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ORIGINAL ARTICLE

Eosinophilia in pediatric uncomplicated appendicitis is a time stable pattern

Josephine Reismann[1] · D. Schädlich[1] · M. I. Minderjahn[1] · K. Rothe[1] · M. Reismann[1]

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Abstract

Purpose We have recently shown that uncomplicated phlegmonous appendicitis is characterized by independent inflammatory patterns based on significant eosinophilia in children aged 7–17 years. However, clinical decision-making based on inflammatory values is not easy, especially due to the dynamics of inflammation over time. The present study was performed to evaluate the basic distinguishability of the inflammatory entities by laboratory values over time based on an extended patient number with children aged 0–17 years.

Methods All patients aged 0–17 years, who underwent appendectomy from January 2008 until June 2016, were retrospectively reviewed. Special attention was paid to cellular subpopulations within full blood counts within compartments of time (onset of symptoms – blood sampling): 0–12 , > 12–24 , > 24–36 , > 36–48 , > 48–72 , > 72 h.

Results 1041 appendectomies were included in the study. The inflammatory course in patients with complicated appendicitis ($n = 369$) was characterized by continuously increased mean leukocytes, neutrophil and monocyte counts compared with patients with phlegmonous appendicitis ($n = 489$). In contrast, continuous relative eosinophilia was found in uncomplicated appendicitis within the inflammatory process. In cases of negative appendectomies ($n = 183$), again, distinct independent inflammatory patterns were found.

Conclusion: Eosinophilia is a constant and independent pattern in children with uncomplicated appendicitis, which, thus, can be distinguished throughout the inflammatory process.

Keywords Children · Appendicitis · Eosinophilia · Time course

Introduction

Acute appendicitis is one of the major causes for urgent hospital admission and surgical intervention in childhood [1]. Clinically and histopathologically, two forms of appendicitis are distinguished: Complicated appendicitis is given when there is evidence of gangrenous or perforated appendix, an intraabdominal abscess, or peritonitis. Uncomplicated appendicitis is diagnosed when a phlegmonous appendix is found histopathologically. The crucial histopathological parameter for differentiation of the two forms is the presence of necrosis [2, 3].

The ability to specify the form of appendicitis already before the start of treatment is of clinical relevance because especially complicated appendicitis dictates decisions regarding special management [2]. The urgency and kind of therapy is dependent on the respective level of inflammation. Patients with complicated disease should probably be treated surgically, while uncomplicated appendicitis might be suitable for non-operative antibiotic treatment [2].

Together with clinical parameters laboratory values are of special relevance within the diagnosis and decision making within the disease. Next to classical laboratory values like C-reactive protein (CRP) and leukocytes, serum sodium concentrations, the neutrophil-to-lymphocyte ratio and new inflammatory markers like chemokines and special enzymes might be used for the discrimination of complicated appendicitis [4, 5]. However, clinical decision-making just on biomarkers is still not feasible because of great interindividual differences in the inflammatory process [6]. The special significance of the inflammatory

✉ Josephine Reismann
Josephine.reismann@charite.de

[1] Department of Pediatric Surgery, Charité, Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany

time course in septic disease has already been addressed to [6]. Still, this matter has not been investigated in detail—especially not in the case of acute appendicitis.

We have recently shown that patients with uncomplicated phlegmonous appendicitis—compared with those affected by complicated disease—represent a distinct patient group, which is characterized by a significant eosinophilic shift of the cellular composition within full blood counts [7].

In the current study, we wanted to investigate the differences in the temporal evolution of CRP and cellular parameters over time in children and adolescents with complicated and uncomplicated disease. In comparison to our previous investigation including patients with an age younger than 7 years, we increased the patient number. Additionally, a control group was established by including patients with negative histopathological findings after appendectomy. The primary hypothesis was that the investigated laboratory values are distinguishable throughout the inflammatory process.

Methods

Study population

All patients aged from 0 to 17 years who underwent appendectomy from January 2008 till June 2016 with subsequent histologically confirmed acute appendicitis and negative histopathological findings were reviewed retrospectively. At hospital admission, informed consent for pseudonymized retrospective evaluation of clinical data had been obtained routinely from all individual participants and their parents included in the study. Ethical approval was provided by the local ethics committee (No. EA2/169/18). Two researchers independently reviewed the records. A senior scientist performed control of results. In case of mistakes or conflicting data, the results were corrected, if necessary.

