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The utility of leukergy test in gastroenteritis with *Campylobacter* in children

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ABSTRACT

Gastroenteritis with *Campylobacter* has proven in the last decade to be one of the most common causes of bacterial enteritis in children.

Objectives. The objective of the current study was to highlight the usefulness of using the leukergy test, a test characterized by the aggregation capacity and increased adhesiveness of leukocytes in the case of bacterial infections, in order to establish as early as possible, the bacterial etiology of campylobacteriosis compared to the time it takes to gather other bacteriological evidence.

Materials and methods. This paper was based on a single center prospective case-control study conducted between June 2020 and May 2021 at Ponderas Academic Hospital, which involved 45 children hospitalized with medium and severe forms of gastroenteritis with *Campylobacter Jejuni,* and a control group of 40 children who are clinically and biologically healthy. The property of aggregation, adhesiveness of leukocytes in peripheral blood of the 85 children included in the research was studied using the leukergia test. The results of the leukergia test were subsequently compared with other acute phase reactants, collected from the same blood sample: total leukocyte count (WBCC), total neutrophil count (NEUT), C-reactive protein (CRP).

Results. Sensitivity, Specificity and Positive Predictive Value had values of: 93.3%, 82.50%, 85.71% for leukergy (%); 93.3%, 85%, 87.5% for WBCC with normal values; 88.89%, 82.5%, 85.11% NEUT and 95.56%, 97.5%, 97.7% for CRP.

Conclusions. The leukergy test is a good predictor in the early establishment of bacterial etiology in the case of gastroenteritis with *Campylobacter Jejuni,* being a quick and effective test price quality ratio. The leukergy test can be of real help through the additional information that it brings also in medical centers that do not have advanced investigative capabilities.

Keywords: leukergy test, leukocyte response, inflammatory syndrome, *Campylobacteriosis*

INTRODUCTION

Gastroenteritis with *Campylobacter jejuni* are common worldwide, especially in developing countries, where Campylobacter infection is very common in the first 2 years of life. In high-income countries, the incidence has been estimated to be 4.4 to 9.3 per 1000 population [1].

Differentiating bacterial gastroenteritis from the viral ones can be difficult at the onset of the disease when the child may present, in both cases, common clinical symptoms such as: fever, inappetence, diarrheal stools, vomiting. Since Campylobacter is an atypical bacterium [1], the leukocyte response in most cases of campylobacteriosis is weak or absent, even more so if antibiotics were administered be-

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fore biological samples were collected. C-reactive protein (CRP) is one of the most sensitive acute phase reactants used in the examination and monitoring of diseases, its level in the blood being a response to tissue damage, stress and many other inflammatory stimuli. Although the CRP level is in many cases increased in Campylobacter gastroenteritis, its determination should not be used as a strict criterion to differentiate a bacterial gastroenteritis from a viral one as long as high levels of CRP can also be found in viral infections with *Herpes simplex*, *Cytomegalovirus*, *Influenza A* or *B*, *Enterovirus* [3,4].

We consider improving the panel of investigations with the additional use of the Leukergia test, which is based on the adhesive and aggregation properties of leukocytes, leukergy being the first reaction that occurs in an organism invaded by an antigen [2,5], nonspecific to viral infections [6,7] and present even if antibiotics are administered [2,8]. That is why we considered that the leukergia test is much more likely to indicate the early presence of bacterial infection in campylobacteriosis, until results can be obtained from the other laboratory investigations.

MATERIALS AND METHODS

The study carried out is a case-control type performed on a group of 210 patients hospitalized or investigated in the emergency room, at Ponderas Academic Hospital in Bucharest, between 1st of June 2020 and 31st of May 2021, from which 85 subjects were selected and divided into 2 study lots. First, a group of 45 patients diagnosed with acute gastroenteritis with Campylobacter, a bacterial infection documented through a positive culture and a typical clinical picture. The second batch is the control group consisting of 40 children with no signs of acute disease, who have performed clinical consultations and routine blood tests in order to perform surgical interventions, these having all the biological parameters investigated within normal limits.

