Using pentraxin-3 for diagnosing acute appendicitis and predicting perforation: A prospective comparative methodological study

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ABSTRACT

BACKGROUND: In this study, we aimed to investigate the diagnostic performance of pentraxin-3 for acute appendicitis, and the predictive performance for perforation in patients with acute appendicitis, compared with white blood cell count, high-sensitivity C-reactive protein and interleukin-6 (IL-6).

METHODS: This study was a prospective methodological study, in which we studied the accuracies of the serum levels of pentraxin-3, white blood cell count, interleukin-6 and high-sensitivity C-reactive protein in estimating acute appendicitis, and in estimating perforation in patients with acute appendicitis. We designed the control group with the patients diagnosed inguinal hernia and admitted for elective surgery. Receiver operating characteristics analysis was used to compare the diagnostic accuracies and predictive performances.

RESULTS: Receiver operating characteristics analysis revealed that the Pentraxin-3 level >3.67 ng/mL showed the sensitivity of 95.5% and specificity of 100.0% for diagnosing acute appendicitis, with an area under the curve of 0.993 (95% CI 0.967–1.000). Also, the Pentraxin-3 level >9.56 ng/mL showed the sensitivity of 92.9%, and the specificity of 87.1% for the prediction of the perforation, with an area under the curve of 0.820 (95% CI 0.736–0.886).

CONCLUSION: The diagnostic performance of Pentraxin-3 for acute appendicitis and the predictive performance for perforation were higher than white blood cell count, high-sensitivity C-reactive protein and interleukin-6.

Keywords: Acute appendicitis; high-sensitivity C-reactive protein; interleukin-6; pentraxin-3; white blood cell count.

INTRODUCTION

Acute abdominal pain is one of the main complaints in patients presenting to the emergency department (ED).1 Acute appendicitis (AA) is one of the most common clinical diagnoses requiring surgical intervention in patients with abdominal pain, with a reported prevalence of 8% in the general population.2,3 Distinguishing AA from the other causes of abdominal pain before the specific surgical treatment is still a challenge.1 On the other hand, the timely accurate diagnosis before the appendectomy is necessary because the prolonged diagnosis process puts the patient at risk of perforation, and that may lead to prolonged hospitalization and broad-spectrum antimicrobial treatment.4-6 White blood cell (WBC) count, inflammatory cytokines, such as C reactive protein (CRP) and Interleukins (ILs), and imaging techniques, such as ultrasonography (US) and computed tomography (CT) are widely used to make correct diagnosis and used to decide which patient should be taken to surgery.5,7,8

WBC count is the most commonly measured markers in patients with the infectious or non-infectious presumed diagnosis. However, its rational use is generally to support the suspected clinical conditions, and it is neither sensitive nor
specific for the diagnosis of AA.\textsuperscript{[2–7]} CRP is also a widely used inflammatory marker and more specific than the WBC count. However, the diagnostic performance of the CRP for the determining severity of disease or complication is lower in the early phase of the infection because the elevation of CRP requires time approximately 48 hours or over. Interleukin-6 is a cytokine with an extensive range of biological activities. On the immune system, it has a wide range of impact, and it can affect the homeostatic process by having hormone-like characteristics. Interleukin-6 is widely used in clinical intervention as a diagnostic tool. Interleukin-6 is a cytokine with an extensive range of biological activities. On the immune system, it has a wide range of impact, and it can affect the homeostatic process by having hormone-like characteristics. Imaging techniques have improved accuracy for the diagnosis of AA, but the qualified personal for the US are not available in all health care institutions. The inability to visualize the normal appendix is considered a major weakness of using the US in examining patients with suspected AA.\textsuperscript{[9]} CT is an advanced technique that is not available at all EDs and associated with exposure to the radiation. Thus, these characteristics negatively affect the widely usage in clinical practice.\textsuperscript{[10]}

