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The Diagnostic Value of Surface Markers in Acute Appendicitis; A Diagnostic Accuracy Study

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ABSTRACT

Objective: To determine the diagnostic value of blood cells surface markers in patients with acute appendicitis.
Methods: In this cross-sectional study, 71 patients who underwent appendectomy following a diagnosis of appendicitis were recruited during a one-year period. The patients were divided into two groups: patients with histopathologically confirmed acute appendicitis and subjects with normal appendix. Blood cell surface markers of all patients were measured. Univariate and multivariate analytical methods were applied to identify the most useful markers. Receiver operating characteristics (ROC) curves were also used to find the best cut-off point, sensitivity, and specificity.
Results: Overall we included 71 patients with mean age of 22.6±10.7 years. Of the 71 cases, 45 (63.4%) had acute appendicitis while 26 (36.6%) were normal. There was no significant difference between two study groups regarding the age (p=0.151) and sex (p=0.142). The initial WBC count was significantly higher in those with acute appendicitis (p=0.033). Maximum and minimum area under the ROC curve in univariate analysis was reported for CD3/RA (0.71) and CD38 (0.533), respectively. Multivariate regression models revealed the percentage of accurate diagnoses based on the combination of γ/δ TCR, CD3/RO, and CD3/RA markers to be 74.65%. Maximum area under the ROC curve (0.79) was also obtained for the same combination.
Conclusion: the best blood cell surface markers in the prediction of acute appendicitis were HLA-DR+CD19, α/β TCR, and CD3/RA. The simultaneous use of γ/δ TCR, CD3/RA, and CD3/RO showed the highest diagnostic value in acute appendicitis.

Keywords: Blood cell surface markers; Acute appendicitis; Diagnostic accuracy; ROC curve.

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Introduction

Appendicitis, occurring as a result of an obstruction in the appendiceal lumen, is one of the most common acute abdominal pains requiring surgical interventions. Timely diagnosis of appendicitis is critical considering its deadly complications, such as appendiceal rupture and peritonitis [1]. As definitive clinical diagnosis of appendicitis is commonly challenging even for experienced surgeons, the negative appendectomy rate has been reported as 10-30% [2-4]. Ultrasonography (US) and computed tomography (CT) have been widely applied to the diagnosis of appendicitis. There are substantial differences between US and CT. US requires considerable skill and experience and it is difficult to recognize the normal appendix. CT is relatively operator independent but exposes patients to increase risks of ionizing radiation and the consequent cancer risks in adults and particularly in children [5,6]. Flun *et al*. indicated the use of CT and US did not decline incidence of negative appendectomy over 2 decades and it could be related to the low sensitivity of CT/US [7]. For these reasons, alternative diagnostic approaches are required.

Despite the use of white blood cell (WBC) count in the diagnosis of appendicitis; its limited sensitivity has necessitated the application of other laboratory tests. Following medical advances, new instruments have been used in the diagnosis of acute appendicitis. However, no particular diagnostic tool can definitely confirm or reject the presence of appendicitis [8]. Thus a combination of various tests is required to achieve maximum accuracy, sensitivity, specificity, and diagnostic value. It has been shown that adding appendicitis inflammatory response (AIR) scores to common diagnostic variables could provide an efficient screening method for identification of patients at risk of appendicitis. However, other inflammatory markers could not lead to more reliable diagnosis [9]. In addition it has been demonstrated that interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) have higher diagnostic value than WBC count for acute appendicitis [10]. It has also been hypothesized that some types of lymphocyte in the appendiceal tissue might contribute to intestinal inflammation based on the finding that patoents with ulceratice colitis and acute appendicitis have elevated levels of lymphocytes in the tissue [11]. Mononuclear cells, especially cluster of differentiation 19 (CD19) has been found to be increased in various stages of acute appendicitis [12].

Previous studies have compared the numbers of various cells in individuals with acute appendicitis and healthy subjects [9-17]. The aim of the current study was to determine the diagnostic value of blood cell surface markers in acute appendicitis. It also sought to identify the most important markers and their best combination to obtain maximum sensitivity and specificity in classification and prediction of patients with acute appendicitis.