The general criteria for inclusion were: age 0-18 years, signed informed consent by the legal guardian of the child that involves the collection from the minor patient of biological samples necessary for any hospital admission, the presence of diarrheal stools and/or vomiting, +/- fever, inappetence at the time of presentation at the emergency room, mandatory complete blood count (CBC), CRP, faecal analysis for clinical cases, as well as written consent to participate in clinical trials.

General exclusion criteria: age over 18 years; refusal of the minor's official guardian to collect biological samples from the child that are necessary to establish a diagnosis, patients who, at the time of admission for signs of gastroenteritis, were previously diagnosed with a disease of a bacterial nature other than enterocolitis, for which they are undergoing antibiotic treatment, patients undergoing systemic treatment with corticoids [2,9], patients with chronic diarrhea or malignant diseases [7].

In addition to the leukergy test, the inflammatory reaction quantification was carried out, by determining the C-reactive protein (CRP), CBC, the leukocyte formula in the peripheral blood. For the determination of the etiological agent of gastroenteritis, coproculture, coprocytograms, quick tests for the detection of *Campylobacter*, *Rotavirus*, *Adenovirus*, *Enterovirus*, *Norovirus*, Gastrointestinal Multiplex RT PCR Panel, Covid 19 RT PCR - SARS-CoV-2 RNA RT PCR testing were performed. The degree of dehydration was established according to the clinical status and values determined with the Astrup method. All laboratory investigations were carried out on standardized and approved devices within the clinic.

The leukergy test (leukocyte aggregation/adhesiveness) was performed according to the modified L. Fleck method [10] by sampling with a Pasteur pipette two three drops of the venous blood collected at admission in a vacutainer coated with anticoagulant used for the blood count, after mixing it for homogenization. The 2-3 drops of blood were allowed to slide for a few seconds on a glass slide positioned at 45 degrees, placed on a special slide support. When the blood covered the surface of the entire blade it was left to dry in a horizontal position at the laboratory temperature or in the incubator, then it was frozen for 10 minutes at a temperature of -10 -18 degrees Celsius, to achieve erythrocyte hemolysis (thus, freezing and thawing not affecting the granulocytes) [9,11,12]. The slide removed from the freezer was later allowed to dry at room/incubator temperature. The smear that was obtained in this manner was then stained with May-Grunwald (3 minutes) and Giemsa (10 minutes) [5]. After these manipulations, the smear slide was gently rinsed with distilled water to remove traces of dyes, left to dry at room/incubator temperature, then examined on a 400 X immersion microscope [11,12]. For a higher accuracy of the results, 1-2 distinct slides with 2-3 strips of blood each, as equal as possible in thickness, were made for each patient, which were examined individually and the final result is a simple average of the 2 readings. The leukergia test was considered positive, when neutrophil leukocytes appeared grouped 3-4-5-6, the distance between the nuclei being less than the diameter of a cell, the percentage of aggregation being more than 10% (Figure 1 – Positive leukergia in *Campylobacter* infection). The calculation of leukergia (%) was made using the formula: Leukergy % = [(number of aggregate leuko-

cytes)/300]x 100. The leukocyte aggregation percentage on the slides was established by counting 300 leukocytes. If 30 of the 300 leukocytes were aggregated/grouped, the percentage of leukocyte aggregation was 10%. Depending on the percentage of aggregate leukocytes, the degree of leukergy (from 1 to 4) is also established, grade 0 meaning the negative leukergy test, according to Table 1 [5,14].

TABLE 1. Degree of Leukergy

Aggregate leukocytes per 300 leukocytes counted	Leukergy percentage (%)	Degree of leukergy
<30	<10 %	0
30-32	10%	1
33-59	11-19%	2
60-104	20-34%	3
>105	> 34%	4