Pentraxin-3 (PTX 3) is the prototype of a long pentraxin group, and it is synthesized locally at the site of inflammation in response to inflammatory cytokine and microbial component.\textsuperscript{[1,11–13]} Both CRP and PTX-3 exhibit low plasma levels in healthy humans and their levels rise in inflammatory situations.\textsuperscript{[14]} However, the rate of increase and the time to reach the maximum level show differences, with CRP reaching its maximum peak at 48 hours and PTX-3 in 6–8 hours.\textsuperscript{[1,15,16]} The delayed increase of the CRP in the inflammatory situations affects negatively the diagnostic and discriminative performance. More rapid increase seen in PTX-3 is likely due to its local production by many cells, as well as the release of the PTX-3 stored in specific granules in the neutrophils and monocytes.\textsuperscript{[1–17]} This distinctive characteristic makes it a popular inflammatory marker for severe infectious diseases. An increase in PTX 3 in the circulation during the early phase of sepsis, septic shock and various infectious disorders and a correlation between its elevation and the severity of infection were shown in the literature.\textsuperscript{[1,11–18–21]}

There are few published studies that have investigated the diagnostic performance of PTX 3 for AA in patients with abdominal pain, and quite rare studies investigating the predictive performance for complication. Considering the above-mentioned characteristics of PTX 3, in this study, we aimed to investigate its diagnostic accuracy for AA and predictive performance for perforation by comparing WBC count, CRP and IL 6.

MATERIALS AND METHODS

Study Design and Setting

This study was a prospective methodological study, in which we studied the accuracies of the serum levels of PTX-3, WBC, IL-6 and hs-CRP in estimating AA in patients with abdominal pain, and in estimating perforation in patients with AA. Patients were informed about this study and its procedures and written informed consents were collected from the participants before their inclusion in the study. Our research was conducted in accordance with Good Clinical Practice standards and according to the Standards for the Reporting of Diagnostic accuracy studies (STARD).\textsuperscript{[22]} The study was approved by the Ethics Committee. This study was carried out between 01.04.2017 to 30.11.2017 at a State Hospital. The hospital is a secondary level hospital with a 200 bed capacity, and approximately nearly 600000 patients are admitted annually.

Participants

Patients who admitted to our general surgery clinic with prediagnosis of AA between the study period were evaluated for eligibility as cases. Patients with symptoms, such as acute abdominal pain, nausea, vomiting, anorexia and right lower quadrant tenderness, were evaluated with a medical history, physical examination, blood test, abdominal ultrasonography (USG) and abdominal computerized tomography (CT) to pre-diagnose AA. Patients diagnosed with inguinal hernia and admitted to our clinic for elective surgery were defined as candidate controls. Patients under 18 years old, over 65 years old, pregnant, with immune system disorders and/or with active infection were excluded from both case and control arms of this study.

Test Methods

All the serum markers were measured in the same serum sample concurrently. The serum levels of PTX3, WBC, IL-6 and hs-CRP were used data in estimating AA, and in estimating perforation in patients with AA.

Venous blood samples taken from the patient group and control group into hemogram test tubes containing EDTA were centrifuged at 15 minutes at 1000 x g within 30 minutes of collection, and then, they were stored at -40°C until the day that the analysis was performed. Pentraxin 3 (Human Pentraxin 3/ TSG-14 Immunoassay Kit) and IL-6 (Human IL-6 Quantikine Elisa Kit) were measured by RT-2100 C Microplate Reader (Rayto Life and Analytical Sciences Co., Ltd., China). CRP levels were measured by the nephelometric method in the RADIM Delta nephelometer (Radim Diagnostics, Pomezia, Italy). WBC count was measured by an automated hematology analyzer (Sysmex Corporation, Kobe, Japan).

There were two reference standards of this study: (1) diagnosed AA, (2) perforated AA. AA was defined as inflammation of the vermiform appendix, and perforated AA was defined as a hole in the appendix or a presence of fecalith in the abdomen. AA and perforated AA were diagnosed with surgical exploration and pathologic examination.

Data Analysis

We did not calculate a priori required sample size. We collected the data of the patients admitted to our clinic between
study period, who had inclusion criteria, who did not have exclusion criteria, and who accepted to involve in this study.