Materials and Methods

Study Population

In this cross-sectional study, of the initial sample of 81 patients [12] who underwent appendectomy due to a diagnosis of acute appendicitis, only 71 remained after exclusion of those with incomplete marker data. All surgeries were performed in Vali Asr Hospital affiliated with Arak University of Medical Sciences (Arak, Iran) during a one-year period from March 2011 and March 2012. Since complete data from all markers were required for analysis, those with incomplete marker data (for whatever reason) were excluded. The patients were only included if they had acute abdominal pain (suspected appendicitis) and underwent appendectomy and was finally diagnosed to have acute appendicitis based on the histopathological examination. The gold standard for diagnosis of the acute appendicitis was considered to be the histopathogy report by the same pathologist. A surgeon examined the patients and completed a questionnaire accordingly. The criteria used to establish the diagnosis of acute appendicitis were CT scan and periappendiceal inflammatory changes such as leukocytosis and C-reactive protein. An appendix larger than 6-mm in transverse diameter were considered abnormal. The study protocol was approved by the institutional review board and the medical ethics committee of Arak University of Medical Sciences. All the patients provided their informed written consents before inclusion in the study.

Study Protocol

About 10-cc venous blood sample was drawn from each patient under sterile conditions. Appendectomy was performed based on standard protocols and the removed appendix was sent to the laboratory where lymphocyte culture was conducted and the severity of appendicitis was assessed. The phenotypic characteristics of lymphocyte subsets in peripheral blood (before and 48-72 hours after appendectomy) and in appendix tissue were analyzed by three color-flow cytometry. The proliferative response of mononuclear cells was assessed by MTT method [12]. Once the markers were examined and counted, levels of 14 markers were recorded for each patient. All the removed appendixes were graded based on histopathological criteria as the gold standard [18]. The patients were divided into two groups: acute appendicitis and normal appendix.

Statistical Analysis

In univariate methods, the sensitivity and specificity of all markers were determined after determining the optimal cut-off point. The predictive value and accuracy of each marker were also assessed by calculating the AUC. In multivariate methods,

binary logistic regression analysis was applied to evaluate the combinations of markers. The percentage of accurate diagnoses and the AUC were used to determine the best combination of markers along with the diagnostic value and accuracy of each combination. All statistical analyses were conducted using MedCalc 13.1.2 (MedCalc Software, Belgium).

Results

The levels of 14 markers were recorded for all 71 patients. Of the 71 cases, 45 (63.4%) had acute appendicitis while 26 (36.6%) were normal. There was no significant difference between two study groups regarding the age (p=0.151) and sex (p=0.142). The initial WBC count was significantly higher in those with acute appendicitis ($p=0.033$). The baseline characteristics of patients in both study groups are summarized in Table 1.

The α/β T cell receptor (α/β TCR) showed maximum sensitivity (86.7) at the cut-off point 60 and γ/δ TCR had the highest levels of specificity (92.3) at the cut-off point 6.7 in univariate analyses. While maximum AUC was observed in case of CD3/RA (0.709), α/β TCR, HLA-DR+CD19, and CD3 were also found to have high AUC (0.696, 0.691, and 0.679, respectively). Overall, CD3/RA, CD3/RO, and CD19 had relatively acceptable sensitivity (82.2, 75.6, and 71.1, respectively) and specificity (61.5, 57.7, and 69.2, respectively) (Table 2).

In order to perform multivariate stepwise logistic regression analysis, all cell markers were first entered into the binary logistic regression analysis. Three markers, i.e. γ/δ TCR (p=0.021), CD3/RA (p=0.017), and CD3/RO (p=0.023), eventually remained in the model (Table 3). Evaluating the diagnostic value of different combinations of markers showed that the regression model had the highest diagnostic value in the presence of γ/δ TCR, CD3/RA, and CD3/RO (LR1 model) (AUC=0.788). The AUC for the regression model was 0.738 in the presence of γ/δ TCR and CD3/RA (LR3 model) and 0.735 for two

Table 1. Demographic and clinical characteristics of 71 patients undergoing appendectomy based on the histopathological examination.

