MEDICAL UNIVERSITY "PROF. PARASKEV STOYANOV" OF VARNA
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DEPARTMENT OF PEDIATRICS

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SOME CONTEMPORARY DIAGNOSTIC ASPECTS OF INFLAMMATORY BOWEL DISEASES IN CHILDREN AND ADOLECENTS

AUTHOR'S DISSERTATION SUMMARY

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CONTENTS

ABBREVIATIONS USED

ESR	erythrocyte sedimentation rate
incl.	inclusive
min.	minutes
RNA	ribonucleic acid
fig.	figure
US $	United States dollars
ANOVA	analysis of variance
c-ANCA	cytoplasmic anti-neutrophil cytoplasmic antibodies
CI	confidence interval
mg	milligram
mL	milliliter
ng	nanogram
p-ANCA	perinuclear anti-neutrophil cytoplasmic antibodies
PCDAI	pediatric Crohn disease activity index
PUCAI	pediatric ulcerative colitis activity index

1. INTRODUCTION

Usually, inflammatory bowel diseases begin in childhood, are characterized by chronic course and complicate with advancing age. In spite of the real progress during the recent years, the effectiveness of the conservative treatment of the patients does not meet the contemporary requirements yet. Both incidence and prevalence rates of Crohn's disease, ulcerative colitis and unspecific collitis permanently increase worldwide and in our country as well. Their timely and precise diagnosis in children and adolescents represents a serious challenge because of the great variety of the clinical symptoms as well as of the dynamics of the laboratory parameters and findings from the imaging examinations.

The constellations of traditional clinical and laboratory examinations used nowadays in combination with more and more popular specific markers not only for the diagnosis of Crohn's disease, ulcerative colitis and unspecific colitis but also for the definitive distinction between themselves and seemingly similar functional gastroduodenal disorders along with certain more common and more rare benign and malignant large bowel diseases in children and adolescents represent the object of intensive research during the last several years.

Some specific dynamic alterations of the values of several diagnostic and differential-diagnostic parameters of the severity of anemia and inflammation during the attacks and remissions of these chronic diseases are outlined which contribute to a different extent to determination of the final diagnosis. Several contemporary biomarkers, among which fecal calprotection, too, are more and more widely used in our country, too. The positive findings from the imaging examinations being already a routine practice play a crucial role. While the diagnostic aspects of the chronic ulcerative colitis, Crohn's disease and unspecific colitis in adult patients have been investigated by a series of Bulgarian authors, the studies among the children and adolescents in Bulgaria are scanty and fragmentary. This is the principal stimulus of ours to carry out the present complex investigation of the contemporary methods for diagnosis of these diseases based on our own great experience in the field of pediatric gastroenterology.

3. PURPOSE AND TASKS

The purpose of the present study is to analyze the individualized diagnostic behaviour in children and adolescents with inflammatory bowel diseases with of view to its perfectioning.

The tasks for accomplishment of this purpose are the following:

1. To study the clinical peculiarities of Crohn's disease, ulcerative colitis and unspecific colitis in children and adolescents.
2. To assess the diagnostic and differential-diagnostic value of the laboratory diagnosis in children with Crohn's disease, ulcerative colitis and unspecific colitis.

3. To study the expression of several microRNAs in children with Crohn's disease and ulcerative colitis as the samples are taken at the first hospitalization on the occasion of exacerbation.

4. To evaluate the diagnostic and differential-diagnostic value of the imaging and pathohistological diagnosis of these diseases in childhood and adolescence.

5. To elaborate an algorithm for the diagnosis of the inflammatory bowel diseases in childhood and adolescence.

WORKING HYPOTHESIS

The original constellation of modern methods for the diagnosis and differential diagnosis presents with a sufficient effectiveness and applicability in children and adolescents with inflammatory bowel diseases.

3. MATERIAL AND METHODS

3.1. Material

The present retrospective/prospective monocentre investigation was carried out in the Department of Pediatrics of the Faculty of Medicine at the Medical University of Varna "Professor Paraskev Stoyanov" during the period between January 1, 2008 and July 31, 2020 incl. The examination of the microRNA started after the permission of the Commission for Research Ethics.

The study covered a total of 76 children hospitalized on the occasion of inflammatory bowel diseases. They were at a mean age of 13,54±3,13 years (range, two to 17 years). There were 41 boys at a mean age of 13,54±3,49 years (range, two to 17 years) while there were 35 girls at a mean age of 13,54±2,69 years (range, two to 17 years). Three diseases were considered, i.e. Crohn's disease with 27 patients, ulcerative colitis with 36 patients and unspecific colitis with 13 patients.

The distributions of all the hospitalized children, the boys and girls according to the diagnosis of the disease can be seen in Fig. 1 through Fig. 3.

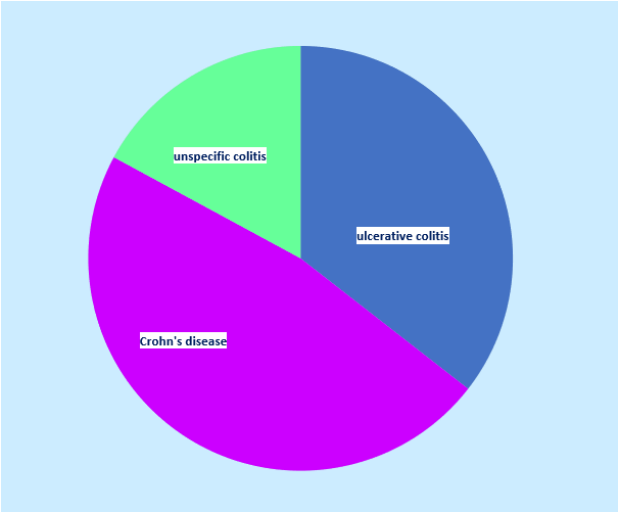

Fig. 1. Distribution of all the hospitalized children according to the diagnosis of the disease

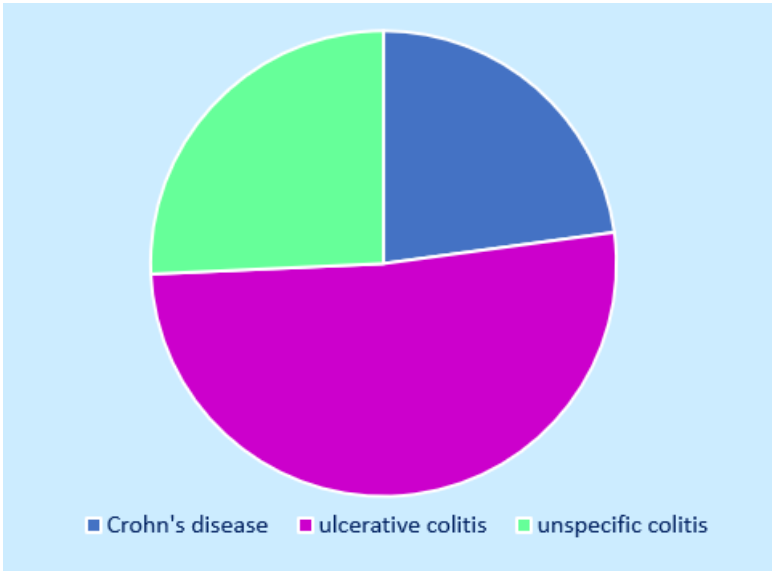

Fig. 2. Distribution of the boys according to the diagnosis of the disease

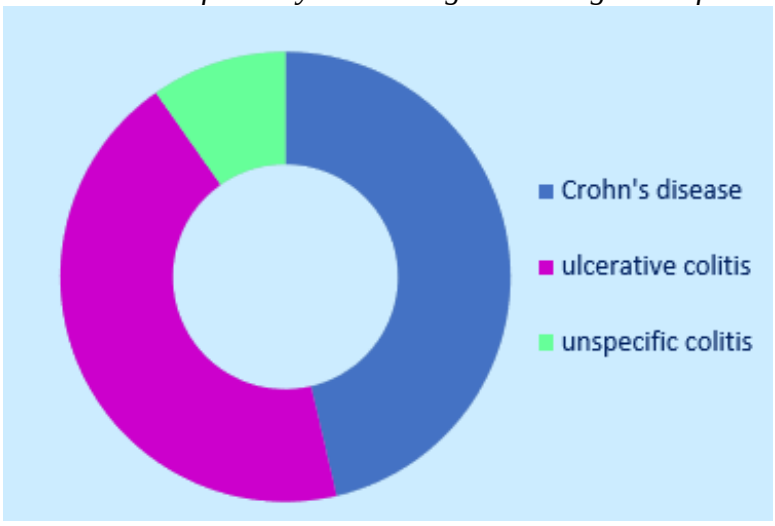

Fig. 3. Distribution of the girls according to the diagnosis of the disease

The distribution of all the hospitalized children according to gender and age groups is demonstrated in Fig. 4.

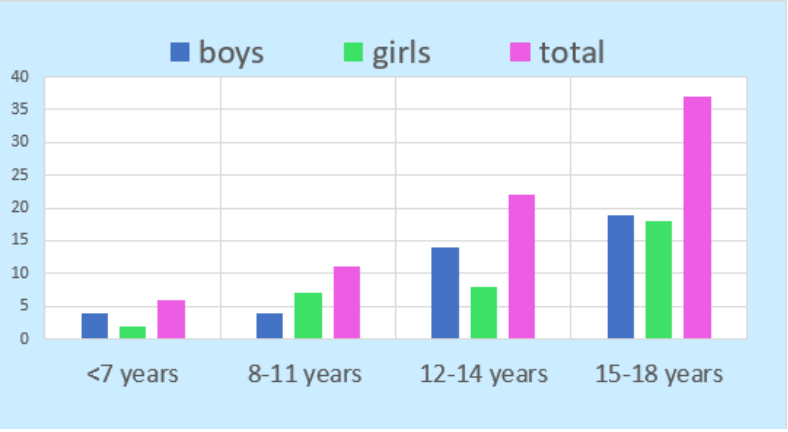

Fig. 4. Distribution of all the hospitalized children according to gender and age groups

The distributions of the hospitalized children with Crohn's disease, ulcerative colitis and unspecific colitis are illustrated in Fig. 5 through Fig. 7.

