Original Contribution

Pentraxin-3: A strong novel biochemical marker for appendicitis in children

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Introduction: Appendicitis is the most common surgical disease evaluated by pediatric surgeons in the emergency department. Despite the history, physical examination, laboratory tests and imaging methods, the misdiagnosis may be observed often in children. Pentraxin-3 (PTX-3) is an acute phase protein which is produced directly in the inflammatory tissue. Our aim was to investigate the diagnostic value of PTX-3 levels in appendicitis in pediatric patients and compare it with the other serum parameters.

Methods: Eighty-eight patients (aged <18 years) were included in this study [Group 1 (n = 28) healthy volunteers, Group 2 (n = 28) patients with non-specific abdominal pain, Group 3 (n = 34) patients underwent appendectomy]. Serum white blood cell (WBC), absolute neutrophil count (ANC), neutrophil/lymphocyte ratio (NLR), C-reactive protein (CRP) and PTX-3 values were measured.

Results: Median serum levels of WBC were higher in Group 2 and 3 than Group 1. ANS, NLR, CRP and PTX-3 were higher in Group 2 than Group 1 and were higher in Group 3 than the other groups. The highest sensitivity was found in NLR >3.5 [94.1 (95% CI = 80.3–99.3)] and PTX-3 > 5.6 ng/mL [91.8 (95% CI = 76.3–98.1)]. PTX-3 has the highest specificity among all of the parameters [90.7 (95% CI = 79.7–96.9)]. The area under the ROC curve showed that the diagnostic value of PTX-3 was greater than any other parameter [0.979 (95% CI = 0.92–0.99)].

Conclusion: In this study, we have shown that PTX-3 is very useful with high sensitivity and specificity in the diagnosis of appendicitis compared to WBC, ANS, NLR and CRP as a first in the literature.
In light of the abovementioned information, we planned to investigate the value of PTX-3 in the diagnosis of appendicitis in a pediatric population, and this is the first study to do so in the literature. Additionally, we aimed to determine whether this biomarker was superior to any other biomarkers currently being used in clinical practice, such as the complete blood count variables [white blood cell (WBC) count, absolute neutrophil count (ANC), and neutrophil/lymphocyte (N/L) ratio] and the CRP level.

2. Methods

2.1. Patient variables

This prospective study was conducted in the pediatric surgery department of our university after receiving approval from the institution’s Human Interventional Ethics Committee (approval number: 15022018/21). From February 2018 to July 2018, 88 patients <18 years old were consecutively included in this study. Group 1 consisted of otherwise healthy volunteers who were admitted to our clinic for outpatient surgery (n = 28). The patients with abdominal pain were divided into two groups. One group was made up of patients with nonspecific abdominal pain (NSAP) who were observed for 24–48 h and discharged (Group 2, n = 28). Those patients were contacted by phone 2 weeks after discharge in order to make sure that appendicitis did not develop during the follow-up period. The patients with obvious symptoms, like urinary tract infections, gastroenteritis, and respiratory system infections, were excluded from the study. Finally, the patients who underwent appendectomies were included in Group 3 (n = 34). The exclusion criteria for this group were as follows: pregnancy, prior surgery for abdominal conditions, pelvic inflammatory disease, concomitant ovarian pathologies, and chronic diseases.

2.2. Biochemical analyses

Upon admission to the clinic, 5 mL of blood was taken from each patient, and the WBC, ANC, PTX-3, and CRP values were obtained. The N/L ratio was calculated by dividing the absolute number of neutrophils by the absolute lymphocyte number. The blood samples were centrifuged for 5 min at 3000 rpm, and a volume of 2 cc of serum from each sample was separated into an Eppendorf tube and stored at −80°C for the PTX-3 evaluation. After reaching a sufficient sample size, the PTX-3 levels of the serum samples were tested simultaneously in the microbiology laboratory of our university. This testing was conducted under double-blind conditions using a microenzyme-linked immunosorbent assay (micro-ELISA), consistent with the quantitative standards based on the Human PTX-3 ELISA Kit (Boster Biological Technology, Pleasanton, CA, USA) protocol. Finally, the samples were measured using an ELx808 Absorbance Microplate Reader (Bio Tek Instruments, Winooski, VT, USA) at a 450-nm wavelength.