Statistical analyses were performed using SPSS version 23 (IBM Corp. in Armonk, NY) and Medcalc version 16 (MedCalc Software bvba, Ostend, Belgium). Descriptive statistics were presented as frequency (n) and percentage (%) for categorical variables and median with interquartile range (IQR) for non-normally distributed variables. Pearson chi-square test or Fisher’s exact test was used for comparing categorical variables, and the Mann-Whitney U test was used for comparing non-normally distributed continuous variables among the study groups. The ROC analysis was used for comparing the accuracy of the serum levels of PTX-3, WBC, IL-6 hs-CRP in estimating AA, and in estimating perforation in patients with AA. The area under ROC curves (AUCs) for these markers was calculated, and DeLong et al. [23] method was used for comparing AUCs. Youden J index was used for estimating the best cut-off values. Sensitivity, specificity, positive likelihood ratio (+LR), negative likelihood ratio (-LR), accuracy with 95% confidence intervals (CIs) were calculated for those estimated best cut-off values. P<0.05 was considered as statistically significance level.

RESULTS

From 01.04.2017 to 30.11.2017, 143 patients admitted to our general surgery clinic with pre-diagnosis of AA, 25 were excluded from this study, and 118 patients were considered eligible cases for this study. Between the same period, 114 patients admitted to our clinic with an inguinal hernia for undergoing elective surgery, 49 of them were excluded, and finally, 65 patients were defined as candidate controls. All the eligible cases (n=118) and the controls (n=65) accepted to include in this study after giving information about this study and its procedures. The serum levels of the PTX-3, WBC, IL-6 and hs-CRP were measured at the admission in all of the eligible patients (n=183). Finally, we followed up all pre-diagnosed AA cases for the presence of acute appendicitis (AA) and/or for the occurrence of perforation. Of 118 cases that underwent surgery, six were negative appendicitis, and five of them were mesenteric lymphadenitis, and one case was Meckel’s diverticulitis (Fig. 1).

![Flow diagram of this study. PTX-3: Pentraxin 3; WBC: White blood cell counts; IL-6: Interleukin 6; hs-CRP: High sensitivity C-reactive protein.](image-url)
Of the 177 patients who were included in the analysis, 65 were the patients without AA, and 112 were the patients with AA. Of the patients with AA, 84 had non-perforated AA, and 28 had perforated AA. The median age was 33.0 year in the non-AA group, 27.0 year in the AA group. This difference was found statistically significant (p=0.002). The ages of the non-perforated and perforated groups were similar. The sex ratio of the groups was also found similarly. The time of pain onset was substantially less than eight hours in the non-perforated group; however, more than eight hours in the perforated group. Both of the serum levels of the PTX-3, WBC, IL-6 and hs-CRP were statistically significantly higher in AA group than non-AA group (p<0.001, p<0.001, p<0.001, p<0.001, respectively). Likewise, all the marker levels were statistically significantly higher in the perforated group than non-perforated group (p<0.001, p<0.001, p<0.001, p<0.001, respectively) (Table 1, Fig. 2).

The areas under ROC curves (AUCs) were 0.993 for PTX-3, 0.870 for WBC, 0.927 for IL-6 and 0.978 for hs-CRP in estimating acute appendicitis. The difference between AUCs for PTX-3 and WBC, and for PTX-3 and IL-6 were statistically significant (p<0.001 and p=0.001, respectively). Besides, the difference between AUCs for PTX-3 and hs-CRP was statistically significantly similar. The best cut-off points for PTX-3, WBC, IL-6 and hs-CRP , estimated by Y ouden J indexes (0.955, 0.648, 0.784 and 0.870, respectively) were >3.67 ng/mL, >11.0 x10^9/L, >55.2 pg/mL and >9.7 mg/L, respectively (Fig. 3 and Table 2).

