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Original Research Article

Granulocyte to monocyte ratio: leucokinetics in hematic biometry for appendicitis screening

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ABSTRACT

Background: Acute appendicitis diagnosis is still challenging and is established with clinical history, physical exam, laboratory tests, and imaging studies. It´s crucial to analyze the blood biometry with physiopathological asses and not with a statistical approach for establishing diagnostic items. The interaction between neutrophils with monocytes is noticeable in the blood biometry, so its relationship could be explained as directly proportional to the neutrophils count because of cellular recruitment and inversely proportional to the monocyte count because of cellular migration.
Methods: A retrospective, transversal and analytic study was conducted, admission blood biometry of all patients (n=160) that went through appendectomy in Pemex´s North Central Hospital between 2014 and 2019 were dissected. Statistical correlation between sensibility and likelihood ratio of granulocyte/monocyte ratio (GMR), leucocyte elevation, and left shift of neutrophil count were contrasted. IBM® SPSS© statistical software performed the statistical analysis.
Results: GMR (1.00) sensitivity showed to be superior to leukocytosis (0.785) and percentage neutrophils (0.846). the same for the negative likelihood ratios (LR-) calculated for GMR (0.00), leukocytosis (0.59) and percentage of neutrophils (0.579).
Conclusions: GMR showed higher sensibility for detecting immune response in a patient with suspected acute appendicitis compared to leucocyte elevation and left shift of neutrophil count or both.

Keywords: Appendicitis, Hematic biometry, Leukocytosis, Neutrophilia

INTRODUCTION

Acute appendicitis (AA) is defined as inflammation of the vermiform appendix secondary to obstruction of its lumen.[1] Worldwide, it represents 7%-11% of the causes of abdominal pain in emergency departments, with an annual incidence of 11 cases per 10.000 inhabitants and a lifetime risk of presenting appendicitis of 7%.[2,3] The perforation rate is highly variable and ranges from 16% to 40% with a bimodal distribution in young patients and those over 50 years old.[4] AA diagnosis continues to be a challenge and is based on the interpretation of clinical history, laboratory, and imaging studies. Multiple diagnostic scales have been proposed for many years and some of these have been integrated into diagnostic algorithms for predicting the risk of appendicitis; however, none have been fully accepted.[5]

Aims and objectives

To demonstrate that the interaction between leukocyte populations using the granulocyte/monocyte ratio (GMR) is more efficient for the diagnosis of AA than leukocyte elevation and neutrophil left shift.

METHODS

A retrospective, cross-sectional, analytical study was conducted. All cases of appendectomies performed in the Central North Hospital of PEMEX from 2014 to 2019, a total of 160 cases were included. Cases with any other cause or suspicion preoperative different from appendicitis were excluded. The absence or incomplete data in the medical records caused elimination from the trial. The admission hematological biometry (first recorded) before the surgical event was evaluated. The granulocyte/monocyte index was defined by the following formula

$$GMR = \frac{N}{L+M}$$

Where *N* is the number of neutrophils, *L* is the number of lymphocytes, and *M* is the number of monocytes. The cut-off values for leukocyte count and left shift were defined according to the adult appendicitis score cut-off points at eleven thousand cells per milliliter for leukocyte elevation, 75% for neutrophil left shift, and for GMR were set at 1.8. AA was defined by a histopathology report. Statistical analysis was performed with IBM® SPSS© Statistics software. The protocol was approved by a central ethics committee, permission: DCAS-SSS GSM-HCN-INV-030-2022. The study was conducted in consistent with the Declaration of Helsinki, Informed consent was obtained from all enrolled participants. Patient anonymity was preserved rigorously.

RESULTS

In current study 160 cases of appendectomy patients were analyzed, of which 79 patients were male and 81 were females, between the ages of 3 and 97 years old, with an average age of 39 years and a median of 42 years. The histopathology report concluded 11 negative appendicitis cases and 149 positive appendicitis cases with a white appendectomy rate of 6.87% (Table 1).