	Acute appendicitis (n=45)	Normal Appendix (n=26)	*p* value
Age (years)	24.0±11.5	20.2±9.5	0.142
Sex			
Men (%)	25 (55.5%)	10 (38.4%)	0.151
Women (%)	20 (44.5%)	16 (61.6%)	
Initial WBC count (×10³ cells/mL)	14.4±3.3	12.8±3.9	0.003

Table 2. Sensitivity, Specificity, cut-off-point and AUC for blood cell surface markers in appendectomy patients

Markers	AUC	Cut off point	Sensitivity (CI[a])	Specificity (CI)
CD[b] 3	0.6790	≤55	48.9 (33.7-64.2)	80.8 (60.6-93.4)
CD 4	0.6390	≤32	44.4 (29.6-60.0)	80.8 (60.6-93.4)
CD 8	0.6130	≤19	64.4 (48.8-78.1)	61.5 (40.6-79.8)
α/βTCR[c]	0.6960	≤60	86.7 (73.2-94.9)	53.8 (33.4-73.4)
γ/δTCR[d]	0.6170	>6.7	31.1 (18.2-46.6)	92.3 (74.9-99.1)
CD3/RA	0.7090	≤33	82.2 (67.9-92.0)	61.5 (40.6-79.8)
CD3/RO	0.6780	≤28	75.6 (60.5-87.1)	57.7 (36.9-76.6)
CD 19	0.6690	>15	71.1 (55.7-83.6)	69.2 (48.2-85.7)
CD 5	0.6760	≤52	53.3 (37.9-68.3)	80.8 (60.6-93.4)
CD19+CD5	0.5470	>0.7	48.9 (33.7-64.2)	73.1 (52.2-88.4)
CD3+CD38	0.5740	≤7.5	82.2 (67.9-92.0)	38.5 (20.2-59.4)
CD 38	0.5330	≤32	60.0 (44.3-74.3)	57.7 (36.9-76.6)
HLA-DR[e]	0.6460	>18	82.2 (67.9-92.0)	46.2 (26.6-66.6)
HLA-DR +CD19	0.6910	>15	68.9 (53.4-81.8)	73.1 (52.2-88.4)

[a] CI: Confidence Interval; [b]CD: Cluster of differentiation; [c] α/βTCR : Alph/Beta T cell receptor; [d] γ/δTCR: Gamma/Delta T cell receptor; [e] HLA-DR: Human leukocyte antigen- D-related HLA locus in humans

Table 3. The result of multivariate logistic regression analysis

Variable	Coefficient	Odds ratio	95% CI	*p* value[a]
γ/δTCR	0.25324	1.2882	(1.04 to 1.60)	0.0212
CD3/RA	-0.07949	0.9236	(0.87 to 0.99)	0.0170
CD3/RO	-0.08670	0.9170	(0.85 to 0.99)	0.0230
Constant	4.0193			

[a] p value <0.05 is significant

models with CD3/RA and CD3/RO (LR2 model) and γ/δ TCR and CD3/RO (LR4 model) (Table 4, Figure 1). The percentage of accurate diagnoses using the LR1 regression model was 74.65%. The pairwise comparisons of the models based on the AUC did not reveal any significant differences between the models. Also, Power of study was found 99% for the model with 3 markers.

Discussion

The current research attempted to examine the diagnostic value of blood cell surface markers in acute appendicitis. According to the results of univariate analysis, none of the markers had an acceptable diagnostic value, i.e. the greatest diagnostic value was less than 0.71 [ROC (CD3/RA)=0.709]. This value was lower than the diagnostic values of rebound tenderness (ROC=0.84) and WBC count (ROC=0.89) calculated by Andersson *et al*., [9]. However, simultaneous use of markers increased the diagnostic value by 8%. The combination of γ/δ TCR, CD3/RA, and CD3/RO had a diagnostic value of 0.79.

Univariate analysis results indicated α/β TCR to have the highest sensitivity and γ/δ TCR to have the highest specificity. Multivariate analyses, on the other hand, revealed the most powerful regression model (LR1) to be the combination of γ/δ TCR, CD3/RA, and CD3/RO (accuracy=76%, sensitivity=80%, and specificity=73%). Various studies have suggested certain tests and variables for accurate diagnosis of patients with acute appendicitis. Malone *et al*. found unenhanced computed tomography (CT) to be an accurate imaging method for the diagnosis of acute appendicitis (accuracy=93%, sensitivity=87%, and specificity=97%). However, the high costs of this method prevented its wide application [19]. Farooqui *et al*. used logistic regression analysis and AUC to assess the diagnostic value of a set of serological markers in the diagnosis of acute appendicitis. They calculated an AUC of 0.745 for the optimal combination of the markers [17]. Andersson *et al*. obtained maximum AUC when a combination of inflammatory markers (MPO, SSA, and MMP9) were considered (AUC=0.71) [9]. Maximum AUC (0.79) using blood cell surface markers in the present study was respectively 5% and 4% higher than the values reported by Farooqui et al. [20] and Andersson *et al*., [9].