For the calculated best cut-off points, the sensitivity and specificity of PTX-3 were 95.5% and 100.0%; the sensitivity and specificity of WBC count were 67.9% and 96.9%; the sensitivity and specificity of IL-6 were 93.8% and 84.6%; and the sensitivity and specificity of hs-CRP were 94.6% and

### Table 1. Demographics and clinical characteristics of the participants

| Variables                        | Non–AA (n=65) | AA (n=112) | p     | Non–perforated (n=84) | Perforated (n=28) | p   |
|----------------------------------|---------------|------------|-------|-----------------------|------------------|-----|
| Age (years), Median (IQR)        | 33.0 (25.5–40.0) | 27.0 (22.3–35.0) | 0.002 | 27.0 (22.3–35.0)      | 27.0 (22.3–40.5) | 0.709 |
| Female, n (%)                    | 30 (46.2)     | 53 (47.3)  | 0.881 | 41 (48.8)             | 12 (42.9)        | 0.585 |
| Time of pain onset, n (%)        |                |            |       |                       |                  |     |
| Less than 8 hours                | 80 (95.2)     | 2 (7.1)    | <0.001|                      |                  |     |
| More than 8 hours                | 4 (4.8)       | 26 (92.9)  |       |                      |                  |     |
| PTX-3 (ng/mL), Median (IQR)      | 0.98 (0.56–1.57) | 7.65 (5.44–10.61) | <0.001| 7.09 (4.91–8.88)      | 13.89 (9.07–17.45) | <0.001|
| WBC (×10^9/L), Median (IQR)      | 7.8 (6.7–9.7) | 12.3 (9.9–14.5) | <0.001| 12.0 (9.8–13.0)       | 14.5 (12.3–17.3)  | <0.001|
| IL-6 (pg/mL), Median (IQR)       | 30.0 (20.4–46.8) | 294.6 (120.4–534.3) | <0.001| 250.8 (87.6–436.8)    | 532.2 (251.3–737.3) | <0.001|
| hs-CRP (mg/L), Median (IQR)      | 2.5 (1.5–3.9) | 51.2 (22.5–89.4) | <0.001| 42.2 (21.1–81.1)      | 88.7 (41.9–122.9) | 0.001 |

AA: Acute appendicitis; PTX-3: Pentraxin-3; WBC: White blood cell; IL-6: Interleukin 6; hs-CRP: High sensitivity C–reactive protein.
92.3%, respectively, in estimating AA although the accuracies of PTX-3, WBC count, IL-6 and hs-CRP were 97.2%, 78.5%, 90.4% and 93.8%, respectively (Table 3).

The areas under ROC curves (AUCs) were 0.820 for PTX-3, 0.744 for WBC, 0.729 for IL-6 and 0.711 for hs-CRP in estimating perforation in patients with AA. The differences between AUCs for PTX-3 and for the other markers were statistically significantly similar. The best cut-off points for PTX-3, WBC, IL-6 and hs-CRP, estimated by Youden J indexes (0.560, 0.417, 0.405 and 0.381, respectively) were >9.56 ng/mL, >13.0 x109/L, >492.0 pg/mL and >82.0 mg/L, respectively (Fig. 4 and Table 4).

For the calculated best cut-off points, the sensitivity and specificity of PTX-3 were 92.9% and 87.1%; the sensitivity

| Test                | Sensitivity (95% CI) | Specificity (95% CI) | +LR (95% CI) | -LR (95% CI) | Accuracy |
|---------------------|----------------------|----------------------|--------------|--------------|----------|
| PTX-3 >3.67 (ng/mL) | 95.5 (89.9–98.5)     | 100.0 (94.5–100.0)   | –            | 0.05 (0.02–0.10) | 97.2     |
| WBC >11.0 (x109/L)  | 67.9 (58.4–76.4)     | 96.9 (89.3–99.6)     | 22.1 (5.6–86.8) | 0.33 (0.30–0.40) | 78.5     |
| IL-6 >55.2 (pg/mL)  | 93.8 (87.5–97.5)     | 84.6 (73.5–92.4)     | 6.1 (3.4–10.8)  | 0.07 (0.04–0.20) | 90.4     |
| hs-CRP >9.7 (mg/L)  | 94.6 (88.7–98.0)     | 92.3 (83.0–97.5)     | 12.3 (5.3–28.6) | 0.06 (0.03–0.10) | 93.8     |

LR: Likelihood ratio; CI: Confidence interval; PTX-3: Pentraxin 3; WBC: White blood cell counts; IL-6: Interleukin 6; hs-CRP: High sensitivity C-reactive protein. Note: Accuracy is the sum of the number of true positive and the number of true negative patients.