Histopathological classification

The classification of appendicitis was based on histopathology. According to the histopathological diagnosis, the patients were classified into the following groups: missing histopathological signs for appendicitis (negative, control group), phlegmonous (= uncomplicated) appendicitis and gangrenous/perforated (= complicated) appendicitis. *Phlegmonous appendicitis* was defined by transmural neutrophilic infiltration of the appendix without gangrene or perforation. *Gangrenous appendicitis* was characterized by ischemic areas with transmural myonecrosis, while perforation was defined by the presence of a transmural defect [3].

Clinical and laboratory data

Diagnostics for acute appendicitis included a full peripheral white blood cell count (WBC). They included the following mature leukocyte subpopulations: neutrophilic granulocytes (neutrophils), eosinophilic granulocytes (eosinophils), basophilic granulocytes (basophils), immature granulocytes, lymphocytes and monocytes. Further, routine diagnostics included C-reactive protein (CRP, mg/L). Blood was taken at the time of hospital admission in the pediatric emergency department.

To ensure comparability of the patients in the different groups, following parameters were documented: age (years), gender and total time period from onset of symptoms until admission and from admission until start of surgery (exact calculation based on patient charts), respectively. Onset of symptoms was defined as the first perception of abdominal pain, which continuously persisted until presentation at the hospital.

To establish a reliable retrospective timeline with sufficient numbers of patients, the time courses were subdivided into 12 h intervals after the first perception of symptoms: 0–12 , > 12–24 , > 24–36 , > 36–48 , > 48–72 , > 72 . 12 h intervals have been previously successfully used to retrospectively investigate clinical outcomes [8]. The minimum number of patients within these categories was 10.

Statistical analysis

After exclusion of normal distribution via box-plot analysis (asymmetric distributions in all groups), Mann–Whitney *U* test was performed for analysis of continuous variables and chi-square test for categorical variables. For judgment of the distinguishability of complicated and uncomplicated appendicitis due to specific values/parameters, ROC (receiver operator characteristic) curve analysis was performed. If the area under the curve exceeded a value of 0.5, the respective parameter was assumed to distinguish complicated from phlegmonous appendicitis sufficiently [9]. Data analysis was performed with GraphPad Prism software (version 6.0f, La Jolla, CA). Data are shown as mean values ± standard deviation and standard error of the mean. $p \leq 0.01$ was considered significant.

Exclusion criteria

Patients were excluded from further analysis due to secondary appendectomies, missing histopathology, missing data, elective appendectomy, and secondary or independent histopathological abnormalities like oxyuriasis or carcinoid.

Results

Epidemiology

1448 appendectomies were performed between January 2008 and June 2016 in children aged 0–17 years. 407 patients were excluded due to inconsistent or missing clinical/temporal data, due to independent histopathological anomalies (esp. oxyuriasis/ carcinoid) and due to unavailable hemogram.

Uncomplicated appendicitis was found in 489 of included patients (47%), complicated disease (co) in 369 patients (35.4%) (gangrenous appendicitis (gg) $n=88$, 8.5%, perforated appendicitis (pf) $n=281$, 27%). A negative histopathological finding (neg) was present in 183 cases (17.6%). Male gender was slightly more common ($n=596$, 57.3%). There were also no significant differences between the groups [uncomplicated (uc), complicated (co) and negative (neg) appendicitis] regarding mean age [10.6 ± 3.0 (uc) vs. 9.6 ± 3.8 (co) vs. 11.7 ± 4.1 (neg); years, mean ± SD]. Mean time period from onset of symptoms until surgery was comparable in patients with uncomplicated and complicated appendicitis (9.2 ± 8.7 vs. 7.8 ± 9.8 h; $p=$ n.s.), but both groups revealed significant differences compared with patients with negative findings (16.4 ± 18.2 h; $p<0.01$). Table 1 outlines patient demographics and relevant time periods for the groups.