2.3. Statistical analysis

The sample size was calculated using the G*Power 3.1 software program (Heinrich Heine University Düsseldorf, Düsseldorf, Germany). In a study of PTX-3 performed on adult patients, Aygun et al. found that the median PTX-3 levels are 3.28 ng/mL in patients with appendicitis and 0.97 ng/mL in patients without appendicitis [9]. Using these values, we calculated a sample size of minimum 16 individuals needed to be included in each group for 95% power and a 5% error level. The data analysis was carried out using IBM SPSS Statistics for Windows (version 21.0; IBM Corp., Armonk, NY, USA) and MedCalc statistical software (version 15.0; MedCalc Software bvba, Ostend, Belgium). The descriptive statistics were given as the number of units (n), percentile (%), mean ± standard deviation or median with interquartile range. The normal distribution of the numerical variables was determined by using the Shapiro-Wilk normality test. If the data complied with a normal distribution, the statistical differences between the groups were evaluated using the one-way analysis of variance and post hoc tests. If the data did not comply with a normal distribution, the Kruskal-Wallis and Mann-Whitney U tests were used. A p value of <0.05 was considered to be statistically significant.

The cut-off values were obtained using a receiver operating characteristic (ROC) analysis in order to differentiate between the non-appendicitis and appendicitis groups. The clinical performance parameters were defined with regard to the sensitivity, specificity, and likelihood ratio.

3. Results

This study included 60 patients who were admitted to the pediatric surgery department due to abdominal pain and 28 healthy volunteers. The demographic information and the WBC, ANC, N/L ratio, CRP, and PTX-3 values are shown in Table 1. The WBC serum level medians were higher in Groups 2 and 3 than in Group 1. The ANC, N/L ratio, CRP, and PTX-3 values were higher in Group 2 when compared to Group 1, and they were higher in Group 3 when compared to the other two groups. The distributions of all of the parameters between the groups are shown in Figs. 1 and 2.

The N/L ratio at >3.5 and PTX-3 level at 5.6 ng/mL exhibited the highest sensitivity, and the PTX-3 exhibited the highest specificity among all of the parameters (Table 2). The area under the ROC curve (AUC) showed that the diagnostic value of the PTX-3 was greater than any of the other biochemical parameters (AUC = 0.979) (Fig. 3).

4. Discussion

It is crucial to make the correct diagnosis of acute appendicitis in order to prevent further complications, like perforations, as well as to prevent unnecessary surgery. The diagnosis must be done also promptly, because the perforation risk is increased in a linear fashion with the duration of symptoms as stated in the study of Narsule et al. [10]. Surgeons rely on the clinical history and physical examination; however, especially in young or uncooperative children, the presumed diagnosis must be supported with additional laboratory tests or imaging modalities [11–13].

In this study, we investigated the role of PTX-3 in the diagnosis of acute appendicitis in children. We found that PTX-3 was very effective for distinguishing appendicitis with a high sensitivity and specificity. It was also evident that PTX-3 was superior to all
of the other markers used in clinical practice (i.e., WBC, ANC, N/L ratio, and CRP values), with a high accuracy (AUC = 0.979).