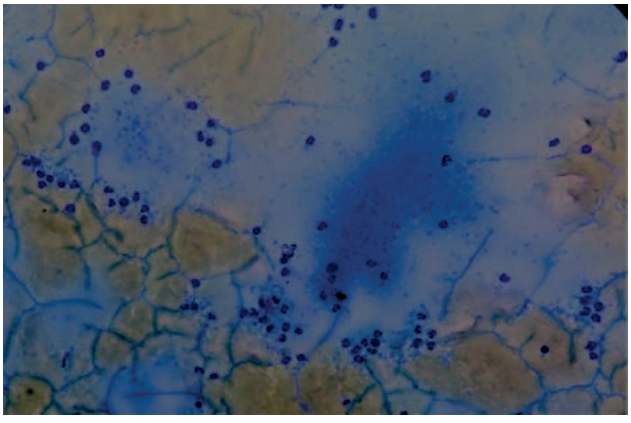

FIGURE 1. Positive Leukergy

The statistical analysis was performed using STATA 13/MP statistics software, using: Square Chi Test, Student t Test, ANOVA Variance Analysis, Odds Ratio Relative Risk (OR), ROC Curve, Area Under the Curve (AUC).

RESULTS

The study included a group of 85 children (32 girls and 53 boys), aged between 2 months and 13 years, out of which 45 children confirmed with gastroenteritis with Campylobacter and 40 children from control group, clinically and paraclinically healthy. The average age of the children confirmed with Campylobacter was mediana = 2 years, (min= 0,2 years, max=7 years) Test t-p=0,0001. A percentage of 91% of the sick patients presented fever, the average temperature being 38,78± 0,92 C degrees. All the children in the control group (M) had a normal temperature between 36-36,9 C degrees. Chi squared p=0,0001. A parallel of the values of the four parameters included in the study are represented in Tables 2, 3, 4 and 5. The comparison between leukergy and infection with Campylobacter, WBCC, NEUT and CRP are shown in Tables 6 and Figure 2. The sensitivity, the specificity, the positive predictive value, were slightly superior for the CRP. Leukergy correlated very well with the presence of bacterial infection, normal leukocyte count and increased CRP, the most accurate being the leukergy test (p=0,001) and CRP (p=0,001) (Table 2).

DISCUSSIONS

Differentiating between viral and bacterial gastroenteritis represents one of the most common issues faced by pediatricians, especially at the onset of the disease, when they both have similar clinical signs (fever, altered stools, vomiting, inappetence) and when the time to obtain a faeces sample can take hours. The only means of diagnosis remain the total leukocyte number (WBC), neutrophil count (NEUT), C-reactive protein (CRP), which are normally elevated in any bacterial infection. In the case of atypical bacteria, such as Campylobacter, the situation may be different because, as it results from our study, the leukocyte response is absent in most cases, normal values of the C-reactive protein may be present, even if in most cases of campylobacteriosis the CRP level is above the normal limit. Also, in the case of gastroenteritis that are partially treated with antibiotics before the collection of biological sam-

TABLE 2. Biological parameters in the study group

	Patients with Campylobacter	control group-healthy children	Normal values infant	Normal values children	Test t student
Test	n = 45	n = 40			p
Leukergy No. of aggregated cells	47,53 ± 17,15 (30 – 120)	7,43± 8,85 (0-32)	<30	<30	0,0001
CRP (mg/dl)	4,56 ± 4,39 (0,01-18,04)	0,09 ± 0,03 (0,01 - 0,2)	<0,5	<0,5	0,0001
Total leukocytes (10^3/µl)	9,76 ± 3,37 (3,55 – 16,05)	8,14 ± 2,45 (5,02 – 13,6)	6-16	5-15	0,0141
Total neutrophil no. (10^3/µl)	5,73 ± 3,32 (0,78 – 13,5)	3,58 ± 4,24 (1,28 – 2,28)	1-6	2-8	0,0106
Total lymphocyte no. (10^3/µl)	3,03 ± 1,64 (0,59 - 6,17)	4,20 ± 1,83 (1,42 – 9,77)	4-12	3-11	0,0026

TABLE 3. Patients confirmed with Campylobacter who received antibiotherapy before admission