Analysis of blood work and CRP values

Differences of CRP cell counts within the investigated cellular subpopulations took place against the background of distinct inflammatory dynamics in the groups over time. Reliable values with sufficient patient numbers within the categories of time could be calculated for time periods of up to 3 days after onset of symptoms. Values of patients with a longer period of abdominal pain at hospital admission were summarized in the category ">72 h". The inflammatory course in patients with complicated appendicitis was characterized by a rapid increase of mean CRP, starting with a mean value of 11.4 ± 3.2 mg/L within the first twelve hours after first perception of symptoms, finally resulting in a mean value of 105.1 ± 83.8 mg/L > 72 h after onset of symptoms. Patients with uncomplicated phlegmonous appendicitis initially showed a different course, already starting from a significant lower value of 3.4 ± 2.2 mg/L within 0–12 h up to a maximum of 25.6 ± 17.1 mg/L (> 24–36 h). The inflammatory course in this group was comparable with that in patients with negative findings: minimum 1.5 ± 1.3 mg/L, maximum 13.6 ± 13.1 mg/L (no significant difference). With respect to measures of time, mean leukocytes were stable over time in patients with complicated inflammation (0–12 h: $17.3\pm3.7\times10^9$/L, > 72 h: $16.1\pm3.1\times10^9$/L), but were continuously above those values in patients with uncomplicated appendicitis (0–12 h: $15.8\pm4.1\times10^9$/L, > 72 h: $13.4\pm5.8\times10^9$/L) and with those with negative findings (0–12 h: $12.1\pm4.4\times10^9$/L, > 72 h: $9.5\pm5.0\times10^9$/L). The inflammatory course and particular significant differences are visualised in Figs. 1 and 2.

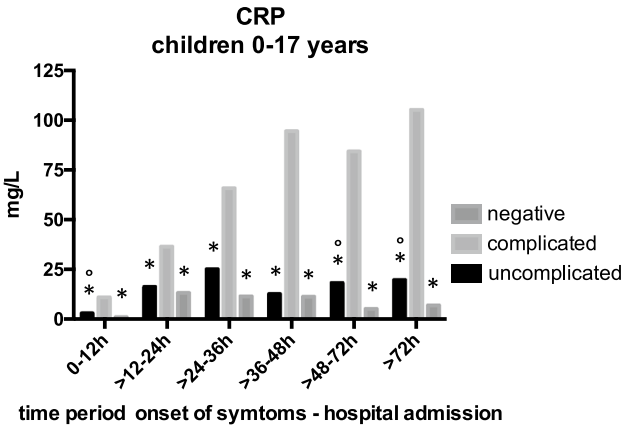

Fig. 1 CRP values of patients with complicated appendicitis, uncomplicated appendicitis and patients with negative histopathological findings within the particular temporal categories; *mg/L* mean values; level of significance $p\leq0.01$, °: vs. negative, *: vs. complicated appendicitis

Table 1 Epidemiology, clinical time courses and mean CRP values of all patients

Histological subgroup	Total number	Gender	Age	Time admission-surgery (h)	CRP (mg/L)
Negative	$n=183$ (17.6%)	♂77 ♀106	11.7 ± 4.1	16.4 ± 18.2*	21.6 ± 47.3*
Uncomplicated	$n=489$ (47.0%)	♂290 ♀199	10.6 ± 3.0	9.2 ± 8.7	19.6 ± 35.4*
Complicated	$n=369$ (35.4%)	♂229 ♀140	9.6 ± 3.8	7.8 ± 9.8	74.6 ± 78.6

Mean values ± SD; n = 1041

CRP vs. complicated appendicitis

*$p<0.01$, time admission-surgery: vs. uncomplicated and complicated appendicitis

