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Mast Cell Essential Roles: Will it be a Novel Tool for Differentiating the Severity of Pediatric Appendicitis?

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Abstract

BACKGROUND: Appendicitis is an undesirable diagnosis, and the Alvarado score (AS) alone is insufficient in recognizing it. Many novel laboratory indicators, such as leukocytes and mast cells, differentiate.

AIM: Our objective was to demonstrate that mast cells had a greater influence on appendicitis severity than the AS, neutrophils, and platelets.

MATERIALS AND METHODS: An observational research in which 35 individuals with acute appendicitis at our institutions were identified for the appendix grade and allocated to one of two groups: Complicated or non-complicated. The AS and white blood cell count were calculated using the neutrophil-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), thrombocyte-to-neutrophil ratio (TNR), and total leukocyte. Mast cell count and granulation are evaluated using pathological anatomy.

RESULTS: TLR could not distinguish between severe appendicitis and others, but some showed potential. NLR, TNR, total leukocyte, ASs, mast cell count, and degranulation demonstrated good sensitivity-specificity values.

CONCLUSIONS: Mast cells have the potential to become a promising approach for predicting complicated pediatric appendicitis.

Introduction

Acute appendicitis is frequent in children and adolescents [1]. The exact pathophysiology of appendicitis is unclear, however, it may develop if not properly identified. Appendectomy [2] and histological examination [3] are now the most common procedures, particularly among the young.

The Alvarado score (AS) is a screening test for acute appendicitis, which may cause serious morbidity if not detected early. However, AS is inadequately sensitive to diagnose appendicitis, resulting in unnecessary surgery or misdiagnosis [4]. Although appendix ultrasonography has high accuracy, there are still issues and limits for detecting uncomplicated appendicitis [5], and it has a relatively poor negative predictive value [6]. The neutrophil profile has been widely examined in appendicitis; however, the primary role determinants in the neutrophil ratio have never been addressed.

Although the mechanisms are uncertain, increased mast cells are responsible for initiating neutrophil and lymphocyte infiltration in the appendicitis immune response [7], [8], [9]. As a result, we sought to demonstrate the importance of mast cells over the AS, neutrophils, and platelets in the severity of appendicitis.

Material and Methods

Patients with uncomplicated and complicated appendicitis who were not identified to be comorbid,

aged 0–18 years, consented to be research participants in the study at Ulin Hospital, Banjarmasin, Indonesia, from August to December 2020. To calculate the minimum samples for each group, the formula for a two-sided continuous result [10] is used:

$$n = \left(\frac{(Z_a + Z_\beta)}{0.5 \ln\left(\frac{1+r}{1-r}\right)} \right)^2 + 3 = \left(\frac{1.64 + 0.84}{0.5 \ln\left(\frac{1+0.6}{1-0.6}\right)} \right)^2$$

$$+3 = 15.8 = 16$$

Uncomplicated appendicitis, also known as 1st grade appendicitis, is characterized as inflamed appendix (simple), <10,000 leukocytes, and no fever. The lower right abdominal pain with localized/generalized peritonitis, leukocytes >10,000, and fever are all symptoms of complicated appendicitis, which can be classified as second grade (gangrenous), third grade (ruptured with localized free fluid), fourth grade (perforated with a regional abscess), and fifth grade (perforated with diffused peritonitis) (Figure 1) [11].

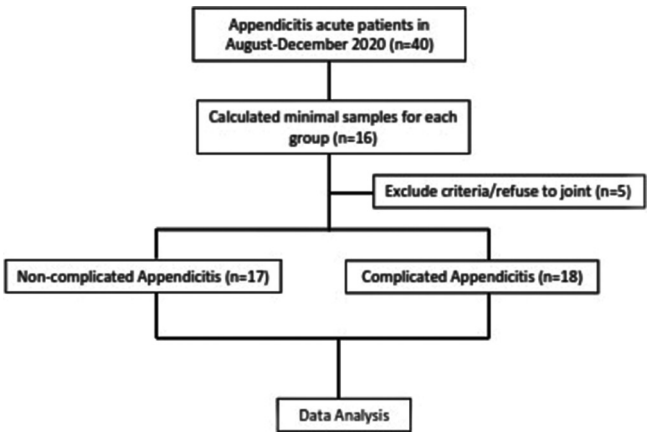

Figure 1: Research step of the 40 patients, 35 were willing to be research subjects, 17 were non-complicated, and 18 had complicated appendicitis

The leukocytes, differential count, neutrophil-to-leukocyte ratio, platelet-to-leukocyte ratio, platelet-to-neutrophil ratio, and leukocyte count profile were measured using venous blood for surgery preparation. According to Gomes *et al.*, the patient was classified into five stages of appendicitis after surgery, and pathological examinations were performed to quantify the quantity and degranulation of mast cells in the mesoappendix, stained with toluidine blue, and assessed by two blinded pathologists [12].

