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## Original Article

### Diagnostic Test of Blood Eosinophil Level as a Marker of *Ascaris lumbricoides* Infection

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#### ABSTRACT

*A. lumbricoides* infection (Ascariasis) is one of 17 neglected tropical diseases in Indonesia. Ironically, many cases of Ascariasis in Indonesia have not been diagnosed properly. This is because stool examination with Kato-Katz's method still rarely done. Therefore, it needs an alternative examination that more simple, easily done and can be routinely used in order to *Ascaris* diagnosing. This study was a diagnostic test for blood eosinophil levels as a marker in *A. lumbricoides* infection. This study was conducted in a private hospital at Medan regency. This study involved 63 children in pre-school and school age who had their parent approval. The stool was examined by Kato-Katz method as a gold standard and blood eosinophil levels was examined as an index in this study. The results showed sensitivity level of blood eosinophilia as a marker is 25.00% (CI95%: 5.49-57.19%) and specificity 96.08% (CI95%: 86.54-99.52%). The index also showed positive predictive value 60% (CI95%: 21.93-88.90%), negative predictive value 84.48% (CI95%: 79.63-88.35%), positive likelihood ratio 6.38 (CI95%: 1.19-34.04) and negative likelihood ratio 0.78 (CI95%: 0.56-1.09). The conclusion is elevated blood eosinophil levels cannot be used as an alternative test Kato-Katz in diagnosing Infection of *A. lumbricoides*. With its low specificity, blood eosinophil does not able to exclude Ascariasis, so it can not be used as a screening. Even though has low specificity, blood eosinophilia has high predictive value that can help practitioner in order to diagnosing.

**Keywords:** Eosinofil, *A. Lumbricoides*, Diagnostic test, Sensitivity, Spesificity.

**Highlights:** This study aims to find a simple examination to help establish the diagnosis of *Ascaris lumbricoides* infection. The results of this study indicate that blood eosinophil levels can help establish the diagnosis, but are less sensitive to rule out differential diagnoses.

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## INTRODUCTION

*Ascaris lumbricoides* infection, which also called *Ascariasis*, is one of the most neglected disease. World Health Organization (WHO) shows the number of *Ascariasis* and other helminthiasis that are transmitted through soil (*soil transmitted diseases*) in 17 *neglected tropical diseases*. The number of people infected by *A. lumbricoides* has reached more than 1.2 billion people worldwide<sup>1</sup>.

According to the data, Indonesia, as one of the country in South East Asia, has high incidence of *Ascariasis*. The data shows that 60%-69% from all children at the age of 2-12 years (school and pre-school age) were suffering from *A. lumbricoides* infection<sup>2</sup>.

This high number of *Ascariasis* is still assumed to have not show the real number, because there are still many cases that have not been diagnosed properly by doctors as medical practitioner. To diagnose an *A. lumbricoides* infection, a *Kato-Katz* test is needed as gold standard<sup>3</sup>. This test is done to find the helminth egg inside the patient's stool. However it is not routinely done.

The human body has a defense mechanism to prevent *A. lumbricoides* to enter and develop inside the body. There are 2 defense mechanism that play a role in preventing helminthiasis, 1.) to prevent chronicity of the infection by killing the parasites and 2.) to prevent the recurrence of infection from the same species<sup>4,5</sup>. Both of these are done by the specific and non-specific immune system.

The specific defense mechanism is done by activating Th2 cells which will then release IL-4 and IL-5. This activity will stimulate IgE formation, eosinophils formation and other inflammatory mediators. Helminthiasis is the main cause of the persistent increase in blood eosinophil (eosinophilia)<sup>4,6</sup>. Regarding to this, there has been no study about the sensitivity and the specificity of blood eosinophilia as a marker to determine helminthiasis, especially *Ascariasis*.