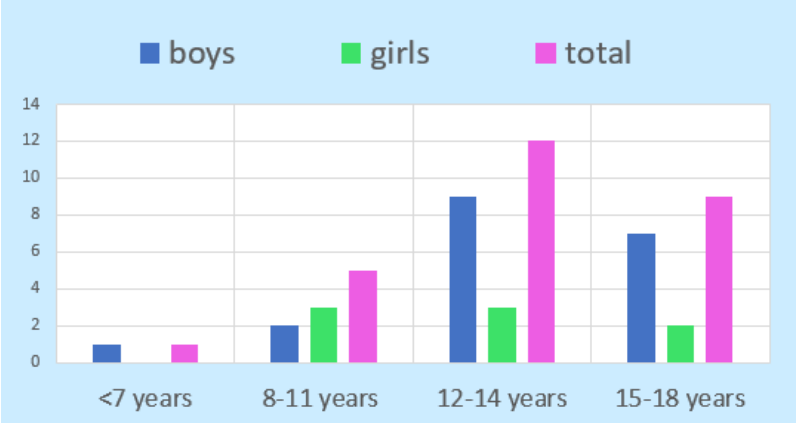

Fig. 5. Distribution of the hospitalized children with Crohn's disease according to gender and age groups

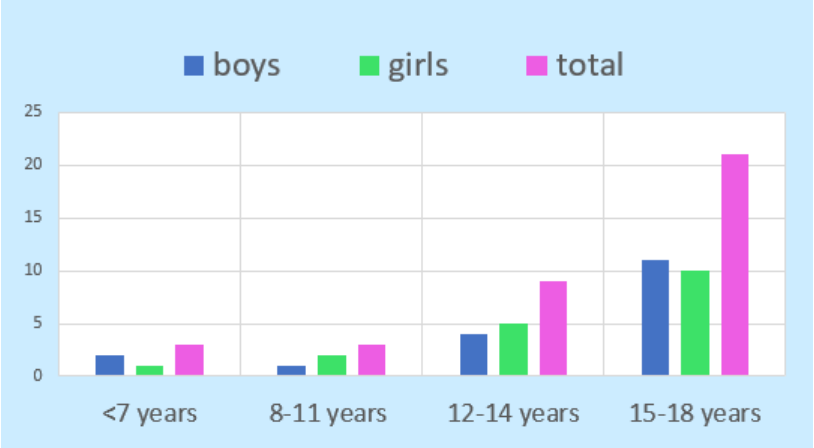

Fig. 6. Distribution of the hospitalized children with ulcerative colitis according to gender and age groups

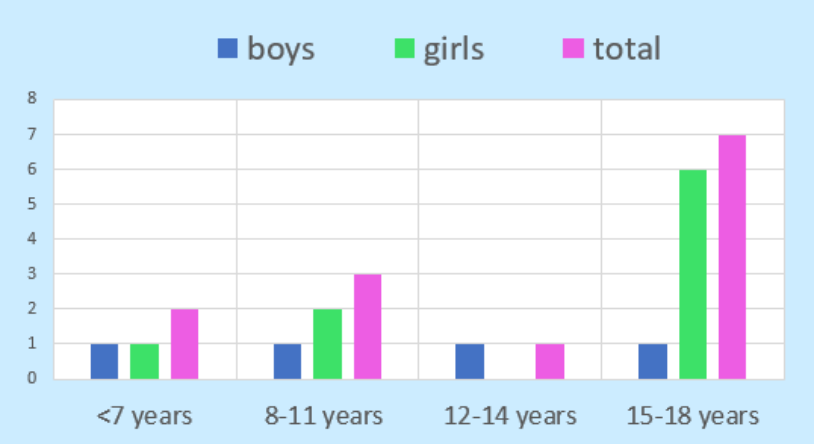

Fig. 7. Distribution of the hospitalized children with unspecific colitis according to gender and age

3.2. Methods

During the analysis of children's records, we paid a special attention to the constellation of various clinical, laboratory (i.e. hematological and biochemical

parameters and markers) and imaging diagnostic examinations. We rendered an account of at least two values of the concrete parameters - at children's admission to hospital and discharge from hospital.

It pertained to the objective assessment of the diagnostic value of the following parameters and examinations:

clinical peculiarities of the disease: severity of the disease (slight, medium severe and severe); value of the pediatric Crohn's disease activity index (PCDAI) and of the pediatric ulcerative colitis activity index (PUCAI); number of hospitalizations; number of serious accompanying diseases as well as organ localization of the inflamatory process;

hematological parameters: hemoglobin concentration; erythrocyte number and leukocyte number;

biochemical parameters: concentration of serum iron, fibrinogen and C-reactive protein;

biological markers: fecal calprotectin; perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA); cytoplasmic anti-neutrophil cytoplasmic antibodies (c-ANCA); expression of 11 different serum microRNAs which were examined by using real-time polymerase chain reaction in 22 children with Crohn's disease, 13 children with ulcerative colitis and 20 clinically healthy children. These microRMNAs were the following: miRNA16-a, miRNA21, miRNA122, miRNA125-a, miRNA131-a, miRNA143-3p, miRNA196-b, miRNA642-3b, miRNA155, miRNA195, and miRNA223;

imaging examinations: abdominal echography; computed tomography; fibrogastroduodenoscopy; fibrocolonoscopy and histopathological examination of intestinal tract biopsy specimens.

The results were statistically processed by means of descriptive, variation, correlation (Pearson's coefficient and χ^2) and unifactorial dispersion analysis ANOVA and Post Hoc Scheffe's test as well as by using paired test. The statistical reliability according to Student-Fisher *t*-criterion was read at significance level of $p<0,05$. The SPSS software package, version 22.0 was applied.

4. OWN RESULTS

4.1. Clinical peculiarities of inflammatory bowel diseases

We analyzed some essential features of the clinical manifestation of these three inflammatory bowel diseases in the hospitalized children and adolescents.

4.1.1. Severity of inflammatory bowel diseases and comorbidity

Children's distributions according to the severity of the diseases and the number of hospitalizations are presented in Table 1 and Table 2.

There exist statistically reliable differences concerning the frequency not only of the slight form of ulcerative colitis and unspecific colitis ($t=3,28$; $p<0,001$), but also of the medium severe form of Crohn's disease and unspecific colitis ($t=2,02$; $p<0,05$) as well as of ulcerative colitis and unspecific colitis ($t=2,07$; $p<0,05$).

Table 1. Children's distributions according to the severity of the diseases

Disease	slight form		medium severe form		severe form	
	n	%	n	%	n	%
Crohn's disease	0	0	17	62,96	10	37,04
ulcerative colitis	11	30,56	19	52,78	6	16,66
unspecific colitis	10	76,92	3	23,08	0	0
total	21	27,63	39	51,32	16	21,05

Table 2. Children's distributions according to the number of hospitalizations

Disease	one		two-three		four-six		eight-eighteen	
	n	%	n	%	n	%	n	%
Crohn's disease	13	48,16	9	33,33	2	7,41	3	11,10
ulcerative colitis	18	50,00	12	33,33	4	11,11	2	5,56
unspecific colitis	11	84,62	2	15,38	0	0	0	0
total	42	55,26	23	30,25	6	7,89	5	6,60

The values of the number of hospitalizations of the children with these three diseases are juxtaposed in Table 3 and Table 4.

Table 3. Number of hospitalizations of the children with three diseases

Disease	arithm. mean	stand. deviat.	stand. error	95% CI		minim.	maxim.
				from	to		
Crohn's disease	2,96	3,75	0,72	1,48	4,45	1	18
ulcerative colitis	2,47	2,56	0,43	1,61	3,34	1	12
unspecific colitis	1,15	0,38	0,10	0,93	1,38	1	2
total	2,42	2,89	0,33	1,76	3,08	1	18

Табл. 4. Comparison of the mean number of children's hospitalizations between three diseases

Patients (I)	Patients (J)	mean diff. (I-J)	stand. error	95% CI		p
				from	to	
Crohn's disease	ulcerative colitis	0,49	0,73	-1,33	2,31	0,80
	unspecific colitis	1,81	0,96	-0,60	4,22	0,18
ulcerative colitis	Crohn's disease	-0,49	0,73	-2,31	1,33	0,80
	unspecific colitis	1,32	0,92	-0,99	3,63	0,37
unspecific colitis	Crohn's disease	-1,81	0,96	-4,22	0,60	0,18
	ulcerative colitis	-1,32	0,92	-3,63	0,99	0,37

Children's distribution according to the number of accompanying diseases is indicated in Table 5.

Table 5. Children's distribution according to the number of accompanying diseases

Disease	one		two		three		four-six	
	n	%	n	%	n	%	n	%
Crohn's disease	6	31,59	6	31,59	4	21,05	3	15,77
ulcerative colitis	10	45,45	6	27,27	3	13,64	3	13,64
unspecific colitis	4	33,33	5	41,67	2	16,67	1	8,33
total	20	37,73	17	32,08	9	16,98	7	13,21

The most common accompanying diseases of the children with these three diseases are shown in Table 6.

Table 6. Frequency of accompanying diseases of the children with three diseases

Accompanying diseases	Crohn's disease	ulcerative colitis	unspecific colitis	total
Clostridium difficile-caused enterocolitis	3	6	1	10
duodenitis	5	1	1	7
acute pancreatitis	3	3	0	6
mesenteric lymphadenitis	5	0	1	6
chronic antral gastritis	5	0	0	5
psotiatric arthritis	1	1	0	2
hemorrhoidal disease	0	2	0	2
liver steatosis	1	0	0	1
celiac disease	1	0	0	1
cholelithiasis	0	0	1	1
total - diseases	8	5	4	10
total - patients	24	13	4	41

The values of the the number of accompanying diseases of the children with these three diseases are juxtaposed in Table 7.

Table 7. Number of accompanying diseases of the children with three diseases

Disease	arithm. mean	stand. deviat.	stand. error	95% CI		minim.	maxim.
				from	to		
Crohn's disease	2,42	1,54	0,35	1,68	3,16	1	6
ulcerative colitis	2,00	1,19	0,25	1,47	2,53	1	5
unspecific colitis	2,00	0,95	0,27	1,39	2,61	1	4
total	2,15	1,28	0,17	1,80	2,50	1	6

The mean number of accompanying diseases of the children with these three diseases is juxtaposed in Table 8.

