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Vascular Changes in Ulcerative Colitis and Leśniowski-Crohn's Disease

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The aim of the report was an attempt at assessing the role of vessels in morphological changes of the intestinal wall affected by ulcerative colitis and Leśniowski–Crohn's disease, as well as determining the character of vascular changes and comparing them in both the afore-mentioned diseases.

The investigations included archival surgical materials originating from 42 patients with ulcerative colitis and 30 individuals with Leśniowski-Crohn's disease.

A histological analysis was performed, along with an immunohistochemical assessment (reactions with antibodies against ICAM-1, VCAM-1, CD34, FVIII and UEA-1). The results were analyzed statistically.

The investigations allowed for determining that vascular changes occurred in both diseases, being of a similar nature in ulcerative colitis and Leśniowski-Crohn's disease. The difference was chiefly quantitative.

The detection of inflammatory in filtrates surrounding the vessels situated in otherwise unchanged or mildly changed intestinal segments proved the leading role played by vessels in both diseases. It should be stressed that perivascular inflammatory infiltrations involved all layers of the intestinal wall – not only in Leśniowski-Crohn's disease, but also in ulcerative colitis.

The authors demonstrated a difference in expression intensity of various investigated vascular markers. In ulcerative colitis, higher values were obtained for the ICAM-1, CD34 and UEA-1 expression, while in Leśniowski-Crohn's disease, higher expression values were characteristic of VCAM-1 and FVIII.

Numerous correlations were also detected in the expression of the investigated markers; the said correlations differed in both diseases.

The results indicate that some histological differences (especially these involving the condition of vessels situated in the mucosa), as well as differences in the expression of immunohistochemical markers may be helpful in differentiating between the two diseases, and mostly in evaluating surgical materials.

Introduction

For many years now, Leśniowski-Crohn's disease and ulcerative colitis have been the focus of interest of both clinicians and pathomorphologists. The recently published literature reports vascular changes occurring in both these diseases. Thus, in Leśniowski-Crohn's disease, characteristic signs are commonly believed to include distension of lymphatic vessels, slight or mild hyperaemia and the presence of inflammatory in filtrates in the arterial and venous walls [3, 8, 15, 18]. On the other hand, in cases of ulcerative colitis, characteristic signs are represented by mucosal and submucosal hyperaemia, as well as the presence of thrombi within mucosal capillaries. Ten percent of patients are described as demonstrating *endarteriitis obliterans* markers, with or without capillary thrombi [2, 11].

The reported information on vascular changes is markedly general in character. No comparison of such changes in both diseases has been presented. There is a lack of more extensive reports on the tissue expression of vascular markers and adhesion molecules. For this reason, the present authors have deemed it worthwhile to concentrate on this subject.

Objectives

The objectives of the investigation were as follows:

1) to evaluate the role of vessels in morphological changes involving intestinal walls in ulcerative colitis and Leśniowski-Crohn's disease, and to determine the character of such changes;
2) to assess the possible role of selected adhesion molecules in vascular changes;
3) to compare vascular changes in both diseases.

Material and Methods

The investigations included archival surgical materials originating from 42 patients with ulcerative colitis and 30 patients with Leśniowski-Crohn's disease. A histological and immunohistochemical analysis was performed. The controls (10 cases) consisted of sections collected from the incision line of the colons resected due to carcinomas.

Based on hematoxylin-eosin stained preparations, the authors selected for further immunohistochemical tests paraffin blocks containing sections with the most characteristic changes. To demonstrate the expression of the investigated vascular markers, the streptavidin-biotin-peroxidase method was employed using an LSAB kit manufactured by DAKO. To facilitate the reaction between the antigen and the antibody, the antigens were denuded in a microwave in a citrate buffer (pH 6.0) at the temperature of 96°C over 12 minutes. The activity of endogenous peroxidase was blocked by 3% H_2O_2. Subsequently, the preparations were incubated at room temperature for 60 minutes with the following antibodies: ICAM-1 (Novocastra NCL-CD54-307), VCAM-1 (DAKO M7106), CD34 (DAKO M7165), and FVIII (DAKO M0616). Determinations were also made of UEA-1 lectin ligands (DAKO X921).

