Intestinal tuberculosis (ITB) as part of abdominal TB has prevalence of
43 approximately 3–16%.²⁻⁴

44 Currently, ITB diagnosis is established based on clinical manifestation, Ziehl-Neelsen
45 (ZN) acid fast bacilli (AFB) stain of the stool, fecal culture, colonoscopy, and
46 histopathology.^{2,6-10} The limitations of the respective laboratory tests make it difficult to
47 diagnose ITB, and hence, providing opportunities for various other laboratory tests to
48 diagnose ITB.

49 Adenosine deaminase (ADA) is an enzyme that is involved in purine metabolism to
50 catalyze the conversion of adenosine to inosine. Proliferation and differentiation of
51 lymphocytes and maturation of monocytes require ADA enzymes.^{11,12} In TB disease, an
52 increase of ADA in the blood or body fluid is reported due to stimulation of T lymphocytes
53 by *Mycobacterium tuberculosis* (MTB) antigen.^{11,12} Varma et al¹³ and Salmanzadeh et al¹⁴
54 reported activity of ADA with significant difference in patients with pulmonary TB and
55 normal controls.

56 In ITB, there is acute or chronic inflammation due to infection process. Haematological
57 parameters as markers of inflammation or infection include leukocyte count, leukocyte
58 differential count (basophile, eosinophile, neutrophile, lymphocyte, and monocyte),
59 Neutrophile to Lymphocyte Ratio (NLR), Monocyte to Lymphocyte Ratio (MLR), and
60 Erythrocyte Sedimentation Rate (ESR). Haematology tests are often requested by clinicians
61 in patients suspected of having ITB.^{2,6,15} Currently, some inflammatory markers have been
62 known, such as Neutrophile to Lymphocyte Ratio (NLR), Monocyte to Lymphocyte Ratio
63 (MLR), and Erythrocyte Sedimentation Rate (ESR).

64 The role of ADA serum and haematology tests in ITB patients are still not widely
65 known yet. The objective of this study is to determine the role of ADA and haematology tests
66 in patients suspected with ITB. Should there be any significant result of ADA and
67 haematology tests, it is hoped that they can be used to assist in diagnosing ITB.

68

69 **Methods**

70 **Study Design and Subjects**

71 Subjects were recruited at the Gastroenterology Outpatient Clinic, Department of
72 Internal Medicine, Gastrointestinal Endoscopy Center of XXX. The study was conducted
73 from December 2020 until December 2022. The inclusion criteria of the study subjects were
74 adult patients aged ≥ 18 years suspected of ITB, who came to the Gastroenterology Outpatient
75 Clinic, Department of Internal Medicine in Gastrointestinal Endoscopy Center XXX, and/or
76 histopathology test. The exclusion criteria of the study subjects were subjects who underwent
77 treatment with anti-tuberculosis drugs > 3 months or post-treatment with anti-tuberculosis
78 drugs < 6 months. Diagnosis of suspected ITB was made by an internal medicine specialist
79 based on the medical history and physical examination, i.e., if there are 3 of 4 main clinical
80 findings and 1 of 3 additional history. Main clinical complaints include weight loss, non-
81 specific abdominal pain, fever of unknown origin, and chronic diarrhea or constipation.
82 Additional history includes history of pulmonary TB, active pulmonary TB with or without
83 anti-tuberculosis drugs treatment (< 6 months of treatment), and positive contact with TB
84 patients. The study subjects were given an explanation and asked for their willingness to
85 participate by signing an informed consent form. History taking was performed to determine
86 whether the subject complies with the inclusion and exclusion criteria.

87

88

89 **Colonoscopy Examination**

90 A colonoscopy examination was performed by a clinician of Gastroenterology
91 Division, Department of Internal Medicine in Gastrointestinal Endoscopy Center XXX.
92 Before a colonoscopy examination, the patient is asked to empty the digestive tract by
93 consuming laxatives at night or in the morning. Laxatives have been prescribed by a doctor.
94 Patients are given sedative drugs and pain relievers. The patient lies in a supine position and
95 the knees are bent towards the chest. Next, the doctor will insert a colonoscope into the anus
96 while pumping air into the large intestine to expand the intestine, so that the intestinal wall
97 can be seen clearly. Patients are also asked to fasting until the colonoscopy is completed. The
98 colonoscopy procedure takes about 30–60 minutes. The doctor will take the necessary
99 pictures. If any abnormalities are found, the doctor will take a sample of intestinal tissue for
100 histopathological examination. Colonoscopy in patient suspected of having ITB may find
101 various features, including ulceration, nodules, polyps, narrowing of the lumen, and irregular
102 multiple fibrous bands. The intestinal area affected by ITB can be found from ileum to
103 caecum, in the form of continuous lesions or single or skip lesions.¹⁶ If abnormalities found
104 in the colonoscopy, a biopsy will be done and the specimen obtained will be sent to the
105 Anatomic Pathology Laboratory of XXX for histopathology test.

106

107 **Histopathology Examination**

108 Histopathology examination is using biopsy material from colonoscopy. In ITB, the
109 gut is harvested from at least 5 granulomas from biopsies originating from one segment.
110 Histopathological procedure were tissue cutting, tissue fixation, grossing, embedding,
111 trimming, fishing/plating, staining of tissue with hematoxylin (Epredia, Kalamzoo, US) and
112 eosin (Merck, Darmstadt, Germany), and finally mounting. Prepared tissue slides will be read
113 by an anatomic pathology specialist. The histopathological result in ITB is formed of

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114 submucosal granulomatous inflammatory features. TB granuloma is characterized by lesions
115 in the submucosa in the form of epithelioid cells, Datia Langhans giant cells, lymphocytes at
116 the edges of granulomas, and caseous necrosis.¹⁷

117

118 **ADA Test**

119 ADA and haematology tests were performed in the Clinical Pathology Laboratory of
120 XXX. Vein blood sample was used for ADA and haematology tests. 9 mL of venous blood
121 was taken from the cubital vein which was collected in a 3 mL EDTA tube for complete
122 hematological examination and ESR, 6 mL non-anticoagulant tube for ADA examination.
123 Blood in a tube non-anticoagulant, allowed to stand at room temperature for about 30
124 minutes, until a clot forms. Then immediately centrifuge 3,000 rotates per minute (rpm) for
125 15 minutes to separate the serum contained in the top layer of the tube.

126 Quantitative test of blood ADA was performed by Mindray BS-200 automatic clinical
127 chemistry. The principle of ADA test is enzymatic colorimetric method. Adenosine
128 deaminase irreversibly converts adenosine into inosine. Inosine is then released from ribose
129 by purine nucleoside phosphorylase (PNP) so that hypoxanthine is formed. Hypoxanthine is
130 oxidized by xanthine oxidase (XOD) to uric acid and hydrogen peroxide (H₂O₂). Hydrogen
131 peroxide along with N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methyl aniline (EHSPT) and 4-
132 amino and imipramine Lin (4-AAP) is converted into coloured benzoquinone by peroxidase
133 (POD). The oxidation rate of benzoquinone is equivalent to the activity of ADA in the
134 sample, which is determined by measuring the increase of absorbance photometrically at a
135 wavelength of 546 nm.¹⁸

136

137 **Haematology Test**

138 Haematology test was performed by Sysmex XN-2000 haematology analyzer with
139 semiconductor laser flowcytometry principle. K3EDTA blood is diluted by reagent that lyses
140 erythrocytes and make holes in the cytoplasmic membrane of all nucleated cells, so that
141 fluorescence dye can enter the cells and bind to the DNA/RNA of the cells. The diluted
142 sample goes into a central core whose outer envelope is coated by a very strong fluid flow
143 (sheath fluid). Afterward, the laser beam will be fired towards the cell from 3 sides, i.e.,
144 Forward Scattered Light (FSC), Side Scattered Light (SSC), and Side Fluorescent Light
145 (SFL). Forward Scattered Light (FSC) informs the size of the cell. Side Scattered Light (SSC)
146 informs the complexity or characteristics of the cell. Side Fluorescent Light (SFL) informs
147 the contents of nucleic acids and cell organelles.¹⁹ ESR inspection was performed using
148 Starrsed RS ESR automatic analyzer.²⁰

149 The results of colonoscopy, histopathology, and response towards treatment with anti-
150 tuberculosis drugs are used as the gold standard in diagnosing ITB. This study was approved
151 by the Health Research Ethics Committee – XXX with Ethical Clearance number XXX.

152

153 **Statistics**

154 Data processing using statistical product and service solution (SPSS) program version
155 20 and Microsoft Excel 2016. The characteristics of the study subject are presented
156 descriptively. The data distribution is checked by Kolmogorov-Smirnov test. **The**
157 **distribution of ADA activity and haematology data were abnormal, therefore the data were**
158 **presented in medians and ranges.** Bivariate analysis to investigate the relationship between
159 two variable. Analysis of the difference between the two groups in the abnormal data
160 distribution was done by using the Mann Whitney U Test. The level of significance used is α
161 = 0.05.

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162 The calculation of cut-off and Area Under the Curve (AUC) is obtained from the ROC
 163 (Receiver Operating Characteristic) curve, and then the sensitivity and specificity of the
 164 examination to the gold standard were calculated (combined results of histopathology,
 165 colonoscopy, and therapeutic response).

166
 167 **RESULTS**

168 **Baseline Characteristics**

169 A total of 143 subjects, 16 (11.2%) subjects were diagnosed with ITB, and 127 (88.8%)
 170 subjects were non-ITB. The other baseline characteristics are summarized in Table 1.

Table 1. Baseline Characteristics of Subjects

Characteristics	Total (N=143, 100%)	ITB (N=16, 11.2%)	Non-ITB (N=127, 88.8%)	<i>p</i> *
Age (years)	43 (± 16.8)	37 (± 17.9)	44 (± 16.6)	0.12
Sex				
- Male, N(%)	50 35.0%	10 7.0%	40 28.0%	0.02
- Female, N(%)	93 65.0%	6 4.2%	87 60.8%	
Clinical manifestation				
- Weight loss N(%)	82 57.3%	13 9.1%	69 48.3%	0.06
- Night sweat N(%)	6 4.2%	1 0.7%	5 3.5%	0.51
- Cough N(%)	7 4.9%	2 1.4%	5 3.5%	0.17
- Fever N(%)	21 14.7%	5 3.1%	16 12.6%	0.06
- Loss of appetite N(%)	50 35.0%	9 6.3%	41 28.7%	0.09
- Non-specific abdominal pain N(%)	123 86.0%	12 8.4%	111 77.6%	0.24
- Chronic diarrhea N(%)	86 60.0%	13 9.1%	73 51.0%	0.10
- Constipation N(%)	95 66.4%	13 9.1%	82 57.3%	0.26
- Diarrhea and constipation N(%)	85 59.4%	13 9.1%	72 50.3%	0.06
- Blood in stool N(%)	77 53.8%	8 5.6%	69 48.3%	0.79
- Mucus in stool N(%)	78 54.5%	8 5.6%	70 49.0%	0.79
- Blood and mucus in stool N(%)	76 53.1%	8 5.6%	68 47.6%	0.79
TB history				
- History of contact with TB patient N(%)	2 1.4%	1 0.7%	1 0.7%	0.21
- History of pulmonary and non-pulmonary TB N(%)	12 8.4%	2 1.4%	10 7.0%	0.62

**p* value: ITB group to non-ITB group

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171

172

173 The results of laboratory test in ITB and non-ITB groups are summarized in Table 2.
 174 There were significant differences in blood ADA activity and ESR, with p values from Mann
 175 Whitney U test were 0.01 and 0.00, respectively.

176 **Table 2. The Results of Laboratory Test of Subjects in ITB and Non-ITB Groups**

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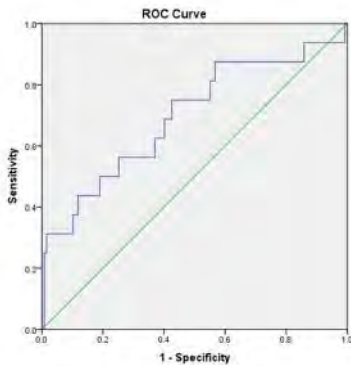
	Total n=143, 100%	Diagnosed with ITB n =16, 11.2%	Non-ITB n =127, 88.8%	<i>p</i> *
ADA level in blood (IU/L)	12.15 (2.95-45.57)	16.51(5.76-44.89)	12.11 (2.95-45.57)	0.01
Leukocyte (10 ³ /μL)	6.99 (2.09-23.84)	7.63(2.09-11.85)	6.98(2.90-23.84)	0.65
Basophil (%)	0.5 (0-2)	0.50(0.00-1.40)	0.5 (0.00-2.00)	0.35
Eosinophil (%)	1.9 (0-24)	1.80(0.00-8.10)	1.9 (0-24)	0.94
Neutrophil (%)	61.30(33.10-87.70)	63.85(38.50-86.60)	61.00(33.10-87.70)	0.52
Lymphocyte (%)	28.30(5.60-53.20)	25.70(7.00-51.60)	29.10(5.60-53.20)	0.52
Monocyte (%)	6.6 (3.3-15.9)	7.20(3.30-12.90)	6.60(3.50-15.90)	0.67
NLR	2.15(0.67-15.37)	2.48(0.75-11.40)	2.12(0.67-15.37)	0.49
MLR	0.22 (0.09-2.84)	0.29(0.09-1.79)	0.22 (0.09-2.84)	0.40
ESR (mm/hour)	28 (1-140)	65(9-140)	26(1.00-134.00)	0.00

180 *p value: ITB group to non-ITB group

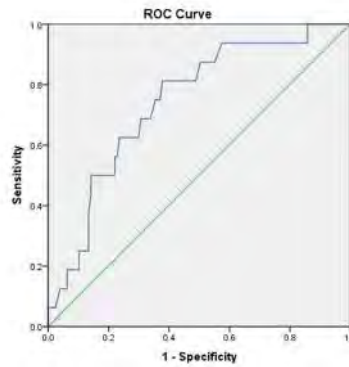
181 **The Result of Blood ADA Test**

182 The median of ADA activity in blood of all subjects, ITB group, and non-ITB group
 183 were 12.15 (2.95-45.57) IU/L, 21.38 (± 12.79) IU/L, and 12.11 (2.95-45.57) IU/L,
 184 respectively. Blood ADA activity in ITB and non-ITB groups were higher and significantly
 185 different (p=0.01). In the ROC curve analysis, and AUC of 0.695 was obtained with a cut-off
 186 of 12.56 IU/L, sensitivity of 75%, and specificity of 57% (Figure 1(a) and Table 3).