Table 8. Comparison of the mean number of accompanying diseases of the children between three diseases

Patients (I)	Patients (J)	mean diff. (I-J)	stand. error	95% CI		p
				from	to	
Crohn's disease	ulcerative colitis	0,42	0,40	-0,59	1,44	0,58
	unspecific colitis	0,42	0,47	-0,77	1,62	0,68
ulcerative colitis	Crohn's disease	-0,42	0,40	-1,44	0,59	0,58
	unspecific colitis	0,00	0,46	-1,16	1,16	1,00
unspecific colitis	Crohn's disease	-0,42	0,47	-1,62	0,77	0,68
	ulcerative colitis	0,00	0,46	1,00	-1,16	1,16

The comparison of PCDAI values of the children with Crohn's disease and of PUCAI ones of the children with ulcerative colitis reveal statistically significant

differences between patients' hospitalization and discharge from hospital ($p<0,001$ and $p<0,0001$, respectively) (Table 9).

Table 9. Values of PCDAI and PUCAI at admission and discharge from hospital

Parameter	Examination at	arithm. mean	stand. deviat.	diff. of stand. error	*t*	p
PCDAI	admission	61,82	11,68	3,52	4,56	<0,001
	discharge	31,14	13,25	5,99		
PUCAI	admission	52,50	16,86	4,87	5,03	<0,0001
	discharge	23,33	11,35	3,28		

4.1.2. Organ localization of the diseases

The localization of the inflammatory process in the large and small bowel of the children with Crohn's disease, ulcerative colitis and unspecific colitis is demonstrated in Table 10. It deals with a wide dissemination of the pathological process in our children and adolescents.

Table 10. Localization of the inflammatory process in the children with three diseases

Localization	ulcerative colitis		Crohn's disease		unspecific colitis	
	n	%	n	%	n	%
large bowel	10	27,78	12	44,44	12	92,31
rectum and sigmoid colon	18	50,00	0	0	0	0
intestine	0	0	11	40,74	0	0
small and large bowel	5	13,88	4	14,82	0	0
rectum	2	5,56	0	0	1	7,69
colon and rectum	1	2,78	0	0	0	0
total	36	100,00	27	100,00	13	100,00

4.2. Laboratory diagnosis in children with Crohn's disease, ulcerative colitis and unspecific colitis

4.2.1. Diagnosis of anemia

The distribution of the children with Crohn's disease, ulcerative colitis and unspecific colitis in whom pathologically diminished values of erythrocytes, hemoglobin and serum iron at admission and discharge from hospital have been established is indicated in Table 11.

Table 11. Children's distribution with pathologically diminished values of erythrocytes, hemoglobin and serum iron at admission and discharge from hospital

Disease	at admission		at discharge	
	n	%	n	%
erythrocytes				
Crohn's disease	4	18,18	1	8,33
ulcerative colitis	11	36,67	2	28,57

unspecific colitis	1	8,33	0	0
total	16	25,00%	3	14,29
hemoglobin				
Crohn's disease	17	62,96	6	28,57
ulcerative colitis	19	52,78	10	34,48
unspecific colitis	2	15,38	1	33,33
total	38	50,00	17	32,08
serum iron				
Crohn's disease	18	85,71	13	76,47
ulcerative colitis	28	87,50	10	58,82
unspecific colitis	6	54,55	0	0
total	52	81,25	23	63,89

The mean number of erythrocytes and the mean concentrations of hemoglobin and serum iron of the children with Crohn's disease, ulcerative colitis and unspecific colitis are juxtaposed in Table 12 through Table 14.

Table 12. Comparison of the mean number of erythrocytes of the children between three diseases at admission and discharge from hospital

Patients (I)	Patients (J)	mean diff. (I-J)	stand. error	95% CI		p
				from	to	
erythrocytes at admission						
Crohn's disease	ulcerative colitis	0,15	0,13	-0,17	0,47	0,51
	unspecific colitis	-0,10	0,16	-0,51	0,31	0,84
ulcerative colitis	Crohn's disease	-0,15	0,13	-0,47	0,17	0,51
	unspecific colitis	-0,25	0,16	-0,64	0,14	0,29
unspecific colitis	Crohn's disease	0,10	0,16	-0,31	0,51	0,84
	ulcerative colitis	0,25	0,16	-0,14	0,64	0,29
erythrocytes at discharge						
Crohn's disease	ulcerative colitis	0,20	0,17	-0,26	0,66	0,51
	unspecific colitis	-0,08	0,28	-0,82	0,65	0,96
ulcerative colitis	Crohn's disease	-0,20	0,17	-0,66	0,26	0,51
	unspecific colitis	-0,29	0,29	-1,06	0,49	0,62
unspecific colitis	Crohn's disease	0,08	0,28	-0,65	0,82	0,96
	ulcerative colitis	0,29	0,29	-0,49	1,06	0,62

A statistically significant difference in terms of the frequency of the pathologically diminished values of erythrocytes at patients' discharge from hospital between ulcerative colitis and unspecific colitis has been established (t=2,43; p=0,03). A statistically reliable difference in terms of the hemoglobin values in the children with single diseases at patients' admission has been observed (χ^2=9,50; p=0,04). There is a moderate positive, statistically significant correlation dependence (ρ=0,29; p=0,01). It deals with statistically significant difference in terms of the frequency of the pathologically diminished hemoglobin values at patients' admission not only between Crohn's disease and unspecific colitis (t=4,46; p<0,001), but also between ulcerative colitis and unspecific colitis (t=3,74; p<0,001).

Table 13. Comparison of the mean hemoglobin concentration of the children between three diseases at admission and discharge from hospital

Patients (I)	Patients (J)	mean diff. (I-J)	stand. error	95% CI		P
				from	to	
hemoglobin at admission						
Crohn's disease	ulcerative colitis	-0,13	0,13	-0,45	0,19	0,61
	unspecific colitis	-0,48	0,17	-0,91	0,05	0,03
ulcerative colitis	Crohn's disease	0,13	0,13	-0,19	0,45	0,61
	unspecific colitis	-0,35	0,16	-0,76	0,07	0,12
unspecific colitis	Crohn's disease	0,48	0,17	-0,05	0,91	0,03
	ulcerative colitis	0,35	0,16	-0,07	0,76	0,12
hemoglobin at discharge						
Crohn's disease	ulcerative colitis	0,06	0,14	-0,29	0,41	0,91
	unspecific colitis	0,05	0,30	-0,70	0,79	0,99
ulcerative colitis	Crohn's disease	-0,06	0,14	-0,41	0,29	0,91
	unspecific colitis	-0,01	0,29	-0,75	0,72	1,00
unspecific colitis	Crohn's disease	-0,05	0,30	-0,79	0,70	0,99
	ulcerative colitis	0,01	0,29	-0,72	0,75	1,00

Табл. 14. Comparison of the mean serum iron concentration of the children between three diseases at admission and discharge from hospital

Patients (I)	Patients (J)	mean diff. (I-J)	stand. error	95% CI		P
				from	to	
serum iron at admission						
Crohn's disease	ulcerative colitis	0,02	0,12	-0,28	0,31	0,99
	unspecific colitis	-0,40	0,16	-0,80	0,01	0,04
ulcerative colitis	Crohn's disease	-0,02	0,12	-0,31	0,28	0,99
	unspecific colitis	-0,42	0,15	-0,79	0,05	0,02
unspecific colitis	Crohn's disease	0,40	0,16	0,01	0,80	0,04
	ulcerative colitis	0,42	0,15	0,05	0,79	0,02
serum iron at discharge						
Crohn's disease	ulcerative colitis	-0,18	0,34	-1,04	0,68	0,87
	unspecific colitis	-4,27	0,73	-6,14	-2,39	0,0001
ulcerative colitis	Crohn's disease	0,18	0,34	-0,68	1,04	0,87
	unspecific colitis	-4,09	0,73	-5,97	-2,21	0,0001
unspecific colitis	Crohn's disease	4,27	0,73	2,39	6,14	0,0001
	ulcerative colitis	4,09	0,73	2,21	5,97	0,0001

The differences concerning the frequency of the pathologically diminished serum iron values at patients' admission not only between Crohn's disease and unspecific colitis (t=2,64; p<0,02), but also between ulcerative colitis and unspecific colitis (t=3,97; p<0,01) are statistically significant. This reliability is much more strongly expressed at patients' discharge from hospital (p<0,0001) (Table 14).

The mean values of these three parameters of the anemia in Crohn's disease and ulcerative colitis at childrens' admission and discharge from hospital can be seen in Table 15.

Table 15. Mean values of these three parameters of the anemia in Crohn's disease and ulcerative colitis at admission and discharge from hospital

Parameter	Examination at	arithm. mean	stand. deviat.	diff. of stand. error	*t*	P
Crohn's disease						
erythrocytes	admission	4,56	0,89	0,26	-2,79	0,017
	discharge	5,07	0,62	0,18		
hemoglobin	admission	109,48	16,9	3,70	-5,07	<0,001
	discharge	123,90	15,00	3,37		
serum iron	admission	4,41	3,95	1,02	-1,71	0,11
	discharge	6,58	3,97	1,03		
ulcerative colitis						
erythrocytes	admission	4,17	0,55	0,21	-0,55	0,60
	discharge	4,22	0,57	0,21		
hemoglobin	admission	108,14	18,59	3,45	-5,44	<0,001
	discharge	121,24	15,76	2,93		
serum iron	admission	5,23	5,01	1,21	-3,18	0,006
	И discharge	9,95	6,19	1,50		

Following the hospital treatment, there is a statistically significant enhancement not only of the mean erythrocyte number and the mean hemoglobin concentration in the children with Crohn's disease (t=-2,79; p=0,017 and t=-5,07; p<0,001, respectively), but also of the mean concentrations of hemoglobin and serum iron in the children with ulcerative colitis (t=-5,44; p<0,001 and t=-3,18; p=0,006, respectively).

4.2.2. Inflammation parameters

The distribution of the children with Crohn's disease, ulcerative colitis and unspecific colitis in whom pathologically enhanced values of leukocytes, fibrinogen and C-reactive protein at admission as well as diminished values at discharge from hospital have been established is displayed in Table 16.

