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The use of selected neutrophil protein plasma concentrations in the diagnosis of Crohn's disease and ulcerative colitis – a preliminary report*

Zastosowanie stężenia wybranych białek neutrofili w osoczu w diagnostyce choroby Leśniowskiego-Crohna i wrzodziejącego zapalenia jelita grubego – doniesienie wstępne

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: Difficulties in diagnosis of inflammatory bowel disease (IBD) motivate the search for new diagnostic tools, including laboratory tests. The aim of this study was to evaluate concentrations of the neutrophil (NEU) proteins leukocyte elastase (HLE-α1AT), lactoferrin and calprotectin as potential biomarkers used in the diagnosis and assessment of clinical activity of Crohn's disease (CD) and ulcerative colitis (UC).

Material/Methods: The study included 27 patients with CD, 33 patients with UC and 20 healthy controls. Plasma concentrations of calprotectin, lactoferrin and HLE-α1AT were measured using ELISA.

Results: In patients with CD higher concentrations of HLE-α1AT (64.3±43.1 vs. 30.1±7.7 ng/l, P<0.001), calprotectin (151.6±97.8 vs. 69.9±22.1 ng/l, P<0.001) and lactoferrin (243.2±102.0 vs. 129.7±32.7 ng/l, P<0.001) than in the control group were found. In patients with UC higher plasma concentrations of HLE-α1AT (62.0±30.9 vs. 30.1±7.7 ng/l, P<0.001), calprotectin (149.6±72.3 vs. 69.9±22.1 ng/l, P<0.001) and lactoferrin (242.6±107.5 vs 129.7±32.7 ng/l, P<0.001) than in the control group were found. HLE-α1AT/NEU and lactoferrin/NEU ratios in patients with UC were significantly higher compared with patients with CD. Calprotectin (P=0.010) and lactoferrin (P=0.023) levels were higher in patients with the active compared with inactive phase of CD.

Conclusions: The diagnostic characteristics of plasma granulocyte protein concentrations indicate the usefulness of these tests in the diagnosis of IBD. Higher HLE-α1AT and lactoferrin/NEU ratios in patients with UC than with CD may suggest the usefulness of these ratios in differential diagnostics. Plasma calprotectin and lactoferrin levels may be useful in CD activity assessment.

Keywords: **inflammatory bowel disease • calprotectin • lactoferrin • leukocytes elastase**

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Introduction

The prevalence of inflammatory bowel disease (IBD) has increased in recent years and continues to grow [1,17,18]. In Poland, according to data from the National Register, in July 2015 there were 6130 people with Crohn's disease (CD) [20]. No data on the number of patients with ulcerative colitis (UC) in Poland are available. The pathogenesis of IBD is not fully elucidated; genetic predisposition, immunological and intestinal microbiota changes are taken into consideration. IBD requires long-term treatment to achieve remission and to prevent relapses and cancer, and in both CD and UC the prognosis is unfavourable. However, in many patients only a transient remission can be achieved. These diseases significantly reduce the quality of the patient's life, limiting the ability to perform many daily and professional activities, often leading to chronic disability, not only physical but also psychosocial [3,21]. Therefore, in order to improve treatment methods new diagnostic tools are developed to ensure early diagnosis, risk stratification and treatment monitoring. New laboratory tests that can be easily available diagnostic tools assessing IBD activity and clinical severity, and monitoring the treatment seem to be necessary [16,21].

Neutrophils (NEU) are involved in the innate immune response and have a large arsenal of antibacterial mechanisms including the ability to phagocytose and produce NET (neutrophil extracellular traps). As cells participating in the inflammatory response, neutrophils are involved in the pathogenesis of IBD, as evidenced by the results of experimental and histopathology studies revealing their presence in the inflamed intestinal wall [15]. In light of the other data, neutrophils' involvement in the pathogenesis of IBD should be considered in the context of their dysfunction, which is one of the immune system abnormalities observed in this disease. Multiple proteins released from cell granules including elastase, calprotectin and lactoferrin, which are easily measurable in faeces and plasma, can reflect neutrophils' activation. These measurements may be useful as an alternative or tests added to the routinely used inflammatory markers in the diagnosis of IBD [2,23,25].

The aim of this study was to evaluate the usefulness of measurements of selected neutrophil protein concentrations in the diagnosis of IBD, differential diagnostics of CD and UC, and in assessing the severity of these diseases.