In the course of the subsequent, 30-minute incubation, a biotinylated antibody was used and subsequently incubated with the peroxidase-streptavidin complex. Between particular incubation sessions, the preparations were rinsed in the TBS buffer (pH 7.6). The antigen was located using DAB-3.3 (DAKO) as a chromogen, which is a substrate for peroxidase. The preparations were then stained with hematoxylin and – following dehydration – closed with a cover glass.

A reaction without the primary antibody was performed as a negative control in immunocytochemical determinations (CD34, FVIII).

In all the control reactions, the material consisted of pyogenic granuloma (*granuloma teleangiectaticum*) sections.

In H&E-stained sections, as well as in sections originating from the same paraffin blocks, in which immunohistochemical reactions had been performed, the number of vessels in all the layers of the colonic wall was counted, the character of changes in these vessels was determined, as well as the presence or absence of expression of the investigated markers. The counts were performed in 10 high power fields (400x) of vision under a high magnification (400 x) in each intestinal wall layer.

To evaluate the *vascular expression* of the investigated markers, the authors employed the five-point scale system proposed by Bernstein et al. [1], where:

0 = absence of expression,
1+ = reaction products appear within the vessels in less than 50% of the investigated fields and the expression is low,
2+ = expression is discernible in one to five vessels in each investigated field,
3+ = reaction products are observed in six to nine vessels in each field of vision,
4+ = expression is seen in more than ten vessels in all the investigated fields.

In the same fields of vision where the vascular expression was determined, further determinations were made of the *degree of expression intensity* within inflammatory infiltrations. The employed system was also reported by Bernstein et al. [1]

Similarly as in the case of vascular expression, the system is a five-point scale, where:
0 = no expression in infiltrate cells,
1+ = less than 50% of ten investigated fields contains in filtrates with reaction products; weak expression,
2+ = expression is seen within 1–10 cells in a field of vision (but is detected in all fields),
3+ = expression is seen in 11–30 cells in a field of vision (in all fields),
4+ = expression is present in more than 30 cells in a field of vision (in all ten fields).

The assessment of *the intensity of inflammatory changes* was based on the method developed by Sandborn et al. to evaluate the degree of inflammatory change intensity in intestinal pouches [12]. The classification includes three intensity levels in inflammatory changes:

grade I – low intensity
grade II – moderate intensity
grade III – marked intensity.

Determinations of inflammatory change intensity were based on the assessment of leukocyte infiltrate density and the degree of intestinal surface involvement by the inflammatory process. The following five-point scale was adopted:
score 1 – low infiltrate density, no crypt abscesses,
score 2 – moderate infiltrate density, infrequent crypt abscesses,
score 3 – high infiltrate density, numerous crypt abscesses.

Depending on the extent of changes, another three-point scoring scale was used, where:
score 1 – involvement of less than 25% of colonic surface,
score 2 – involvement of 25%-50% of the surface,
score 3 – involvement of more than 50% of the surface.

Inflammation of **grade I** intensity was diagnosed when the sum of both scores equaled or was lower than **4**, **grade II** – when the sum was **5**, and **grade III** – when it equaled **6**.

The results were analyzed statistically. To compare the degree of expression of the investigated parameters between the experimental groups and between each experimental group and the controls, the authors employed non-parametric methods (the comparison of the mean number of vessels and the expression of CD34, F VIII, UEA-1, ICAM-1, and VCAM-1- the Mann-Whitney test; correlations between the degree of marker expression in each group – the Spearman's correlation coefficient).

The statistical significance was accepted at $p< 0.05$.

Results

The comparison of vascular changes in the two experimental groups failed to demonstrate any significant qualitative differences. Quantitative differences were noted, however.

The statistical analysis showed a significant difference ($p<0.001$) between the number of mucosal vessels in the experimental groups (13.98±5.88 in ulcerative colitis vs. 9.23±4.77 in Leśniowski-Crohn's disease). No such differences were observed in other layers of the intestinal wall. The number of vessels in the controls was significantly lower. The comparison between the number of vessels noted in both experimental groups and between the experimental groups and the controls is presented in Tables 1, 2 and 3.