## MATERIALS AND METHODS

This study used a cross sectional approach. The objective of this study is to prove the diagnostic value of blood eosinophil level as a marker for *Ascariasis*. This study is conducted in a private hospital at Medan Regency. In total, there were 63 children in preschool & school age (2-12 years old) had involved as participants. Any factor that can affect eosinophil levels such as atopic history, steroid or anti-histamine uses, intensive disease, and anthelmintics that might affect the examinations are already being excluded.

Infection status of *A. lumbricoides* was determined by the finding of helminth egg using Kato Katz test. Stool tests were done at Parasitology Lab of UMSU. Blood eosinophil level was measured by using absolute eosinophil count test in hospital lab where the study is being conducted.

### Kato Katz Test for Stool Examination

Kato method for stool examination is done to find worm egg as its diagnostic phase. This test is a qualitative measure, different from its modification, Kato-Katz method can be used as quantitative test. The test has been done according to standard procedure. Stool samples that have been taken were immediately tested in Parasitology Lab in FK UMSU. About a mung bean size stool was taken using a wooden applicator stick, placed on an object glass. The object glass was covered with cellophane tape that have already been soaked overnight in Kato solution. Stool examination taken on after 30-60 minutes. Each stool sample was made into three preparations and then examined by two experienced laborant separately. If the stools can't be examined immediately, it will be preserved with formalin 10%. This preservation method can make the stool viable for testing even after a few days without affecting the test result<sup>7</sup>.

## Blood Eosinophil Level Count

Blood Eosinophil Level Count is done by measuring absolute blood eosinophil from peripheral blood sample. The sample is indicated as eosinophilia if the increase in blood eosinophil level is more than  $0.45 \times 10^3$  cells/ $\mu\text{L}$ <sup>8,9</sup>. Blood samples that have been taken by experienced phlebotomist are analysed in the hospital laboratory where they are being admitted. The blood was diluted using Dungey's solution with the ratio 1:20, this solution will stain the eosinophil and simultaneously breakdown erythrocytes and leukocytes. After that Improved Neubauer counting chamber will be filled with the solution, let the chamber be for 5 minutes until eosinophil filled and settled inside the counting chamber. This process was done inside a petri dish layered with moisturized absorption paper to prevent evaporation. Under the microscope, eosinophils was counted on four leukocyte square. The calculation must be accomplished before one hour mark to reduce error.

## RESULTS AND DISCUSSION

Table 1 shows the result of stool examination using Kato Katz methods. From 63 samples, 14 samples were infected by helminth. Most of them was suffered Ascariasis.

Based on table 2, from 63 samples examined at the hospital where study was held, the number of samples who suffered single *A. lumbricoides* infection occur more frequently on male (7 out of 33 patients (23.3%)) compared to female (5 out of 30 (16.7%)). There is no correlation between patient's sex and *A. lumbricoides* infection due to miniscule difference in lifestyle, behaviour and habit in male and female at this particular age group. It means that both male and female have the same chance to be infected by *A. lumbricoides*. Another study in North Sumatra in 2010 that did not separate *A. lumbricoides* infections from other soil-borne helminth infections, also showed that there is no correlation between sex and helminthiasis.<sup>11</sup>

Table 2 shows the number of patients who suffered single *A. lumbricoides* infection occurs more in school age group at 6-12 years old (4 out of 18 patients (22.2%)). It is more than patients in pre-school age group (8 out of 45 patients (17.8%)). The number of *A. lumbricoides* infection in this study does not show its correlation with school age children due to adequate sanitation quality in Medan Regency, which cause less soil to be infected by *A. lumbricoides* egg. This situation will make children in those area do not get infected even if they play on the soil.