Methods

The study included a group of 60 patients with IBD (33 with UC and 27 with CD, aged from 18 to 75 years) and 20 healthy subjects in the control group. The patients were hospitalized in the Department of Gastroenterology, Hepatology and Infectious Diseases of the University Hospital, Krakow, Poland in the years 2010 to 2013. The diagnosis of CD and UC was based on the clinical assessment including patient's history, physical examination and endoscopy with the histological evaluation of intestinal mucosal biopsy specimens as well as results of imaging studies. The criteria for exclusion from the study were coexisting malignancy, endocrine disorders, diabetes, obesity, ischemic heart disease and systemic diseases. The control group consisted of 20 healthy subjects aged 20 to 61 years. The UC patients were divided into the active (23 patients) and inactive (10 patients) phase of the disease subgroups according to the Truelove-Witts index based on the number of bowel movements per day and systemic abnormalities [24]. The UC patients with mild disease and remission in the Truelove-Witts scale were assigned to the inactive subgroup whereas the patients classified as having moderate or severe disease were assigned to the active subgroup. The CD patients, according to the Crohn's disease activity index (CDAI), were divided into active for CDAI>150 (16 patients) and for CDAI<150 inactive (11 patients) phase of disease subgroups [4].

The Bioethical Committee of the Jagiellonian University in Krakow, Poland approved the study and all patients expressed their consent to participate in the study.

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Venous blood was collected using S-Monovette tubes (Sarstedt, Germany). Plasma was separated and aliqu-

oted into polypropylene tubes for deep-freezing, and stored at – 70°C until assayed. C-reactive protein (CRP) was measured using immunonephelometric assay on the Nephelometer II Analyzer (Siemens Healthcare Diagnostics). The rest of the selected parameters were determined using the ELISA method, using the appropriate kits: leukocyte elastase – alpha-1 antitrypsin complex (HLE-α1AT) (PMN Elastase ELISA, BioVendor, Czech Republic), plasma calprotectin (Hycult Biotechnology Company, USA), plasma lactoferrin (Hycult Biotechnology Company, USA).

Normality of distribution of the results was assessed using the Kolmogorov-Smirnov test. Normally distributed results were presented as means and standard deviations, and means were compared using the t test. Nonparametrically distributed results were presented as medians and inter-quartile differences, and medians were compared using the Mann-Witney U-test. The significance level $p<0.05$ was applied. Based on the ROC (receiver operating characteristics) curves analysis, relevant cut-off values were selected, and diagnostic accuracy, sensitivity, specificity, positive predictive values, negative predictive values, and likelihood ratios for positive and negative results were calculated. Furthermore, ratios of granulocyte protein concentrations to leukocyte (WBC) and neutrophil (NEU) counts were evaluated as parameters reflecting neutrophil activity.

Results

Table 1 shows a comparison of the analyzed results in patients with CD, CU and the control group. CRP, plasma lactoferrin, lactoferrin/WBC ratio, calprotectin concentration, calprotectin/WBC ratio, HLE-α1AT level, and HLE-α1AT/WBC ratio were significantly higher in patients with CD. The median concentrations of lactoferrin, calprotectin, HLE-α1AT, and neutrophil proteins/

Table 1. Comparison of the test results and calculated ratios in control group and patients with inflammatory bowel disease

		Studied Groups			P		
		Control group (n=20)	Crohn's disease (n=27)	Ulcerative colitis (n=33)	Control group vs Crohns disease	Control group vs Ulcerative Colitis	Crohns disease vs CU
CRP	[mg/l]	1.1 (0.51-2.14)	6.4 (0.5-20.5)	2.3 (1.1-19.6)	0.028	0.012	NS
WBC	10^3/uL]	5.7 (4.9-6.6)	8.3 (6.2-10.0)	6.4 (5.3-8.9)	0.003	NS	NS
NEU	[10^3/uL]	3.02 (2.35-3.75)	6.25 (4.14-7.44)	4.00 (3.20-5.58)	>0.001	0.015	0.023
Lactoferrin	[ng/mL]	129.7±32.7	243±102	243±108	>0.001	>0.001	NS
Lactoferrin WBC		21.9 (17.1-25.2)	32.9 (24.0-35.4)	29.9 (24.6-40.7)	0.008	0.003	NS
Lactoferrin NEU		40.0 (32.2-59.2)	43.5 (31.7-52.6)	48.6 (42.5-70.3)	NS	0.049	0.033
Calprotectin	[ng/mL]	69.9±22.1	151.6±97.8	149.6±72.3	>0.001	>0.001	NS
Calprotectin WBC		12.7 (8.4-17.4	20.0 (13.7-25.7)	15.8 (12.9-28.8)	0.007	0.010	NS
Calprotectin NEU		19.7 (16.1-33.2)	23.4 (16.8-42.6)	23.2 (18.7-51.6)	NS	NS	NS
HLE-α1AT	[ng/mL]	30.1±7.7	64.3±43.1	62.0±30.9	0.001	>0.001	NS
HLE-α1AT WBC		5.3 (4.2-6.7)	6.7 (5.8-9.2)	8.4 (6.5-9.9)	0.006	>0.001	NS
HLE-α1AT NEU		9.9 (7.4-13.2)	10.3 (7.2-13.4)	12.8 (10.9-15.9)	NS	0.012	0.049