187



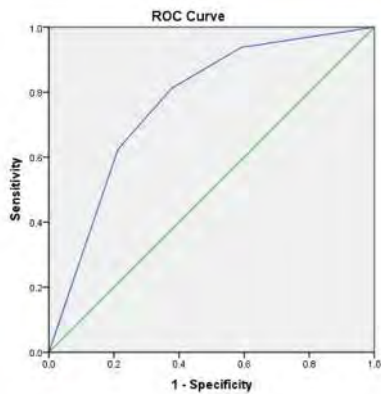
(a)



(b)

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(c)

Figure 1. ROC curve for (a) blood ADA test, (b) ESR test, and (c) ADA+ ESR tests

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Table 3. Blood ADA Diagnostic Test

Result of Blood ADA (cut-off 12.56 IU/L)	Gold Standard		
	ITB	Non-ITB	Total
Positive	12	54	66
Negative	4	73	77
Total	16	127	143
- Sensitivity	= 75%		
- Specificity	= 57%		
- Positive Predictive Value (PPV)	= 18%		
- Negative Predictive Value (NPV)	= 95%		

192

Haematology Test Results

193
194 The result of leukocyte count from all subjects, ITB group, and non-ITB group showed
195 the following median and mean respectively 6.99 (2.09-23.84) $10^3/\mu\text{L}$, 7.6 (\pm 3.2) $10^3/\mu\text{L}$,
196 and 7.7 (2.9-23.84) $10^3/\mu\text{L}$ (Table 2). There was no significant difference between the ITB
197 and non-ITB groups in leukocyte count, leukocyte differential count (for basophil, eosinophil,
198 neutrophil, lymphocyte, monocyte), NLR and MLR with p value 0.65, 0.35, 0.94, 0.52, 0.52,
199 0.67, 0.49 and 0.40, respectively (Table 2).

200 The median of ESR of all subjects, ITB group, and non-ITB group were 28(1-140)
201 mm/hour, 65.87(\pm 38.1) mm/hour, and 36.75(\pm 33) mm/hour, respectively (Table 2). ESR
202 result in ITB and non-ITB groups were higher and significantly different ($p=0.00$). In the

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203 ROC curve analysis, an AUC of 0.741 was obtained with a cut-off of 32.5 mm/hour,
204 sensitivity of 81%, and specificity of 62% (Figure 1 (b) and Table 4).

Table 4. Blood ESR Diagnostic Test

ESR Test Result (cut-off 32.5 mm/hour)	Gold Standard		
	ITB	Non-ITB	Total
Positive	13	48	61
Negative	3	79	82
Total	16	127	143

- Sensitivity = 81%
- Specificity = 62%
- Positive Predictive Value (PPV) = 21%
- Negative Predictive Value (NPV) = 96%

205

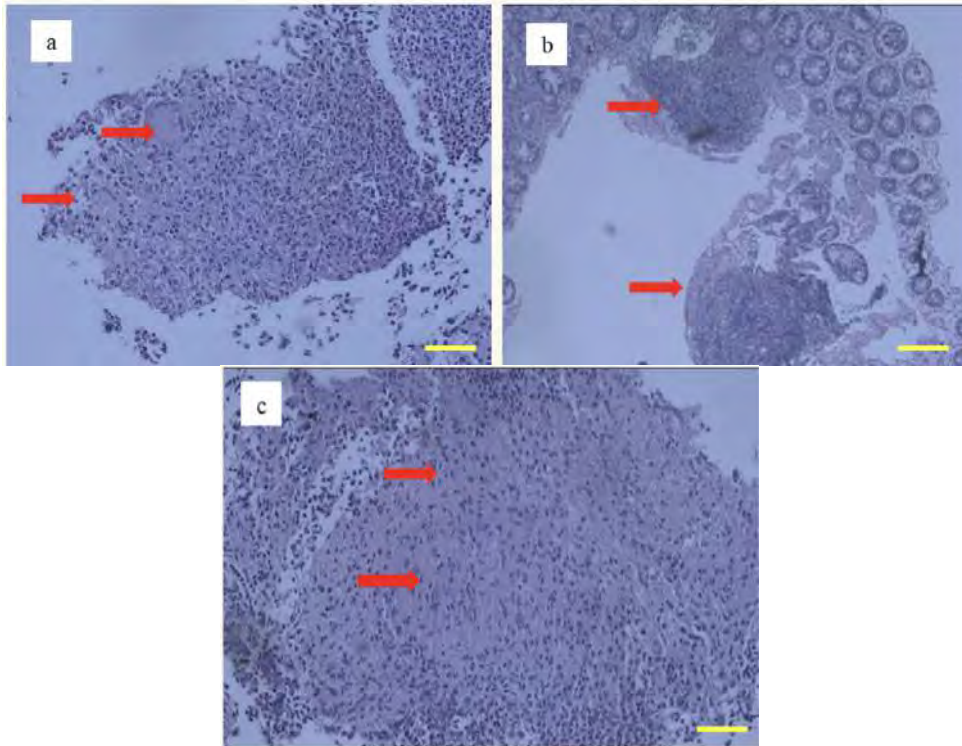
206 **Combination of Blood Biomarkers**

207 From the blood ADA and ESR tests, a combination of tests was performed to diagnose
208 ITB with a cut-off of 12.56 IU/L and 32.5 mm/hour, respectively. Subsequently, ROC curve
209 analysis was performed and an AUC of 0.768 (CI 0.656-0.880) (Figure 1 (c)), sensitivity of
210 81.3%, and specificity of 62.2 % were obtained.

211

212 **Histopathological Result**

213 The histopathological result several patients with confirmed ITB showed complete
214 overview of ITB markers in the form of granulomatous inflammation, epitheloid cells, giant
215 cells (Datia Langhans), and lymphocyte aggregates (Figure 2), while some other subjects
216 showed incomplete and varied overview.



217

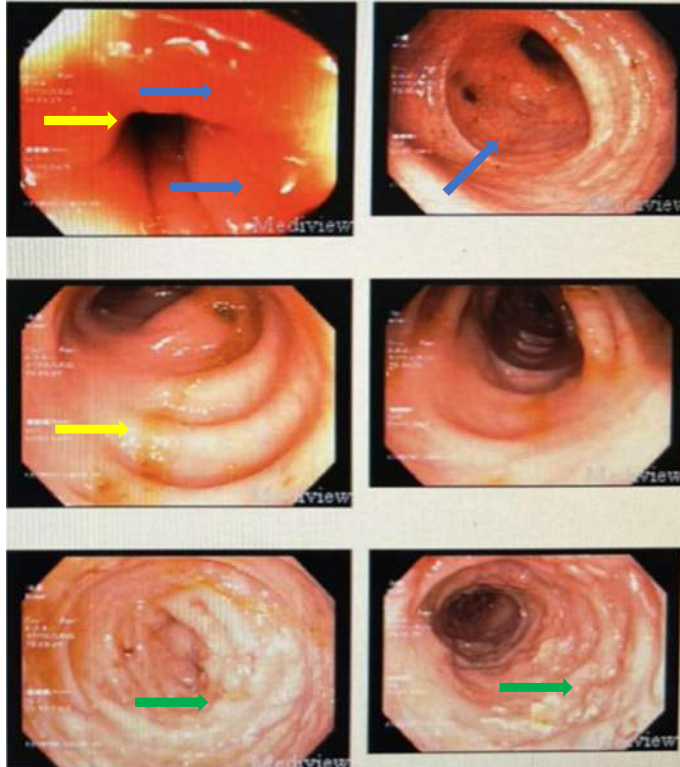
218

219 **Figure 2.** Histopathological result of subjects with confirmed ITB a. Overview of Datia Langhans
220 cells (red arrows) b. Overview of lymphoid aggregates (red arrows) c. Overview of epitheloid areas (red
221 arrows). Yellow bar: 20x magnification.

222

223 Colonoscopy Result

224 The colonoscopy result of patients with ITB in this study showed multiple ulceration,
225 oedema, and hyperaemic mucosa in various areas, such as descending colon, transverse
226 colon, ascendent colon, caecum, and terminal ileum as seen in Figure 3.



227

228 **Figure 3.** Colonoscopy result in patient with ileocolitis who were suspected of having TB showed multiple
229 ulceration (green arrow), oedema (yellow arrow), and hyperaemic intestinal mucosa (blue arrow).
230

231 Discussion

232 A total of 143 subjects, 16 (11.2%) subjects were diagnosed with ITB and 127 (88.8%)
233 subjects were non-ITB. The proportion of ITB in this study was 11.2%, which is accordance
234 with the ITB prevalence of 3-16%.¹ The proportion of ITB in XXX in this study is almost the
235 ~~same as Suparmin et al study, i.e., 8/60 (13.3%).⁵~~

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236 The age and sex characteristics of the subjects in this study is almost the same as
237 Suparmin et al. The mean age of the subjects in TB and non-TB colitis groups in the study
238 conducted by Suparmin et al were 38.6 (\pm 15.76) years and 49.7 (\pm 17.96) years. The mean
239 age of the subjects of this study in TB and non-TB colitis groups were 37 (\pm 17.9) years and
240 44 (\pm 16.6) years. The majority of sex characteristic in this study were females (65%), same
241 with Suparmin et al (55%)⁵.

242 In this study, there were significant differences in blood ADA enzyme activity between
 243 ITB and non-ITB groups (p= 0.011) with ADA activity of 21.38 (± 12.79) IU/L and 12.11
 244 (2.95-45.57) IU/L, respectively. The sensitivity and specificity of blood ADA test were 75%
 245 and 57%, respectively, with a cut-off of 12.56 U/L. There are limited reports regarding ADA
 246 test in patients with ITB. Most reports were reporting ADA test in patients with pulmonary
 247 TB and abdominal TB with ascites (Table 5).

248 The comparison of sensitivity and specificity of blood ADA test in this study and other
 249 studies can be seen in Table 5. The result of sensitivity and specificity of blood ADA test in
 250 this study are in line with the result obtained by Salmanzadeh et al¹⁴ in pulmonary TB cases,
 251 i.e., 77% and 70% with ADA cut-off point of 15.5 U/L. However, the result of sensitivity and
 252 specificity in this study are different with Stevanovic et al²¹, i.e., 56% and 89% in
 253 extrapulmonary TB subjects with a cut-off of 24 U/L. The differences might be Stevanovic et
 254 al used the reference value from the test kit (24 U/L) and did not use a new cut-off that might
 255 be optimal for ADA test in extrapulmonary TB.

256

257 **Table 5. Comparison of ADA activity in various TB patients**

258

259 **DISCUSSION WITH TABLE?**

260

No.	Study	Patient	Specimen	Cut-off (U/L)	Sensitivity	Specificity
1	This study	Intestinal TB	Serum	12.56	75%	57%
2	Salmanzadeh et al ¹⁴	Pulmonary TB	Serum	15.5	77%	70%
3	Stevanovic et al ²¹	Extrapulmonary TB (Peritonitis TB, Spondylitis TB, lymph node TB, ovarian, Tuba fallopi, renal, etc.)	Serum	24	56%	89%
4	Pandey R et al ²⁴	Pulmonary TB, extrapulmonary TB, healthy controls	Serum	25	90.7%	100%

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262 ADA consists of 2 isoenzymes, namely ADA1 and ADA2. ADA1 is an intracellular
263 protein that is expressed mainly by T, B lymphocytes, and natural killer cells. ADA1 plays a
264 role in the immune mechanism. ADA2 is an intracellular protein that is expressed primarily
265 by monocytes and macrophages. ADA2 activity is increased in tuberculous pleural effusion,
266 cerebrospinal fluid, and autoimmune disease. ADA activity in T lymphocytes was 10-12
267 times higher than in B lymphocytes. ADA activity was reported to increase in TB patients
268 due to T cell stimulation by MTB antigen.^{22,23}

269 In ITB patients, when granulomas rupture and release MTB, macrophages in intestinal
270 tissue will phagocytize MTB and present MTB antigen to T lymphocytes. Subsequently, T
271 lymphocytes will be activated to produce ADA at the time of proliferation and differentiation
272 of T lymphocytes. Apart from T lymphocytes, ADA is also produced by monocytes in the
273 process of maturation into macrophages. Therefore, ADA can be used to diagnose ITB.