In both experimental groups, mucosal congestion was observed, being clearly more pronounced in ulcerative colitis. These patients often demonstrated sinusoidal distension of the capillaries. In Leśniowski-Crohn's disease, the intensity of congestion was visibly lower, and the capillaries were characterized by narrowed lumens. Only in 11 cases did the authors note focal sinusoid distension of the mucosal capillary vessels.

In both experimental groups, the submucosal membrane showed numerous vessels with markedly sinusoidally distended lumens. Such a phenomenon involved various types of vessels – the arteries, veins, capillaries and lymphatic vessels.

It should be emphasized that both groups demonstrated perivascular inflammatory infiltrates in all the layers of the intestinal wall. In patients with ulcerative colitis, in deeper layers of the intestinal wall, the infiltrations were observed solely around the vessels, and in the majority of cases, they

TABLE 1
Comparison of the number of vessels in patients with ulcerative colitis and in the controls

	M-W test	p	No.	No.
Mucosal vessels	**4.8763**	<0.0001	42	10
Submucosal vessels	0.03484	0.972207	42	10
Muscular layer vessels	1.56954	0.116523	42	10
Serous membrane vessels	**2.230421**	0.02572	42	10

TABLE 2
Comparison of the number of vessels in patients with Leśniowski-Crohn's disease and in the controls

	M-W test	p	No.	No.
Mucosal vessels	4.535	<0.071594	30	10
Submucosal vessels	-1.07813	0.280977	30	10
Muscular layer vessels	**2.01113**	0.044313	30	10
Serous membrane vessels	**2.12343**	0.033719	30	10

TABLE 3
Comparison of the number of vessels in patients with ulcerative colitis and Leśniowski-Crohn's disease

	M-W test	p	No.	No.
Mucosa	**3.83**	0.001	42	30
Submucosa	1.61	0.10	42	30
Muscular layer	-0.90	0.36	42	30
Serous membrane	-0.40	0.68	42	30

TABLE 4
Comparison of the expression of the investigated markers in ulcerative colitis and Leśniowski-Crohn's disease

ICAM	M-W test	p	No.	No.
Infiltrates	**4.39065**	0.000011	42	30
Mucosal vessels	**2.34755**	0.018903	42	30
Submucosal vessels	**3.72427**	0.000196	42	30
Muscle layer vessels	**4.06829**	0.000047	42	30
Serous membrane vessels	**3.2159**	0.001302	42	30
VCAM	M-W test	p	No.	No.
Infiltrates	-1.182207	0.237186	42	30
Mucosal vessels	-1.09406	0.273937	42	30
Submucosal vessels	**-2.41089**	0.015919	42	30
Muscle layer vessels	**-3.06985**	0.002143	42	30
Serous membrane vessels	**-2.90533**	0.003672	42	30
CD34	M-W test	p	No.	No.
Infiltrates	**5.71839**	<.00001	42	30
Mucosal vessels	**-2.12091**	0.033937	42	30
Submucosal vessels	**-2.57735**	0.009961	42	30
Muscle layer vessels	**-3.22722**	0.001251	42	30
Serous membrane vessels	**-3.15251**	0.00162	42	30
FVIII	M-W test	p	No.	No.
Infiltrates	**-2.10404**	0.035383	42	30
Mucosal vessels	-1.51033	0.130969	42	30
Submucosal vessels	-1.2711	0.203703	42	30
Muscle layer vessels	-1.8718	0.061244	42	30
Serous membrane vessels	**-2.91027**	0.003614	42	30
UEA	M-W test	p	No.	No.
Infiltrates	**3.02714**	0.002471	42	30
Mucosal vessels	0.38284	0.701842	42	30
Submucosal vessels	-0.24144	0.809217	42	30
Muscle layer vessels	-0.37104	0.710612	42	30
Serous membrane vessels	**-2.0925**	0.036402	42	30

(Statistically significant differences are given in bold).

were limited in size. In Leśniowski-Crohn's disease, the perivascular infiltrations were clearly visible in the regions where extravascular inflammatory changes were less intensive or in areas where there were no such extravascular infiltrates.

In both groups, but especially in patients with Leśniowski-Crohn's disease, some arteries showed wall thickening accompanied by inflammatory infiltrates involving the vessels. Single vessels demonstrated ***endarteritis obliterans***-type changes.