Results are presented in the form of mean±standard deviation and median (first quartile-third quartile). Abbreviations: CRP - C-reactive protein, WBC - White blood Cells, NEU - Neutrophils HLE-α1AT - leukocytes elastase - alfa 1 antytripsine, P – level level of statistical ignificance,NS - not significant of statistical significance, NS - not significant

Table 2. Diagnostic characteristics of the evaluated tests and ratios in the diagnosis of Crohn's disease

		Cut-off value	Accurancy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV %	LR+
CRP	[mg/L]	4.7	48.5	80.0	60.4	70.0	48.5	2.31
WBC	[10^3/uL]	6.5	73.3	72.0	75.0	78.3	68.2	2.88
NEU	[10^3/uL]	3.8	80.0	85.0	75.0	77.3	83.3	3.40
Lactoferrin	[ng/mL]	161.7	87.2	85.2	90.0	92.0	81.8	8.52
Lactoferrin / WBC		26.6	73.3	68.0	80.0	81.0	66.7	3.40
Lactoferrin / NEU		23.9	55.0	100.0	10.0	52.6	100.0	1.11
Calprotectin	[ng/mL]	99.1	83.0	77.8	90.0	91.3	75.0	7.78
Calprotectin / WBC		18.5	71.1	56.0	90.0	87.5	62.1	5.60
Calprotectin / NEU		8.2	55.0	100.0	10.0	52.6	100.0	1.11
HLE-α1AT	[ng/mL]	40.1	85.1	81.5	90.0	91.7	78.3	8.15
HLE-α1AT / WBC		5.2	71.1	88.0	50.0	68.8	76.9	1.76
HLE-α1AT / NEU		5.1	52.5	100.0	25.0	51.3	100.0	1.05

Abbreviations: CRP - C-reactive protein, WBC - White blood Cells, NEU - Neutrophils , HLE-α1AT - leukocytes elastase - alfa 1 antytripsine, PPV- positive predictive value, NPV- negative predictive value, LR+ - likelyhood ratio for positive result

WBC ratios were twice as high in patients with CD as in the control group. The median lactoferrin/NEU ratio, calprotectin/NEU ratio and HLE-α1AT/NEU ratio did not differ significantly between these two groups.

CRP, plasma lactoferrin, lactoferrin/WBC ratio, lactoferrin/NEU ratio, plasma calprotectin, calprotectin/WBC ratio, and HLE-α1AT level were significantly higher in patients with UC compared with the control group. The medians of lactoferrin, calprotectin and HLE-α1AT levels were in patients with UC twice as high as in the control group. No significant differences in calprotectin/NEU ratio between patients with UC and controls were found. Only neutrophil count, lactoferrin/NEU ratio and HLE-α1AT/NEU ratio were significantly higher in patients with UC than in CD patients.

Figure 1 shows the ROC curves analysis for tests evaluated in patients with CD and controls. Areas under the ROC curves (AUCs) above 0.8 were found for neutrophil count (0.850), plasma calprotectin (0.854), lactoferrin (0.872) and HLE-α1AT (0.900). For granulocyte proteins/WBC ratios AUCs below 0.8 were found, and for the granulocyte proteins/NEU ratios AUCs were less than 0.6. Table 3 shows diagnostic characteristics of the tests evaluated in CD patients and controls. High accuracy, sensitivity, specificity, predictive values, and likelihood ratios (LR+) were found for lactoferrin, calprotectin and HLE-α1AT. Neutrophil count was characterized by high accuracy, sensitivity and negative predictive value whereas for granulocyte proteins/NEU ratios low accuracy (approximately 50%), high sensitivity and negative predictive value were found. High positive predictive value was found only for lactoferrin/WBC ratio and calprotectin/WBC ratio.

Figure 2 presents the ROC curves for the evaluated tests in patients with UC and controls. The largest areas under the curves were found for neutrophil count (0.708), plasma calprotectin (0.824), lactoferrin (0.855), and HLE-α1AT (0.900). For granulocyte proteins/WBC ratios between 0.7 and 0.8 were found, while for granulocyte proteins/NEU ratios AUCs below 0.7 were found.