The complete blood count is the most commonly ordered laboratory test for children suspected of having appendicitis. The most commonly used diagnostic scoring systems (the Alvarado score and Pediatric Appendicitis Score) include an elevated WBC count and left shift (neutrophil predominance, absolute neutrophil count >75%) [14,15]. However, the WBC results are usually nonspecific, because elevations may be seen in numerous infectious diseases [16]. In our study, the WBC values of the healthy controls (Group 1) were lower than those in the other two groups, but there was no difference between the group with nonspecific abdominal pain and the appendicitis group. Kessler et al. reported a 69% elevation in the WBC count of appendicitis patients and a 56% elevation in non-appendicitis patients, with a sensitivity of 77% and a specificity of 63% at a level above 10,000 cells/µL [17]. In their study, Allister et al. reported that the appendicitis patients had elevated WBC values that were greater than those of the control subjects (14,200 cells/µL vs. 10,600 cells/µL) [18]. Therefore, we believe that the WBC count has a limited diagnostic utility for discriminating appendicitis in patients with right lower quadrant pain.

Fig. 1. Comparison of the serum WBC, ANC, N/L ratio, CRP levels between healthy controls (Group 1), non-specific abdominal pain (Group 2) and appendicitis patients (Group 3). * = p < 0.05.

Fig. 2. Comparison of the serum PTX-3 levels between three groups. * = p < 0.05.
In the ANC values, there was a difference between the nonspecific abdominal pain group (Group 2) and the appendicitis group (Group 3), which was also reported in the studies by Khan et al. and Benito et al. [19,20]. In a study conducted by Fergusson et al., a neutrophil count of >11,000 cells/mm² revealed a 59% sensitivity and 88% specificity, while Kharbanda et al. found similar results with 69% sensitivity and 75% specificity [21,22]. Our study revealed similar percentages with regard to a moderate power of discrimination between the appendicitis patients and those with NSAP.

The diagnostic value of the N/L ratio has been investigated by many researchers. Our study revealed a significant difference between the three groups with regard to the N/L ratio, which is in accordance with the results seen in the literature. In a former study, we found a 90% sensitivity and an 88% specificity at a level of >3.5 for the N/L ratio, which suggests that an N/L ratio of 3.5 may be used as a diagnostic cut-off value for children with appendicitis [23]. When investigating the N/L ratio, Nazik et al. found significantly higher values in the appendicitis patients (mean = 6.06 ± 4.1) than in the control patients (mean = 1.24 ± 0.8) [24].

The CRP is the most characteristic and widely used acute phase reactant in humans [25]. We found an increasingly high sensitivity and specificity, reaching up to a mean of 15.80 mg/L in the appendicitis group. Previously, sensitivities of 0.58–0.85 and specificities of 0.33–0.82 were reported [26-29]. Mikaelsson et al. found that children with a CRP level of 25 mg/L or higher were more likely to have appendicitis, while Rodriguez-Sanjuan et al. found that children being admitted to the hospital was the reason for the higher PTX-3 levels in our study.

Fig. 3. ROC curves for WBC, ANC, N/L ratio, CRP and PTX-3 to discriminate appendicitis from non-appendicitis.
the complaints to blood draw is not investigated (disease progression bias). In the study of Wu et al., they showed that inflammatory markers (WBC and CRP) may provide a favorable diagnostic value at different time points in the diagnosis of appendicitis [37]. Also, Benito et al. stated that the diagnostic performance of the tests (WBC, CRP, ANC, procalcitonin, calprotectin, APPY1) was superior in children in whom the onset of pain was >24 h [19]. As we showed that PTX-3 increases in patients with appendicitis, the correlation of elevation of PTX-3 with time may be investigated also.

5. Conclusions

In this study, we showed that the PTX-3 levels were significantly higher in the pediatric appendicitis patients than in the abdominal pain and healthy patients, with a high accuracy. These findings imply that a high PTX-3 level is a strong indicator of appendicitis and may help distinguish children with appendicitis from children with non-specific abdominal pain.

Further studies should be planned to investigate the timely increase in the PTX-3 level with regard to its quick response to inflammation in order to diagnose these patients faster, and to monitor the clinical progression of suspected appendicitis patients. Additionally, the differences between perforated and nonperforated appendicitis cases should also be investigated, with larger patient numbers in each of the groups.

Conflict of interest

The authors declare that they have no conflict of interest.

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