Moreover, in patients with Leśniowski-Crohn's disease, the authors often encountered typical granulomas situated in the closest vicinity of the vessels. However, their direct association with the adventitia was demonstrated in one case only.

Both in ulcerative colitis and in Leśniowski-Crohn's disease, the perivascular inflammatory infiltrates were not associated with a specific type of changes within the vessel walls.

In the majority of cases of ulcerative colitis, immunohistochemistry revealed the expression of all the investigated markers within the inflammatory infiltrates; in the greatest number of cases, the phenomenon involved ICAM-1, VCAM-1 and UEA-1. In patients with Leśniowski-Crohn's disease, a high intensity of expression of these markers within the inflammatory infiltrates was noted in a lower number of cases-with the exception of VCAM-1.

The highest number of cases showing a considerable intensity of vascular expression was noted in both groups with respect to CD34 and UEA-1, and the phenomenon was seen in all the intestinal wall layers. However, it should be stressed that the highest number of vessels was demonstrated in the reaction with the anti-CD34 antibody, while the lowest number was seen in the case of the anti-F VIII antibody.

Differences were noted between both experimental groups in the intensity of expression of particular markers. In the case of ulcerative colitis, the authors noted a higher intensity of CD34 and UEA-1 vascular expression, while in patients with Leśniowski-Crohn's disease, this was true for F VIII. Differences were also found in the intensity of adhesion molecules expression. In ulcerative colitis, the expression of ICAM-1 was clearly intensified, while in patients with Leśniowski-Crohn's disease, it was the expression of VCAM-1. The differences were statistically significant. The comparison of the expression of the investigated markers in both experimental groups is presented in Table 4.

In the case of ulcerative colitis, a significant correlation was observed between the vascular expression of VCAM-1 and UEA-1 in the mucosa, and between the vascular expression of CD34 and F VIII, as well as F VIII and UEA-1 in the mucous membrane. No such correlations were noted in patients with Leśniowski-Crohn's disease. On the other hand, these individuals demonstrated a correlation of the expression of ICAM-1 and CD34, as well as the expression of ICAM-1 and UEA-1 within the inflammatory infiltrates. Such correlations were not found in ulcerative colitis.

In both the experimental groups, the authors noted a significant correlation between the vascular expression of ICAM-1 and VCAM-1 within the mucosa.

The correlation between the expression of ICAM-1 and VCAM-1 in each experimental group (and the inter-group differences) is understandable. The increase in adhesion molecules expression is dependent on inflammation intensity. In the present material, in the majority of cases, the intensity was high (grade III according to Sandborn).

In the controls, the expression of all the investigated markers was significantly lower.

Discussion

The results have demonstrated that vascular changes do occur both in ulcerative colitis and in Leśniowski-Crohn's disease, and in both these entities, such changes differ mainly quantitatively. The differences are associated with the number of vessels and the prevalence of various changes, as well as the expression of particular vascular markers.

The highest, significant differences in the number of vessels were noted in the mucosa. The number of mucosal vessels in the controls was distinctly lower as compared to both groups manifesting inflammatory changes. Yet, the most striking differences were observed in the number of vessels in patients with ulcerative colitis and Leśniowski-Crohn's disease. In ulcerative colitis, the vessel number was significantly higher. These differences may be to a degree associated with the fact that in ulcerative colitis, the predominant type of vessels demonstrates clear (even sinusoidal) distensions, owing to which they are well visible. On the other hand, in Leśniowski-Crohn's disease, in view of considerable capillary narrowing, the vessels were frequently unidentifiable in HE-stained preparations. They were much more visible in immunohistochemical tests (especially in the reaction with the antibody against CD34 and UEA-1). Nevertheless, in immunohistochemical preparations, the number of vessels was significantly lower as compared to histological preparations.

In the ulcerative colitis group, no narrowed vessels were encountered in the mucosa. Vessels with edematous endothelium were also more infrequently noted in comparison to Leśniowski-Crohn's disease.