In Table 3 diagnostic characteristics of the tests evaluated in patients with UC and the control group are

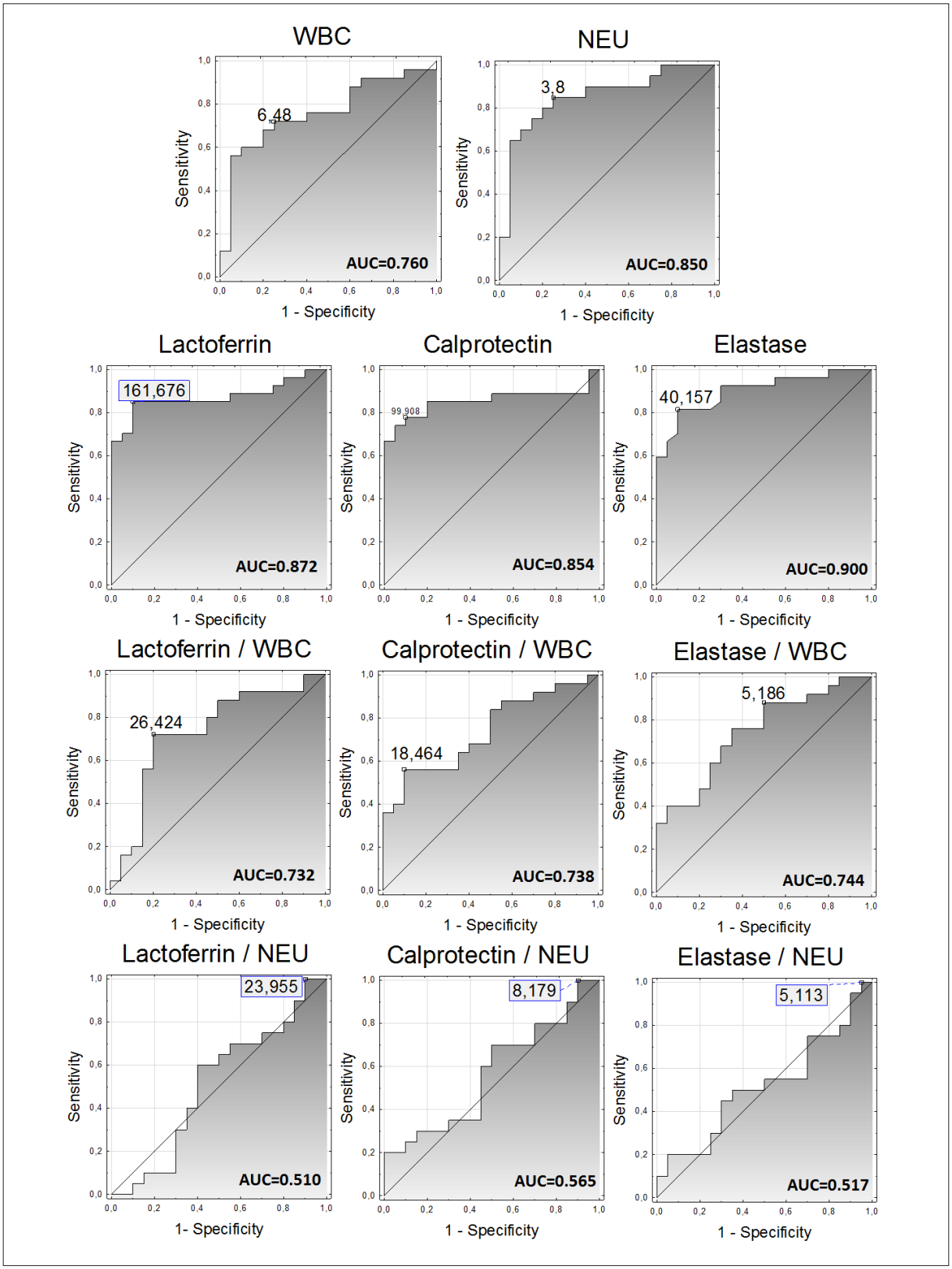

Fig. 1. ROC curves analysis for the test results and calculated ratios in patients with Crohn's disease and control group. (Abbreviations: ROC – receiver operating characteristics NEU – neutrophils, WBC – white blood cells)