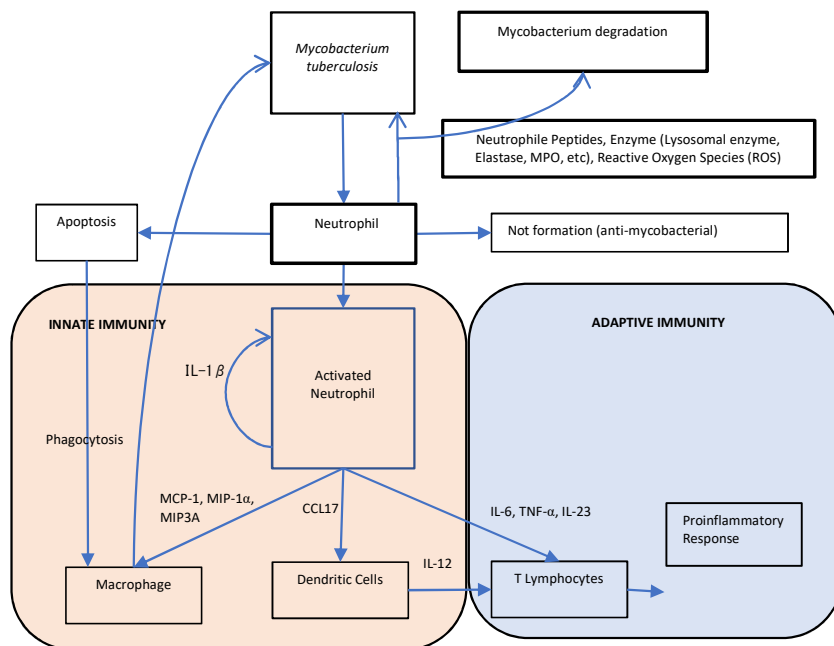
274 The cut-off value of ADA test have a lot of varies. Therefore, it is need to be determine
275 a suitable cut-off value according to the patient population. The cut-off value of the ADA kit
276 in this study was the same with Stevanovic et al²¹, i.e., 24 U/L, while Salmazadeh et al¹⁴ and
277 this study obtained cut-off values of 15.5 U/L and 12.56 U/L, respectively. Pandey et al²⁴
278 used a higher cut-off value for ADA serum, i.e., 25 U/L.

279 There were no significant differences in leukocyte count, leukocyte differential count,
280 neutrophil to lymphocyte ratio (NLR), and monocyte to lymphocyte ratio (MLR) between
281 ITB and non-ITB groups. Reports on leukocyte count, leukocyte differential count, NLR, and
282 MLR in ITB cases are still rare. Most reports on leukocyte count, leukocyte differential
283 count, NLR, and MLR are in pulmonary TB cases. The results of leukocyte count and
284 leukocyte differential count in this study are in line with Abay et al.²⁵ The results of
285 leukocyte count and leukocyte differential count that reported by Abay et al were not
286 significantly different.²⁵

287 The NLR result of this study did not show any significant difference between ITB and
 288 non-ITB groups. It could possibly because in this study, the ITB cases were chronic which
 289 were supported by high ESR results of the subjects, while NLR plays more role in early cases
 290 of acute inflammation/infection or early TB infection. The results of this study is in line with
 291 those obtained by Rees et al²⁶ in subjects with pulmonary TB, but it is not in accordance with
 292 the report of Sulastri et al²⁷ who got a higher and significant NLR in the pulmonary TB
 293 group.

294 Neutrophil to lymphocyte ratio (NLR) is an indicator of acute inflammation. Neutrophil
 295 plays a role in the MTB killing process in the early days of infection. MTB germs that enter
 296 the body will stimulate neutrophils and phagocyte MTB. Neutrophils kill MTB by secreting
 297 various enzymes and peptides that are present in its granules (lysosomal enzymes, neutrophil
 298 peptides, and reactive oxygen species (ROS)). Activated neutrophils produce IL-8, IL-1 β ,
 299 and IFN- γ cytokines which in turn activate monocytes/ macrophages, dendritic cells, and
 300 lymphocytes. Neutrophils will release Neutrophil Extracellular Traps (NET) to the circulation
 301 which will trap MTB germs. Neutrophil response is related to the degree of inflammation and
 302 tissue damage. Inadequate neutrophil phagocytosis of MTB will result in damage to host cells
 303 causing tissue necrosis. Inflammatory processes that last a long time will result in chronic
 304 inflammation and damage to body tissues. (Figure 4).²⁸

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Figure 4. The role of neutrophils in TB infection (modification).²⁸

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There were no significant differences in MLR result between ITB and non-ITB groups in this study. The results of this study are in line with those reported by Rees et al²⁶ who did not find any significant differences in MLR. However, a systematic review by Adane et al³¹ reported significant differences of MLR in patients with TB. These varied results of MLR studies depended on the origin of subject's location, existence of any comorbidities (HIV, diabetes mellitus), age, TB treatment, and the selected control group.²⁶

Monocytes play a role in natural/nonspecific immune responses, while lymphocytes play a role in specific immune responses. Monocytes or macrophages in ITB play a role in non-specific immune responses in the form of phagocytosis against MTB. Subsequently, activated macrophages secrete pro-inflammatory cytokines to recruit monocytes and dendritic cells to the site of infection.²⁹⁻³² Monocytes further differentiate into blood-derived macrophages, so that MTBs can be internalized, but not destroyed. In that phase, blood-derived macrophages accumulate, and MTB grows logarithmically.³³ Dendritic cells activate the immune response by carrying MTB to the lymph nodes and presenting it to T lymphocytes. Furthermore, IL-12 secreted by macrophages activates lymphocytes T (especially the TH1 subset of CD4+ T lymphocytes) to produce IFN- γ , so that macrophages can kill MTB (Figure 4). The accumulation of blood-derived macrophages and activation of T lymphocytes will induce the formation of granulomatous lesions in the form of epithelioid tubercles.³³

337 ESR test result in the ITB group was higher and significant when compared to the non-
 338 ITB group with results of 65.87 (\pm 38.1) mm/hour and 36.75 (\pm 33) mm/hour, respectively.
 339 The sensitivity and specificity of ESR test was 81% and 62%, respectively, with a cut-off of
 340 32.5 mm/hour. ESR test with high sensitivity (81%) can be used as a screening test in ITB.
 341 ESR report in ITB cases is still rare. Most ESR tests were reported in pulmonary TB cases.
 342 The overall ESR test results from various studies can be seen in Table 5.

343

344 **Table 5. Comparison of ESR result in various TB patients**

No	Study	Patient (mm/hour)	Comparator mm/hour	<i>p</i> *
1	This study	ITB patients 65.87 (\pm 38.1)	Non-ITB patients 36.75 (\pm 33)	0.00
2	Khalil et al ³⁴	Before pulmonary TB treatment 49.41 mm/hour	After pulmonary TB treatment 33.85 mm/hour	0.02
3	Bashir et al ³⁵	Pulmonary TB patients 115.17	Control 11.08	0.00
4	Chong ³⁶	Pulmonary TB 57 \pm 39	Abdominal TB 46 \pm 28	0.305

345

346 ESR test has been in use for a long time to assess acute phase proteins in chronic
 347 diseases, including TB disease. Pro-inflammatory cytokines produced in ITB disease will
 348 stimulate the liver to produce acute phase proteins, such as fibrinogen which can increase
 349 ESR result. ESR test has limitations, such as affected by anemic conditions and
 350 hypoalbuminemia which cause a false increase of ESR. Therefore, when using ESR test in
 351 patients with ITB, it is necessary to pay attention to the condition of anemia and
 352 hypoalbuminemia.³⁴⁻³⁶

353

354 **Combination of Blood Biomarkers**

355 The combination of ADA serum and ESR test with cut-offs of 12.56 IU/L and 32.5
 356 mm/hour, respectively, did not result in a significant increase in sensitivity and specificity

357 when compared to each of ADA or ESR. Therefore, each ADA or ESR test can be used
358 independently to assist in diagnosing ITB.

359

360 **Conclusion**

361 Blood ADA and ESR tests were significantly higher among ITB compared to Non ITB
362 groups. Blood ADA and ESR test have a high sensitivity value so that they are potential as
363 screening biomarkers for intestinal TB patients. There were no significant differences in
364 leukocyte count, leukocyte differential count, neutrophil to lymphocyte ratio (NLR), and
365 monocyte to lymphocyte ratio (MLR) between ITB and non-ITB groups.

366

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Reviewer 2



Manuscript Review Form

Reviewer	: Reviewer 3
Manuscript #	: M2023128
Manuscript Title	: Blood Adenosine Deaminase and Complete Hematology Test as Potential Candidates for Laboratory Tests in Intestinal Tuberculosis

No.	Manuscript Components	Yes	No
1.	Does this manuscript present new ideas or results that have not been previously published?		√
	Notes: Other research that has been published: Diagnostic performance of adenosine deaminase for abdominal tuberculosis: A systematic review and meta-analysis. https://doi.org/10.3389/fpubh.2022.938544 Assessment of adenosine deaminase levels and lymphocyte counts in tuberculosis ascites. https://doi.org/10.53730/ijhs.v6nS3.7494		
2.	Are the title and abstract of the manuscript appropriate?	√	
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8.	Is there a logical flow of argument in the Discussion which elucidate all the presented/obtained data?	√	
	Notes:		
9.	Are the conclusions and interpretations valid and supported by the data?	√	
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10.	Is the manuscript clear, comprehensible, and written in a good English structure?	√	
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Reviewer 3



Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2023128 Editor Decision Round 1 - Resubmit for Review

Ferry Sandra <ferry@trisakti.ac.id>

Tue, Aug 15, 2023 at 9:32 AM

To: Secretariat of InaBJ <secretariatinabj@gmail.com>

Dear Secretariat of The Indonesian Biomedical Journal,

Please find the revision of manuscript M2023128. I sincerely apologize for the delay of the revision. I have made a major revision to the manuscript and all the comments from reviewers are incorporated accordingly.

Thank you.

Regards,
Ferry Sandra

[Quoted text hidden]

--

Ferry Sandra, D.D.S., Ph.D.
Head of Medical Research Center
Universitas Trisakti

**Round 1 Revision from Author.docx**

3817K

1 **High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal**
2 **Tuberculosis Patients**

3
4 **Abstract**

5 **Background:** Currently, laboratory diagnosis of intestinal tuberculosis (ITB) is limited based
6 on clinical manifestations, providing opportunities for alternative laboratory tests to diagnose
7 ITB. At the present time, the role of serum adenosine deaminase (ADA) and hematological
8 tests in ITB patients are not widely known. The objective of this study is to determine the role
9 of ADA and hematological tests in patients suspected with ITB.

10 **Method:** In this study, a total of 143 subjects were recruited. Subjects that were suspected of
11 ITB were classified as ITB group, while subjects with inflammatory bowel disease,
12 hemorrhoid, and intestinal malignancy were classified as non-ITB group. Colonoscopy and
13 histopathological examinations, as well as ADA measurement and hematological test were
14 performed.

15 **Result:** Out of 143 subjects, 16 (11.2%) subjects were diagnosed with ITB and 127 (88.8%)
16 subjects were classified as non-ITB group. ADA level and erythrocyte sedimentation group
17 (ESR) of ITB groups were significantly higher than the ones of non-ITB group ($p<0.05$). Cut-
18 off, sensitivity, and specificity of ADA level were 12.56 IU/L, 75%, and 57%, respectively.
19 Cut-off, sensitivity, and specificity of ESR were 32.5 mm/hour, 81%, and 62%, respectively.
20 Colonoscopy of ITB subjects displayed multiple ulcerations, edema, and hyperemic mucosa.
21 Histopathological examination of ITB subjects exhibited granulomatous inflammation,
22 epitheloid cells, giant cells, and lymphocyte aggregates.

23 **Conclusion:** ADA level and ESR were significantly higher among ITB patients compared
24 with non-ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and
25 ESR tests could be considered as a screening test for ITB.

26 **Keyword:** intestinal tuberculosis, adenosine deaminase, hematological tests
27

28 Introduction

29 According to the Global Tuberculosis Report 2019 from the World Health Organization
30 (WHO), Indonesia was ranked third among the countries with highest burden of tuberculosis
31 (TB).^{1,2} The global prevalence of extrapulmonary TB was 14% and the prevalence in
32 Indonesia was $\leq 9.9\%$. Intestinal tuberculosis (ITB), as a part of abdominal TB, had a
33 prevalence of approximately 3–16%.³⁻⁵

34 Currently, ITB diagnosis was established based on clinical manifestations, Ziehl-
35 Neelsen (ZN) acid fast bacilli (AFB) stain of the stool, fecal culture, colonoscopy, and
36 histopathology.^{3,6-10} The limitations of the respective laboratory pose difficulties to diagnose
37 ITB, and hence, providing opportunities for alternative laboratory tests to diagnose ITB.¹¹

38 Adenosine deaminase (ADA) is an enzyme involved in purine metabolism which
39 catalyzes the conversion of adenosine to inosine. ADA is also involved in the proliferation
40 and differentiation of lymphocytes and maturation of monocytes.^{11,12} Elevation ADA levels
41 in blood or body fluid during tuberculosis infection is due to the stimulation of T
42 lymphocytes by *Mycobacterium tuberculosis* (MTB) antigen.^{12,13} The activity of ADA differs
43 significantly between patients with pulmonary TB and the control group.^{14,15}

44 In ITB, acute or chronic inflammation is present due to infection process.
45 Hematological tests are often requested by clinicians in patients suspected of having ITB, as
46 several parameters can be utilized as markers of inflammation or infection. Some of them
47 those are being included: leukocyte count, leukocyte differential count (basophil, eosinophil,
48 neutrophil, lymphocyte, and monocyte), Neutrophil to Lymphocyte Ratio (NLR), Monocyte
49 to Lymphocyte Ratio (MLR), and Erythrocyte Sedimentation Rate (ESR).^{3,6,16,17} At the
50 present time, the role of serum ADA and hematological tests in ITB patients are not widely
51 known. The objective of this study is to determine the role of ADA and hematological tests in
52 patients suspected with ITB.

53

54 **Methods**

55 **Study Design and Subjects**

56 Subjects were recruited at the Gastroenterology Outpatient Clinic, Department of
57 Internal Medicine, Gastrointestinal Endoscopy Center of Dr. Cipto Mangunkusumo Central
58 General Hospital from December 2020 until December 2022, with the inclusion criteria as
59 follows: ≥ 18 years old and suspected ITB. Meanwhile the exclusion criteria: being treated
60 with anti-tuberculosis drugs for >3 months, post-treatment with anti-tuberculosis drugs for <6
61 months.