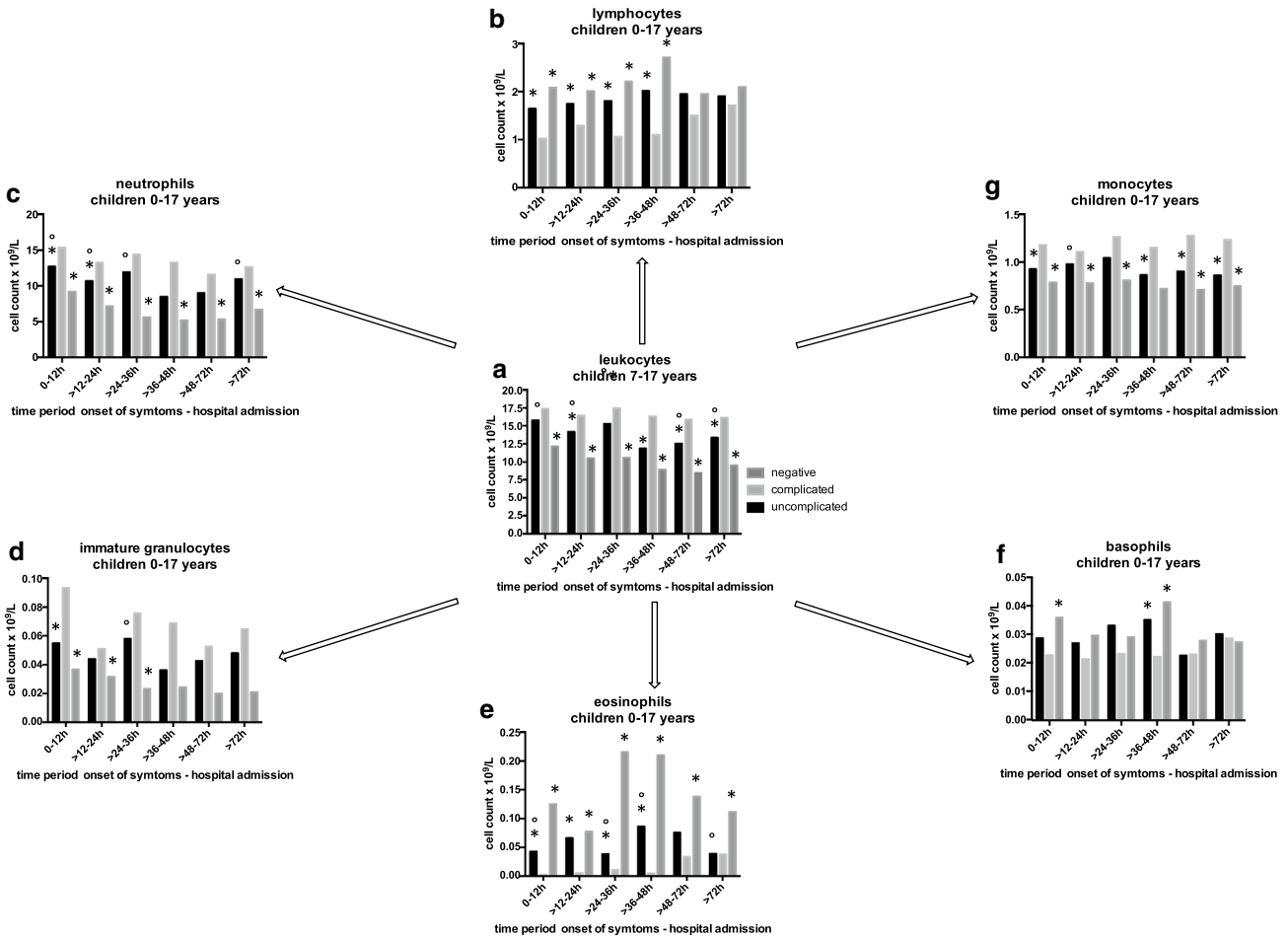

Fig. 2 Cell counts of patients with complicated appendicitis, uncomplicated appendicitis and patients with negative histopathological findings within the particular temporal categories. **a** leukocytes, **b** lymphocytes, **c** neutrophils, **d** immature granulocytes, **e** eosinophils, **f** basophils, **g** monocytes; cell count × 10^9/L, mean values; level of significance $p \leq 0.01$, °: vs. negative, *: vs. complicated appendicitis

Fractionized in the WBCs constituent parts, children with complicated appendicitis showed continuously increased mean neutrophil and monocyte counts compared with patients with uncomplicated appendicitis and negative histopathology [neutrophils 0–12 h: $15.3 \pm 3.3 \times 10^9$/L, > 72 h: $12.6 \pm 4.9 \times 10^9$/L (co) vs. 0–12 h: $12.7 \pm 3.8 \times 10^9$/L, > 72 h: $10.9 \pm 5.7 \times 10^9$/L (uc) vs. 0–12 h: $9.2 \pm 4.0 \times 10^9$/L, > 72 h: $6.7 \pm 3.8 \times 10^9$/L (neg); monocytes 0–12 h: $1.2 \pm 0.6 \times 10^9$/L, > 72 h: $1.2 \pm 0.5 \times 10^9$/L (co) vs. 0-12 h: $0.9 \pm 0.3 \times 10^9$/L, > 72 h: $0.9 \pm 0.4 \times 10^9$/L (uc) vs. 0–12 h: $0.8 \pm 0.4 \times 10^9$/L, > 72 h: $0.8 \pm 0.3 \times 10^9$/L (neg); Fig. 2c, g].