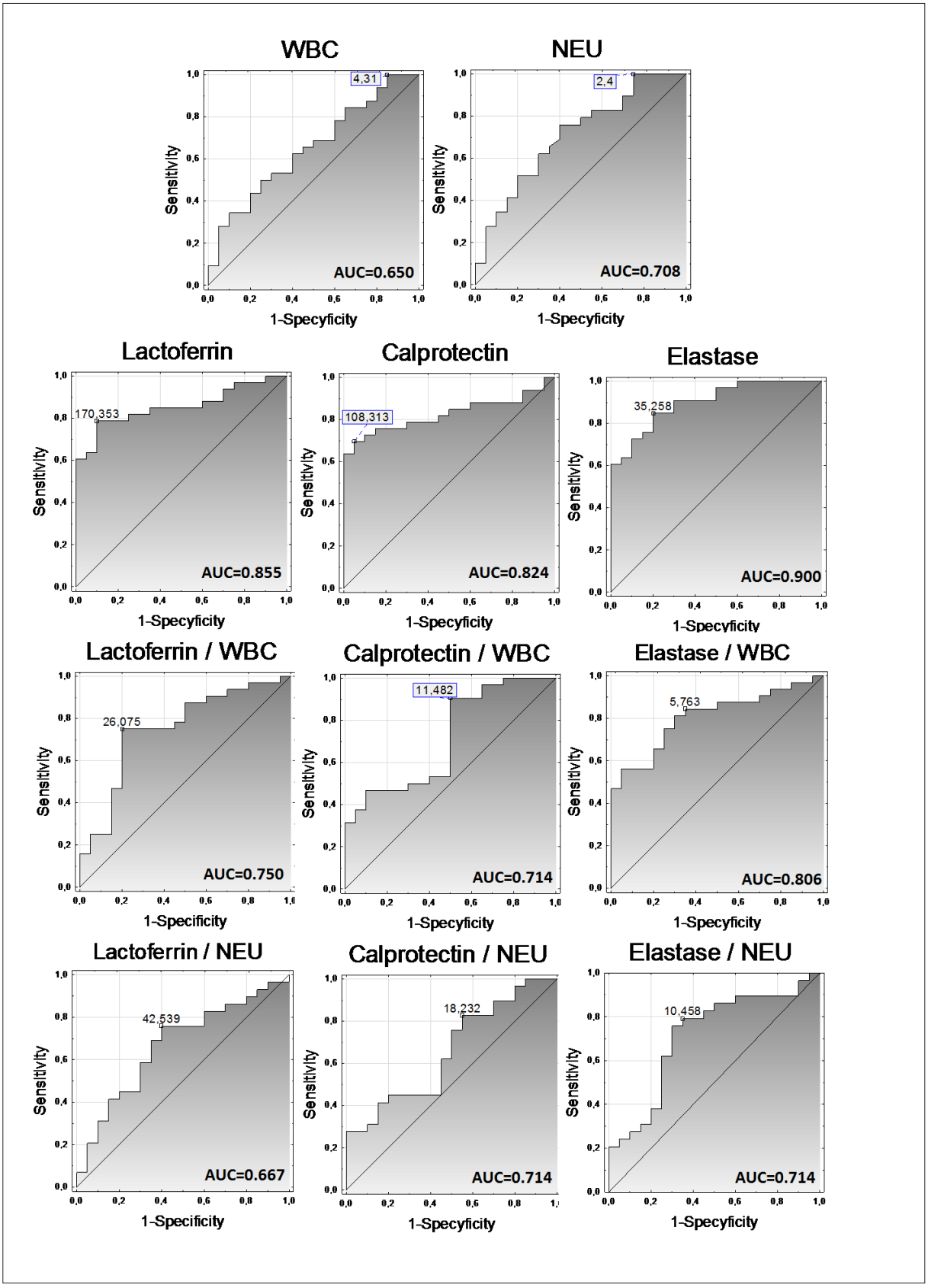

Fig. 2. ROC curves analysis for the test results and calculated ratios in patients with ulcerative colitis and control group. (Abbreviations: ROC – receiver operating characteristics, NEU – neutrophils, WBC – white blood cells)

shown. High accuracy, sensitivity, specificity, and predictive values for lactoferrin, calprotectin, and HLE-α1AT were found. Calprotectin had the highest LR+.

In Table 5 the results of performed tests and calculated ratios in patients with active and inactive CD are compared. In patients with active CD significantly higher levels of lactoferrin and calprotectin were found. The mean lactoferrin and calprotectin concentrations were almost twice as high as in patients in inactive CD. Differences between calprotectin and lactoferrin/WBC and NEU ratios were not significant. The concentration of lactoferrin and calprotectin had little effect on the odds of active disease (increase by 1.4% and 3.5%, respectively) (Table 5). Other tests and calculated ratios were not associated with clinical activity of CD.

The same comparisons between patients with exacerbation and remission of UC were also made (Table 4). In patients with active UC significantly higher leukocyte and neutrophil counts were found. Changes in measured protein concentrations and calculated ratios did not significantly increase the odds of active UC (Table 5).

Discussion

Recent data indicate that the diagnosis of IBD is established up to three years after onset of symptoms [19]. Moreover, the diagnosis in 5-15% of cases is difficult to establish based on the clinical, imaging and histological evaluation [27]. These difficulties motivate the search for new diagnostic tools including laboratory tests, which could be used to diagnose, assess the severity and prognosis, and to monitor the treatment of IBD.

It was observed that CRP was characterized by a low diagnostic sensitivity and specificity, and low predictive values of the results and the likelihood ratio for a positive result, which indicates little use of this test in the diagnosis of CD and UC (Tables 3 and 4). Elevated CRP levels seem to be a good indicator of clinical activity of CD and UC (Table 5). Similar findings were observed by Lehrke et al., who found a higher concentration of CRP in patients with active CD and UC compared to the remission [14].