62 Patients were suspected of ITB if 3 out of 4 main clinical findings were present, and 1
63 out of 3 additional history criteria for ITB was met. The main clinical findings of ITB include
64 weight loss, non-specific abdominal pain, fever of unknown origin, and chronic diarrhea or
65 constipation. The additional history includes history of pulmonary TB, active pulmonary TB
66 with or without anti-tuberculosis drugs treatment (<3 months of treatment), and positive
67 contact history with TB patients. Patients with inflammatory bowel disease (Crohn's disease
68 or ulcerative colitis), hemorrhoid, and intestinal malignancy were classified as non-ITB
69 group.

70 Prior to the recruitment, all subjects were explained and asked for their consents of
71 participation by signing an informed consent form. The research protocol was approved by
72 the Health Research Ethics Committee - Faculty of Medicine Universitas Indonesia and Dr.
73 Cipto Mangunkusumo Central General Hospital (#KET-
74 1498/UM2.F1/ETIK/PPM.00.02/2020).

75

76 **Colonoscopy Examination**

77 After being given laxatives, sedatives and analgesics, subjects lied in a supine position
78 for a colonoscopy insertion. If abnormalities were found, biopsies were taken for

79 histopathological examination. Various features could be identified during colonoscopy of
80 patient with suspected ITB, such as ulceration, nodules, polyps, narrowing of the lumen, and
81 irregular multiple fibrous bands. ITB may affect extensive parts of the intestine, in the form
82 of continuous lesions or single or skip lesions.¹⁶

83

84 **Histopathological Examination**

85 Samples for examination were harvested from at least 5 granulomas originating from
86 a single segment. After tissue fixation, grossing, embedding and slicing, tissue sections were
87 stained with hematoxylin (Epredia, Kalamazoo, MI, USA) and eosin (Merck, Darmstadt,
88 Germany), and mounted. Prepared tissue slides will be read by an anatomic pathologist.

89

90 **ADA Test**

91 Nine mL of venous blood was drawn from the cubital vein. Three mL were collected in
92 an EDTA tube for complete hematological test, while 6 mL in non-anticoagulated tube for
93 ADA test.

94 Blood in a non-anticoagulated tubes were allowed to stand at room temperature for
95 about 30 minutes, until blood clot formed. Immediate centrifugation of 3,000 revolutions per
96 minute (rpm) for 15 minutes was performed to separate the serum from the rest of the
97 remaining sample.

98 Quantitative test of blood ADA was performed by BS-200 automated clinical chemistry
99 analyzer (Mindray, Shenzhen, China). The principle the test used was enzymatic colorimetry.
100 The measurement was determined by the increase of photometric absorbance at a wavelength
101 of 546 nm.

102

103 **Hematological Test**

104 Hematology test was performed by XN-2000 hematology analyzer (Sysmex, Kobe,
105 Hyogo, Japan) with semiconductor laser flowcytometry. K₃EDTA blood was diluted by the
106 reagent that lysed erythrocytes and perforated the cytoplasmic membrane of all nucleated
107 cells. This process allowed fluorescent dyes to enter the cells and bind to the nucleic acids of
108 the cells. ESR measurement was performed using Starrsed RS automated ESR analyzer
109 (Sysmex).

110

111 **Statistics**

112 Data were processed using SPSS software version 20 and Microsoft Excel 2016. Data
113 distribution was checked using Kolmogorov-Smirnov test. Bivariate analysis was performed
114 to investigate the relationship between two measured variables. Analysis of the difference
115 between the two groups in the abnormal data distribution was done by using the Mann-
116 Whitney U Test. The level of significance used was $p < 0.05$.

117 The calculation of cut-off and Area Under the Curve (AUC) was obtained from the
118 ROC (Receiver Operating Characteristic) curve. Sensitivity and specificity of the
119 examination compared to the gold standard (combined results of histopathology,
120 colonoscopy, and therapeutic response) were then calculated.

121

122 **RESULTS**

123 A total of 143 subjects, 16 (11.2%) of them were diagnosed with ITB, and 127 (88.8%)
124 subjects were non-ITB (Table 1). In percentage, the ITB group had higher weight loss, loss of
125 appetite, chronic diarrhea, and constipation.

126

127 **ADA and ESR of ITB and non-ITB groups**

128 From the results of laboratory test, ADA level and ESR of ITB groups were
 129 significantly higher than the ones of non-ITB group (Table 2). Meanwhile, all other
 130 hematological parameters of ITB and non-ITB groups were almost similar.

131 The median of ADA level in blood of ITB and non-ITB groups were 16.51 (11.82-
 132 35.61) IU/L, and 12.11 (8.89-15.99) IU/L, respectively (Table 2). In the ROC curve analysis,
 133 AUC of 0.695 (95% CI 0.542-0.849) was obtained with a cut-off of 12.56 IU/L. Sensitivity of
 134 ADA were 75%, and specificity of 57% (Table 3).

135 The median of ESR of ITB group and non-ITB group were 65 (33.50-92.50) mm/hour
 136 and 26 (13.00-45.00) mm/hour, respectively (Table 2). In the ROC curve analysis, AUC of
 137 0.741 (95% CI 0.623-0.860) was obtained with a cut-off of 32.5 mm/hour. Sensitivity of ESR
 138 was 81%, and specificity of 62% (Table 4).

139 ROC analyses were conducted for the combination of ADA and ESR testing. For the
 140 utilization of ADA and ESR in diagnosing ITB, the cut-off was 12.56 IU/L and 32.5
 141 mm/hour, respectively. Subsequently, ROC curve analysis was performed and AUC of 0.768
 142 (CI 0.656-0.880), sensitivity of 81.3%, and specificity of 62.2 % were obtained.

143

144 **Table 1. Characteristics of ITB (n=16) and non-ITB (n=127) Groups**

Characteristics	ITB		Non-ITB	
Age (years)	42.19±18.28		43.53 ±16.65	
Gender (n, %)				
- Male	10	62.5%	40	31.5%
- Female	6	37.5%	87	68.5%
Clinical manifestation (n, %)				
- Weight loss	13	81.3%	69	54.3%
- Night sweat	1	6.3%	5	3.9%
- Cough	2	12.5%	5	3.9%
- Fever	5	31.3%	16	12.6%
- Loss of appetite	9	56.3%	41	32.3%
- Non-specific abdominal pain	12	75.0%	111	87.4%

- Chronic diarrhea	13	81.3%	73	57.5%
- Constipation	13	81.3%	82	64.6%
- Blood in stool	8	50.0%	69	54.3%
- Mucus in stool	8	50.0%	70	55.1%
- Blood and mucus in stool	8	50.0%	68	53.5%
TB history (n, %)				
- History of contact with TB patient	1	6.3%	1	0.8%
- History of pulmonary and non-pulmonary TB	2	12.5%	10	7.9%

145

146 **Table 2. Laboratory Results of ITB (n=16) and Non-ITB (n=127) Groups**

Parameter	ITB	Non-ITB	<i>p</i>
ADA level (IU/L)	16.51 (11.82-35.61)	12.11 (8.89-15.99)	0.011*
Leukocyte (10 ³ /μL)	7.63 (4.85-10.96)	6.98 (5.58-8.45)	0.656
Basophil (%)	0.50 (0.30-0.70)	0.50 (0.40-0.80)	0.358
Eosinophil (%)	1.80 (0.80-4.15)	1.90 (1.00-3.70)	0.941
Neutrophil (%)	63.85 (49.97-74.22)	61.0 (54.10-71.00)	0.522
Lymphocyte (%)	25.70 (16.65-36.82)	29.10 (21.70-34.90)	0.520
Monocyte (%)	7.20 (4.62-8.32)	6.60 (5.40-7.90)	0.670
NLR	2.48 (1.44-4.49)	2.12 (1.56-3.31)	0.495
MLR	0.29 (0.15-0.44)	0.22 (0.19-0.29)	0.401
ESR (mm/hour)	65 (33.50-92.50)	26 (13.00-45.00)	0.002*

147 Mann-Whitney U test; **p*<0.05. NLR: Neutrophil to Lymphocyte Ratio, MLR: Monocyte to
 148 Lymphocyte Ratio, ESR: Erythrocyte Sedimentation Rate.

149

ADA Result	ITB	Non-ITB	Total
Positive	12	54	66
Negative	4	73	77
Total	16	127	143

- Sensitivity = 75%
- Specificity = 57%
- Positive Predictive Value (PPV) = 18%
- Negative Predictive Value (NPV) = 95%

150

151

152 **Table 4. ESR Results of ITB (n=16) and Non-ITB Groups (n=127) with cut-off: 32.5 mm/hour**

ESR Result	ITB	Non-ITB	Total
Positive	13	48	61
Negative	3	79	82
Total	16	127	143

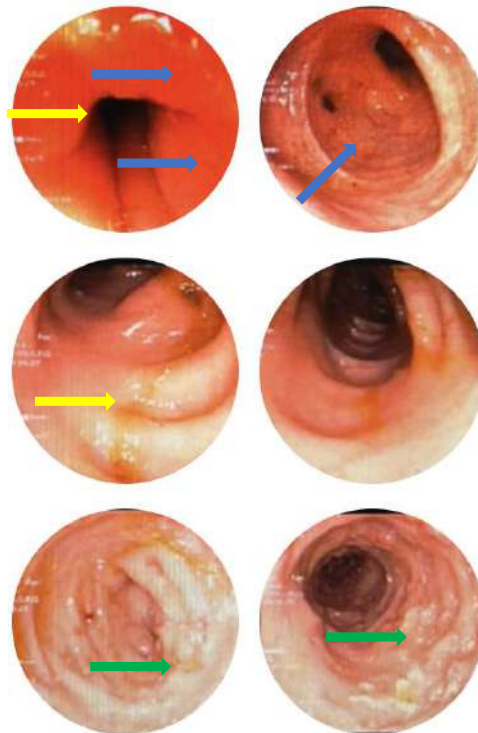
- Sensitivity = 81%

- Specificity = 62%
- Positive Predictive Value (PPV) = 21%
- Negative Predictive Value (NPV) = 96%

153
154

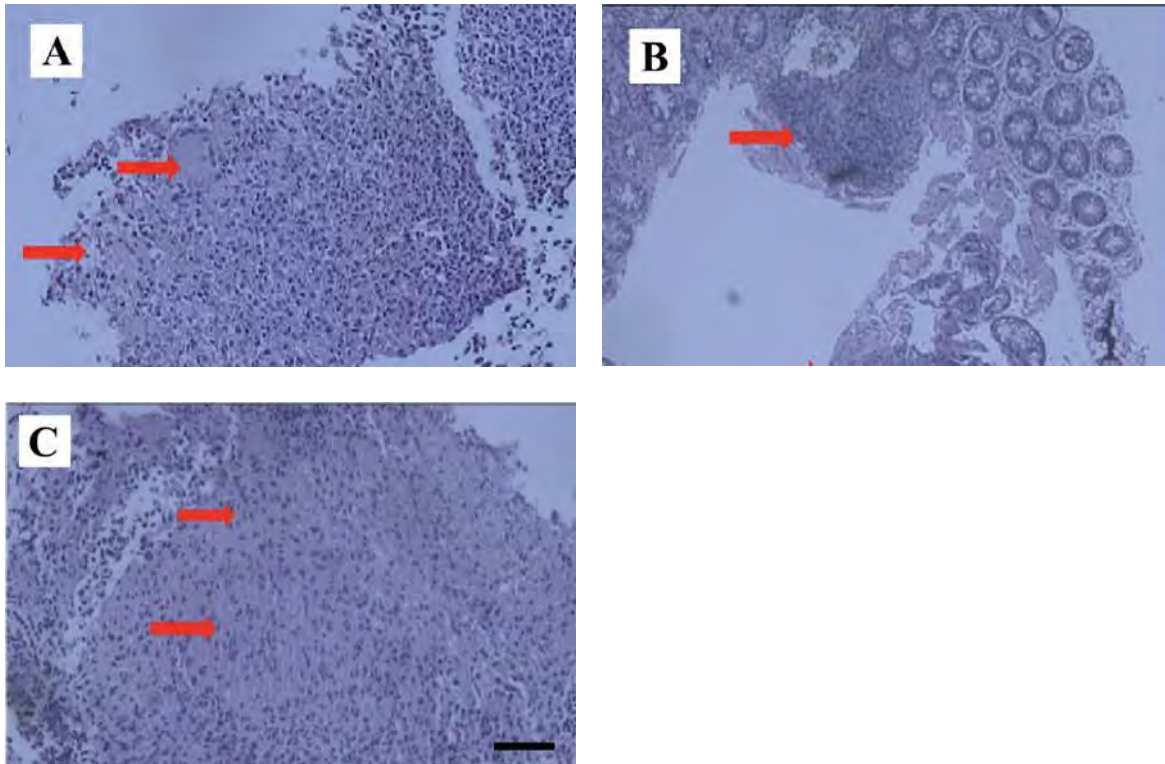
155 Colonoscopic and Biopsy Histopathological Appearances of ITB Subjects

156 In this study, colonoscopy of subjects with ITB displayed multiple ulcerations, edema,
157 and hyperemic mucosa in various areas, such as descending colon, transverse colon,
158 ascendent colon, caecum, and terminal ileum (Figure 1). Meanwhile, histopathological
159 examination of patients with confirmed ITB exhibited granulomatous inflammation,
160 epithelioid cells, giant cells (Datia Langhans), and lymphocyte aggregates (Figure 2). Some of
161 the other subjects showed incomplete and varied overview.



162

163 **Figure 1.** Colonoscopic results of ITB-suspected-subjects with ileocolitis. Blue arrow: hyperemic intestinal
164 mucosa; Yellow arrow: edema; Green arrow: ulceration.