Table 16. Distribution of the children with pathologically enhanced values of leukocytes, fibrinogen and C-reactive protein at admission and diminished values at discharge from hospital

Disease	at admission		at discharge	
	n	%	n	%
leukocytes				
Crohn's disease	13	48,15	6	35,30
ulcerative colitis	22	61,11	9	31,03
unspecific colitis	2	15,39	0	0
total	37	48,68	15	30,61
fibrinogen				
Crohn's disease	7	29,17	4	36,36
ulcerative colitis	8	36,36	2	15,38
unspecific colitis	0	0	0	0
total	15	26,78	6	24,00
C-reactive protein				
Crohn's disease	18	72,00	8	53,33
ulcerative colitis	15	57,69	7	58,33
unspecific colitis	2	28,57	0	0
total	35	60,34	15	51,72

Some statistically significant differences in terms not only of the values of the leukocytes in the children with single diseases at patients' admission (χ^2=11,74; p=0,019), but also of the frequency of their pathologically enhanced values between Crohn's disease and unspecific colitis at patients' admission (t=2,36; p<0,05) have been observed.

The mean number of leukocytes and the mean C-reactive protein concentrations of the children with Crohn's disease, ulcerative colitis and unspecific colitis are juxtaposed in Table 17 and Table 18.

Table 17. Comparison of the mean number of leukocytes of the children between three diseases at admission and discharge from hospital

Patients (I)	Patients (J)	mean diff. (I-J)	stand. error	95% CI		p
				from	to	
leukocytes at admission						
Crohn's disease	ulcerative colitis	-0,13	0,13	-0,45	0,19	0,60
	unspecific colitis	0,41	0,17	-0,02	0,83	0,06
ulcerative colitis	Crohn's disease	0,13	0,13	-0,19	0,45	0,60
	unspecific colitis	0,53	0,16	0,13	0,94	0,006
unspecific colitis	Crohn's disease	-0,41	0,17	-0,83	0,02	0,06
	ulcerative colitis	-0,53	0,16	-0,94	-0,13	0,006
leukocytes at discharge						
Crohn's disease	ulcerative colitis	0,02	0,16	-0,40	0,43	0,99
	unspecific colitis	0,29	0,34	-0,56	1,15	0,69
ulcerative colitis	Crohn's disease	-0,02	0,16	-0,43	0,40	0,99
	unspecific colitis	0,28	0,33	-0,55	1,10	0,70
unspecific colitis	Crohn's disease	-0,29	0,34	-1,15	0,56	0,69
	ulcerative colitis	-0,28	0,33	-1,10	0,55	0,70

There is a statistically significant difference in terms of the frequency of the pathological C-reactive protein values between Crohn's disease and unspecific colitis at patients' admission (t=3,35; p<0,01).

Table 18. Comparison of the mean C-reactive protein concentration of the children between three diseases at admission and discharge from hospital

Patients (I)	Patients (J)	mean diff. (I-J)	stand. error	95% CI		p
				from	to	
C-reactive protein at admission						
Crohn's disease	ulcerative colitis	-0,14	0,13	-0,48	0,20	0,57
	unspecific colitis	-0,43	0,21	-0,95	0,09	0,12
ulcerative colitis	Crohn's disease	0,14	0,13	-0,20	0,48	0,57
	unspecific colitis	-0,29	0,21	-0,81	0,23	0,37
unspecific colitis	Crohn's disease	0,43	0,21	-0,09	0,95	0,12
	ulcerative colitis	0,29	0,21	-0,23	0,81	0,37
C-reactive protein at discharge						
Crohn's disease	ulcerative colitis	0,05	0,20	-0,46	0,56	0,97
	unspecific colitis	-0,53	0,38	-1,52	0,45	0,39
ulcerative colitis	Crohn's disease	-0,05	0,20	-0,56	0,46	0,97
	unspecific colitis	-0,58	0,39	-1,59	0,42	0,34
unspecific colitis	Crohn's disease	0,53	0,38	-0,45	1,52	0,39
	ulcerative colitis	0,58	0,39	-0,42	1,59	0,34

The mean values of these three parameters of the inflammation in Crohn's disease and ulcerative colitis at childrens' admission and discharge from hospital are demonstrated in Table 19.

Table 19. Mean values of these three parameters of the inflammation in Crohn's disease and ulcerative colitis at admission and discharge from hospital

Parameter	Examination at	arithm. mean	stand. deviat.	diff. of stand. error	t	p
Crohn's disease						
leukocytes	admission	12,06	7,66	1,86	1,80	0,09
	discharge	9,16	2,64	0,64		
fibrinogen	admission	4,26	1,29	0,39	-0,70	0,50
	discharge	6,73	11,28	3,40		
C-reactive protein	admission	40,34	34,53	8,95	4,26	<0,001
	discharge	12,88	18,51	4,78		
ulcerative colitis						
leukocytes	admission	13,72	6,78	1,25	4,09	<0,001
	discharge	9,13	3,02	0,56		
fibrinogen	admission	4,68	1,89	0,53	1,83	0,09
	discharge	3,42	1,64	0,46		
C-reactive protein	admission	131,35	28,87	8,71	1,42	0,19
	discharge	7,69	7,27	2,19		

The mean number of leukocytes in the children with ulcerative colitis is statistically significantly greater than that in those with unspecific colitis (p=0,006) not only at their admission (Table 17), but also at their admission than at their discharge from hospital (p<0,001) (Table 19). The mean C-reactive protein values of the children with Crohn's disease are statistically reliably greater at patients' admission than at their discharge from hospital (p<0,001) (Table 19).

The mean fibrinogen concentration of the children between these three diseases at admission is compared in Table 20.

Table 20. Comparison of the mean fibrinogen concentration of the children between three diseases at admission

Patients (I)	Patients (J)	mean diff. (I-J)	stand. error	95% CI		p
				from	to	
Crohn's disease	ulcerative colitis	-0,16	0,15	-0,54	0,23	0,59
	unspecific colitis	0,31	0,19	-0,18	0,79	0,29
ulcerative colitis	Crohn's disease	0,16	0,15	-0,23	0,54	0,59
	unspecific colitis	0,46	0,20	-0,03	0,96	0,07
unspecific colitis	Crohn's disease	-0,31	0,19	-0,79	0,18	0,29
	ulcerative colitis	-0,46	0,20	-0,96	0,03	0,07

Fecal calprotection presents with pathologically enhanced concentrations in all 27 examined children, namely in 16 children with Crohn's disease, ten children with ulcerative colitis and one child with unspecific colitis.

4.2.3. Microribonucleic acids in children with inflammatory bowel diseases

The results from the examination of the expression of 11 microRNAs in 22 children with Crohn's disease, 13 children with ulcerative colitis and 20 clinically healthy children are systemnatized in Table 21 through Table 23.

Table 21. Values of 11 microRNAs in the children with Crohn's disease

MicroRNA	arithm. mean	stand. deviat.	maximal value	minimal value
miRNA122	1,97	2,39	9,17	0,07
miRNA142-3p	2,15	2,58	12,56	0,38
miRNA196-b	1,46	1,75	8,67	0,36
miRNA642-3b	1,88	2,00	8,39	0,13
miRNA155	1,44	1,63	7,64	0,18
miRNA131-a	2,62	3,96	19,18	0,19
miRNA125-a	1,58	2,08	10,31	0,17
miRNA16-a	0,88	2,22	4,59	0,29
miRNA21	1,78	2,86	13,70	0,26
miRNA223	3,21	5,56	22,62	0,26
miRNA195	0,71	0,54	2,82	0,07

Table 22. Values of 11 microRNAs in the children with ulcerative colitis

MicroRNA	arithm. mean	stand. deviat.	maximal value	minimal value
miRNA122	3,38	5,07	17,91	0,18
miRNA142-3p	1,49	1,42	4,01	0,11
miRNA196-b	1,69	1,45	4,24	0,19
miRNA642-3b	1,46	2,02	7,92	0,32
miRNA155	1,58	1,64	5,91	0,16
miRNA131-a	1,45	1,43	4,62	0,22
miRNA125-a	1,59	1,62	5,42	0,19
miRNA16-a	2,19	2,28	8,29	0,13
miRNA21	1,58	2,01	7,48	0,08
miRNA223	1,61	1,45	5,57	0,12
miRNA195	1,92	2,11	7,49	0,19

The value of microRNA195 is statistically significantly higher (by 2,70 times) in the children with ulcerative colitis than in those with Crohn's disease (t=2,03; p<0,05). The value of microRNA16-a is by 2,49 times higher while that of microRNA122 is by 1,72 times higher in the patients with ulcerative colitis than in those with Crohn's disease. The value of microRNA223 is by 1,99 times higher and that of microRNA131-a is by 1,81 times higher while that of microRNA142-3p is by 1,44 times higher in the patients with Crohn's disease than in those with ulcerative colitis. The values of the expression of all the microRNAs are higher in the patients than in the healthy children. Only the values of microRNA142-3p and microRNA642-b3 are statistically significantly higher in the children with Crohn's disease than in the healthy children (t=2,05; p<0,05 and t=2,00; p<0,05, respectively).

Table 23. Values of 11 microRNAs in the healthy children

MicroRNA	arithm. mean	stand. deviat.	maximal value	minimal value
miRNA122	0,90	1,19	3,72	0,04
miRNA142-3p	1,00	0,57	2,35	0,01
miRNA196-b	1,00	0,69	2,69	0,12
miRNA642-3b	1,00	0,43	3,14	0,17
miRNA155	1,00	0,80	3,63	0,05
miRNA131-a	1,00	0,92	3,59	0,04
miRNA125-a	1,00	0,81	2,79	0,11
miRNA16-a	1,00	1,20	5,16	0,05
miRNA21	0,95	0,95	4,55	0,04
miRNA223	1,00	1,42	5,82	0,06
miRNA195	1,00	1,40	6,40	0,06

4.3. Imaging and pathohistological diagnosis in children with Crohn's disease, ulcerative colitis and unspecific collitis

The results from the imaging and pathohistological examinations of the children with these three diseases are presentd in Table 24.