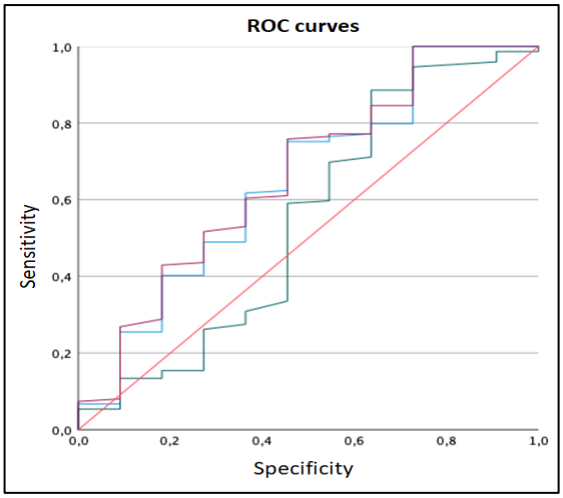

Figure 1: ROC curve: IGR, leukocytosis, and neutrophilia (IGR: blue line, leukos: green line, neutros: red line, reference: orange line).

Normality was demonstrated by the Kolmogorov-Smirnov test ($p>0.05$); ROC curves were calculated where the area under the curve (AUC) was documented with no significant statistical difference between GMR (0.650), leukocytosis (0.546) and percentage of neutrophils (0.664) (Figure 1). The sensitivity calculated for GMR (1.00) was shown to be superior to the sensitivity of leukocytosis (0.785) and percentage neutrophils (0.846), the same for the negative likelihood ratios (LR) calculated for GMR (0.00), leukocytosis (0.59) and percentage of neutrophils (0.579) with their corresponding post-test effect on Fagan's nomograms (Figure 2).

Table 1: Demographic data (n=160).

Cases	Males % (N=79)	Females % (N=81)	Mean age (SD)
	49.37	50.62	39.14 (±22.58)

The GMR showed a greater diagnostic impact compared to leukocytosis and percentage neutrophils (Table 2). The correlation was calculated through Pearson's correlation test between GMR, leukocytosis, and neutrophil percentage, with the diagnosis of AA obtaining a higher correlation between GMR and AA ($p<0.001$, $r=0.414$) compared to leukocytosis and neutrophil percentage (Table 3).

Table 2: Sensitivity and likelihood ratio comparison between GMR, leukocytosis and neutrophilia.

Parameters	Sensitivity	Pre-test	LR +/LR -	Pos-Test +	Pos-test -
GMR	1.000	0.931	1.22/0.00	0.94	0.00
Leukos	0.785	0.931	1.23/0.59	0.94	0.89
Neutros	0.846	0.931	1.16/0.57	0.94	0.89

GMR: Granulocyte/Monocyte Index, Leukos: Leukocytosis, Neutros: Neutrophilia, LR+: Positive likelihood ratio, LR-: Negative likelihood ratio.

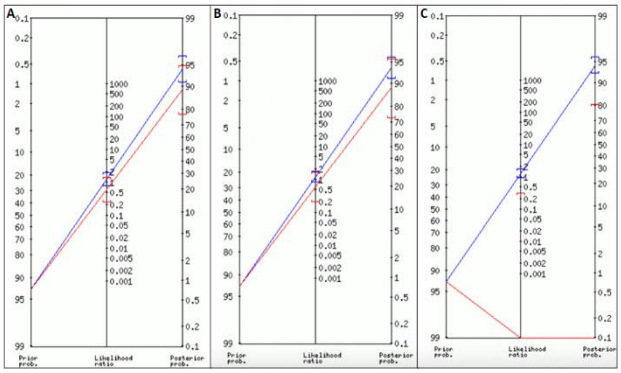

Figure 2: Fagan´s nomogram; A) Leukocytosis, B) Neutrophilia, C) IGR, LR+: blue line, LR-: red line.

Table 3: Person´s correlation test (r) for GMR, leukocytosis, and neutrophilia with AA.

Parameters	R value	P value	N
GMR	0.414*	<0.001	160
Leukos	0.090**	0.257	160
Neutros	0.081**	0.307	160

GMR: Granulocyte/Monocyte Index, Leukos: Leukocytosis, Neutros: Neutrophilia, *considerable relationship with regular correlation. **very weak relationship with little correlation.