In contrast, relative eosinophilia was found in uncomplicated inflammation over time compared with complicated disease (0-12 h: $0.003 \pm 0.005 \times 10^9$/L, > 72 h: $0.038 \pm 0.055 \times 10^9$/L (co) vs. 0-12 h: $0.043 \pm 0.042 \times 10^9$/L, > 72 h: $0.039 \pm 0.047 \times 10^9$/L (uc) as shown in Fig. 2e. The inflammatory course with respect to basophils is characterized by relative basophilia over time as shown in Fig. 2f.

The mean lymphocyte counts over time were comparably reduced in patients with complicated appendicitis [0–12 h: $0.023 \pm 0.013 \times 10^9$/L, > 72 h: $0.029 \pm 0.015 \times 10^9$/L (co) vs. 0–12 h: $1.646 \pm 0.758 \times 10^9$/L, > 72 h: $1.91 \pm 0.898 \times 10^9$/L (uc) vs. 0–12 h: $2.087 \pm 1.326 \times 10^9$/L, > 72 h: $2.096 \pm 0.885 \times 10^9$/L (neg); Fig. 2b].

ROC curve analysis

The area under the curve (AUC) exceeded a value of 0.5 for CRP and all investigated cellular subpopulations in almost all cases within the particular temporal categories and in total (Fig. 3).