No. crt.	T grade C	CRP (mg/dl)	Degree leukergia	No aggregate cells	% leukergia	Leukocytes (10^3/µl)	Neutrophils %	Neutrophils (10^3/µl)
1	*36,8*	*0,01*	2	40	13,33	5,87	13,3	0,78
2	**39**	**8,29**	2	40	13,33	12,73	59,4	7,56
3	**38,5**	*0,32*	2	55	18,33	5,01	18,9	0,95
4	**40,7**	**9,06**	4	120	40,00	11,62	**86**	**9,99**
5	**38,5**	**2,6**	2	55	18,33	**14,28**	**71,5**	**10,21**
6	**38,6**	**14,16**	2	55	18,33	**16,05**	**60,6**	**9,72**
7	**39,4**	**2,8**	2	40	13,33	10,58	46,7	5,07
8	**38,8**	**5,29**	2	45	15,00	8,11	43,8	3,55
9	**40**	**4,36**	1	32	10,66	6,11	84,4	5,16
10	**39,3**	**1,51**	2	40	13,33	4,76	34,1	1,62
11	**38,5**	**2,48**	2	45	15,00	6,85	64	4,38

TABLE 4. Children in the control group with positive leukergy who received antibiotherapy recently before hospital admission

No. crt.	T grade C	CRP(mg/dl)	Degree leukergy	No aggregate cells	% leukergy	Leukocytes (10^3/µl)	Neutrophils %	Neutrophils (10^3/µl)
1	36,5	0,1	1	32	10,66	8,4	35,9	3,02
2	36,4	0,1	1	31	10,33	12,29	40,5	4,97

TABLE 5. CRP value variation

CRP value (mg/dl)	Campylobacter cases (+)	Control group	Total	%
Normal < 0,5	7	40	47	55,3
Slightly increased 0.5-4.9	24	0	24	28,2
Moderately grown 5,9-9	8	0	8	9,4
High increased > 10	6	0	6	7,1
	45	40	85	100

TABLE 6. Specificity and Sensitivity of the studied parameters

	leukergy (aggregate cells)		Total No. Leukocytes		Total No. Neutrophils		CRP	
	M	C	M	C	M	C	M	C
Campylobacter (+) (n=45)	7	42	6	42	7	40	1	43
Control group (-) (n=40)	33	3	34	3	33	5	39	2
Sensitivity (%)	93,3		93,3		88,9		95,6	
Specificity (%)	82,5		85		82,5		97,5	
Positive predictive walue (%)	85,7		85,5		85,1		97,7	

ples, the possibility of relying on bacteriological results in establishing a diagnosis greatly decreases. In this sense, a rapid and low-cost test such as the leukergia test, can be of real help to any doctor in his decision-making analysis for the purpose of therapeutic management.

Leukergy (leukocyte aggregation/adhesiveness test), studied and originally described by Ludwik Fleck between 1942-1952, is a non-specific marker of inflammation, a fact that has been confirmed by its presence in a variety of inflammatory processes researched over the years in various diseases in both humans and animals [15]. More specifically, L. Fleck observed this phenomenon in 1942, after intravenously administering bacteria into the blood of animals, noting a new property of neutrophil leukocytes to aggregate into groups of cells, an increase in their oxidative turnover, their migration and last but not least the increase in their phagocytic capacity, demonstrating that there is no definite causal link between leukergy and leukocytosis. He concluded that the leukergy was positive a few hours after the injection of the bacteria, due to the increase in fever but before the onset of leukocytosis and the increase in the rate of blood sedimentation (ESR), and that the leukergyc state lasted longer than the duration of fever, leukocytosis and normalization of the value (ESR) [16]. Taking into account the above, we have introduced in this paper the study of four variables (CRP, WBCC, NEUT, leukergy), to verify