DISCUSSION

Diagnostic algorithms based on risk scales have been shown to optimize the usefulness of imaging studies and avoid delays in surgical management. Risk classification systems are sufficiently sensitive by themselves. The most useful of them is the AIR (appendicitis inflammatory score), and the AAS (adult appendicitis score) has shown a specificity of 93% for patients classified as high risk. In the design of the AAS, two variables were selected concerning blood biometry (total leukocytes and percentage of neutrophils), and cut-off points were assigned based on the receiver operating characteristic curve (ROC), dividing these variables into four categories.[6,7] However, it is essential to analyze the behavior of blood biometry through a pathophysiological analysis and not a statistical behavior to determine diagnostic variables, for this it is important to consider the following: In the absence of inflammatory states, there are reservoirs of mature non-activated neutrophils ready for recruitment; and the lung is the most important of these reservoirs.[8,9] Although it is well documented that the half-life of circulating neutrophils is 5.4 days, when responding to inflammatory cytokines, activated neutrophils extend their half-life and persist in circulation to migrate to the site of inflammation.[10-15]

The process of leukocyte migration is complex and involves multiple factors, of which the main integrin related to immune activation and migration is CD11b, the expression of which is directly proportional to the intensity of the immune stimulus. On the other hand, neutrophils are differentiated into two phenotypes, pro-inflammatory (CD11b$^+$/GR-1$^+$/CXCR4) and pro-angiogenic (MMP9$^+$/CXCL2) that participate in different phases of leukocyte kinetics and their differentiation is influenced by monocytes depending on their functional polarization.[16] Classical leukocyte kinetics describes that neutrophils are the first leukocyte population to be recruited and their activation allows monocytes to be recruited, but the monocyte population in circulation is differentiated into two types: classical and non-classical, and, recent studies support that non-classical monocytes circulate parallel to the endothelium and upon the signal of tissue injury initiate processes of transcription and expression of cytokines that recruit neutrophils from the systemic reserves and circulating neutrophils.[17-22] Once the leukocyte recruitment process is initiated, tissue-resident monocytes express proinflammatory cytokines that induce neutrophil migration to the site of injury and/or infection.[23] In the case of AA, neutrophil migration is not limited to the tissue but extends to the peritoneal space, where the abundant fat-associated lymphoid clusters (FALCs) found in the omental tissue participate in the leukocyte kinetics with greater importance in the advanced stages of AA.[24] FALCs are the immunological unit in the peritoneal cavity and are composed of classical mesothelial cells (Krt19$^+$/Msln$^+$), immunomodulatory mesothelial cells (CxCL13$^+$ and Ifit1$^+$), fibroblasts (Mant2$^+$ and Ccl11$^+$), extracellular trap-forming neutrophils, macrophages, adipocytes, and B cells.[25]

In leukocyte kinetics, the interaction between neutrophil and monocyte cell lines is defined in four phases:[26,27] Phase 1: Activation of monocytes (non-classical and resident), expression of proinflammatory cytokines, and neutrophil recruitment. Phase 2: Neutrophil migration and lymphocyte recruitment. Phase 3: Control of neutrophil influx by monocytes and increased monocyte infiltrate. Phase 4: Anti-inflammatory regulation of monocytes and neutrophil clearance by apoptosis. Therefore, leukocyte kinetics will be evident in the cellular numbers of blood biometry and their relationship can be defined as directly proportional to the number of neutrophils by recruitment and inversely proportional to the number of mononuclear cells by migration. Clinical criteria are not sensitive enough to confirm or rule out AA individually. In combination, right lower quadrant pain, duration of pain, hypersensitivity, rebound, and leukocytosis are statistically significant to infer appendicitis; however, no clinical criteria can rule out AA.[28,29] The LR- of leukocytosis and neutrophilia (LR->0.01) are not statistically significant, on the contrary, the LR- of GMI (LR-<0,01) is statistically a useful and effective tool for the diagnostic process of AA as demonstrated in our study.[28] In recent years, the use of the neutrophil-lymphocyte ratio (NLR) developed in Slovakia in 2001 has been evaluated to identify systemic inflammatory response in intensive care unit patients.[30] The last meta-analysis that evaluated the NLR in the general population for the diagnosis of AA reported a sensitivity of 88,89% and specificity of 90,91%; on the other hand, another meta-

analysis evaluated the NLR in the pediatric population and reported a sensitivity of 82% and specificity of 76%.[31,32]

Limitations

The principal limitation of this study is the retrospective focus, results may not be generalized and further research is needed.

CONCLUSION

The GMR reflects leukocyte kinetics and therefore allows identifying systemic inflammatory responses in the absence of elevated leukocyte numbers or left shift of the neutrophil population. This applied in the diagnostic process of AA facilitates the diagnosis in the early stages or modified by drugs expediting the decision of early laparoscopic or open exploration and thus decreasing the morbidity of patients with AA. More studies are needed to define whether GMR is useful in clinical practice. However, the understanding of leukocyte kinetics and interactions between leukocyte populations increasingly allows for accurate assessments of the pathophysiological status of patients.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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