Based on growing evidence for the involvement of neutrophils in the pathogenesis of IBD we evaluated plasma granulocyte protein levels in patients with CD and UC. We found significantly higher concentrations of the studied granulocyte proteins in patients with CD and UC compared with the control group (Table 1). The largest areas under the ROC curves were found for HLE-α1AT in both diseases (Figures 1 and 2). In the diagnosis of CD, taking the cut-off value of 40 ng/mL HLE-α1AT had diagnostic sensitivity, specificity and positive predictive value above 80%, and negative predictive value close to 80% (Table 2). Similar diagnostic characteristics for HLE-

Table 3. Diagnostic characteristics of the evaluated tests and ratios in the diagnosis of ulcerative colitis

		Cut-off value	Accurancy (%)	Sensitivity (%)	Specificity (%)	PPV %	NPV %	LR+
CRP	[mg/L]	3.0	48.5	80.0	60.4	80.0	48.5	2.42
WBC	[10^3/uL]	5.1	81.3	35.0	63.5	66.7	53.8	1.25
NEU	[10^3/uL]	3.0	79.3	45.0	65.3	67.6	60.0	1.44
Lactoferrin	[ng/mL]	170.4	78.8	90.0	83.0	92.9	72.0	7.88
Lactoferrin / WBC		26.1	75.0	80.0	76.9	85.7	66.7	3.75
Lactoferrin / NEU		42.5	75.9	60.0	69.4	73.3	63.2	1.90
Calprotectin	[ng/mL]	108.3	69.7	95.0	79.2	95.8	65.5	13.94
Calprotectin WBC		11.5	90.6	50.0	75.0	74.4	76.9	1.81
Calprotectin NEU		18.2	82.8	45.0	67.3	68.6	64.3	1.50
HLE-α1AT	[ng/mL]	35.3	84.8	80.0	83.0	87.5	76.2	4.24
HLE-α1AT / WBC		5.8	81.3	65.0	75.0	78.8	68.4	2.32
HLE-α1AT / NEU		10.5	79.3	65.0	73.5	76.7	68.4	2.27

Abbreviations: CRP - C-reactive protein, WBC - White blood Cells , NEU - Neutrophils, HLE-α1AT - leukocytes elastase - alfa 1 antytripsine, PPV- positive predictive value, NPV- negative predictive value, LR+ - likelyhood ratio for positive result

Table 4. Comparison of the test results and calculated ratios in patients with active and inactive Crohn's disease and ulcerative colitis

		Crohn's disease			Ulceratibe colitis		
		Inactive CDAI: 108±30 N=11	Active CDAI: 292±130 N=16	P	Inactive N=10	Active N=23	P
CRP	[mg/L]	1.60 (0.13-6.41)	16.41 (1.73-24.04)	0.0239	0.87 (0.49-1.87)	12.70 (1.37-26.19)	0.0245
WBC	[10^3/uL]	7.6 (5.2-9.5)	8.8 (6.5-10.0)	NS	5.3 (4.6-6.4)	7.2 (5.8-9.1)	0.029
NEU	[10^3/uL]	5.1 (3.8-7.0)	6.5 (4.7-7.6)	NS	3.3 (2.6-3.5)	4.2 (3.5-5.9)	0.025
Lactoferrin	[ng/mL]	186.3±45.2	276.7±112.0	0.023	199.8±72.0	258.6±115.3	NS
Lactoferrin WBC		24.0 (17.2-35.3)	33.2 (28.7-38.8)	NS	29.9 (23.2-43.5)	30.0 (26.1-37.4)	NS
Lactoferrin NEU		32.5 (24.9-47.3)	44.2 (41.0-54.3)	NS	57.9 (40.6-81.2)	47.9 (42.5-65.0)	NS
Calprotectin	[ng/mL]	90.6±42.0	187.5±104.2	0.010	113.2±72.8	163.3±68.6	NS
Calprotectin WBC		13.8 (9.9-21.4)	21.9 (15.5-31.3)	NS	13.0 (11.9-17.0)	18.6 (13.5-30.7)	NS
Calprotectin NEU		17.7 (14.3-24.8)	25.9 (19.8-50.7)	NS	21.9 (20.5-43.1)	35.2 (18.3-51.6)	NS
HLE-α1AT	[ng/mL]	50.8±12.9	72.2±53.5	NS	47.0±21.6	67.6±32.3	NS
HLE-α1AT WBC		6.9 (932	6.7 (5.5-9.2)	NS	8.3 (7.0-9.2)	8.9 (6.4-10.1)	NS
HLE-α1AT NEU		10.6 (5.8-9.1)	9.3 (6.8-16.9)	NS	13.2 (11.5-15.2)	12.8 (10.1-16.6)	NS

Results are presented in the form of mean±standard deviation and median (first quartile-third quartile). Abbreviations: CRP - C-reactive protein, WBC - White blood Cells, NEU - Neutrophils, HLE-α1AT - leukocytes elastase - alfa 1 antytripsine, P – level of statistical significance, NS - not significant

α1AT using the cut-off value of 35.3 ng/mL were found in the diagnosis of UC (Table 3). Moreover, large areas under the ROC curves in the diagnosis of CD (Figure 1) and UC (Figure 2) for lactoferrin and calprotectin were found. Diagnostic sensitivity, specificity and predictive values of these tests were close to 80% in the diagnosis of both UC and CD (Tables 2 and 3). Diagnostic characteristics of the evaluated granulocyte proteins indicate that measurement of plasma HLE-α1AT, lactoferrin and calprotectin may be useful in the diagnosis of IBD.