Figure 3. ROC Curves of PTX-3, WBC, IL-6 and hs-CRP for estimating acute appendicitis. PTX-3: Pentraxin 3; WBC: White blood cell counts; IL-6: Interleukin 6; hs-CRP: High sensitivity C-reactive protein.

Figure 4. ROC curves of PTX-3, WBC, IL-6 and hs-CRP for estimating perforation. Note: ROC curves of the IL-6 and CRP were overlapped. PTX-3: Pentraxin 3; WBC: White blood cell counts; IL-6: Interleukin 6; hs-CRP: High sensitivity C-reactive protein.
and specificity of WBC count were 99.3% and 85.2%; the sensitivity and specificity of IL-6 were 60.7% and 79.8%; and the sensitivity and specificity of hs-CRP were 60.7% and 77.4%, respectively, in estimating perforation in patients with AA. Besides, the accuracies of PTX-3, WBC count, IL-6 and hs-CRP were 81.3%, 74.1%, 75.0% and 73.2%, respectively (Table 5).

No adverse event had been seen neither in collecting the serum samples nor in evaluating or managing the patients with AA.

**DISCUSSION**

We found that WBC count, CRP, IL6 and PTX 3 levels were significantly higher in the patients with AA than in the patients without AA. To evaluate the clinical significant diagnostic performance of these studied parameters, we applied a ROC curve analysis. The AUC of the PTX 3 value was the highest among all studied markers in our study.

Nowadays, public health interventions at the individual and population levels aim to prevent disease, protect and promote health are more popular than the classical diagnosis and treatment approaches. However, the burden on health care institutions increases day by day, and ED crowding is one of the major global healthcare issues. For optimal use of healthcare resources in the EDs, early identification of definitive diagnosis, recognition of disease severity and prediction of complication is of major importance.

AA is one of the most common surgical emergencies encountered in the EDs. The traditional approach in the diagnosis of AA largely depends on clinical history, physical examination of patients and assessment of non-specific inflammatory markers. VBC count and CRP are the most frequently used inflammatory cytokines, but the sensitivity and specificity for diagnostic accuracy were reported between 47%–74% and 55%–89% for WBC count and 39%–73% and 58%–97% for CRP, respectively in a meta-analysis. It was reported that the negative appendectomy rate could reach up to 10 per cent in patients with performed appendectomy. On the other hand, the delay in diagnosis of AA is related to the complications, such as perforation, and the mortality rate of uncomplicated AA is reported as 0.3%, it increases up to 6.0% in perforated cases. Thus, new diagnostic and prognostic biochemical markers could benefit the assessment and management of patients with abdominal pain to have better guide the diagnosis of AA.

PTX 3 is an acute-phase protein that is rapidly synthesized and released in response to inflammatory cytokines, and its production directly reflects the affected tissue inflammatory response occurs. It has been considered as a novel early diagnostic and prognostic biomarker in infectious illnesses, and our findings supported that PTX 3 is present very low level (<2 ng/mL) in normal humans.

The correlation between the level of the PTX 3 and disease severity was shown in the clinically important infectious conditions. It was reported that the concentration of PTX 3 reached the maximum level in the early phase of patients with acute pancreatitis and then allowing distinction...
of the patients as mild, moderate and severe on the first day of disease. Hamed et al. showed that the plasma level of PTX 3 was able to discriminate patients with sepsis or septic shock significantly on the first day of infection. Hansen et al. reported that a high PTX 3 level in patients with necrotizing soft tissue infection at the time of admission was associated with septic shock, amputation and risk of death. They emphasized that PTX 3 might be used to distinguish high-risk patients who require aggressive surgical treatment. Using ROC curve analysis, we demonstrated that PTX 3 has the highest positive likelihood ratio for the occurrence of the perforation, and this finding supports the importance of PTX 3 to foresee the severity of the disease.