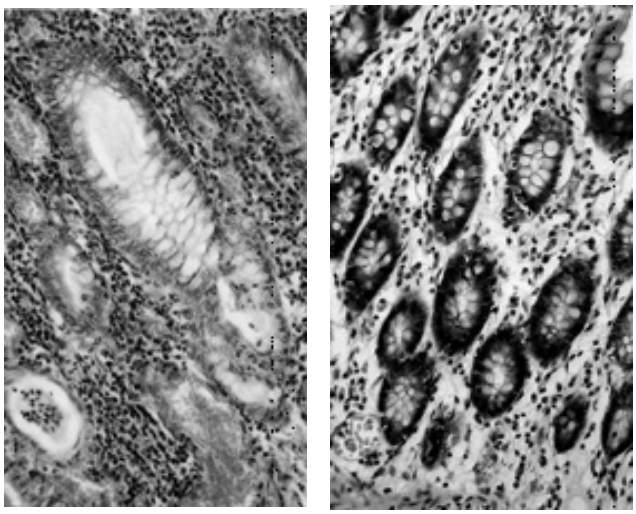

Fig. 1a. Ulcerative colitis. Distended mucosal vessels. Blood congestion. HE × 50. 1b. Control group. A scant number of blood-filled vessels seen in the mucosa. HE × 20.

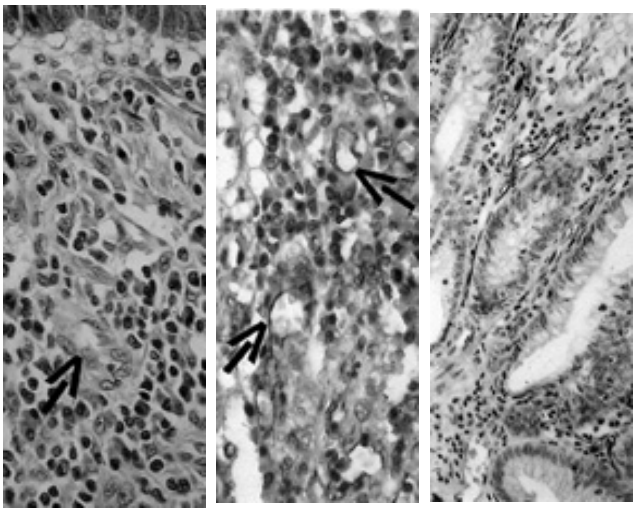

Fig. 2. Leśniowski-Crohn's disease.
a) A scant number of narrowed vessels in the mucosa of the region involved by inflammatory infiltration. HE × 90.
b) The same case as above. UEA-1 expression demonstrates the presence of a large number of vessels within the mucosa with inflammatory changes. × 50.
c) CD 34 expression in mucosal capillaries. × 25.

The presented differences may be helpful in evaluating endoscopic materials. Nevertheless, this statement is true only with respect to the latter changes. The presence of vessels with narrowed lumen and/or edematous endothelial cells indicates Leśniowski-Crohn's disease rather than ulcerative colitis. No such diagnostic role is ascribed to detection of distended vessels. Although, as a rule, they occur in ulcerative colitis, their presence has been also observed in moderately numerous cases of Leśniowski-Crohn's disease.

Dhillon et al. [2] drew attention to distended mucosal capillaries in ulcerative colitis. However, a search of the literature on the subject has failed to locate a report that would characterize vascular changes in Leśniowski-Crohn's disease, despite the fact that such changes are ascribed an important role in the pathogenesis of the disease [7, 13]. The present authors have not succeeded in finding in the literature a comment on the validity of the vascular status assessment in diagnostic management of endoscopic materials. The only observation on vascular changes in Leśniowski-Crohn's disease was associated with the relationship between vessels and granulomas.