We also evaluated the relationship of granulocyte protein concentrations with the activity of CD and UC (Table 4). In patients in the active phase of CD higher lactoferrin and calprotectin levels than in patients in remission were found (Table 4). Logistic regression analysis showed that elevated lactoferrin and calprotectin concentrations significantly increase the odds of CD exacerbation (Table 5). In the UC group all granulocyte protein levels did not significantly differ between patients in the active phase of the disease and patients in remission (Table 4). In patients with active UC significantly higher leukocyte and neutrophil counts compared with patients in inactive UC were found (Table 5). Altogether, these results indicate poor suitability of plasma granulocyte protein measurements for the assessment of UC clinical activity. It should be noted, however, that the evaluation of these relationships was influenced by the small number of patients in remission of both CD and UC.

Leucocyte elastase is involved in the pathogenesis of IBD, and the relationship between IBD activity and the concentration of this enzyme in both plasma and faeces has recently been reported [8,22]. As shown in Tables 1 and 2, high plasma concentration of the HLE-α1AT complex in patients with CD and UC may reflect increased neutrophil activity. This seems to be confirmed by the significantly increased neutrophil count in patients with IBD. Gouni-Berthold et al. reported increased plasma HLE-α1AT concentrations in patients with UC, which correlated with

Table 5. Relationship between the test results and calculated ratios and clinical activity of Crohn's disease and ulcerative colitis – logistic regression analysis

		Crohn's disease		Ulcerative Colitis	
		Odds ratio (95% confidence interval)	**P**	**Odds ratio (95% confidence nterval)**	**P**
CRP	[mg/L]	1.148 (1.012-1.303)	0.033	1.011 (1.002-1.218)	0.049
WBC	[10^3/uL]	1.123 (0.777-1.622)	NS	1.560 (0.925-2.632)	NS
NEU	[10^3/uL]	1.190 (0.729-1.943)	NS	1.970 (0.828-4.690)	NS
Lactoferrin	[ng/mL]	1.014 (1.001-1.028)	0.045	1.007 (0.997-1.017)	NS
Lactoferrin / WBC		1.103 (0.987-1.232)	NS	0.995 (0.951-1.042)	NS
Lactoferrin / NEU		1.077 (0.977-1.187)	NS	0.994 (0.968-1.021)	NS
Calprotectin	[ng/mL]	1.035 (1.005-1.066)	0.022	1.011 (0.998-1.024)	NS
Calprotectin / WBC		1.122 (0.992-1.270)	NS	1.010 (0.940-1.085)	NS
Calprotectin / NEU		1.065 (0.974-1.165)	NS	1.004 (0.962-1.048)	NS
HLE-α1AT	[ng/mL]	1.018 (0.987-1.049)	NS	1.035 (0.993-1.079)	NS
HLE-α1AT / WBC		1.057 (0.851-1.314)	NS	1.035 (0.837-1.279)	NS
HLE-α1AT / NEU		1.053 (0.886-1.251)	NS	1.035 (0.837-1.279)	NS

Results are presented in the form of mean±standard deviation and median (first quartile-third quartile) Abbreviations: CRP - C-reactive protein, WBC - White blood Cells, NEU - Neutrophils, HLE-α1AT - leukocytes elastase - alfa 1 antytripsine, P – level of statistical significance, NS - not significant

disease activity [8]. Diagnostic characteristics of HLE-α1AT similar to those obtained in our study were reported by Kuno et al., who found that this enzyme measured in plasma is a useful indicator of IBD activity, better identifying patients in remission than ESR and CRP [11]. We found that HLE-α1AT levels in plasma of patients with the active disease were higher than in patients with inactive disease, but the difference was not statistically significant. Altogether, our results confirm the usefulness of plasma HLE-α1AT in the diagnosis of IBD, but did not confirm its relationship with clinical activity of both CD and UC. As mentioned above, the limitation of the last analysis was the small number of patients in remission.

Determination of calprotectin and lactoferrin in faeces and plasma has been the subject of numerous studies. Leach et al. found that plasma calprotectin levels, both in patients with UC and CD, were higher than in the control group [20]. In this study calprotectin had high diagnostic sensitivity and specificity (91% and 71%, respectively) in the diagnosis of IBD [13]. Similar diagnostic characteristics for calprotectin were obtained in our study. Another confirmation of the involvement of neutrophils in the pathogenesis of IBD and diagnostic capabilities of calprotectin is the study by Foell et al., who evaluated the release of calprotectin in the gastrointestinal tract of patients with CD, UC, irritable bowel syndrome (IBS) and in the control group [6]. They observed higher release of calprotectin in the inflamed tissue, but found no differences between the release of calprotectin in the active and inactive CD.