The first grade of appendicitis is characterized as uncomplicated appendicitis, whereas the subsequent grades are classified as complicated appendicitis, resulting in two classes that are analyzed using unpaired independent t-test statistic methods. The averages and standard deviations of data acquired from total leukocyte and mast cell count investigations will be examined. Sensitivity and specificity for each variable were computed for each data set.

The primary variables, total leukocyte, TNR, TLR, NLR, and mast cell degranulation and count are investigated using the spearman rho correlation to see whether they are involved in challenging, complicated, or uncomplicated appendicitis. The outcome of linear regression stated as a coefficient will demonstrate either a strong or mild influence on appendicitis, which was previously categorized into two categories.

This research was authorized by the Institutional Review Board of Universitas Lambung Mangkurat, Banjarmasin, Indonesia (No. 265/KEPK.FK ULM/EC/VIII/2020).

Results

This research involved 40 participants with pathologist confirmed acute appendicitis, five of whom were left out due to their reluctance to participate. Seventeen of the 35 individuals had uncomplicated appendicitis, which was paired with 18 patients in the complicated appendicitis group.

The patients' average age is 7.25 years, with the majority being between the ages of 1 and 17. There has never been a case of complicated appendicitis in a child under the age of 1 year (Table 1).

Table 1: Primary characteristic data of the patient

Variable	Uncomplicated appendicitis (n = 17) N,%	Complicated appendicitis (n = 18) N,%	p-value
Gender			
Male	9 (52.94)	15 (83.33)	0.46
Female	8 (47.06)	3 (16.67)	0.12
Age (years)			
<1	4 (23.53)	0 (0)	NA
1–17	8 (47.06)	11 (61.11)	
>17	5 (29.41)	7 (38.89)	
Appendicitis grade			
1st grade	17 (100)	0	NA
2nd grade	0	3 (16.67)	
3rd grade	0	8 (44.44)	
4th grade	0	5 (27.78)	
5th grade	0	2 (11.11)	

*p-value considered significant if p<0.05.

To obtain the mean and standard deviation of data, total leukocyte, TNR, TLR, and NLR leukocyte profiles were studied; thus, it was analyzed using an unpaired independent T-test, which was then tested for sensitivity and specificity for two classes differences, resulting in the analysis of a receiver operating characteristic (ROC) curve.

There are no significant variations across TLR classes in ROC curve analysis, and the lowest sensitivity and specificity are 61.1% and 58.8%, respectively. While the mast cell profile differs significantly between the two groups, the distinction between complicated and uncomplicated appendicitis is clear (Table 2).

Except for TNR, which has a negative correlation, the Spearman rho test shows that other

Table 2: Significance and single ROC analysis identifying sensitivity and specificity of the test alone

Variable	Appendicitis non-complicated (n = 17)	Appendicitis complicated (n = 18)	Significance[a] (p)	ROC analysis
Leukocyte profile	Mean ± SD	Mean ± SD		
Leukocyte count	9.582.35 ± 2.350.59	18.627.8 ± 5.195.56	0.000	Sensitivity 88.9% Specificity 88.2%
Thrombocyte-to-neutrophil ratio (TNR)	103.17 ± 55.11	26.8 ± 15.24	0.000	Sensitivity 94.1% Specificity 94.4%
Thrombocyte-to-lymphocyte ratio (TLR)	263.65 ± 225.20	280.7 ± 145.83	0.350	Sensitivity 61.1% Specificity 58.8%
Neutrophil-to-lymphocyte ratio (NLR)	4.43 ± 5.51	12.6 ± 7.18	0.000	Sensitivity 77.8% Specificity 76.5%
Mast cell profile				
Total mast cells (cells/100 μm2)	1.14 ± 1.01	7.1 ± 2.58	0.000	Sensitivity 100% Specificity 100%
Mast cells degranulation (%)	1 ± 2.24	31.3 ± 8.6	0.007	Sensitivity 100% Specificity 100%
Alvarado score	4.53 ± 2.40	8.5 ± 0.86	0.000	Sensitivity 88.9% Specificity 94.1%

[a]Analysis was done using independent t-test. *p-value considered significant if p<0.05.

indications indicate a complex appendicitis diagnosis. It implies that when the TNR is higher, individuals are more likely to get uncomplicated appendicitis [13] rather than complicated appendicitis. Of strength, total leukocyte, NLR, and TLR are three additional leukocyte profiles that might signal the chance of complicated appendicitis. Mast cell profiles, such as mast cell count and granulation, have been associated with complicated appendicitis and the AS (Table 3).