**Table 1.** Stool examination result from Kato Katz test.<sup>15</sup>

No	Stool Test Result	Sample (n)	%
1	Single <i>Ascaris lumbricoides</i> infection	12	19.0
2	Mixed infection <i>A. lumbricoides</i> & <i>T. Trichiura</i>	2	3.2
3	Single <i>T. Trichiura</i> infection	2	3.2
4	No helminth eggs found	47	74.6
Total		63	100

**Table 2.** Frequency Distribution of Characteristic and Analysis Result of the Correlation between Sex, Age, Nutritional Status, Blood Hemoglobin Levels and Blood Eosinophil Level

No	Characteristic	Stool examination result using Kato Katz test		N	p-value
		Eggs	Eggs		
		<i>A. lumbricoides</i> (+)	<i>A. lumbricoides</i> (-)		
1	Sex				
	Male	7 (11.1%)	26 (41.3%)	33 (52.4%)	0.6463
	Female	7 (11.1%)	23 (36.5%)	30 (47.6%)	
2	Age				
	Pre-school age	9 (14.3%)	36 (57.1%)	45 (71.4%)	0.6848
	School age	5 (7.9%)	13 (20.6%)	18 (28.6%)	
3	Blood eosinophil level				
	Normal	10 (15.9%)	48 (76.2%)	58 (92.1%)	0.0150
	Eosinophilia	4 (6.3%)	1 (1.6%)	5 (7.9%)	

UNICEF's survey in Indonesia showed the number of *A. lumbricoides* infection is higher in pre-school age group (63.7%) than in school age group (53.0%)<sup>10</sup>.

Table 2 shows the number of patients who single *A. lumbricoides* infection followed by blood eosinophilia occurs in 4 out of 5 patients (80%). This number is more than Ascariasis patients without blood eosinophilia (10 out of 58 patients (17.1%)). It shows correlation between Ascariasis and blood eosinophilia.

A study taken on elementary student shows helminth infection severity will

followed by eosinophil blood elevation.<sup>12</sup> Blood eosinophilia are common in asymptomatic helminthiasis in rural area.<sup>13</sup>

Furthermore, the patient of single *A. lumbricoides* infection taken out to see correlation between single *A. lumbricoides* infection and blood eosinophilia. Based on Table 3, in this study shows that samples with single *A. lumbricoides* infection mostly affect those with blood eosinophilia (3 out of 5 patients) compared to patients without elevated blood eosinophil level (9 out of 58 patients).

**Table 3.** Blood and Stool test result

Blood eosinophil level	Stool examination result using Kato Katz test		Total
	Single infection <i>A. lumbricoides</i>	Mixed Infection with no <i>A. lumbricoides</i> egg	
Blood eosinophilia (+)	3 (4.8%)	2 (3.2%)	5
Blood eosinophilia (-)	9 (14.3%)	49 (77.8%)	58
Total	12	51	63

There is almost no test that qualify as gold standard, that become a reference as comparison in statistic test for other test in parasitology, and no test with 100% accuracy to confirm helminthiasis, including *A. lumbricoides*<sup>14,15</sup>.

WHO still recommends *Kato-Katz* test for surveillance and epidemiological survey in *A. lumbricoides* infection because it is relatively simple, fast, cheap and can be classified based on infection severity<sup>16</sup>. This study compares diagnostic test results of elevated blood eosinophil level with Kato Katz test result as a gold standard.

Based on the result in table 3, sensitivity will be calculated using the following formula  $\frac{a}{a+c} * 100\%$   $a/(a+c) * 100\%$ . Sensitivity of blood eosinophilia as a marker for Ascariasis is 25.00% (CI 95%: 5.49%–57.19%).

Specificity will be calculated with the formula  $\frac{d}{b+d} * 100\%$  from the data acquired in table 3. Specificity of blood eosinophilia as a marker for Ascariasis is 96.08% (CI 95%: 86.54%–99.52%).

Positive Predictive Value will be calculated using the formula  $\frac{a}{a+b} * 100\%$

from the data acquired in table 3. Positive predictive value for blood eosinophilia as marker to indicate Ascariasis is: 60% (CI 95%: 21.93%–88.90%).