Table 24. Most common diagnoses and findings from the imaging examinations of the children with three diseases

Diagnosis and finding	ulcerative colitis	Crohn's disease	unspecific colitis	total
	abdominal echography			
aerocolia	20	9	9	38
thickened intestinal loops	0	2	0	2
total - findings	1	2	1	2
total - patients	20	11	9	40
	computed tomography			
thickened wall of ileum	0	3	0	3
terminal ileitis	1	1	0	2
total - diagnosis and finding	1	2	0	2
total - patients	1	4	0	5
	fibrogastroduodenoscopy			
chronic duodenitis	0	5	1	6
erythemic and antral gastritis	0	5	0	5
chronic gastroduodenitis	0	3	0	3
total - diagnoses	0	3	1	3
total - patients	0	13	1	14
	fibrocolonoscopy			
chronic unspecific colitis	0	0	9	9
hyperemic mucosa	2	0	5	7
ulcerative lesions	6	0	0	6
edematous intestinal mucosa	0	1	5	6
erosions	0	5	1	6
total - diagnosis and findings	2	2	4	5
total - patients	8	6	20	34
	pathohistology			
chronic ulcerative colitis	3	0	0	3
unspecific colitis	0	2	0	2
lymphoplasmocytic infiltration	2	0	0	2
total - diagnoses and finding	2	1	0	3
total - patients	5	2	0	7

One can see the pathological findings and concrete diagnoses when making use of the abdominal echography, computed tomography, fibrogastroduodenoscopy, fibrocolonoscopy, and pathohistology in the children with Crohn's disease, ulcerative colitis and unspecific colitis. It considers a great number of concrete diseases of the gastrointestinal tract and pathological findings in the children with these three definitely established inflammatory bowel diseases. This proves the important

diagnostic and differential-diagnostic role of these methods of imaging diagnosis in these diseases in childhood and adolescence.

4.4. Correlation dependences between single analyzed parameters

The results from Pearson's correlation analysis of the dependence between PCDAI values in the children with Crohn's disease and PUCAI values in the children with ulcerative colitis, on the one hand, and the mean values of the parameters of the severity of the anemia and inflammation at children's admission and discharge from hospital, on the other hand, are generalized in Table 25 and Table 26.

Table 25. Correlation analysis of the parameters of the anemia and the values of PCDAI and PUCAI at admission and discharge from hospital

	erythrocytes		hemoglobin		serum iron	
	admission	discharge	admission	discharge	admission	discharge
	Crohn's disease					
	PCDAI examination at admission					
patients (n)	13	6	13	11	11	9
Pearson (r)	-0,25	0,09	-0,58	-0,58	-0,33	-0,05
p	0,41	0,85	0,004	0,06	0,32	0,89
	PCDAI examination at discharge					
patients (n)	11	4	11	9	9	8
Pearson (r)	0,18	0,61	0,57	0,53	-0,36	-0,46
p	0,59	0,39	0,07	0,14	0,34	0,25
	ulcerative colitis					
	PUCAI examination at admission					
patients (n)	13	5	13	10	12	7
Pearson (r)	-0,34	-0,82	-0,52	-0,61	-0,17	-0,61
p	0,03	0,09	0,07	0,06	0,59	0,78
	PUCAI examination at discharge					
patients (n)	12	4	12	9	11	7
Pearson (r)	0,05	-0,24	-0,13	-0,14	-0,07	-0,24
p	0,89	0,76	0,68	0,73	0,84	0,60

Table 26. Correlation analysis of the parameters of the inflammation and the values of PCDAI and PUCAI at admission and discharge from hospital

	leukocytes		fibrinogen		C-reactive protein	
	admission	discharge	admission	discharge	admission	discharge
	Crohn's disease					
	PCDAI examination at admission					
patients (n)	13	8	12	5	13	9
Pearson (r)	-0,19	-0,41	0,37	0,03	0,17	0,03
p	0,53	0,31	0,23	0,96	0,57	0,94
	PCDAI examination at discharge					
patients (n)	11	6	10	4	11	7
Pearson (r)	0,24	0,62	-0,18	-0,35	-0,09	0,27
p	0,47	0,19	0,62	0,65	0,77	0,56

	ulcerative colitis					
	PUCAI examination at admission					
patients (n)	13	10	12	7	11	6
Pearson (r)	0,33	-0,35	0,09	-0,42	0,21	0,32
p	0,28	0,33	0,78	0,35	0,53	0,54
	PUCAI examination at discharge					
patients (n)	12	9	11	6	10	6
Pearson (r)	-0,34	0,19	-0,24	-0,52	-0,30	-0,23
p	0,28	0,61	0,48	0,29	0,39	0,66

We observe not only a strong negative, statistically significant correlation dependence between PCDAI value and mean hemoglobin concentration at admission (r=-0,58; p=0,004), but also a strong positive, statistically insignificant correlation dependence between PCDAI value and mean leukocyte number at children's discharge from hospital (r=0,62; p=0,19).

We establish not only a moderate negative, statistically significant correlation dependence between PUCAI value and mean erythrocyte number at admission (r=-0,34; p=0,03), but also a strong negative, statistically insignificant correlation dependence between this value and mean erythrocyte number at children's discharge from hospital (r=-0,82; p=0,09).

The correlation dependences between PUCAI values and mean hemoglobin concentrations at children's admission and discharge from hospital are strong negative, statistically insignificant, too (r=-0,52; p=0,07 and r=-0,61; p=0,06, respectively).

The results from Pearson's correlation analysis of the dependence between the values of PCDAI and PUCAI in the children with Crohn's disease and ulcerative colitis at admission and discharge from hospital, on the one hand, and mean fecal calproptectin values, on the other hand, are compared in Table 27.

Table 27. Correlation analysis of the values of fecal calproptectin, PCDAI and PUCAI at admission and discharge from hospital

	PCDAI		PUCAI	
	Examination at			
	admission	discharge	admission	discharge
patients (n)	8	7	8	7
Pearson (r)	0,79	-0,48	0,57	-0,14
p	0,02	0,27	0,14	0,77

We establish not only a strong positive, statistically significant correlation dependence between PCDAI value and mean fecal calproptectin concentration in the children with Crohn's disease at admission (r=0,79; p=0,02), but also a strong positive, statistically insignificant correlation dependence between PUCAI value and mean fecal calproptectin concentration in the children with ulcerative colitis at admission (r=0,57; p=0,14).

The results from Pearson's correlation analysis of the dependence between the values of PCDAI and PUCAI in the children with Crohn's disease and ulcerative colitis at admission and discharge from hospital, on the one hand, and the number of accompanying diseases in the single patient, on the other hand, are juxtaposed in Table 28.

Table 28. Correlation analysis of the number of accompanying diseases and the values of PCDAI and PUCAI at admission and discharge from hospital

	PCDAI		PUCAI	
	Examination at			
	admission	discharge	admission	discharge
patients (n)	10	8	10	9
Pearson (r)	0,14	-0,60	0,58	0,49
p	0,71	0,12	0,08	0,18

We establish not only a strong negative, statistically insignificant correlation dependence between PCDAI value in the children with Crohn's disease and the number of accompanying diseases at discharge from hospital (r=-0,60; p=0,12), but also a strong positive, statistically insignificant correlation dependence between PUCAI value in the children with ulcerative colitis at admission (r=0,58; p=0,08).

The results from Pearson's correlation analysis of the dependence between the values of PCDAI and PUCAI in the children with Crohn's disease and ulcerative colitis, on the one hand, and the mean values of p-ANCA and c-ANCA at admission and discharge from hospital, on the other hand, are compared in Table 29.

Table 29. Correlation analysis of the values of ther parameters p-ANCA, c-ANCA, PCDAI and PUCAI at admission and discharge from hospital

	p-ANCA	c-ANCA
	Crohn's disease	
	PCDAI examination at admission	
patients (n)	9	9
Pearson (r)	0,46	-0,08
P	0,21	0,83
	PCDAI examination at discharge	
patients (n)	8	8
Pearson (r)	-0,51	0,23
P	0,19	0,57
	ulcerative colitis	
	PUCAI examination at admission	
patients (n)	8	8
Pearson (r)	0,23	0,00
P	0,59	1,00
	PUCAI examination at discharge	
patients (n)	8	8
Pearson (r)	-0,25	-0,42
P	0,55	0,29

There exist not only a moderate positive and a moderate negative statistically insignificant correlation dependence between PCDAI value in the children with Crohn's disease at admission and discharge from hospital and p-ANCA parameter value (r=0,46; p=0,21 and r=-0,51; p=0,19, respectively), but also a moderate negative, statistically insignificant correlation dependence between PUCAI value in the children with ulcerative colitis and mean c-ANCA parameter value at discharge from hospital (r=-0,42; p=0,29).

We have compared the values of six microRNAs such as microRNA16-a, microRNA122, microRNA131-a, microRNA142-3p, microRNA195 and microRNA223 with two parameters of the anemia such as hemoglobin and serum iron concentrations as well as with two parameters of the inflammation such as leukocyte number and C-reactive protein concentration in the patients with both diseases and the control group of 20 healthy children. A moderate positive, statistically significant correlation dependence between microRNA131-a values, on the one hand, and leukocyte number (r=0,39; p=0,003) and C-reactive protein concentration (r=0,36; p<0,007), on the other hand, in all the children with Crohn's disease and ulcerative colitis has been established. We observe a weak positive, statistically significant correlation dependence between microRNA16-a values, on the one hand, and the patients with ulcerative colitis towards those with Crohn's disease (r=0,27; p=0,043), on the other hand. There is a moderate positive, statistically significant correlation dependence between microRNA122 values, on the one hand, and C-reactive protein in the control group (r=0,469; p=0,037), on the other hand. A strong negative, statistically significant correlation dependence between the values of hemoglobin concentrations in the patients with ulcerative colitis, on the one hand, and the values of microRNA122 (r=-0,662; p=0,014), microRNA131a (r=-0,632; p=0,02) and microRNA223 (r=-0,642; p=0,018), on the other hand, has been proved. In the children with Crohn's disease, there are no any statistically significant correlation dependences between the values of these six microRNAs, on the one hand, and the four analyzed parameters of the anemia and inflammation (p>0,05), on the other hand.

4.5. Diagnostic algorithm in inflammatory bowel diseases in childhood and adolescence

Our original diagnostic algorithm for the inflammatory bowel diseases in childhood and adolescence is presented in Fig. 8.