The diagnosis accuracy of markers described in the present research was comparable to the rates reported by previous studies [9,19,20]. Based on our findings, blood cell surface markers were cost-effective tools to predict acute appendicitis as accurately as common variables and tests could do. The percentage of accurate diagnosis (74.65%) in our

Table 4. The percent of correct classification and AUC for four regression models (LR1, LR2, LR3, LR4)

Model	Sensitivity	Specificity	AUC	Percent of cases correctly classified
LR 1	80.0	73.1	0.788	74.65 %
LR 2	77.78	61.54	0.735	66.20 %
LR 3	55.60	92.30	0.738	64.79 %
LR 4	73.3	65.40	0.735	71.83 %

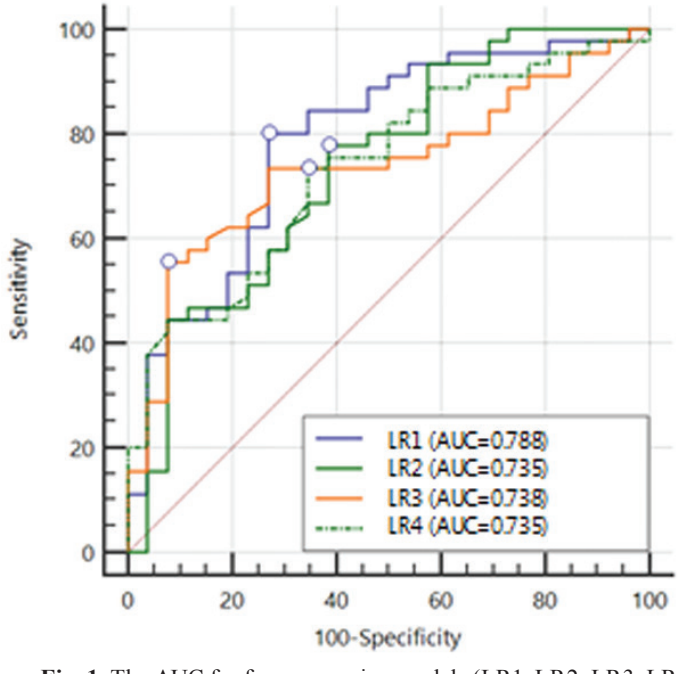

Fig. 1. The AUC for four regression models (LR1, LR2, LR3, LR4)

study was higher than the most accurate diagnostic method (AIR score, accuracy=58.3%) suggested by Andersson *et al*., [9]. Since the importance of these markers in the diagnosis of appendicitis has received little attention, future studies in this regard are recommended. Furthermore, adjustments for age and sex may lead to more favorable results about the diagnostic values of the studied markers. Therefore, further studies are warranted to assess the accuracy of the mentioned markers in different age groups of male and female patients along with conventional diagnostic tests.

One of the strong points of this study is the diagnostic accuracy of markers is similar to other methods (according to results from other studies). Thus, markers provide a new method for diagnosis of acute appendicitis, and since only a blood sample is required, risks and complications due to radiation are significantly reduced for high-risk people (pregnant women, infants, and the elderly) compared to CT and ultrasound. There were several limitations to this study. First, the sample size is small. Also, better results may be obtained using these markers along with usual diagnostic tests for appendicitis and variables of age and sex.

In conclusion, based on the results of univariate analyses, the best blood cell surface markers in the prediction of acute appendicitis were HLA-DR+CD19, α/β TCR, and CD3/RA. The simultaneous use of γ/δ TCR, CD3/RA, and CD3/RO showed the highest diagnostic value in acute appendicitis.