165

166

167 **Figure 2.** Colonoscopic biopsy histopathological results of ITB group. A: Datia Langhans cells (red arrow); B:
168 Lymphoid aggregates (red arrow); C: Epitheloid areas (red arrow). Black bar: 50 μ m.
169
170

170

171 Discussion

172 There were 16 ITB subjects among all 143 recruited subjects from the Gastroenterology
173 Outpatient Clinic, Department of Internal Medicine, Gastrointestinal Endoscopy Center of
174 Dr. Cipto Mangunkusumo Central General Hospital. The subject population of 16/143
175 (11.2%) was in accordance with the previous reported ITB prevalence (3-16%).^{1,18}

176 ADA level was significantly higher in ITB group compared with the one of non-ITB
177 group. The sensitivity and specificity of ADA test were 75% and 57%, respectively, with a
178 cut-off of 12.56 IU/L. These data could be useful for further investigation since in the present
179 time there is not any available data of ITB ADA level. Most studies reported ADA results of
180 patients with pulmonary tuberculosis and abdominal tuberculosis.^{15,19} The cut-off value of
181 ADA level in this study was lower than the one in Serbian study on extrapulmonary
182 tuberculosis (24 U/L)¹⁹ and Nepalese study on pulmonary/extrapulmonary tuberculosis (25
183 U/L).²⁰

184 There was no significant difference in leukocyte count and leukocyte differential count
185 between ITB and non-ITB groups in this study. These results are in accordance with the ones
186 in Ethiopian study, reporting that the counts between the two groups were not significantly
187 different.²¹

188 Although NLR may indicate an early TB infection, there was not any significant
189 difference between NLR of ITB and non-ITB groups in this study. This result was similar to
190 the findings reported by previous study.²² However, contradictory findings were reported in a
191 study in Bandung, Indonesia, whereas pulmonary TB subjects exhibited significantly higher
192 NLR.²³

193 There was no significant difference in MLR between ITB and non-ITB groups in this
194 study. Both similar²² and contradictive findings²⁴ in the MLR of patients with TB, have been
195 reported which might be influenced by the study site, existence of any comorbidities (HIV,
196 diabetes mellitus), age, and TB treatment.²²

197 Compared with non-ITB group (26 (13.00-45.00) mm/hour), the ITB group had
198 significant higher ESR (65 (33.50-92.50) mm/hour). Similarly, a previous study reported that
199 ESR was significantly higher in patients with pulmonary TB compared with those without
200 TB.²⁵ In this study, high sensitivity of ESR (81%) was calculated, which could be promoted
201 as a screening test for ITB. ESR test has been used for a long time to assess acute phase
202 proteins in chronic diseases, including TB. Pro-inflammatory cytokines produced in ITB will
203 stimulate the liver to produce acute phase proteins, such as fibrinogen which may lead to an
204 increase in ESR. ESR measurement has limitations, such as being affected by anemic
205 conditions and hypoalbuminemia, which cause a false-high ESR.²⁵⁻²⁷

206 The combination of ADA and ESR test results with cut-offs of 12.56 IU/L and 32.5
207 mm/hour, respectively, did not provide a significant increase in sensitivity and specificity

208 compared to individual ADA or ESR test result. Therefore, each of ADA or ESR test can be
 209 used independently to assist the diagnose of ITB.

210

211 **Conclusion**

212 ADA level and ESR were significantly higher among ITB patients compared with non-
 213 ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests
 214 could be considered as a screening test for ITB.

215

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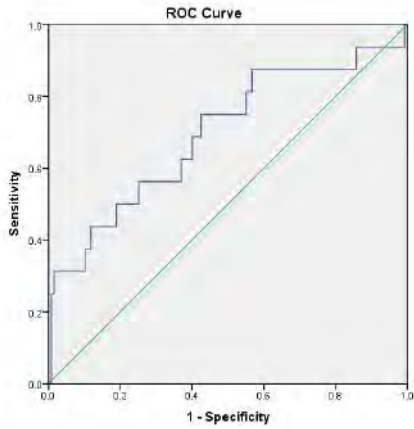
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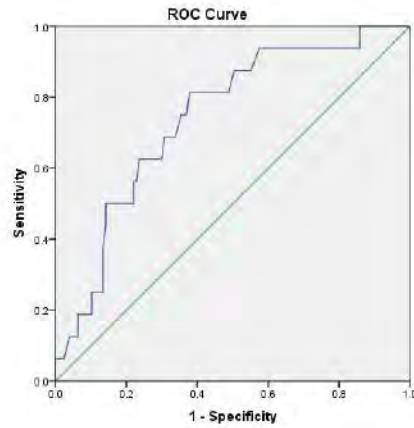
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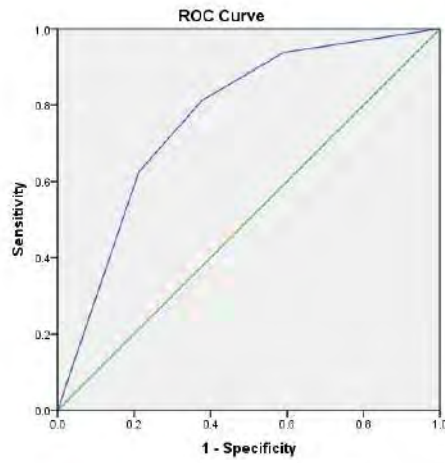
297 **Supplementary 1.**
298 **ROC curve for (a) ADA test, (b) ESR test, and (c) ADA+ ESR tests**
299



(a)



(b)



(c)

300

301

302



Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2023128 Editor Decision Round 2 - Revisions Required

Secretariat of InaBJ <secretariat@inabj@gmail.com>
To: ferry@trisakti.ac.id

Fri, Aug 18, 2023 at 7:30 AM

Dear Dr. Ferry Sandra,

Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "**High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients**".

Our decision is: **Revisions Required.**

Find the file attached to see detailed comments from reviewers. Please make sure you read all the comments and revise the manuscript based on the suggestions given.

Revise this manuscript thoroughly before **August 25, 2023**. Mark/highlighted the revised part of the manuscript, so that the editor will notice the changes.

When you are done, you can upload it in: <https://inabj.org/index.php/ibj/author/submissionReview/2406>, or simply send us an email of your revised manuscript and response letter.

Please let us know when you have received this email. If you have any questions, do not hesitate to contact us. Thank you for your attention. We wish you a nice day.

Best Regards,

--

Secretariat of The Indonesian Biomedical Journal

Prodia Tower 9th Floor

Jl. Kramat Raya No.150, Jakarta 10430, Indonesia

Phone. +62-21-3144182 ext. 3872

Fax. +62-21-3144181

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High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients

Abstract

Background: Currently, laboratory diagnosis of intestinal tuberculosis (ITB) is limited based on clinical manifestations, providing opportunities for alternative laboratory tests to diagnose ITB. At the present time, the role of serum adenosine deaminase (ADA) and hematological tests in ITB patients are not widely known. The objective of this study was to determine the role of ADA and hematological tests in patients suspected with ITB.

Method: In this study, a total of 143 subjects were recruited. Subjects that were suspected of ITB were classified as ITB group, while subjects with inflammatory bowel disease, hemorrhoid, and intestinal malignancy were classified as non-ITB group. Colonoscopy and histopathological examinations, as well as ADA measurement and hematological test were performed.

Result: Out of 143 subjects, 16 (11.2%) subjects were diagnosed with ITB and 127 (88.8%) subjects were classified as non-ITB group. ADA level and erythrocyte sedimentation rate (ESR) of ITB groups were significantly higher than the ones of non-ITB group ($p < 0.05$). Cut-off, sensitivity, and specificity of ADA level were 12.56 IU/L, 75%, and 57%, respectively. Cut-off, sensitivity, and specificity of ESR were 32.5 mm/hour, 81%, and 62%, respectively. Colonoscopy of ITB subjects displayed multiple ulcerations, edema, and hyperemic mucosa. Histopathological examination of ITB subjects exhibited granulomatous inflammation, epithelioid cells, giant cells, and lymphocyte aggregates.

Conclusion: ADA level and ESR were significantly higher among ITB patients compared with non-ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests could be considered as a screening test for ITB.

Keyword: intestinal tuberculosis, adenosine deaminase, hematological tests

Comment [NMD1]: Need some explanation regarding the association of ADA and ITB, why choosing ADA as the marker?

Comment [NMD2]: Suggestions: "In this study, suspected ITB subjects, and non-ITB subjects with inflammatory bowel disease, hemorrhoid, and intestinal malignancy were recruited."

No need to mention the number because in the Methods section, you did not mention the total number of section. Hence, you can mention it in the Results.

Comment [NMD3]: Mention the methods.

28 **Introduction**

29 According to the Global Tuberculosis Report 2019 from the World Health Organization
30 (WHO), Indonesia was ranked third among the countries with highest burden of tuberculosis
31 (TB).^{1,2} The global prevalence of extrapulmonary TB was 14% and the prevalence in
32 Indonesia was $\leq 9.9\%$. Intestinal tuberculosis (ITB), as a part of abdominal TB, had a
33 prevalence of approximately 3–16%.³⁻⁵

34 Currently, ITB diagnosis was established based on clinical manifestations, Ziehl-
35 Neelsen (ZN) acid fast bacilli (AFB) stain of the stool, fecal culture, colonoscopy, and
36 histopathology.^{3,6-10} The limitations of the respective laboratory pose difficulties to diagnose
37 ITB, and hence, providing opportunities for alternative laboratory tests to diagnose ITB.¹¹

38 Adenosine deaminase (ADA) is an enzyme involved in purine metabolism which
39 catalyzes the conversion of adenosine to inosine. ADA is also involved in the proliferation
40 and differentiation of lymphocytes and maturation of monocytes.^{11,12} Elevated ADA levels
41 in blood or body fluid during tuberculosis infection is due to the stimulation of T
42 lymphocytes by *Mycobacterium tuberculosis* (MTB) antigen.^{12,13} The activity of ADA differs
43 significantly between patients with pulmonary TB and the control group.^{14,15}

44 In ITB, acute or chronic inflammation is present due to infection process.
45 Hematological tests are often requested by clinicians in patients suspected of having ITB, as
46 several parameters can be utilized as markers of inflammation or infection. Some of them
47 those are ~~being~~ included: leukocyte count, leukocyte differential count (basophil,
48 eosinophil, neutrophil, lymphocyte, and monocyte), Neutrophil to Lymphocyte Ratio (NLR),
49 Monocyte to Lymphocyte Ratio (MLR), and Erythrocyte Sedimentation Rate (ESR).^{3,6,16,17} At
50 the present time, the role of serum ADA and hematological tests in ITB patients are not
51 widely known. The objective of this study wasis to determine the role of ADA and
52 hematological tests in patients suspected with ITB.

53

Comment [NMD4]: Define the control group based on the previous study. Is it normal healthy people, or is it other type of TB patients?

54 **Methods**

55 **Study Design and Subjects**

56 Subjects were recruited at the Gastroenterology Outpatient Clinic, Department of
57 Internal Medicine, Gastrointestinal Endoscopy Center of Dr. Cipto Mangunkusumo Central
58 General Hospital from December 2020 until December 2022, with the inclusion criteria as
59 follows: ≥ 18 years old and suspected ITB. Meanwhile the exclusion criteria: being treated
60 with anti-tuberculosis drugs for >3 months, post-treatment with anti-tuberculosis drugs for <6
61 months.

62 Patients were suspected of ITB if 3 out of 4 main clinical findings were present, and 1
63 out of 3 additional history criteria for ITB was met. The main clinical findings of ITB include
64 weight loss, non-specific abdominal pain, fever of unknown origin, and chronic diarrhea or
65 constipation. The additional history includes history of pulmonary TB, active pulmonary TB
66 with or without anti-tuberculosis drugs treatment (<3 months of treatment), and positive
67 contact history with TB patients. Patients with inflammatory bowel disease (Crohn's disease
68 or ulcerative colitis), hemorrhoid, and intestinal malignancy were classified as non-ITB
69 group.

70 Prior to the recruitment, all subjects were explained and asked for their consents of
71 participation by signing an informed consent form. The research protocol was approved by
72 the Health Research Ethics Committee - Faculty of Medicine Universitas Indonesia and Dr.
73 Cipto Mangunkusumo Central General Hospital (#KET-
74 1498/UM2.F1/ETIK/PPM.00.02/2020).

76 **Colonoscopy Examination**

77 After being given laxatives, sedatives and analgesics, subjects lied in a supine position
78 for a colonoscope insertion. If abnormalities were found, biopsies were taken for

Comment [NMD5]: Is this inclusion and exclusion criteria only for the ITB group? Or for all subjects?

If it's only for ITB group it should be clarified, for example by adding information "for the ITB subjects, the inclusion criteria was...."

Comment [NMD6]: This exclusion criteria can be moved after the sentences explaining the criteria for suspected ITB.

Comment [NMD7]: With similar characteristics? For example age range, etc?

79 histopathological examination. Various features could be identified during colonoscopy of
80 patient with suspected ITB, such as ulceration, nodules, polyps, narrowing of the lumen, and
81 irregular multiple fibrous bands. ITB may affect extensive parts of the intestine, in the form
82 of continuous lesions or single or skip lesions.¹⁶

83

84 **Histopathological Examination**

85 Samples for examination were harvested from at least 5 granulomas originating from
86 a single segment. After tissue fixation, grossing, embedding and slicing, tissue sections were
87 stained with hematoxylin (EpreDia, Kalamazoo, MI, USA) and eosin (Merck, Darmstadt,
88 Germany), and mounted. Prepared tissue slides will be read by an anatomic pathologist.

89

90 **ADA Test**

91 Nine mL of venous blood was drawn from the cubital vein. Three mL were collected in
92 an EDTA tube for complete hematological test, while 6 mL in non-anticoagulated tube for
93 ADA test. Blood in a non-anticoagulated tubes were allowed to stand at room temperature for
94 about 30 minutes, until blood clot formed. Immediate centrifugation of 3,000 revolutions per
95 minute (rpm) for 15 minutes was performed to separate the serum from the rest of the
96 remaining sample. Quantitative test of blood ADA was performed by BS-200 automated
97 clinical chemistry analyzer (Mindray, Shenzhen, China). The principle the test used was
98 enzymatic colorimetry. The measurement was determined by the increase of photometric
99 absorbance at a wavelength of 546 nm.