Negative Predictive Value will be calculated using the formula  $\frac{d}{(c+d)} * 100\%$  from the data acquired in table 3. Negative predictive value for blood eosinophilia as marker to Ascariasis is: 84.48% (CI 95%: 79.63%–88.35%).

Positive likelihood ratio will be calculated using the formula  $\frac{sensitivity}{1-specificity}$  from the data acquired in table 3. Positive likelihood ratio for blood eosinophilia as marker to indicate Ascariasis is: 6.38 (CI 95%: 1.19–34.04).

Negative likelihood ratio will be calculated using the formula  $\frac{1-sensitivity}{specificity}$  from the data acquired in table 3. Negative likelihood ratio for elevated blood eosinophilia level as marker to indicate *A. lumbricoides* infection is: 0.78 (CI 95%: 0.56–1.09).

**Table 4.** Diagnostic Test Result for Elevated Blood Eosinophil Level

Blood Eosinophil Result	Diagnostic Test Result					
	Sens.	Spes.	PPV	NPV	LR+	LR-
	25.00%	96.08%	60%	84.48%	6.38	0.78

Abbreviation

Sens. = Sensitivity

Spes. = Specificity

PPV = Positive Predictive Value

NPV = Negative Predictive Value

LR+ = Positive Likelihood Ratio

LR- = Negative Likelihood Ratio



According to table 4 result, the sensitivity of elevated blood eosinophil level as diagnostic tool for marking *A. lumbricoides* infection is 25.00% with 96.08% specificity.

The 25.00% sensitivity means that, from observation done to 100 patients infected by *A. lumbricoides* and tested for eosinophilia, only 25 patients can be correctly diagnosed and the remaining 75 patients are failed to be diagnosed although they are really infected. This shows that the number of false negative is high. If there is no elevation in blood eosinophil level (normal blood eosinophil level), this cannot rule out the possibility of *A. lumbricoides* infection.

The 96.08%, specificity means in 100 healthy person that are being tested for blood eosinophil level, about 96 people are correctly indicated as healthy, and about 4 person are indicated as being infected by *A. lumbricoides* even though they are not. This means that the number of false positive is miniscule that it could help in diagnosing *A. lumbricoides* infection if the anamnesis result and physical diagnostic examination support it.

A study in North Argentina, 2012, compared test results of *A. lumbricoides* infection using *Kato-Katz* method, *McMaster* and *Mini-FLOTAC* with flotation salt solution FS2 (NaCl) and FS7 (ZnSO<sub>4</sub>), using the gold standard the result was positive in one of those test. The result of sensitivity test was 87.1 % for *Mini-FLOTAC* with FS7 solution, *Kato-Katz* 84.4%, *Mini-FLOTAC* with FS2 solution 61.3% and *McMaster* only about 48.3%<sup>17</sup>.

A study in Brazil showed that the *A* study in Brazil showed that the sensitivity of faecal egg count *A. lumbricoides* are respectively 97.3%, 94.2% and 69.5% for *Kato-Katz*, *Formalin-Ether Sedimentation* and *McMaster*<sup>18</sup>. A study in several countries, showed that *Kato-Katz* method is better compared to *McMaster* in faecal egg count for *A. lumbricoides*<sup>19</sup>. Another in Ethiopia

show the sensitivity of *Kato-Katz* as a single test is only 67.8%<sup>20</sup>.

In meta-analysis that involves many research, the sensitivity of *Kato-Katz* test is various. Sensitivity of *Kato-Katz* test on 1 sample for 1 slide is 63.8%, sensitivity of *Kato-Katz* test on 1 sample for 2 slides is 64.6%, sensitivity of *Kato-Katz* test for 2 samples taken from 1 patient is 69.2%, sensitivity of *Kato-Katz* test for 3 samples taken from 1 patient is 70.4%. This number looks higher if compared to the sensitivity direct microscopic examination (52.1%), *Formol-Ether Concentration* (56.9%) and *McMaster* (61.1%). Sensitivity of *Kato-Katz* test lies under *FLOTAC* and *mini-FLOTAC* (79.7% dan 75.5%)<sup>21</sup>.