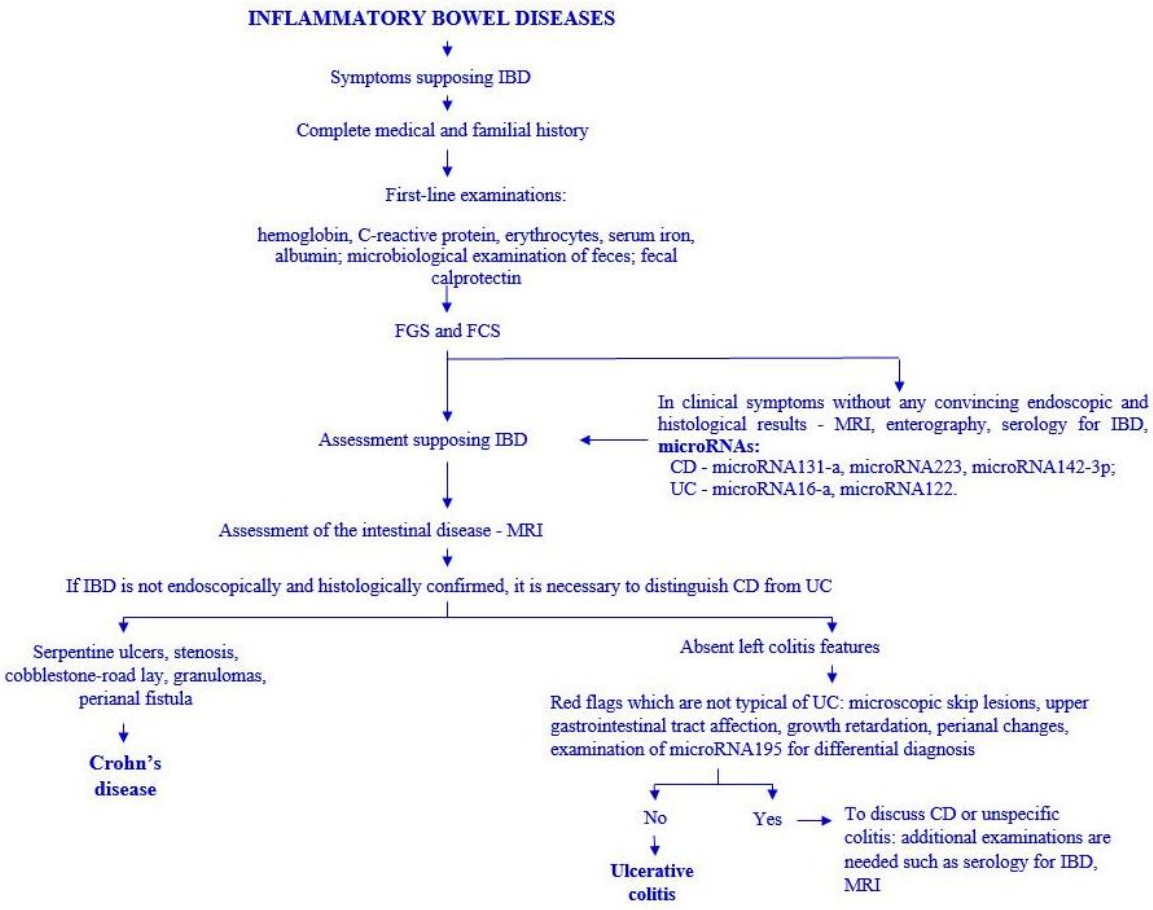

Fig. 8. Diagnostic algorithm for the inflammatory bowel diseases in childhood and adolescence

5. DISCUSSION

5.1. Clinical characteristics of the children with inflammatory bowel diseases

The moderate-sevelely manifested form of the disease prevails not only among all the children with these three diseases (in 51,35%), but also in the children with Crohn's disease (in 64%), in 28,38% of all the children, in 76,92% of the children with unspecific colitis and in 30,56% of the children with ulcerative colitis. Some 20,27% of all the children present with severe form, 36,00% of the children do with Crohn's disease and 16,66% of the children do with ulcerative colitis. The differences in terms of the frequency not only of the slight form of the ulcerative colitis and unspecific colitis (t=3,28; p<0,001), but also of the medium severe form of Crohn's disease and unspecific colitis (t=2,72; p<0,05) as well as of ulcerative colitis and unspecific colitis (t=2,07; p<0,05) are statistically significant.

The number and relative share of the children with one hospitalization only are the greatest (42 or 55,26%) followed by those with two and with three hospitalizations (15 or 19,73% and eight or 10,52% of the cases, respectively). The results in the children with ulcerative colitis are similar - with a frequency of 50,00%, 19,44% and 13,89%, respectively, and in the children with Crohn's disease

with a frequency of 48,16%, 22,22% and 11,11% of the cases, respectively. The majority of the children with unspecific colitis present with one hospitalization only - 11 or 84,62% of the cases. The mean number of hospitalizations is the greatest in the children with Crohn's disease (2,96±3,75) followed by that in the children with ulcerative colitis (2,47±2,56) and that in the children with unspecific colitis (1,15±0,38).

The number and relative share of the children with one serious accompanying disease only are the greatest (20 or 37,73%) followed by those with two and with three accompanying diseases (17 or 32,08% and nine or 16,98% of the cases, respectively). The relative share of the children with ulcerative colitis with one, two and three accompanying diseases is 45,45%, 27,27% and 13,64%, respectively, that of the children with Crohn's disease is 31,59%, 31,59% and 21,05%, and that of the children with unspecific colitis is 33,33%, 41,67% and 16,67%.

A total of 53 children (69,74% of the cases) present with serious accompanying diseases. These diseases present with a greatest number and relative share among the children with unspecific colitis (in 12 children or in 92,31% of the cases). Next follow both Crohn's disease with accompanying diseases in 19 children (or in 70,37%) and ulcerative colitis with accompanying diseases in 22 (or in 61,11% of the cases). The mean number of the serious accompanying diseases is the greatest in the children with Crohn's disease (2,42±1,54) followed by that in the children with ulcerative colitis (2,00±1,19) and by that in the children with unspecific colitis (2,00±0,95). We observe not only a strong positive, statistically insignificant correlation dependence between PCDAI value of the children with Crohn's disease at discharge from hospital and the number of the accompanying diseases (Pearson's coefficient r=-0,60; p=0,12), but also a strong negative, statistically insignificant correlation dependence between PUCAI value of the children with ulcerative colitis at admission and this number (Pearson's coefficient r=0,58; p=0,08).

A statistically significant diminution of the mean PCDAI values of the children with Crohn's disease from 61,82±11,68 at admission down to 31,14±13,25 at discharge from hospital (t=4,56; p<0,001) and of PUCAI ones of the children with ulcerative colitis from 52,50±16,86 down to 23,33±11,35 (t=5,03; p<0,0001, respectively) has been established.

Among the children with ulcerative colitis, the localization of the pathological alterations prevails simultaneously in rectum and sigmoid colon (in 18 patients or in one half of the cases) as well as in ileum and colon (in 10 patients or in 27,78% of the cases). Among the children with Crohn's disease, the pathological alterations prevail only in colon (in 12 patients or in 44,44%) and only in intestine (in 11 patients or in 40,74% of the cases). Among the children with unspecific colitis, the inflammatory process is localized predominantly in colon (in 12 patients or in 92,31% of the cases).

In ten out of a total of 150 patients with Crohn's disease (in 6,67% of the cases), hospitalized during the period between January, 2013 and September, 2015 in the Clinic of Gastroenterology at Tsaritsa Giovanna of Sofia, the diagnosis of the disease is done at the age below 16 years (R. V. Nakov, 2017).

D. Abuquteish and J. Putra (2019) discuss a series of manifestations of the affection of the upper gastrointestinal tract in children with inflammatory bowel diseases. It deals with lymphocytic esophagitis observed predominantly in Crohn's disease, as well as with focal gastritis, duodenal cryptitis and epitheloid granuloma which is a diagnostic peculiarity of Crohn's disease, too.

An inflammatory process has been diagnosed in 17 out of 105 children and adolescents at a mean age of 10,4 years (in 16,19% of the cases) in the course of the examination of 107 biopsies from the ileal mucosa and of 693 biospies from the colonic mucosa (R. M. Najarian *et al*., 2019). In 14 of these children (in 82,25% of the cases), a histologically proved pancolitis has been considered. There is a significant relationship between the histologically and endoscopically proved pancolitis (p=0,02).

5.2. Laboratory diagnosis in the children with inflammatory bowel diseases

At patient's admission, erythrocyte number is below the normal range in 25% of all the children, in 36,67% of the children with ulcerative colitis, in 18,18% of the children with Crohn's disease and only in 8,33% of the children with unspecific colitis. The difference in terms of the frequency of these erythrocyte values between ulcerative colitis and unspecific colitis is statistically significant (t=2,43; p=0,03). At patient's discharge from hospital, erythrocyte number is below the normal range in 14,29% of all the children, in 28,57% of the children with ulcerative colitis and in 8,33% of the children with Crohn's disease.

A moderate negative, statistically significant correlation dependence between PUCAI value of the children with ulcerative colitis at admission and mean erythrocyte number has been detected (Pearson's coefficient r=-0,34; p=0,03).

At patient's admission, hemoglobin concentration is below the normal range in one half of all the children, in 62,96% of the children with Crohn's disease, in 52,78% of the children with ulcerative colitis and in 15,38% of the children with unspecific colitis. The difference in terms of hemoglobin values in the children with single diseases is statistically significant (χ^2=9,50; p=0,04). A moderate positive, statistically significant correlation dependence between these parameters has been observed (ρ=0,29; p=0,01). The differences in terms of the frequency of these hemoglobin values between Crohn's disease and unspecific colitis as well as between ulcerative colitis and unspecific colitis are statistically significant (t=4,46; p<0,001 and t=3,74; p<0,001, respectively). At patient's discharge from hospital, hemoglobin concentration is below the normal range in 32,08% of all the children, in 34,48% of the children with ulcerative colitis, in 28,57% of the children with Crohn's disease and in 33,33% of the children with unspecific colitis. There is a strong negative, statistically significant correlation dependence between PCDAI value of the children with Crohn's disease and mean hemoglobin concentration at patient's admission (Pearson's coefficient r=-0,58; p=0,004).