100

101 **Hematological Test**

102 Hematology test was performed by XN-2000 hematology analyzer (Sysmex, Kobe,
103 Hyogo, Japan) with semiconductor laser flowcytometry. K₃EDTA blood was diluted by the

Comment [NMD8]: It will be better if the information about parameters examined for the hematological test are included in this sub-section as well.

104 reagent that lysed erythrocytes and perforated the cytoplasmic membrane of all nucleated
105 cells. This process allowed fluorescent dyes to enter the cells and bind to the nucleic acids of
106 the cells. ESR measurement was performed using Starrsed RS automated ESR analyzer
107 (Sysmex).

108

109 **Statistics**

110 Data were processed using SPSS software version 20 ([IBM Corporation, Armonk, NY,](#)
111 [USA](#)) and Microsoft Excel 2016 ([Microsoft Corporation, Redmond, WA, USA](#)). Data
112 distribution was checked using Kolmogorov-Smirnov test. Bivariate analysis was performed
113 to investigate the relationship between two measured variables. Analysis of the difference
114 between the two groups in the abnormal data distribution was done by using the Mann-
115 Whitney U Test. The level of significance used was $p < 0.05$.

116 The calculation of cut-off and Area Under the Curve (AUC) was obtained from the
117 ~~ROC~~ (Receiver Operating Characteristic [\(ROC\)](#) curve. Sensitivity and specificity of the
118 examination compared to the gold standard (combined results of histopathology,
119 colonoscopy, and therapeutic response) were then calculated.

120

121 **RESULTS**

122 A total of 143 subjects, 16 (11.2%) of them were diagnosed with ITB, and 127 (88.8%)
123 subjects were non-ITB (Table 1). In percentage, the ITB group had higher weight loss, loss of
124 appetite, chronic diarrhea, and constipation.

125

126 **ADA and ESR of ITB and non-ITB groups**

127 From the results of laboratory test, ADA level and ESR of ITB groups were
 128 significantly higher than the ones of non-ITB group (Table 2). Meanwhile, all other
 129 hematological parameters of ITB and non-ITB groups were almost similar.

130 The median of ADA level in blood of ITB and non-ITB groups were 16.51 (11.82-
 131 35.61) IU/L, and 12.11 (8.89-15.99) IU/L, respectively (Table 2). In the ROC curve analysis,
 132 AUC of 0.695 (95% CI 0.542-0.849) was obtained with a cut-off of 12.56 IU/L. Sensitivity of
 133 ADA were 75%, and specificity of 57% (Table 3).

134 The median of ESR of ITB group and non-ITB group were 65 (33.50-92.50) mm/hour
 135 and 26 (13.00-45.00) mm/hour, respectively (Table 2). In the ROC curve analysis, AUC of
 136 0.741 (95% CI 0.623-0.860) was obtained with a cut-off of 32.5 mm/hour. Sensitivity of ESR
 137 was 81%, and specificity of 62% (Table 4).

138 ROC analyses were conducted for the combination of ADA and ESR testing. For the
 139 utilization of ADA and ESR in diagnosing ITB, the cut-off was 12.56 IU/L and 32.5
 140 mm/hour, respectively. Subsequently, ROC curve analysis was performed and AUC of 0.768
 141 (CI 0.656-0.880), sensitivity of 81.3%, and specificity of 62.2 % were obtained
 142 ([Supplementary 1](#)).

144 **Table 1. Characteristics of ITB (n=16) and non-ITB (n=127) Groups**

Characteristics	ITB		Non-ITB	
Age (years), <u>mean±SD</u>	42.19±18.28		43.53 ±16.65	
Gender (n, %)				
- Male	10	62.5%	40	31.5%
- Female	6	37.5%	87	68.5%
Clinical manifestation (n, %)				
- Weight loss	13	81.3%	69	54.3%
- Night sweat	1	6.3%	5	3.9%
- Cough	2	12.5%	5	3.9%
- Fever	5	31.3%	16	12.6%
- Loss of appetite	9	56.3%	41	32.3%

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M2023128 – High ADA and ESR of Intestinal Tuberculosis

- Non-specific abdominal pain	12	75.0%	111	87.4%
- Chronic diarrhea	13	81.3%	73	57.5%
- Constipation	13	81.3%	82	64.6%
- Blood in stool	8	50.0%	69	54.3%
- Mucus in stool	8	50.0%	70	55.1%
- Blood and mucus in stool	8	50.0%	68	53.5%
TB history (n, %)				
- History of contact with TB patient	1	6.3%	1	0.8%
- History of pulmonary and non-pulmonary TB	2	12.5%	10	7.9%

145

146 **Table 2. Laboratory Results of ITB (n=16) and Non-ITB (n=127) Groups**

Parameter	ITB	Non-ITB	p
ADA level (IU/L)	16.51 (11.82-35.61)	12.11 (8.89-15.99)	0.011*
Leukocyte (10 ³ /μL)	7.63 (4.85-10.96)	6.98 (5.58-8.45)	0.656
Basophil (%)	0.50 (0.30-0.70)	0.50 (0.40-0.80)	0.358
Eosinophil (%)	1.80 (0.80-4.15)	1.90 (1.00-3.70)	0.941
Neutrophil (%)	63.85 (49.97-74.22)	61.0 (54.10-71.00)	0.522
Lymphocyte (%)	25.70 (16.65-36.82)	29.10 (21.70-34.90)	0.520
Monocyte (%)	7.20 (4.62-8.32)	6.60 (5.40-7.90)	0.670
NLR	2.48 (1.44-4.49)	2.12 (1.56-3.31)	0.495
MLR	0.29 (0.15-0.44)	0.22 (0.19-0.29)	0.401
ESR (mm/hour)	65 (33.50-92.50)	26 (13.00-45.00)	0.002*

147 Mann-Whitney U test; **p*<0.05. NLR: Neutrophil to Lymphocyte Ratio, MLR: Monocyte to
 148 Lymphocyte Ratio, ESR: Erythrocyte Sedimentation Rate. [Data were presented in median](#)
 149 [\(min-max\)](#).

150

[Table 3...?](#)

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ADA Result	ITB	Non-ITB	Total
Positive	12	54	66
Negative	4	73	77
Total	16	127	143

- Sensitivity = 75%
- Specificity = 57%
- Positive Predictive Value (PPV) = 18%
- Negative Predictive Value (NPV) = 95%

151

152

153 Table 4. ESR Results of ITB (n=16) and Non-ITB Groups (n=127) with cut-off: 32.5 mm/hour

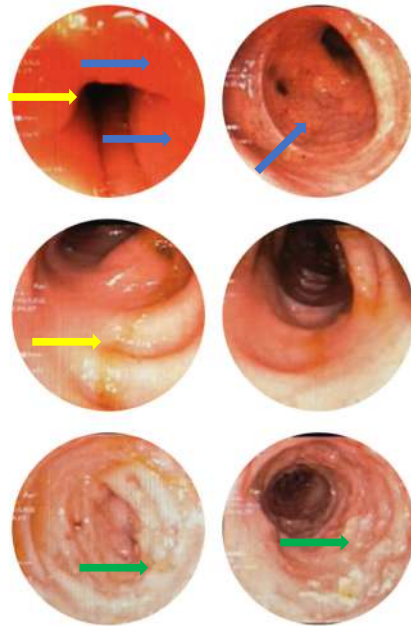
ESR Result	ITB	Non-ITB	Total
Positive	13	48	61

	Negative	3	79	82
	Total	16	127	143
-	Sensitivity	=	81%	
-	Specificity	=	62%	
-	Positive Predictive Value (PPV)	=	21%	
-	Negative Predictive Value (NPV)	=	96%	

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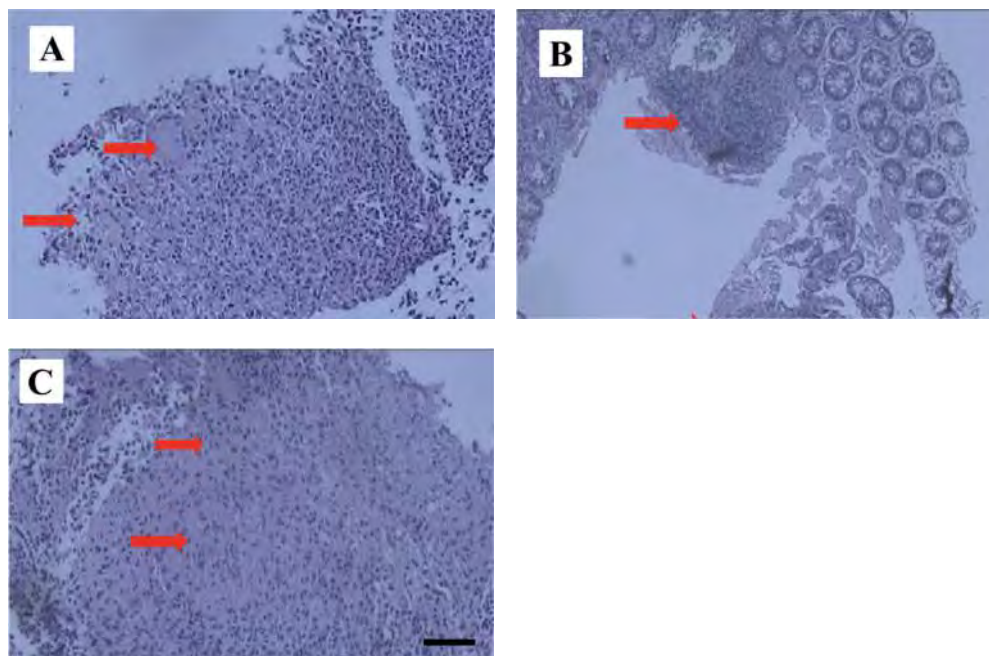
156 **Colonoscopic and Biopsy Histopathological Appearances of ITB Subjects**

157 In this study, colonoscopy of subjects with ITB displayed multiple ulcerations, edema,
158 and hyperemic mucosa in various areas, such as descending colon, transverse colon,
159 ascendent colon, caecum, and terminal ileum (Figure 1). Meanwhile, histopathological
160 examination of patients with confirmed ITB exhibited granulomatous inflammation,
161 epitheloid cells, giant cells (Datia Langhans), and lymphocyte aggregates (Figure 2). Some of
162 the other subjects showed incomplete and varied overview.



163

164 **Figure 1.** Colonoscopic results of ITB-suspected-subjects with ileocolitis. Blue arrow: hyperemic intestinal
165 mucosa; Yellow arrow: edema; Green arrow: ulceration.



166

167

168 **Figure 2.** Colonoscopic biopsy histopathological results of ITB group. A: Dardia Langhans cells (red arrow); B:
169 Lymphoid aggregates (red arrow); C: Epithelioid areas (red arrow). Black bar: 50 μ m.

170

171

172 Discussion

173 There were 16 ITB subjects among all 143 recruited subjects from the Gastroenterology
174 Outpatient Clinic, Department of Internal Medicine, Gastrointestinal Endoscopy Center of
175 Dr. Cipto Mangunkusumo Central General Hospital. The subject population of 16/143
176 (11.2%) was in accordance with the previous reported ITB prevalence (3-16%).^{1,18}

177 ADA level was significantly higher in ITB group compared with the one of non-ITB
178 group. The sensitivity and specificity of ADA test were 75% and 57%, respectively, with a
179 cut-off of 12.56 IU/L. These data could be useful for further investigation since in the present
180 time there is not any available data of ITB [patients'](#) ADA level. Most studies reported ADA
181 results of patients with pulmonary tuberculosis and abdominal tuberculosis.^{15,19} The cut-off
182 value of ADA level in this study was lower than the one in Serbian study on extrapulmonary
183 tuberculosis (24 U/L)¹⁹ and Nepalese study on pulmonary/extrapulmonary tuberculosis (25
184 U/L).²⁰

185 There was no significant difference in leukocyte count and leukocyte differential count
186 between ITB and non-ITB groups in this study. These results are in accordance with the ones
187 in Ethiopian study, reporting that the counts between the two groups were not significantly
188 different.²¹

189 Although NLR may indicate an early TB infection, there was not any significant
190 difference between NLR of ITB and non-ITB groups in this study. This result was similar to
191 the findings reported by previous study.²² However, contradictory findings were reported in a
192 study in Bandung, Indonesia, whereas pulmonary TB subjects exhibited significantly higher
193 NLR.²³

194 There was no significant difference in MLR between ITB and non-ITB groups in this
195 study. Both similar²² and contradictive findings²⁴ in the MLR of patients with TB, have been
196 reported which might be influenced by the study site, existence of any comorbidities (HIV,
197 diabetes mellitus), age, and TB treatment.²²

198 Compared with non-ITB group (26 (13.00-45.00) mm/hour), the ITB group had
199 significant higher ESR (65 (33.50-92.50) mm/hour). Similarly, a previous study reported that
200 ESR was significantly higher in patients with pulmonary TB compared with those without
201 TB.²⁵ In this study, high sensitivity of ESR (81%) was calculated, which could be promoted
202 as a screening test for ITB. ESR test has been used for a long time to assess acute phase
203 proteins in chronic diseases, including TB. Pro-inflammatory cytokines produced in ITB will
204 stimulate the liver to produce acute phase proteins, such as fibrinogen which may lead to an
205 increase in ESR. ESR measurement has limitations, such as being affected by anemic
206 conditions and hypoalbuminemia, which cause a false-high ESR.²⁵⁻²⁷

207 The combination of ADA and ESR test results with cut-offs of 12.56 IU/L and 32.5
208 mm/hour, respectively, did not provide a significant increase in sensitivity and specificity

209 compared to individual ADA or ESR test result. Therefore, each of ADA or ESR test can be
210 used independently to assist the diagnose of ITB.