A study in Philipines around 2004-2005, showed *Kato-Katz* test have good sensitivity and specificity, but this level of sensitivity and specificity may vary from day to another. Around 8.8% samples in that study with *Kato-Katz* test generated change in the result, this can be from negative results to positive result (4.9%) or positive result to negative (3.9%)<sup>15</sup>. Elevated blood eosinophil level in Ascariasis will be more stable because the increase of blood eosinophil level will remain for some time in blood and tissue because the helminths have life cycle that cross the host's tissue<sup>4,8</sup>.

*Kato-Katz* test for faecal egg count specifically for *A. lumbricoides* egg also have weakness. By using *Kato* solution that contain glycerol, hyalin layer will be dried off and causing the inside of the egg to be more visible. But after a few minutes, the hyalin layer may cause distortion and sometimes damage the egg. This will make the egg to be misidentified as another object and reported as no egg were found. This could affect *A. lumbricoides* egg even not as frequent as to hookworm egg<sup>18</sup>.

High specificity of blood eosinophil examination level may be useful in diagnosing Ascariasis. But even though the specificity is high, its sensitivity is low. This means that the elevation of blood eosinophil

level cannot be used as a tool for Ascariasis screening in a population. Diagnostic test for screening purposes must have high sensitivity even its specificity is quite low<sup>22</sup>

The main objective from a diagnostic test is its utilization to confirm the diagnosis. Sensitivity and specificity are not useful to indicate whether if an individual that being examined suffer from a disease or not based on the test result that being used. That is why a probability degree is needed in a test to diagnose a disease. It is called as predictive value<sup>23</sup>. Positive predictive value is a probability an individual really suffer from a disease if the test result is positive. And negative predictive value is the probability an individual does not suffer from a disease if the diagnostic test result is negative<sup>22</sup>.

Table 4 shows the positive predictive value of Blood eosinophilia as a marker for Ascariasis is 60%, and its negative predictive value around 84.48%.

Blood eosinophilia as a marker had 60% positive predictive value means that by observing blood eosinophilia a clinician can convince themselves that their patient have 60% possibility being infected by *A. lumbricoides*. 84.48% negative predictive value means a clinician can convince themselves that their patient has 84.48% possibility not being infected by *A. lumbricoides* if they have normal blood eosinophil level.

In contrast with sensitivity and specificity, predictive value is unstable to be used as diagnostic test. Its value is really fluctuative and depends on disease prevalence<sup>22</sup>. This means that predictive value in this study can only be used on another region with equivalent prevalence for *A. lumbricoides* infection.

The last parameter that has been acquired from diagnostic test using data from table 3 is likelihood ratio. Likelihood ratio is comparison of likelihood to get one specific result from a test done on group of infected samples with group of healthy samples<sup>24,25</sup>.

Based on the statistic test result on table 4, positive likelihood ratio of blood

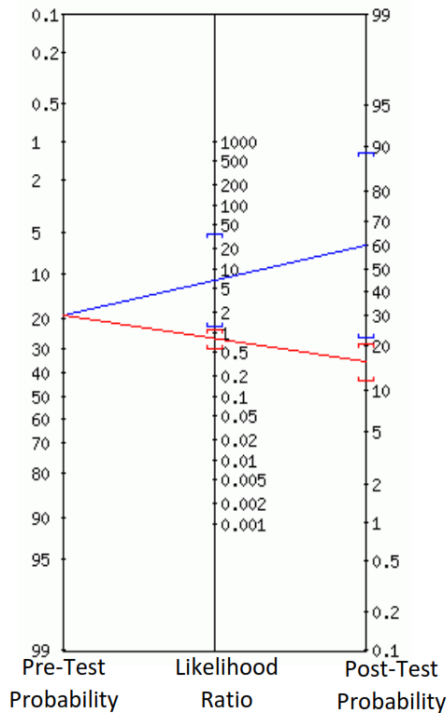
eosinophilia as marker for Ascariasis is 6.38, and its negative likelihood ratio around 0.78.