At patient's admission, serum iron concentration is below the normal range in 81,25% of all the children, in 87,50% of the children with ulcerative colitis, in

85,71% of the children with Crohn's disease and in 54,55% of the children with unspecific colitis. The differences in terms of the frequency of the reduced serum iron concentration between Crohn's disease and unspecific colitis (t=2,64; p<0,02) as well as between ulcerative colitis and unspecific colitis (t=3,97; p<0,01) are statistically significant. At patient's discharge from hospital, serum iron concentration is below the normal range in 63,89% of all the children, in 76,47% of the children with Crohn's disease and in 58,82% of the children with ulcerative colitis.

We observe a statistically significant improvement of the mean values of erythrocyte number (t=-2,79; p=0,017) and not only of hemoglobin concentrations but also of serum iron concentrations in ulcerative colitis (t=-3,18; p=0,006) after the hospital treatment performed.

The mean hemoglobin concentration is statistically significantly higher in the children with Crohn's disease than in those with unspecific colitis at their admission (p=0,03). Besides the mean serum iron concentrations are statistically significantly higher in the children with Crohn's disease and those with ulcerative colitis than in the children with unspecific colitis not only at their admission (p=0,04 and p=0,02, respectively), but also at their discharge from hospital (p<0,0001 and p<0,0001, respectively).

Among a cohort of 427 children with ulcerative colitis at a mean age of 12,7 years (range, four to 17 years), a mean PUCAI value of 49,8±20,1 has been established (D. R. Mack *et al*., 2020). The leukocyte number, erythrocyte sedimentation rate (ESR) and platelet count represent these three laboratory parameters which best forecast the dissemination and severity of the disease.

Within a retrospective multicentre cohort investigation during the period between 2012 and 2018 in the USA, a total of 8007 children and adolescents with inflammatory bowel diseases at a mean age of 15,4 years have been analyzed (A. E. Jacobson-Kelly *et al*., 2020). Serum iron has been examined in 12,6% of the patients with these diseases. Anemia has been diagnosed in 29,8% of the cases at admission. The prevalence of anemia among these patients increases statistically significantly from 24,6% in 2012 up to 32,4% in 2018 (p<0,0001).

Normal values of hemoglobin, albumin, C-reactive protein, platelet count and ESR have been established in 45%, 84%, 68%, 81% and 35% of 31 children with ulcerative colitis as well as in 47%, 40%, 7%, 53% and 0% of 15 children with Crohn's disease who have been examined at the hospital of the University of Kurume in Japan during the period between January, 2008 and December 2015 (Y. Takaki *et al*., 2019).

We establish that at children's admission, leukocyte number is over the normal range in 48,68% of all the children, in 61,11% of the children with ulcerative colitis, in 48,15% of the children with Crohn's disease and in 15,39% of the children with unspecific colitis. The difference in terms of the frequency of these values of leukocytes in the children with single diseases is statistically significant (χ^2=11,74; p=0,019). The difference in terms of the frequency of the pathologically enhanced values of leukocytes between Crohn's disease and unspecific colitis is statistically significant, too (t=2,36; p<0,05). At patient's discharge from hospital, leukocyte

number is over the normal range in 30,61% of all the children, in 35,30% of the children with Crohn's disease and in 31,03% of the children with ulcerative colitis.

At patient's admission, fibrinogen concentration is over the normal range in 26,78% of all the children, in 36,36% of the children with ulcerative colitis, in 29,17% of the children with Crohn's disease but below the normal range in 10,00% of the children with unspecific colitis. At patient's discharge from hospital, fibrinogen concentration is below the normal range in 16,00% of all the children, in 23,08% of the children with ulcerative colitis and in 9,09% of the children with Crohn's disease but over the normal range in 24,00% of all the children, in 36,36% of the children with Crohn's disease and in 15,38% of the children with ulcerative colitis.

At patient's admission, pathologically enhanced C-reactive protein concentrations have been established in 60,34% of all the children, in 72,00% of the children with Crohn's disease, in 57,69% of the children with ulcerative colitis and in 28,57% of the children with unspecific colitis. At patient's admission, the difference in terms of the frequency of the pathological C-reactive protein values between Crohn's disease and unspecific colitis is statistically significant (t=3,35; p<0,01). At patient's discharge from hospital, we observe pathologically enhanced C-reactive protein concentrations in 51,72% of all the children, in 58,339% of the children with ulcerative colitis and in 53,33% of the children with Crohn's disease. There is a statistically significant reduction of the frequency of the pathologically enhanced C-reactive protein concentrations (t=4,26; p<0,001) and of the leukocyte number (t=4,09; p<0,001) after the hospital treatment performed.

The mean leukocyte number is statistically significantly greater in the children with ulcerative colitis than that in those with unspecific colitis at their admission (p=0,006). The comparison of the mean concentrations of C-reactive protein and fibrinogen in the children does not reveal any statistically significant differences in terms of these three diseases between patient's admission and discharge from hospital.

We observe a strong positive, statistically significant correlation dependence between PCDAI value of the children with Crohn's disease and mean fecal calprotectin concentration at patient's admission (Pearson's coefficient r=0,79; p=0,02).

There are not only a moderate positive, but also a moderate negative correlation dependence between PCDAI value of the children with Crohn's disease at patient's admission and discharge from hospital and the parameter of anti-neutrophil cytoplasmic antibodies (ANCA), i.e. of perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA) (Pearson's coefficient r=0,46; p=0,21 and Pearson's coefficient r=-0,51; p=0,19, respectively), however, these correlations are not statistically significant. A moderate negative correlation dependence between PUCAI value of the children with ulcerative colitis at patient's discharge from hospital and the mean value of the cytoplasmic anti-neutrophil cytoplasmic antibodies (c-ANCA) at patient's admission (Pearson's coefficient r=-0,42; p=0,29) has been observed which is statistically insignificant.

Research activity on the diagnostic role of fecal calprotectin is very intensive during the recent several years worldwide. The investigations of some Bulgarian gastroenterologists not only in adult patients (V. Nakov *et al*., 2014; R. Nakov *et al*., 2018), but also in children with inflammatory bowel diseases (R. Shentova *et al*., 2015; R. Shentova *et al*., 2016; R. Shentova *et al*., 2016a; R. R. Shentova-Eneva, 2018; R. Shentova *et al*., 2020) deserve an attention, too.

According to B. Veauthier and J. R. Hornecker (2018), fecal calprotectin is used in order to exclude the diagnosis of Crohn's disease in children and adult patients while endoscopy and cross-sectional imaging diagnosis serve for confirmation of this diagnosis and determination of the dissemination of the disease.

Fecal calprotectin concentrations are examined not only initially but also during the subsequent two to five hospitalizations for 18 months after the treatment with Infliximab carried out in 53 children with Crohn's disease within a prospective, longitudinal cohort investigation (A. J. Foster *et al*., 2019). A clinically manifested relapse is observed in 18 children (in 33,96% of the cases). The basal fecal calprotectin levels are statistically significantly higher in these children (723 μg/g; in the interquartile range between 283 and 1758 μg/g) than in the rest children (244 μg/g; in the interquartile range between 61 and 627 μg/g) (p=0,02). The fecal calprotectin concentrations over 250 μg/g demonstrate a good prognostic accuracy concerning the occurrence of relapse of the disease within three months.

Pathologically enhanced fecal calprotectin concentrations (over 150 ug/g) have been established in 55 children with ulcerative colitis (in 42,97%) and in 26 children with Crohn's disease (in 20,31% of the cases) out of a total of 128 children during the period between 2013 and 2015 in Poland (U. Daniluk *et al*., 2019). Among the children with Crohn's disease only, there has been an association between fecal calprotectin, on the one hand, and hemoglobin (R=-0,53), ESR (R=0,53), albumin (R=-0,52), mean erythrocyte corpusular volume (R=-0,64) and width of erythrocyte distribution (R=0,56), on the other hand.

The comparative analysis of the expression of eleven selected microRNAs in the children with Crohn's disease demonstrates highest values of microRNA223, microRNA131-a and microRNA142-3p but lowest values of microRNA195 and microRNA16-a. In the children with ulcerative colitis, the microRNA122 and microRNA16-a present with highest values while microRNA131-a and microRNA642-3b do with lowest values.

The values of the expression of all the microRNAs are higher among the patients than the healthy children, however, there are statistically significant differences only between the children with Crohn's disease and healthy children concerning microRNA142-3p and microRNA642-b3 (t=2,05; p<0,05 and t=2,00; p<0,05, respectively).

The comparative analysis of the expression of six microRNAs such as microRNA16-a, microRNA122, microRNA131-a, microRNA142-3p, microRNA195 and microRNA223, on the one hand, and the concentrations of hemoglobin, serum iron and C-reactive protein as well as leukocyte number in the patients with both diseases and the control group of 20 healthy children, on the other hand, demonstrates

a moderate positive, statistically significant correlation dependence between microRNA131a values, on the one hand, and leukocyte number (r=0,39; p=0,003) and C-reactive protein concentration (r=0,36; p<0,007), on the other hand, in all the children with Crohn's disease and ulcerative colitis. There exist not only a weak positive, statistically significant correlation dependence between microRNA16a values, on the one hand, and the patients with ulcerative colitis towards those with Crohn's disease (r=0,27; p=0,043), on the other hand, but also a moderate positive, statistically significant correlation dependence between microRNA122 values, on the one hand, and C-reactive protein in the control group (r=0,469; p=0,037), on the other hand. We prove a strong negative, statistically significant correlation dependence between the values of hemoglobin concentrations in the patients with ulcerative colitis, on the one hand, and microRNA122 (r=-0,662; p=0,014), microRNA131a (r=-0,632; p=0,02) and microRNA223 (r=-0,642; p=0,018), on the other hand.

Data of different authors who have studied a series of concrete microRNAs in serum and tissues during the recent years are contradictory.

MicroRNA223 has been identified as a new mediator of interaction between the signal pathway of interleukin-23 and claudin-8, the new target of interleukin-23, during the development of inflammatory bowel diseases (H. Wang *et al*., 2016). MicroRNA223 expression is enhanced in these diseases and its activity is regulated by the signal pathway of interleukin-23. According to the results from the investigation by means of real-time polymerase chain reaction, only microRNA223 is characterized by a significantly higher expression not only in inflamed but also in non-inflamed tissues of ulcerative colitis patients than in healthy persons.