211

212 Conclusion

213 ADA level and ESR were significantly higher among ITB patients compared with non-
214 ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests
215 could be considered as a screening test for ITB.

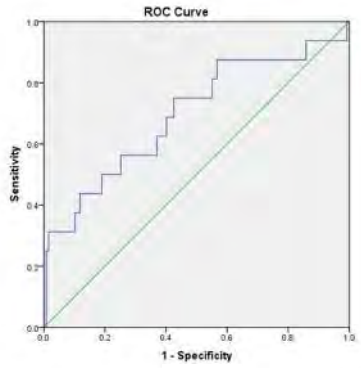
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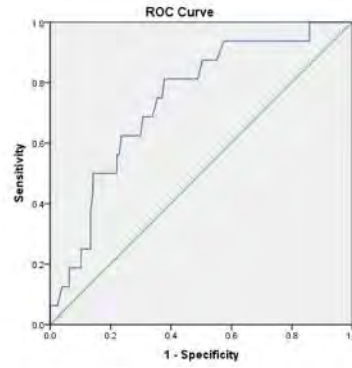
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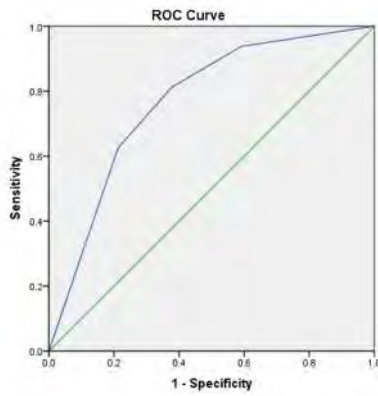
298 **Supplementary 1.**
299 **ROC curve for (a) ADA test, (b) ESR test, and (c) ADA+ ESR tests**
300



(a)



(b)



(c)

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Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2023128 Editor Decision Round 2 - Revisions Required

Ferry Sandra <ferry@trisakti.ac.id>

Fri, Aug 18, 2023 at 1:40 PM

To: Secretariat of InaBJ <secretariat@inabj@gmail.com>

Dear Secretariat of The Indonesian Biomedical Journal,

Thank you for giving us the opportunity to submit our revised manuscript "High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients" for publication in the Indonesian Biomedical Journal. All reviewers' comments in the second round of the peer-review have been addressed in the attached file.

Thank you. We look forward to hearing from you.

Regards,

Ferry Sandra

[Quoted text hidden]

--

Ferry Sandra, D.D.S., Ph.D.

Head of Medical Research Center

Universitas Trisakti



Round 2 Revision from Author.docx

3818K

1 **High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal**
2 **Tuberculosis Patients**

3
4 **Abstract**

5 **Background:** Currently, laboratory diagnosis of intestinal tuberculosis (ITB) is limited based
6 on clinical manifestations, providing opportunities for alternative laboratory tests to diagnose
7 ITB. At the present time, the role of serum adenosine deaminase (ADA) and hematological
8 tests in ITB patients are not widely known. The objective of this study was to determine the
9 role of ADA and hematological tests in patients suspected with ITB.

10 **Method:** Subjects that were suspected of ITB were classified as ITB group, while subjects
11 with inflammatory bowel disease, hemorrhoid, and intestinal malignancy were classified as
12 non-ITB group. Colonoscopy, histopathological examinations, and hematological test were
13 performed. ADA measurement was also performed with clinical chemistry analyzer based on
14 enzymatic colorimetry principle.

15 **Result:** Out of 143 subjects, 16 (11.2%) subjects were diagnosed with ITB and 127 (88.8%)
16 subjects were classified as non-ITB group. ADA level and erythrocyte sedimentation rate
17 (ESR) of ITB group were significantly higher than the ones of non-ITB group ($p<0.05$). Cut-
18 off, sensitivity, and specificity of ADA level were 12.56 IU/L, 75%, and 57%, respectively.
19 Cut-off, sensitivity, and specificity of ESR were 32.5 mm/hour, 81%, and 62%, respectively.
20 Colonoscopy of ITB subjects displayed multiple ulcerations, edema, and hyperemic mucosa.
21 Histopathological examination of ITB subjects exhibited granulomatous inflammation,
22 epithelioid cells, giant cells, and lymphocyte aggregates.

23 **Conclusion:** ADA level and ESR were significantly higher among ITB patients compared
24 with non-ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and
25 ESR tests could be considered as a screening test for ITB.

26 **Keyword:** intestinal tuberculosis, adenosine deaminase, hematological tests
27

28 Introduction

29 According to the Global Tuberculosis Report 2019 from the World Health Organization
30 (WHO), Indonesia was ranked third among the countries with highest burden of tuberculosis
31 (TB).^{1,2} The global prevalence of extrapulmonary TB was 14% and the prevalence in
32 Indonesia was $\leq 9.9\%$. Intestinal tuberculosis (ITB), as a part of abdominal TB, had a
33 prevalence of approximately 3–16%.³⁻⁵

34 Currently, ITB diagnosis was established based on clinical manifestations, Ziehl-
35 Neelsen (ZN) acid fast bacilli (AFB) stain of the stool, fecal culture, colonoscopy, and
36 histopathology.^{3,6-10} The limitations of the respective laboratory pose difficulties to diagnose
37 ITB, and hence, providing opportunities for alternative laboratory tests to diagnose ITB.¹¹

38 Adenosine deaminase (ADA) is an enzyme involved in purine metabolism which
39 catalyzes the conversion of adenosine to inosine. ADA is also involved in the proliferation
40 and differentiation of lymphocytes and maturation of monocytes.^{11,12} Elevated ADA levels in
41 blood or body fluid during tuberculosis infection is due to the stimulation of T lymphocytes
42 by *Mycobacterium tuberculosis* (MTB) antigen.^{12,13} The activity of ADA differs significantly
43 between patients with pulmonary TB and the normal healthy people^{14,15}

44 In ITB, acute or chronic inflammation is present due to infection process.
45 Hematological tests are often requested by clinicians in patients suspected of having ITB, as
46 several parameters can be utilized as markers of inflammation or infection. Some of them are
47 leukocyte count, leukocyte differential count (basophil, eosinophil, neutrophil, lymphocyte,
48 and monocyte), Neutrophil to Lymphocyte Ratio (NLR), Monocyte to Lymphocyte Ratio
49 (MLR), and Erythrocyte Sedimentation Rate (ESR).^{3,6,16,17} At the present time, the role of
50 serum ADA and hematological tests in ITB patients are not widely known. The objective of
51 this study was to determine the role of ADA and hematological tests in patients suspected
52 with ITB.

53

54 **Methods**

55 **Study Design and Subjects**

56 Subjects were recruited at the Gastroenterology Outpatient Clinic, Department of
57 Internal Medicine, Gastrointestinal Endoscopy Center of Dr. Cipto Mangunkusumo Central
58 General Hospital from December 2020 until December 2022, with the inclusion criteria as
59 follows; for the ITB group: ≥ 18 years old and suspected ITB. Meanwhile the exclusion
60 criteria: being treated with anti-tuberculosis drugs for >3 months, post-treatment with anti-
61 tuberculosis drugs for <6 months.

62 Patients were suspected of ITB if 3 out of 4 main clinical findings were present, and 1
63 out of 3 additional history criteria for ITB was met. The main clinical findings of ITB include
64 weight loss, non-specific abdominal pain, fever of unknown origin, and chronic diarrhea or
65 constipation. The additional history includes history of pulmonary TB, active pulmonary TB
66 with or without anti-tuberculosis drugs treatment (<3 months of treatment), and positive
67 contact history with TB patients. Patients with inflammatory bowel disease (Crohn's disease
68 or ulcerative colitis), hemorrhoid, and intestinal malignancy were classified as non-ITB
69 group.

70 Prior to the recruitment, all subjects were explained and asked for their consents of
71 participation by signing an informed consent form. The research protocol was approved by
72 the Health Research Ethics Committee - Faculty of Medicine Universitas Indonesia and Dr.
73 Cipto Mangunkusumo Central General Hospital (#KET-
74 1498/UM2.F1/ETIK/PPM.00.02/2020).

75

76 **Colonoscopy Examination**

77 After being given laxatives, sedatives and analgesics, subjects lied in a supine position
78 for a colonoscopy insertion. If abnormalities were found, biopsies were taken for

79 histopathological examination. Various features could be identified during colonoscopy of
80 patient with suspected ITB, such as ulceration, nodules, polyps, narrowing of the lumen, and
81 irregular multiple fibrous bands. ITB may affect extensive parts of the intestine, in the form
82 of continuous lesions or single or skip lesions.¹⁶

83

84 **Histopathological Examination**

85 Samples for examination were harvested from at least 5 granulomas originating from
86 a single segment. After tissue fixation, grossing, embedding and slicing, tissue sections were
87 stained with hematoxylin (Epredia, Kalamazoo, MI, USA) and eosin (Merck, Darmstadt,
88 Germany), and mounted. Prepared tissue slides will be read by an anatomic pathologist.

89

90 **ADA Test**

91 Nine mL of venous blood was drawn from the cubital vein. Three mL were collected in
92 an EDTA tube for complete hematological test, while 6 mL in non-anticoagulated tube for
93 ADA test. Blood in non-anticoagulated tubes were allowed to stand at room temperature for
94 about 30 minutes, until blood clot formed. Immediate centrifugation of 3,000 revolutions per
95 minute (rpm) for 15 minutes was performed to separate the serum from the rest of the
96 remaining sample. Quantitative test of blood ADA was performed by BS-200 automated
97 clinical chemistry analyzer (Mindray, Shenzhen, China). The principle the test used was
98 enzymatic colorimetry. The measurement was determined by the increase of photometric
99 absorbance at a wavelength of 546 nm.

100

101 **Hematological Test**

102 Hematology test was performed by XN-2000 hematology analyzer (Sysmex, Kobe,
103 Hyogo, Japan) with semiconductor laser flowcytometry for measurement of leukocyte,

104 basophil, eosinophil, neutrophil, lymphocyte, monocyte, NLR, and MLR. K₃EDTA blood
105 was diluted by the reagent that lysed erythrocytes and perforated the cytoplasmic membrane
106 of all nucleated cells. This process allowed fluorescent dyes to enter the cells and bind to the
107 nucleic acids of the cells. ESR measurement was performed using Starrsed RS automated
108 ESR analyzer (Sysmex).

109

110 **Statistics**

111 Data were processed using SPSS software version 20 (IBM Corporation, Armonk, NY,
112 USA) and Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA). Data
113 distribution was checked using Kolmogorov-Smirnov test. Bivariate analysis was performed
114 to investigate the relationship between two measured variables. Analysis of the difference
115 between the two groups in the abnormal data distribution was done by using the Mann-
116 Whitney U Test. The level of significance used was $p < 0.05$.

117 The calculation of cut-off and Area Under the Curve (AUC) was obtained from the
118 Receiver Operating Characteristic (ROC) curve. Sensitivity and specificity of the
119 examination compared to the gold standard (combined results of histopathology,
120 colonoscopy, and therapeutic response) were then calculated.

121

122 **RESULTS**

123 A total of 143 subjects, 16 (11.2%) of them were diagnosed with ITB, and 127 (88.8%)
124 subjects were non-ITB (Table 1). In percentage, the ITB group had higher weight loss, loss of
125 appetite, chronic diarrhea, and constipation.

126

127 **ADA and ESR of ITB and non-ITB groups**

128 From the results of laboratory test, ADA level and ESR of ITB groups were
 129 significantly higher than the ones of non-ITB group (Table 2). Meanwhile, all other
 130 hematological parameters of ITB and non-ITB groups were almost similar.

131 The median of ADA level in blood of ITB and non-ITB groups were 16.51 (11.82-
 132 35.61) IU/L, and 12.11 (8.89-15.99) IU/L, respectively (Table 2). In the ROC curve analysis,
 133 AUC of 0.695 (95% CI 0.542-0.849) was obtained with a cut-off of 12.56 IU/L. Sensitivity of
 134 ADA were 75%, and specificity of 57% (Table 3).

135 The median of ESR of ITB group and non-ITB group were 65 (33.50-92.50) mm/hour
 136 and 26 (13.00-45.00) mm/hour, respectively (Table 2). In the ROC curve analysis, AUC of
 137 0.741 (95% CI 0.623-0.860) was obtained with a cut-off of 32.5 mm/hour. Sensitivity of ESR
 138 was 81%, and specificity of 62% (Table 4).

139 ROC analyses were conducted for the combination of ADA and ESR testing. For the
 140 utilization of ADA and ESR in diagnosing ITB, the cut-off was 12.56 IU/L and 32.5
 141 mm/hour, respectively. Subsequently, ROC curve analysis was performed and AUC of 0.768
 142 (CI 0.656-0.880), sensitivity of 81.3%, and specificity of 62.2 % were obtained
 143 (Supplementary 1).