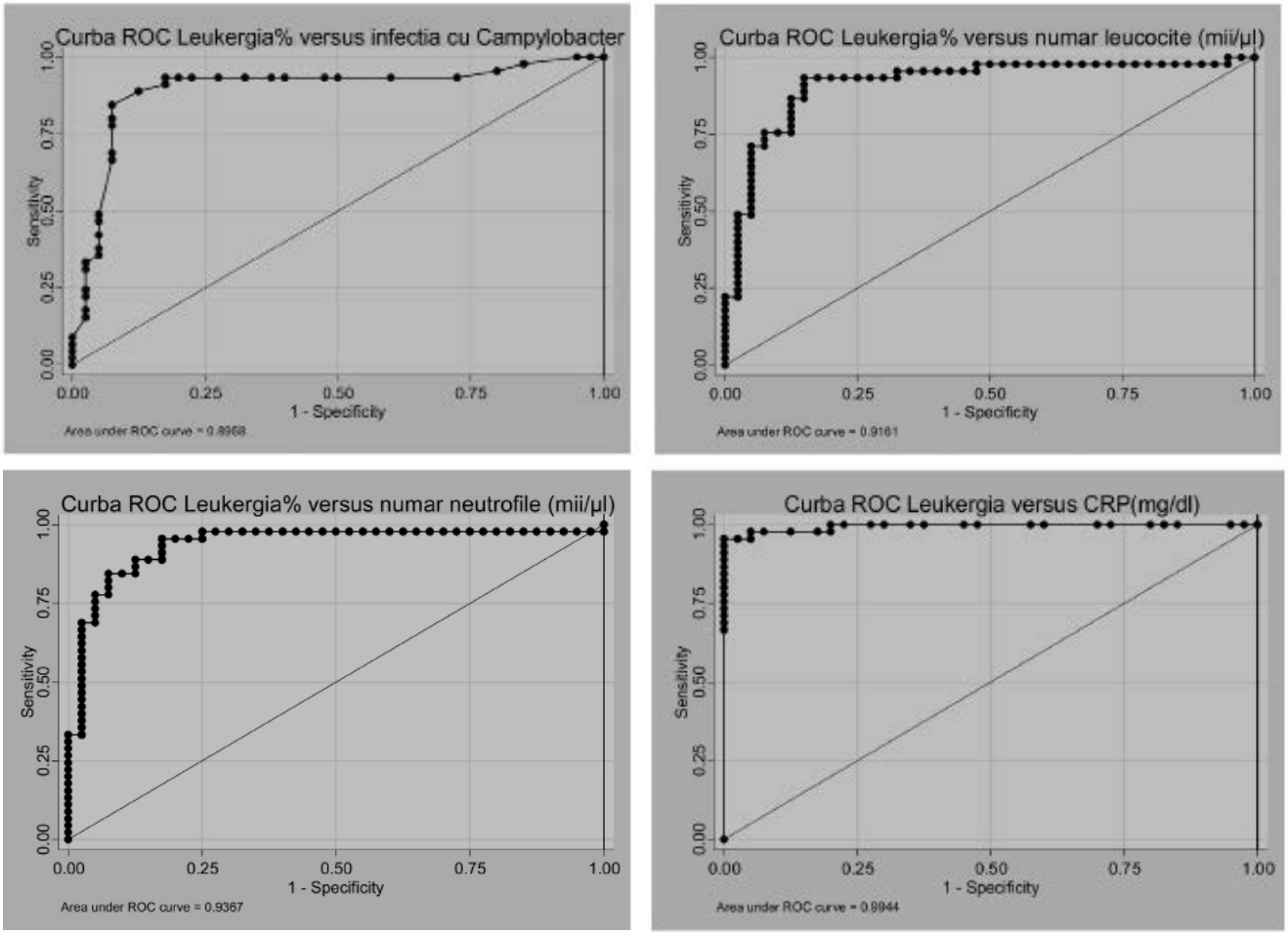

FIGURE 2. Leukergy (%) in patients with *Campylobacter* compared to the control group

whether the four phenomena are interdependent. Regarding the leukocyte response, out of the 85 children included in the study, only 3 showed true leukocytosis, the three cases being from the group of children confirmed with bacterial infection with Campylobacter. Four of them showed a number of leukocytes to the upper limit of normal, which we considered as leukocytosis at onset being that it was also associated with neutrophilia. Neutrophilia was found at (24.4%). Eleven patients positive for Campylobacter received antibiotics before taking biological samples upon admission, with two of the them exhibiting leukocytosis with neutrophilia upon admission (Table 3). Leukergy (leukocyte aggregation) was present in all the patients infected with Campylobacter, both in those with leukocytes and neutrophils within the normal range and in those presenting with leukocytosis with neutrophilia. We conclude from the above, that leukocytosis is not necessary to induce the phenomenon of leukergy in case of infection with Campylobacter, since this bacterium is also present if the leukocytes and neutrophils were within normal limits as has been demonstrated in other studies [17]. In addition, it was found that an increased leukocytosis correlated very well with a high degree of leukocyte aggregation in the case of more severe infections.