In 2008 qualitative point-of-care tests detecting calprotectin and lactoferrin in faeces were introduced for predicting exacerbations of IBD, differentiating IBD from functional disorders and to reduce the need for invasive diagnostic procedures [12]. Langhorst et al. measured calprotectin, lactoferrin and leukocytes in faeces and found that calprotectin and lactoferrin concentrations were significantly higher in patients with IBD than in the group with IBS and healthy controls [12]. As evidenced in our results and published data, measurements of calprotectin and lactoferrin both in plasma and faeces may be useful in the diagnosis of IBD and its differentiation from IBS, and assessment of CD activity, which can be helpful in therapeutic decisions making.

One of the aims of this study was to evaluate leukocyte and neutrophil counts and the ratios of granulocyte protein levels to WBC and NEU counts in patients with IBD. Significantly higher leukocyte and neutrophil counts in patients with CD compared with the control group but comparable granulocyte proteins/WBC and granulocyte proteins/NEU ratios were found (Table 1). Exceptions were lactoferrin/WBC ratio and lactoferrin/NEU ratio, which were significantly higher in patients with CD. Although WBC count did not differentiate patients with UC and the control group, a significantly higher neutrophil count was found in UC patients (Table 1). Moreover, in patients with UC plasma HLE-α1AT, lactoferrin and calprotectin/WBC ratios, and HLE-α1AT and lactoferrin/NEU ratios were significantly higher than in the control group (Table 1). HLE-α1AT and lactoferrin/NEU ratios were in patients with UC significantly higher than in patients with CD (Table 1). From Figures 1 and 2 it is clear that lactoferrin, calprotectin and plasma HLE-α1AT are directly associated with NEU and the AUCs of lactoferrin/NEU, calprotectin/NEU and elastase/NEU close to 0.5 in CD support this concept. In UC it appears slightly different, since the AUCs of ratios are higher than 0.5 and close to 0.7. It may suggest that neutrophils are more activated in UC than in CD. Neutrophils are among the first cells migrating from the blood to the sites of inflammation. The perception of their role in the inflammatory process has significantly changed lately. The opinion that neutrophils are only cells responding to inflammatory mediators has changed after demonstrating the ability of activated neutrophils to act as macrophages [26]. It was observed that the number of neutrophils with polarized cell membrane is higher in patients with IBD compared to healthy subjects [10,12]. What is more, there are data suggesting the role of abnormal neutrophil function in the pathogenesis of IBD. In patients with CD hydrogen peroxide production and phagocytosis were reported to be impaired [5,9]. Neutrophil dysfunction observed in CD seems to be related to the defect in macrophage response. In vitro and in vivo studies on macrophages derived from CD patients showed a decrease in proinflammatory cytokine production compared to cells from healthy subjects [7]. It was also observed that the migration of neutrophils in skin and injured bowel in patients with CD is lower than normal. Nonetheless, our results indicate increased neutrophil count and activity in IBD rather than their dysfunction.

No differences in neutrophil count and calculated ratios between patients with active CD and UC, and patients in remission were observed (Table 4). The parameters of diagnostic characteristics of the studied tests decreased in the order: concentrations of granulocyte proteins – proteins/WBC ratio – protein/NEU ratio (Tables 2 and 3, Figures 1 and 2). Moreover, the AUCs for granulocyte proteins/NEU ratios were small and diagnostic accuracy of about 50% in the diagnosis of CD was found (Table 2) while higher diagnostic accuracy (about 70-80%) was observed in the UC diagnosis (Table 3). Altogether, diagnostic characteristics of neutrophil count was less favourable than that of HLE-α1AT, calprotectin and lactoferrin, which suggests that granulocyte proteins are better candidate markers in the diagnosis of IBD than neutrophil count or ratios calculated in our study.

In summary, we found that plasma HLE-α1AT, calprotectin and lactoferrin levels were significantly higher in patients with IBD than in the control group. Diagnostic characteristics of plasma granulocyte protein concentrations indicate the usefulness of these tests in the diagnosis of IBD. Significantly higher HLE-α1AT/NEU ratio and lactoferrin/NEU ratio in patients with UC than with CD suggest the potential role of these ratios in differential diagnostics. Plasma calprotectin and lactoferrin levels are higher in patients with active disease than in remission of CD.

The main limitation of our study was the small number of patients in the studied groups and the lack of a comparison of plasma levels of granulocyte protein concentration to concentration in faeces. For this reason our results should be considered as preliminary, and they require confirmation in further studies.

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