Table 3: Analysis of the variable effect on diagnosing complicated/uncomplicated appendicitis

Variable	Spearman rho correlation		Linear regression	
	(r)	p-value	β coefficient	p-value
Inflammation response				0.000
Leukocyte profile				0.000
Leukocyte count	0.759	0.000	0.753	0.000
Thrombocyte-to-neutrophil ratio (TNR)	–0.741	0.000	–0.702	0.000
Thrombocyte-to-lymphocyte ratio (TLR)	0.175	0.313	0.046	0.791
Neutrophil-to-lymphocyte ratio (NLR)	0.671	0.000	0.547	0.001
Mast cell profile				0.000
Total mast cells (cells/100 μm^2)	0.870	0.000	0.839	0.000
Mast cells degranulation (%)	0.895	0.000	0.926	0.000
Alvarado score	0.690	0.000	0.754	0.000

In the linear regression, excluding TLR, all factors that strongly correlate to complicated appendicitis are shown in Figure 2. Mast cell profile can be seen in Figure 3.

Discussion

The most prevalent causes of juvenile appendicitis are fecalith blockage [14] and bacterial hematological spread [15]. Obstruction causes dilatation, which promotes bacterial growth in the appendix lumen, resulting in ischemia and necrosis of the appendix wall. Bacterial growth and necrotic tissue reduce the inflammatory response. Pathological samples from one instance of appendicitis in infants under 1 year old were obtained from incidental appendicitis connected to the last operation for Hirschsprung disease. Acute appendicitis in children under the age of 1 year is rare, difficult to diagnose, and lacks typical symptoms. Rotavirus infection, the most prevalent cause of enterocolitis, has been shown to have no connection to the development of appendicitis [16]. Because of easy communication and a variety of low-fiber diets, older children are more likely to be detected with appendicitis [17], [18]. The inflammatory response is a well-coordinated mechanism that eliminates germs and dead tissue.

All innate and adaptive immune response cells are involved in controlling the elimination process. The cells work in tandem on the inflammatory response, with polymorphonuclear (PMNs) dominating initially, followed by macrophages and mast cells. Mast cells have two phases of function: Early and late. Mast cells live for 30 days. Cells produce cytokines, chemokines, and vasoactive chemicals in an autocrine, paracrine, and endocrine way to impact the inflammatory response. Mast cells, which are produced in the bone marrow and contain a large number of cytoplasmic granules [19], are engaged in the inflammatory response that kills pathogens. Mast cells possess a large number of receptors for cytokine activation; hence, they play an essential role in the amplification and resolution of inflammation. Mast cells also function in innate and adaptive immunity as mediators of cell populations and germ eradication. In the early stages of inflammation, mast cells influence neutrophil activation by identifying pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs) as part of toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) [20]. Mast cells degranulate in response to bacterial toxins, antimicrobial peptides, complement proteins, and Fc receptor stimulation. Mast cells generate histamine, proteases, TNF-, *de novo* lipid mediator synthesis, and the transcription and release of antimicrobial and immunomodulatory cytokines and chemokines during degranulation. Mast cells' position in tissues surrounding blood arteries and nerves, the host's external environment interface, and first contact with the pathogen [21] implies an early role in bacterial elimination. Histamine-producing mast cell degranulation causes vasodilation, which promotes chemotaxis and bacterial phagocytosis. TLS activation in the lipopolysaccharide membrane lowers the calcium influx threshold, allowing mast cells to degranulate and release cytokines [20]. The results of mast cell-t-cell crosstalk aid in regulating the inflammatory response. When mast cells degranulate, TNF production through the lymphotoxin receptor (LT-R) stimulates CD4+ T cells and releases IL-4 and IL-6. Mast cells activate CD8+ T lymphocytes through MHC Class I, resulting in more effective phagocytosis [22]. The appendix is a lymphoid organ