While the conservative treatment approach of AA with antibiotics in the pediatric population has been accepted, this concept is controversial in the adult population, and the emergent appendectomy has been widely performed in adult patients presenting to the EDs. In a recent meta-analysis demonstrated that delaying appendectomy for up to 24 hours might be an acceptable alternative for patients that have not risk factors of complicated appendicitis. In addition, some studies reported that higher morbidity and error rates when working or operating at out of hours because of an inadequate number of staff could not be assigned in all EDs or emergency operating rooms at out of hours and required preoperative evaluations might not be performed. If the patients that have a low risk for the complication could be defined, the emergent appendectomy could be postponed and the workload out of hours can be reduced. Several markers or parameters have been studied for prediction of the complication. For example, Shin et al. demonstrated that the delta neutrophil index is a good predictor for the perforation in adult patients with AA (sensitivity 67%, specificity 90%, AUC 0.807). Obinwa et al. composed a clinical model, including a combination of anorexia, rebound tenderness, leukocytosis and pyrexia, to predict advanced appendicitis. They reported that this model might be useful in the early identification of patients with advanced AA, with a 38% sensitivity. Alvarez-Alvarez et al. demonstrated serum fibrinogen as a good predictor for appendicular perforation, with a sensitivity of 86.77%, a specificity of 91.49, a positive predictive value of 93.65 and, a negative predictive value of 82.69. In the presented study, the predictive accuracy of PTX-3 in estimating perforation in patients with AA was 81.3 (the sensitivity and specificity of PTX-3 were 92.9 and 87.1) with a +LR of 7.2 and a −LR of 0.08.

There are several limitations to the present study. Firstly, our study has a relatively small sample size given that this study was conducted in a single center. More than one PTX 3 value could have been measured, and then, the increase and decrease or the timely rate of increase or decrease according to first value might guide the diagnosis or the risk of perforation.

Conclusion

In the present study, we demonstrated that PTX 3 values were significantly higher in patients with AA compared to the patients without AA and significantly higher in patients with perforation than in patients without perforation. In addition, both the diagnostic performance for AA and the predictive performance for perforation were higher than WBC, hs-CRP and IL. The usefulness of PTX 3 as a diagnostic and predictive marker for AA should be validated in prospective randomized controlled studies.

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Gül et al. Using Pentraxin-3 for diagnosing acute appendicitis and predicting perforation

**ORİJİNAL ÇALIŞMA - ÖZET**

Akut apandisit tanısını koymak ve perforasyonu öngörmek için pentraxin-3 kullanımı: İleriye yönelik karşılaştırılmış metodolojik çalışma

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AMAÇ: Biz bu çalışmada, pentraxin-3’ün akut apandisit için tanısal performansını ve perforasyon için prediktif performansını, beyaz küre sayısı, yüksek duyarlıklı C-reaktif protein ve interlökin-6 ile karşılaştırmayı amaçladık.

GEREÇ VE YÖNTEM: İleriye yönelik metodolojik türde olan bu çalışmada, pentraxin-3, beyaz küre sayısı, yüksek duyarlıklı C-reaktif protein ve interlökin-6’in akut apandisit için tanısal performansı ve akut apandisitli hastalarda perforasyon için prediktif performansı karşılaştırıldı. Kontrol grubunu, inguinal herni tanısı alan ve kliniğimize elektif cerrahi için başvuran hastalar oluşturdu. Tanısal doğruluk ve prediktif gücünün karşılaştırılmasında “receiver operating characteristics’ analizi kullanıldı.

BULGULAR: Serum pentraxin-3 düzeyi >3.67 ng/mL olması, akut apandisit tanısı için %95.5 sensitivite ve %100.0 spesifiteye sahipken eğri altın-daki alan %0.99 (95% CI 0.967–1.000) olarak bulundu. Ayrıca pentraxin-3 düzeyi >9.56 ng/mL olması, perfor perfore akut apandisit prediksiyonu için %92.9 sensitivite ve %87.1 spesifiteye sahipken, eğri altında olan 0.820 (95% CI 0.736–0.886) olarak bulundu.

TARTIŞMA: Pentraxin-3’ün akut apandisit için tanısal performansı ve perforasyon için prediktif gücü beyaz küre sayısı, yüksek duyarlıklı C-reaktif protein ve interlökin-6’ya göre yüksek bulunmuştur.

Anahtar sözcükler: Akut apandisit; beyaz küre sayımı; interlökin-6; pentraxin-3; yüksek duyarlıklı C-reaktif protein.

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