MicroRNA223 presents with a higher peripheral blood expression in ulcerative colitis patients than in control subjects (C. Polytarchou *et al*., 2015). The enrichment of the exosomal microRNA223 by human mast cells-1 inhibits claudin-8 expression and disturbs the intestinal barrier function (M. Li *et al*., 2020). These mast cells can represent a new target for the treatment of the inflammatory bowel diseases.

MicroRNA122 expression is higher in macroscopically intact parts of the large bowel in the children with Crohn's disease than in those in the children with ulcerative colitis and in halthy children in Hungary (N. J. Béres *et al*., 2016).

The comparative analysis between the patients with ulcerative colitis or Crohn's disease, on the one hand, and healthy individuals, on the other hand, reveals a considerably stronger expression in the intestinal mucosa of several microRNAs such as microRNA21, microRNA155, microRNA195 and microRNA223 in ulcerative colitis as well as of microRNA21 and microRNA155 in Crohn's disease than in control subjects (X. M. Xu *et al*., 2016).

5.3. Imaging and pathohistological diagnosis in the children with inflammatory bowel diseases

By using fibrocolonoscopy in a total of 36 children with these three inflammatory bowel diseases, we establish a total of five different diagnoses and six specific pathological findings, the main of which are four: a hyperemic mucosa (in

seven), edematous mucosa (in six), ulcerative lesions (in six) and erosions (in six children). In the children with ulcerative colitis, it deals with three diagnoses in a total of six patients and with two pathological findings in eight patients. In the children with Crohn's disease, one diagnosis in two patients and four pathological findings in eight patients have been detected. In the children with unspecific colitis, one diagnosis in one patient and three pathological findings in 11 patients have been established.

By using abdominal ultrasonography in a total of 43 childrten with these three inflammatory bowel diseases, a total of five specific pathological findings have been identified. Of them, aerocolia is the most common - in a total of 38 patients, i.e. in 20 children with ulcerative colitis, nine children with Crohn's disease and other nine children with unspecific colitis. Thickened intestinal loops are detected in two children with Crohn's disease. In the children with Crohn's disease, three pathological findings in 12 patients while in those with unspecific colitis, three pathological findings in 11 patients have been detected.

By using endoscopy in the children with these three inflammatory bowel diseases, five different diagnoses in 15 patients and one specific pathological finding in one child have been identified. In the children with ulcerative colitis, it deals with one diagnosis in one patient, in those with Crohn's disease it does with three diagnoses in five children and one pathological finding in one child while in those with unspecific colitis it deals with one diagnosis in nine patients.

By using computed tomography in a total of ten children with ulcerative colitis and Crohn's disease, four different diagnoses in five patients and three specific pathological findings in other five patients have been detected. In the children with ulcerative colitis, it deals with four diagnoses in four patients while in those with Crohn's disease, it does with one diagnosis in one child and with three pathological findings in five children. Thickened ileal wall is the most frequent finding present in three children with Crohn's disease.

The results from the biopsies demonstrate a total of seven different diagnoses in ten patients and five pathological findings in six ones. In ulcerative colitis, five diagnoses in a total of eight patients and two pathological findings in three children have been established, in Crohn's disease, there are two diagnoses in two children and two pathological findings in two children while in unspecific colitis, there is only one pathological finding in one child.

By using fibrogastroscopy, two diagnoses such as chronic duodenitis in five patients with Crohn's disease and in one patient with unspecific colitis as well as erythemic and antral gastritis in five patients with Crohn's disease along with one pathological finding in one patient with Crohn's disease have been diagnosed.

The examination of 50 children with ulcerative colitis aged between two and 18 yearts by means of colonoscopy identifies pancolitis in 20, left-side colitis in 24, and ulcerative proctitis in six patients (B. Iwańczak *et al*., 2020). There is a strong positive correlation dependence between PUCAI value and fecal calprotectin concentration, on the one hand, and endoscopic activity of the disease, on the other hand, however, such a relationship with C-reactive protein is absent.

Twenty children, 16 of whom are with a proved Crohn's disease, three children are with suspicion of this disease, and one child is with colitis without a specified status have been examined (K. Mudambi *et al*., 2020). In three children with an established Crohn's disease but without evidence of an inflammatory process during the magnetic resonance imaging, a positive finding of an inflammatory process during the contrast-enhanced echography has been detected. The echographic examination of the child presenting with data about colitis proves a considerable fat accumulation around the colon and confirms the diagnosis of Crohn's disease.

6. CONCLUDING REMARKS

We examined a total of 76 patients, of whom 36 (18 boys and 18 girls) are with ulcerative colitis, 27 (19 boys and eight girls) are with Crohn's disease and 13 (four boys and nine girls) are with unspecific colitis. We analyzed in a comparative manner not only some essential clinical features of these three diseases such as severity of the disease, number of hospitalizations and accompanying diseases, and organ localization of the inflammation but also a constellation of strictly selected hematological and biochemical parameters, contemporary biological markers and imaging examinations. For the first time in our country, we studied the expression of 11 microRNAs in the patients with ulcerative colitis and Crohn's disease and in healthy children as well.

We confirmed the diagnostic value of the changes of the parameters of the anemia such as erythrocyte number and concentrations of hemoglobin and serum iron as well as of the inflammation such as leukocyte number and concentrations of C-reactive protein, fibrinogen, fecal calprotectin, p-ANCA and c-ANCA in these diseases. The findings from fibrocolonoscopy, abdominal ultrasonography, computed tomography, endoscopy and fibrogastroscopy are essential for diagnosis specification. The expression of all these microRNAs was higher in the patients than in the healthy children. The values of microRNA142-3p and microRNA642-b3 were statistically significantly higher in the children with Crohn's disease than in the healthy children (t=2,05; $p<0,05$ and t=2,00; $p<0,05$, respectively). The value of microRNA195 was statistically significantly higher in the children with ulcerative colitis than in those with Crohn's disease (t=2,03; $p<0,05$). That was why this modern method could successfully be applied in the pediatric gastroenterological practice in our country, too.

We established a series of statistically significant correlation dependences between concrete examined parameters thus proving their diagnostic and differential-diagnostic value.

With the present study, we succeeded proving the validity of our working hypothesis according to which our constellation of contemporary methods for diagnosis and differential diagnosis of the inflammatory bowel diseases in children and adolescents is sufficiently effective and applicable.

7. CONCLUSIONS

Based on the study performed by us the following main **conclusions** could be drawn:

1. The clinical characteristics and laboratory parameters undergo dynamic alterations during the chronic course of these diseases.

2. The exact diagnosis of Crohn's disease, ulcerative colitis and unspecific colitis in childhood and adolescence is more effective when the constellation of suitable contemporary laboratory methods of examination is used.

2a. There are essential differences in terms of the main parameters of the severity of the anemia such as erythrocyte number and concentrations of hemoglobin and serum iron between Crohn's disease and ulcerative colitis.

2b. There are considerable differences in terms of the main parameters of the severity of the inflammation such as leukocyte number and concentrations of C-reactive protein, fibrinogen, fecal calprotectin, p-ANCA and c-ANCA between Crohn's disease and ulcerative colitis.

3. The expression of 11 microRNAs examined was higher in the children with Crohn's disease and ulcerative colitis than in the healthy children while the expression of microRNA195, microRNA223, microRNA142-3p and microRNA131- could possess a diagnostic and differential-diagnostic value.

4. The modern imaging examinations such as abdominal echography, computed tomography, fibrogastroduodenoscopy, and fibrocolonoscopy played a primary diagnostic and differential-diagnostic role in Crohn's disease, ulcerative colitis and unspecific colitis in childhood and adolescence.

5. The original algorithm for the diagnosis of the inflammatory bowel disease in childhood and adolescence elaborated by us could be applied in the gastroenterological practice in our country.

8. LIST OF PUBLICATIONS RELATED TO THE DISSERTATION

1. **Koleva, K.** International scientific communications concerning the issues of Crohn's disease in children. *Detski i infekts. bolesti MP*, **12**, 2020, No 1, 10-16 (in Bulgarian).
2. **Koleva, K.,** M. Georgieva. Dynamic institutionalization of research on Crohn's disease in childhood. *Detski i infekts. bolesti MP*, **12**, 2020, No 1, 3-9 (in Bulgarian).
3. **Koleva, K.,** M. Georgieva. Microribonucleic acids in children with Crohn's disease. *Varn. med. forum*, **9**, 2020, No 2, 33-37 (in Bulgarian).
4. **Koleva, K.,** M. Georgieva. Microribonucleic acids in children with ulcerative colitis. *Varn. med. forum*, **9**, 2020, No 2, 38-42 (in Bulgarian).
5. **Koleva, K.,** M. Georgieva. Microribonucleic acids in children with inflammatory bowel diseases. *Detski i infekts. bolesti MP*, **12**, 2020, No 2, 3-9 (in Bulgarian).

9. CONTIBUTIONS OF THE DISSERTATION

9.1. Original scientific and applicable contributions

1. The constellation of suitable contemporary laboratory and imaging methods of examination in the children with Crohn's disease, ulcerative colitis and unspecific colitis has been applied for the first time in our country.

2. The examination of the expression of 11 microRNAs as an additional differential-diagnostic method in the children and adolescents with Crohn's diseas and ulcerative colitis has been approved for the first time in our country.

3. The correlation dependences between some microRNAs and standard parameters of the anemia and inflammation in the inflammatory bowel diseases in childhood and adolescence have been established for the first time in our country.

9.2. Contributions of confirmatory nature

1. The diagnostic and differential-diagnostic value of erythrocyte number and concentrations of hemoglobin and serum iron in Crohn's disease, ulcerative colitis and unspecific colitis in childhood and adolescence has been confirmed.

2. The diagnostic and differential-diagnostic value of leukocyte number and concentrations of C-reactive protein, fibrinogen, fecal calprotectin, p-ANCA and c-ANCA in Crohn's disease, ulcerative colitis and unspecific colitis in childhood and adolescence has been confirmed.

3. The diagnostic and differential-diagnostic value of abdominal echography, computed tomography, fibrogastroduodenoscopy, and fibrocolonoscopy in these three diseases in childhood and adolescence has been confirmed.

4. The diagnostic and differential-diagnostic value of microRNA195, microRNA131-a, microRNA142-3p and microRNA223 in Crohn's disease and ulcerative colitis in childhood and adolescence has been confirmed.