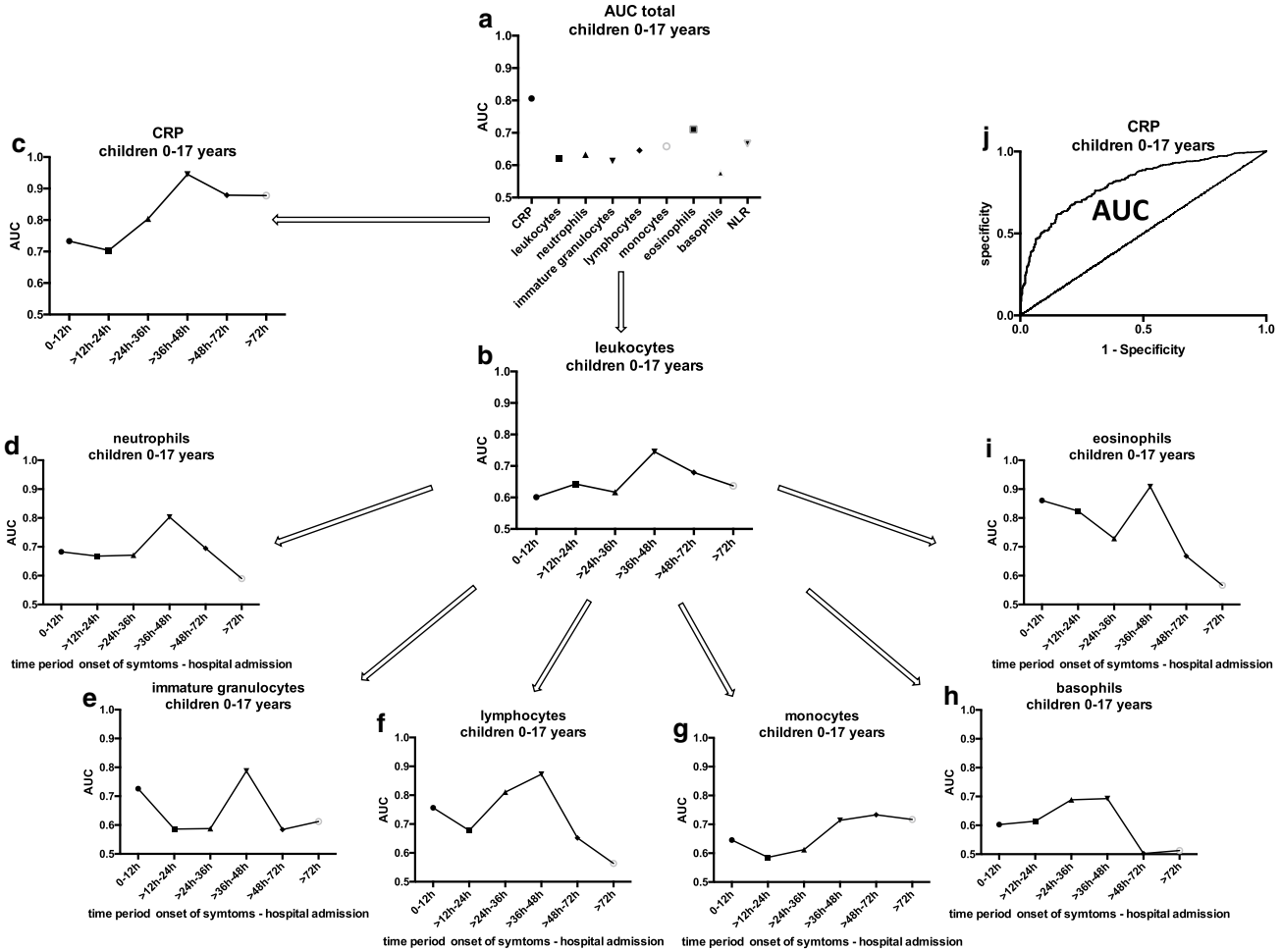

Fig. 3 Area-under-the-curve (AUC) values for CRP, leukocytes and cellular subpopulations in patients with complicated and uncomplicated appendicitis: total (**a**) and particular temporal categories (**b**–**i**). **j** Demonstration of receiver operating characteristic (ROC) curve for CRP: an AUC > 0.5 indicates distinguishability of inflammatory parameters

Discussion

We have previously shown that different inflammatory patterns characterize uncomplicated and complicated appendicitis with special regard to eosinophils [7]. With the present study, the question should be clarified whether the previously found inflammatory patterns are stable over time.

The key finding of our study is that CRP and cellular parameters within uncomplicated and complicated appendicitis in children and adolescents show significant and time stable differences. Gangrenous and perforated appendicitis seem to represent highly inflammatory entities, which are primarily characterized by rapidly increasing CRP, relative neutrophilia and monocytosis, while uncomplicated appendicitis is associated with moderately enhanced CRP and time stable relative eosinophilia.

The continuous distinctness of the two entities is emphasized by AUC values, which significantly exceed values of 0.5 for all parameters over almost all time intervals.

Roland Andersson et al. were the first surgical researchers who published convincing epidemiologic data on the possibility that uncomplicated phlegmonous appendicitis and complicated appendicitis represent independent entities [10].

The pathophysiological background might be explained by immunological studies performed by Marie Rubér et al. who have shown that phlegmonous appendicitis is primarily characterized by Th2 T-helper cell mechanisms, while gangrenous and complicated appendicitis are based on Th1- and Th17 dependent mechanisms [11, 12]. This view is supported by histopathological findings showing eosinophilic infiltration of resected appendices within phlegmonous appendicitis [13]. In addition, we were able to demonstrate eosinophilic inflammation in full peripheral blood counts of respective patients [7].

Common up-regulation of eosinophils and basophils is a typical pattern, which supports the view of a Th2-cell association in case of phlegmonous appendicitis [14, 15]. Th1- and Th17-dependent conditions would be compatible with the observed relative and time stable increase of monocytes and neutrophils in our study [11, 16].

Interestingly, significant differences between the majorities of parameters within the different categories seem to occur quite early within the first 12 h after onset of symptoms. The in-hospital time periods until surgery are comparable between patients with uncomplicated and complicated disease. Complicated appendicitis seems to represent primarily a prehospital development. This result is compatible with other studies on that matter [8, 17].

The weakest point of our analysis lies in its nature as a retrospective study. It may be criticized that retrospective division in temporal categories is difficult. With the presented study design we aimed to counteract this bias by establishment of 12 h and even broader time intervals. We only included patients in the study if reliable classification within these time intervals was possible. The parameters considered were restricted to a limited number of endpoints. However, with the present study design, we did not aim to present definite values for specific time points, but to give semi-quantitative information regarding relative compositions over time. We interpret the comprehensive results as a sign for the success of this approach.

In conclusion, the previously described laboratory patterns within uncomplicated and complicated appendicitis are distinguishable throughout the inflammatory process over days. The hypothesis is confirmed. Attempts to identify and establish suitable time independent and more effective biomarkers seem to be reasonable.

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Compliance with ethical standards

Conflict of interest Josephine Reismann declares that she has no conflict of interest. Dag Schädlich declares that he has no conflict of interest. Maximiliane I. Minderjahn declares that she has no conflict of interest. Karin Rothe declares that she has no conflict of interest. Marc Reismann declares that he has no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (No. EA2/169/18) and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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