Blood eosinophilia as a marker had 6.38 positive likelihood ratio means that the group with Ascariasis have tendency 6.38 times higher to generate elevated blood eosinophil level compared to those without the infection. This number shows the ability of blood eosinophilia to support the diagnosis. Positive likelihood ratio below 10 indicate that the test is not strong enough in confirming the diagnosis<sup>24</sup>.

This tool also had 0.78 negative likelihood ratio means that the likelihood of blood eosinophilia in Ascariasis is only 0.78 times compared to samples without the infection (0.78:1). Hence, the group without Ascariasis only have the likelihood of 1.28 times higher not to have blood eosinophilia compared to the group with the Ascariasis (1:1.28). This number shows the weak ability of negative test result from blood eosinophilia in excluding the diagnosis of Ascariasis. A test will able to exclude a differential diagnosis if the negative likelihood ratio is below 0.1<sup>24</sup>.

Likelihood ratio is not only used to determine the ability of a test in helping the diagnosis or excluding other possible diseases, but also can be used to calculate the probability of a disease after examinations have been done. To calculate the probability of a disease in a patient after observing the possibility of the test result, a tool called *Nomogram Feye* is needed<sup>24,25</sup>.

In Figure 1, simulation using *Nomogram Feye*, shows the likelihood of *A. lumbricoides* infection on pediatric age at the hospital where study was held is initially 19.04% and can increase to 60% if the eosinophil count shows increased level. The likelihood for *A. lumbricoides* infection to occur on pediatric patients decreased to approximately 16% if the blood count does not show any increase in blood eosinophil level. This indicates the usefulness of elevated blood eosinophil level as a marker in supporting the diagnosis of *A. lumbricoides* infection.



**Figure 1.** Pre-test and Post-test probability *A. lumbricoides* infection after blood eosinophil level test.

**CONCLUSIONS**

The prevalence of *A. lumbricoides* infection on pediatric patients at the hospital where study was held is 22.2%. *A. lumbricoides* infection on pediatric patients affect more female patient at school age. Pediatric patients with *A. lumbricoides* infection affect larger number of children with normal nutritional status, children with decreased blood haemoglobin, and children with elevated blood eosinophil level.

Diagnostic test result for elevated blood eosinophil level as an indicator *A. lumbricoides* infection are: sensitivity 28.57% (CI95%: 8.39-58.10%), specificity 97.96% (CI95%: 89.15-99.95%), positive predictive value 80% (CI95%: 32.67-97.06%), negative predictive value 82,76% (CI95%: 77.47-87.02%), positive likelihood ratio 14,00 (CI95%: 1.70-115.40), negative likelihood ratio 0,729 (CI95%: 0.52-1.02).

Specificity of blood eosinophilia may be useful in diagnostic process, but it shows low specificity on the test, so it is unusable in excluding other differential diagnosis. This low sensitivity cannot be used in Ascariasis screening. High predictive value of elevated blood eosinophil is useful to help clinicians in interpreting the test results and diagnose the patient.

In accordance to sensitivity and specificity, its high positive likelihood ratio and low negative likelihood ratio, show that blood eosinophilia is useful in supporting the diagnosis of *A. lumbricoides* infection, but not useful enough in excluding other differential diagnosis.

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**ETHICAL CLEARANCE**

The research protocol was approved by The Research Ethic Committee of Medical Faculty Medicine, University of North Sumatera (KEPK FK USU) by issuing a letter with the number 319/TGL/KEPK FK USU-RSUP HAM/2017.

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**CONFLICT OF INTEREST**

All authors have no conflict of interest.

**AUTHOR CONTRIBUTION**

Writer, literature searcher, collecting data from literature: SMR,TRIP,DE, conceptor and supervision :MP, review and supervision: AAD.

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