The CRP value was within the normal range in all children in the control group included in the study, being increased to 84.4% of cases with positive Campylobacter (most of them having a slightly increased CRP) (Table 5). Seven of the sick children had normal CRP values. Antibiotic treatment administered before investigations in 11 of the children confirmed with Campylobacter, did not alter the result of leukergy, which remained present even after several doses of antibiotic, while the other acute phase reactants undergo changes after antibiotherapy.

From the above data we conclude that CRP is a specific marker of Campylobacter infection, but at the same time we can say that it is not necessary to have an increased level of CRP to induce the phenomenon of leukergy, leukergy also being encountered in the case of campylobacteriosis with normal values of CRP [6,18-20]. Furthermore, the level of CRP is not an inflammatory marker that can always highlight the severity of the infection or the type of microorganism (virus/bacterium). Instead, leukergy indicated every time the presence of bacterial infection (Table 3).

The leukergy test was positive for 49 children out of the 85 included in the study (45 diagnosed with Campylobacter and 4 witnesses) Chi square p=0.0001, even after taking antibiotics before taking biological samples at hospital admission Chi square p=0.013. The degree of leukocyte aggregation in sick children was on average 47.53, which corresponds to a degree of leukergy 2 out of 4 (T-test p=0.0001), a moderately increased degree. In the case of the control group (M), the leukergy test was positive (grade 1) in 4 out of the 40 children (Table 7). Two of them had recently completed antibiotic treatment for conditions in the ENT sphere. One of the children had tight phimosis and a recently cured urinary tract infection, and the 4th had frequent respiratory tract infections followed by otitis. None of them showed clinical signs of acute infection or changes in the blood count, CRP, uroculture upon admission to hospital. The presence of leukergy, of a low degree of leukocyte aggregation (grade 1/10%) in our case, demonstrates the superiority of the leukergy test in detecting traces of a bacterial infection even after the end of the antibiotic treatment, when the patient is no longer clinically symptomatic and with test results within normal limits [5]. Moreover, a high degree of adhesiveness was found in children with present bacterial infection compared to healthy ones (M), highlighted by a longer blood flow time on the slide inclined at 45 degrees when performing the leukergia test, specific for bacterial infections [6]. As described above, in some inflammatory conditions there is a close correlation between leukocytosis, leukergy and CRP while in others there are not, leukergy being the only constant present in all cases of bacterial infection.

The possibility of an associated urinary tract infection was ruled out in all patients diagnosed with Campylobacter gastroenteritis through the collection of urine analysis and uroculture, which came out negative. All sick children showed mild or medium dehydration syndrome with no signs of hemoconcentration in the blood count. In 9 children (20%) Campylobacter infection was accompanied by other gastrointestinal infections of a viral nature (4 with Rotavirus, 3 with Enterovirus, 2 with positive SARS Cov2) and three of them showed other bacterial co-infections (cultures positive to Yersinia, Salmonella and EPEC). In all cases of virobacterial co-infection, the presence of Campylobacter caused the leukergy to be positive, even in cases where all the reactants of the acute phase were within normal limits.

CONCLUSIONS

Induction of the phenomenon of leukergy in gastroenteritis with Campylobacter is not dependent on the leukocyte response or the level of CRP. The degree of leukocyte aggregation is closely correlated with the severity of bacterial infection, not being influenced by the administration of antibiotics before the collection of biological samples. The leukergy test can provide, in a short time, significant information that can complement the other biological investigations which take longer to perform, information that can be of real help for any doctor in his decisional analysis for the therapeutic management in case of an acute gastroenteritis in a child. We also mention that the leukergy test is a rapid and efficient test with a good price-quality ratio, a test that could be useful for detecting a bacterial infection, assessing its severity in medical centers where the services of a clinical laboratory are not available around the clock.

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