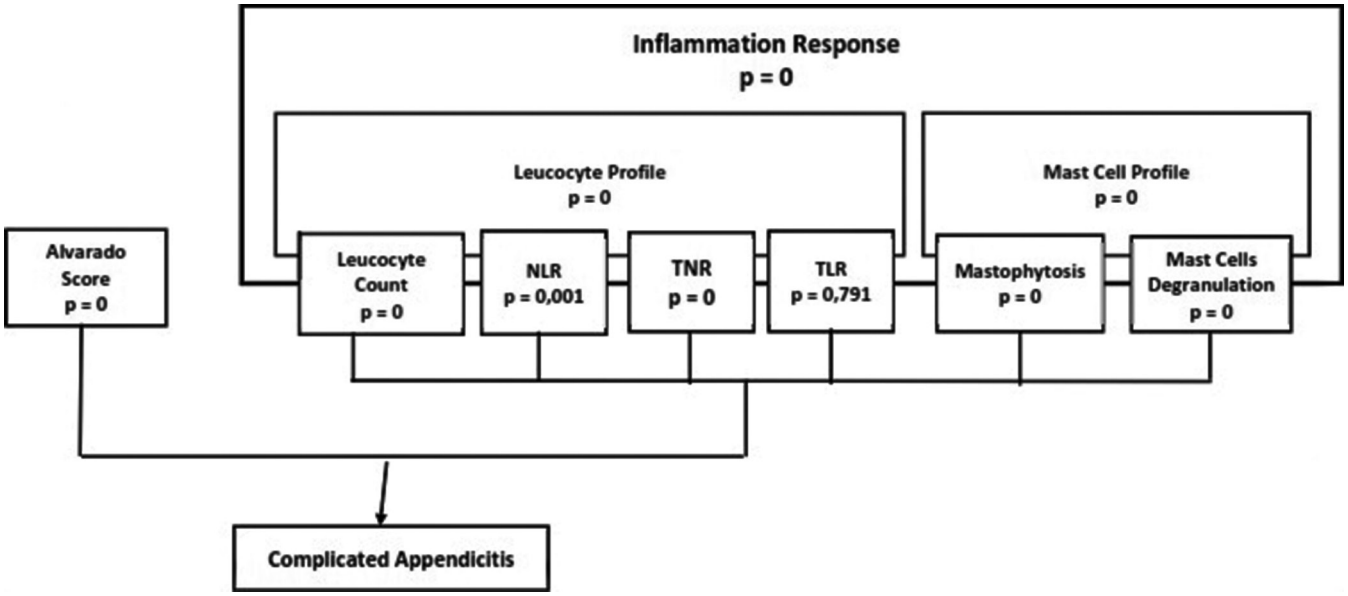

Figure 2: Statistical analysis of dummy variable linear regression. All factors, excluding TLR, have strongly correlated to complicated appendicitis

with a well-developed inflammatory response system that includes lymphocytes and other cells, most notably mast cells.

When an infection progresses to a severe, systemic level, relative thrombocytopenia develops due to decreased platelet synthesis, hemodilution, platelet consumption, sequestration in microvessels, and immune-mediated destruction. TNR is associated with vascular permeability [23], [24], while leukocyte count provides a complete picture of inflammatory leukocyte orchestral variables. In severe infections, lymphocytes employ nitric oxide (NO) to inhibit platelet aggregation [25]. Unlike Ceylan *et al.* [26], the TNR was insignificant, suggesting that the patients with complicated appendicitis were not seriously ill.

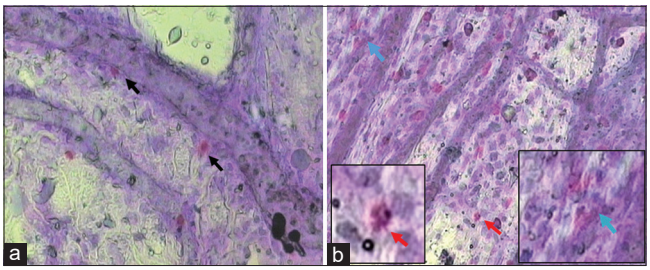

Figure 3: Mast cells profile. (a) From the omentum of an uncomplicated appendix and the mast cells are perivascular, intact with the nucleus and purplish (black arrow). (b) Omentum complicated appendix shows an increase in the number (red arrow) and mast cell degranulation (blue arrow)

The AS in uncomplicated appendicitis was 4.532.40 (the lowest score was two and the highest score was seven), which was consistent with the previous study [4], and the pathological diagnosis was acute appendicitis. This shows that the AS can distinguish between simple and complicated appendicitis and may be used as a therapeutic signal in uncomplicated appendicitis. The AS contains components for leukocyte count and neutrophilia (shift to the left), signaling that further scoring components (mast cell profile) are needed to increase diagnosis accuracy in uncomplicated appendicitis.

Conclusions

Mast cells play an essential role in the immune system because they work with innate and adaptive immune cells. Because of their excellent specificity and sensitivity, mast cells are a one-of-a-kind approach for discriminating between simple and complex appendicitis. Mast cell samples can only be collected after surgery, while what is necessary is completed before surgery. Pre-operative histamine levels as a mast cell product will be studied more in the future.

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