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References

1. Andersson RE. Meta-analysis of the clinical and laboratory diagnosis of appendicitis. *Br J Surg*. 2004;**91**(1):28-37.
2. Owings MF, Kozak LJ. Ambulatory and inpatient procedures in the United States, 1996. *Vital Health Stat 13*. 1998;(139):1-119.
3. Paydar S, Akbarzadeh A, Manafi AR, Ghaffarpasand F. Short-term Outcome of Open Appendectomy in Southern Iran: A Single Center Experience. *Bull Emerg Trauma*. 2013;**1**(3):123-6.
4. Pourhabibi Zarandi N, Javidi Parsijani P, Bolandparvaz S, Paydar S, Abbasi HR. Accuracy of Surgeon's Intraoperation Diagnosis of Acute Appendicitis, Compared with the Histopathology Results. *Bull Emerg Trauma*. 2014;**2**(1):15-21.
5. Hernanz-Schulman M. CT and US in the diagnosis of appendicitis: an argument for CT. *Radiology*. 2010;**255**(1):3-7.
6. Brenner DJ, Hall EJ. Computed tomography--an increasing source of radiation exposure. *N Engl J Med*. 2007;**357**(22):2277-84.
7. Flum DR, McClure TD, Morris A, Koepsell T. Misdiagnosis of appendicitis and the use of diagnostic imaging. *J Am Coll Surg*. 2005;**201**(6):933-9.
8. Ellis H, Natbanson LK. Appendix and appendectomy. In: Zinner MJ, Schwartz SI, Ellis H, editors. Maingot's abdominal operations. 10th ed. Connecticut: Appleton & Lange; 1997. p. 1191-1227.
9. Andersson M, Ruber M, Ekerfelt C, Hallgren HB, Olaison G, Andersson RE. Can new inflammatory markers improve the diagnosis of acute appendicitis? *World J Surg*. 2014;**38**(11):2777-83.
10. Huang D, Han M, Xie Y. Value of blood inflammatory markers in the diagnosis of acute appendicitis in children. *Nan Fang Yi Ke Da Xue Xue Bao*. 2012;**32**(8):1154-6.
11. Jo Y, Matsumoto T, Yada S, Nakamura S, Yao T, Hotokezaka M, et al. Histological and immunological features of appendix in patients with ulcerative colitis. *Dig Dis Sci*. 2003;**48**(1):99-108.
12. Mosayebi G, Alizadeh SA, Alasti A, Amouzandeh Nobaveh A, Ghazavi A, Okhovat M, et al. Is CD19 an immunological diagnostic marker for acute appendicitis? *Iran J Immunol*. 2013;**10**(4):216-28.
13. Kuga T, Taniguchi S, Inoue T, Zempo N, Esato K. Immunocytochemical analysis of cellular infiltrates in human appendicitis. *Surg Today*. 2000;**30**(12):1083-8.
14. Sack U, Biereder B, Elouahidi T, Bauer K, Keller T, Trobs RB. Diagnostic value of blood inflammatory markers for detection of acute appendicitis in children. *BMC Surg*. 2006;**6**:15.
15. Beltran MA, Almonacid J, Vicencio A, Gutierrez J, Cruces KS, Cumsille MA. Predictive value of white blood cell count and C-reactive protein in children with appendicitis. *J Pediatr Surg*. 2007;**42**(7):1208-14.
16. Sand M, Bechara FG, Holland-Letz T, Sand D, Mehnert G, Mann B. Diagnostic value of hyperbilirubinemia as a predictive factor for appendiceal perforation in acute appendicitis. *Am J Surg*. 2009;**198**(2):193-8.
17. Khan I, ur Rehman A. Application of alvarado scoring system in diagnosis of acute appendicitis. *J Ayub Med Coll Abbottabad*. 2005;**17**(3):41-4.
18. Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and cotran pathologic basis of disease, professional edition: expert consult-online. 8th ed. Elsevier Health Sciences; 2009.
19. Malone AJ, Jr., Wolf CR, Malmed AS, Melliere BF. Diagnosis of acute appendicitis: value of unenhanced CT. *AJR Am J Roentgenol*. 1993;**160**(4):763-6.
20. Farooqui W, Pommergaard HC, Burcharth J, Eriksen JR. The diagnostic value of a panel of serological markers in acute appendicitis. *Scand J Surg*. 2014;1-7.