144

145 **Table 1. Characteristics of ITB (n=16) and non-ITB (n=127) Groups**

Characteristics	ITB		Non-ITB	
Age (years), mean±SD	42.19±18.28		43.53 ±16.65	
Gender (n, %)				
- Male	10	62.5%	40	31.5%
- Female	6	37.5%	87	68.5%
Clinical manifestation (n, %)				
- Weight loss	13	81.3%	69	54.3%
- Night sweat	1	6.3%	5	3.9%
- Cough	2	12.5%	5	3.9%
- Fever	5	31.3%	16	12.6%
- Loss of appetite	9	56.3%	41	32.3%

- Non-specific abdominal pain	12	75.0%	111	87.4%
- Chronic diarrhea	13	81.3%	73	57.5%
- Constipation	13	81.3%	82	64.6%
- Blood in stool	8	50.0%	69	54.3%
- Mucus in stool	8	50.0%	70	55.1%
- Blood and mucus in stool	8	50.0%	68	53.5%
TB history (n, %)				
- History of contact with TB patient	1	6.3%	1	0.8%
- History of pulmonary and non-pulmonary TB	2	12.5%	10	7.9%

146

147 **Table 2. Laboratory Results of ITB (n=16) and Non-ITB (n=127) Groups**

Parameter	ITB	Non-ITB	p
ADA level (IU/L)	16.51 (11.82-35.61)	12.11 (8.89-15.99)	0.011*
Leukocyte (10 ³ /μL)	7.63 (4.85-10.96)	6.98 (5.58-8.45)	0.656
Basophil (%)	0.50 (0.30-0.70)	0.50 (0.40-0.80)	0.358
Eosinophil (%)	1.80 (0.80-4.15)	1.90 (1.00-3.70)	0.941
Neutrophil (%)	63.85 (49.97-74.22)	61.0 (54.10-71.00)	0.522
Lymphocyte (%)	25.70 (16.65-36.82)	29.10 (21.70-34.90)	0.520
Monocyte (%)	7.20 (4.62-8.32)	6.60 (5.40-7.90)	0.670
NLR	2.48 (1.44-4.49)	2.12 (1.56-3.31)	0.495
MLR	0.29 (0.15-0.44)	0.22 (0.19-0.29)	0.401
ESR (mm/hour)	65 (33.50-92.50)	26 (13.00-45.00)	0.002*

148 Mann-Whitney U test; **p*<0.05. NLR: Neutrophil to Lymphocyte Ratio, MLR: Monocyte to
 149 Lymphocyte Ratio, ESR: Erythrocyte Sedimentation Rate. Data were presented in median
 150 (min-max).

151

152 **Table 3. ADA Results of ITB (n=16) and Non-ITB (n=127) Groups with cut-off: 12.56**
 153 **IU/L**

ADA Result	ITB	Non-ITB	Total
Positive	12	54	66
Negative	4	73	77
Total	16	127	143
- Sensitivity	= 75%		
- Specificity	= 57%		
- Positive Predictive Value (PPV)	= 18%		
- Negative Predictive Value (NPV)	= 95%		

154

155

156 **Table 4. ESR Results of ITB (n=16) and Non-ITB Groups (n=127) with cut-off: 32.5 mm/hour**

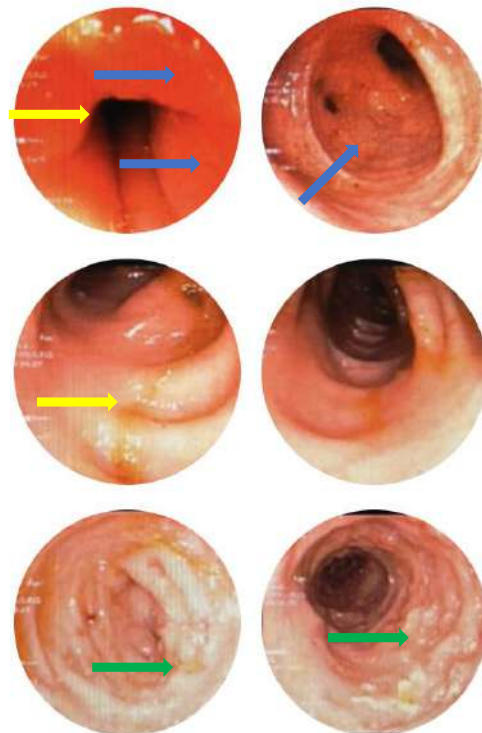
ESR Result	ITB	Non-ITB	Total
Positive	13	48	61
Negative	3	79	82
Total	16	127	143

- Sensitivity = 81%
- Specificity = 62%
- Positive Predictive Value (PPV) = 21%
- Negative Predictive Value (NPV) = 96%

157
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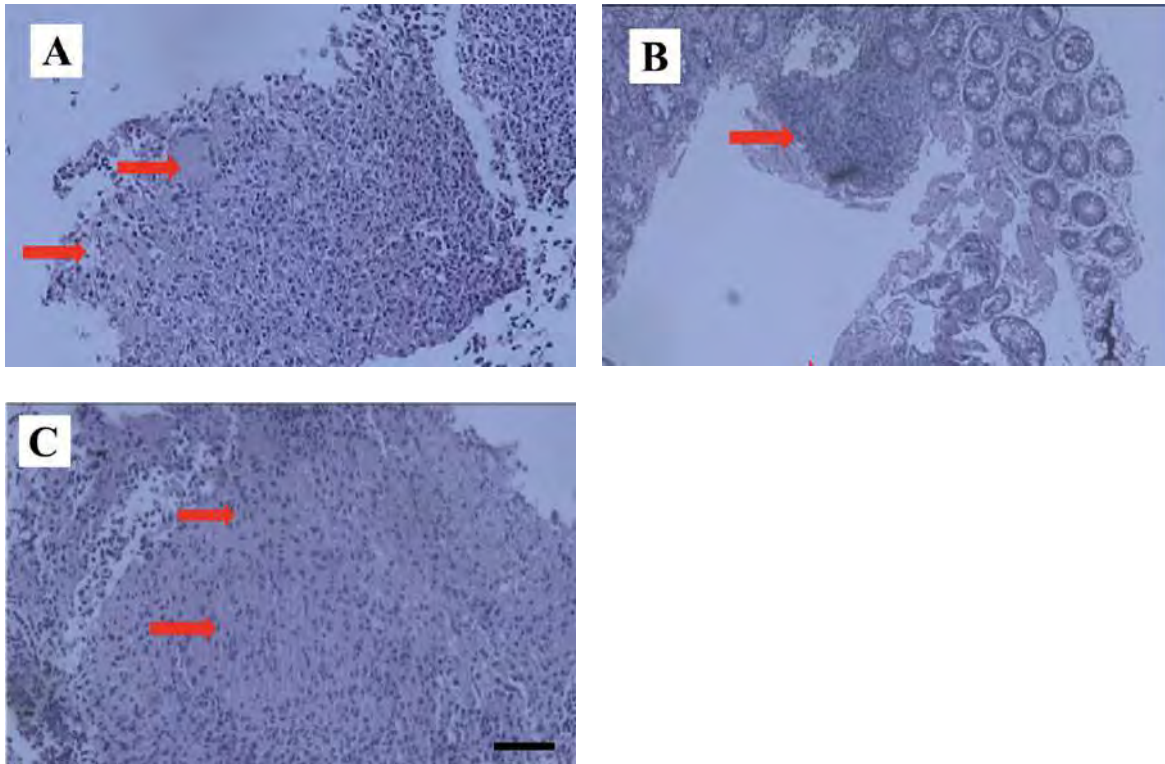
159 **Colonoscopic and Biopsy Histopathological Appearances of ITB Subjects**

160 In this study, colonoscopy of subjects with ITB displayed multiple ulcerations, edema,
161 and hyperemic mucosa in various areas, such as descending colon, transverse colon,
162 ascendent colon, caecum, and terminal ileum (Figure 1). Meanwhile, histopathological
163 examination of patients with confirmed ITB exhibited granulomatous inflammation,
164 epithelioid cells, giant cells (Datia Langhans), and lymphocyte aggregates (Figure 2). Some of
165 the other subjects showed incomplete and varied overview.



166

167 **Figure 1.** Colonoscopic results of ITB-suspected-subjects with ileocolitis. Blue arrow: hyperemic intestinal
168 mucosa; Yellow arrow: edema; Green arrow: ulceration.



169

170

171 **Figure 2.** Colonoscopic biopsy histopathological results of ITB group. A: Datia Langhans cells (red arrow); B:
172 Lymphoid aggregates (red arrow); C: Epitheloid areas (red arrow). Black bar: 50 μ m.
173
174

175 Discussion

176 There were 16 ITB subjects among all 143 recruited subjects from the Gastroenterology
177 Outpatient Clinic, Department of Internal Medicine, Gastrointestinal Endoscopy Center of
178 Dr. Cipto Mangunkusumo Central General Hospital. The subject population of 16/143
179 (11.2%) was in accordance with the previous reported ITB prevalence (3-16%).^{1,18}

180 ADA level was significantly higher in ITB group compared with the one of non-ITB
181 group. The sensitivity and specificity of ADA test were 75% and 57%, respectively, with a
182 cut-off of 12.56 IU/L. These data could be useful for further investigation since in the present
183 time there is not any available data of ITB patients' ADA level. Most studies reported ADA
184 results of patients with pulmonary tuberculosis and abdominal tuberculosis.^{15,19} The cut-off
185 value of ADA level in this study was lower than the one in Serbian study on extrapulmonary
186 tuberculosis (24 U/L)¹⁹ and Nepalese study on pulmonary/extrapulmonary tuberculosis (25
187 U/L).²⁰

188 There was no significant difference in leukocyte count and leukocyte differential count
189 between ITB and non-ITB groups in this study. These results are in accordance with the ones
190 in Ethiopian study, reporting that the counts between the two groups were not significantly
191 different.²¹

192 Although NLR may indicate an early TB infection, there was not any significant
193 difference between NLR of ITB and non-ITB groups in this study. This result was similar to
194 the findings reported by previous study.²² However, contradictory findings were reported in a
195 study in Bandung, Indonesia, whereas pulmonary TB subjects exhibited significantly higher
196 NLR.²³

197 There was no significant difference in MLR between ITB and non-ITB groups in this
198 study. Both similar²² and contradictive findings²⁴ in the MLR of patients with TB, have been
199 reported which might be influenced by the study site, existence of any comorbidities (HIV,
200 diabetes mellitus), age, and TB treatment.²²

201 Compared with non-ITB group (26 (13.00-45.00) mm/hour), the ITB group had
202 significant higher ESR (65 (33.50-92.50) mm/hour). Similarly, a previous study reported that
203 ESR was significantly higher in patients with pulmonary TB compared with those without
204 TB.²⁵ In this study, high sensitivity of ESR (81%) was calculated, which could be promoted
205 as a screening test for ITB. ESR test has been used for a long time to assess acute phase
206 proteins in chronic diseases, including TB. Pro-inflammatory cytokines produced in ITB will
207 stimulate the liver to produce acute phase proteins, such as fibrinogen which may lead to an
208 increase in ESR. ESR measurement has limitations, such as being affected by anemic
209 conditions and hypoalbuminemia, which cause a false-high ESR.²⁵⁻²⁷

210 The combination of ADA and ESR test results with cut-offs of 12.56 IU/L and 32.5
211 mm/hour, respectively, did not provide a significant increase in sensitivity and specificity

212 compared to individual ADA or ESR test result. Therefore, each of ADA or ESR test can be
 213 used independently to assist the diagnose of ITB.

214

215 **Conclusion**

216 ADA level and ESR were significantly higher among ITB patients compared with non-
 217 ITB patients. Since the sensitivities of ADA and ESR tests were high, the ADA and ESR tests
 218 could be considered as a screening test for ITB.

219

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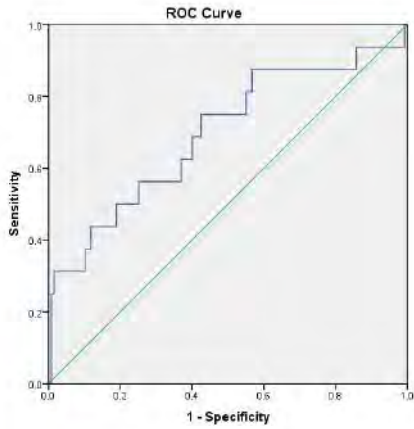
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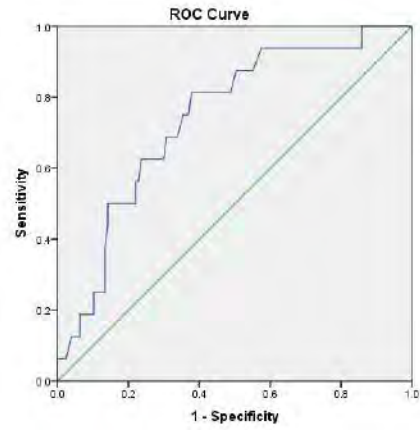
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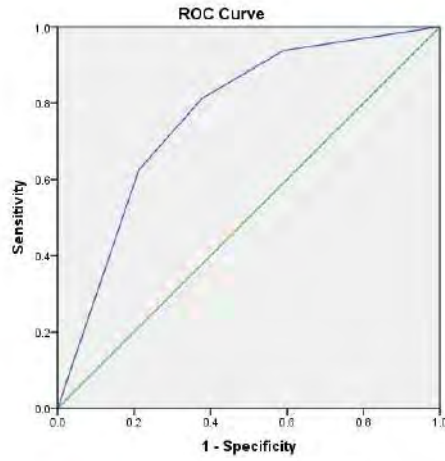
301 **Supplementary 1.**
302 **ROC curve for (a) ADA test, (b) ESR test, and (c) ADA+ ESR tests**
303



(a)



(b)



(c)

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Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2023128 Editor Decision - Manuscript Accepted

Secretariat of InaBJ <secretariat@inabj@gmail.com>
To: ferry@trisakti.ac.id

Mon, Aug 21, 2023 at 11:26 AM

Dear Dr. Ferry Sandra,

Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "**High Adenosine Deaminase Level and Erythrocyte Sedimentation Rate of Intestinal Tuberculosis Patients.**"

Our decision is to: **Accept Manuscript.**

Your manuscript will be sent to our publisher for typesetting and you should receive the proofreading in due course.

Congratulations on your interesting research, and thank you for allowing us to publish this valuable material. Please let us know once you have read this email. We wish you a nice day.

Best Regards,

--

Secretariat of The Indonesian Biomedical Journal

Prodia Tower 9th Floor

[Jl. Kramat Raya No.150, Jakarta 10430, Indonesia](#)

Phone. +62-21-3144182 ext. 3872

Fax. +62-21-3144181

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