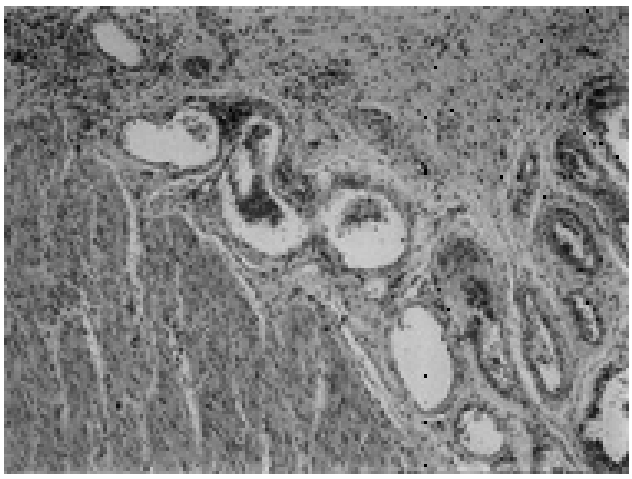

Fig. 3. Ulcerative colitis. Numerous sinusoidally distended vessels in the submucosa. Inflammatory infiltrations are seen in the vicinity of some vessels. HE × 25.

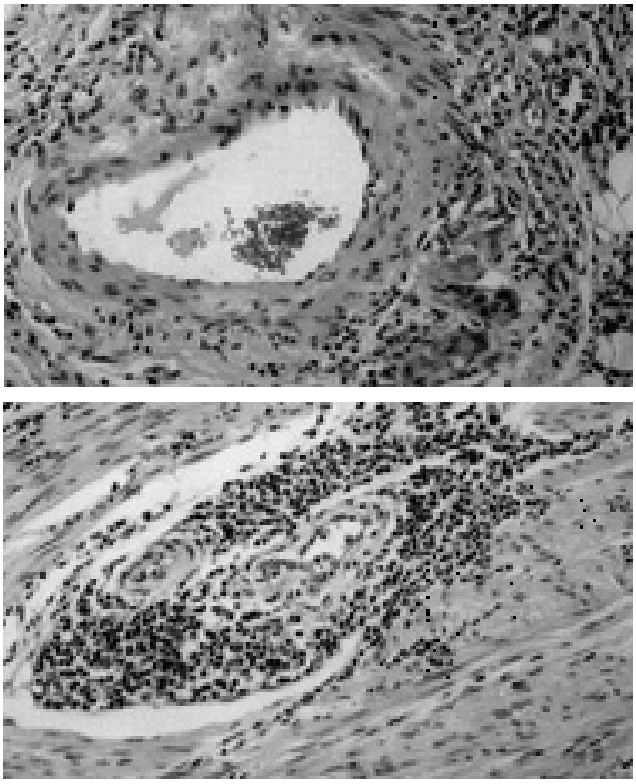

Fig. 4 a. Leśniowski-Crohn's disease. A granuloma in the close vicinity of the vessel wall. HE × 50.
4 b. Ulcerative colitis. A profuse lymphocyte infiltration in the vicinity of muscle layer cells. HE × 50.

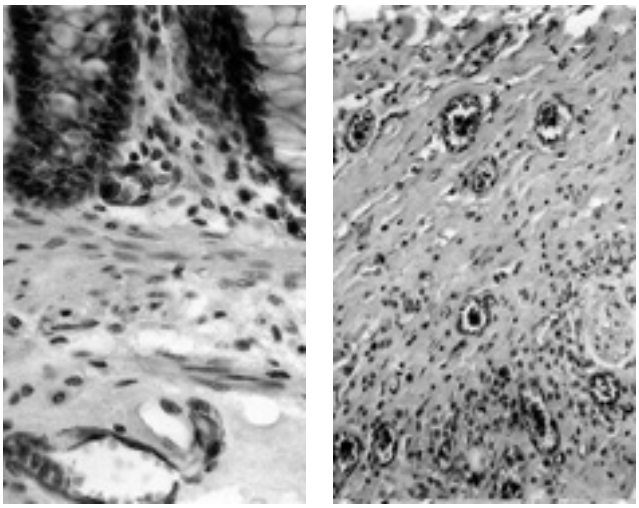

Fig. 5 a. VCAM-1 expression in the endothelium and in single inflammatory infiltrate cells. × 90.
5 b. ICAM-1 expression in the endothelium and in single inflammatory infiltrate cells. × 50.

Such a relationship was emphasized by Matson et al. and Mooney et al. [7, 8]. Matson observed a frequent presence of granulomas directly associated with vessels. He even defined such changes as granulomatous vasculitis. Mooney estimated that the mean percentage of 46.1% of granulomas (range: 15%–90.4%) was seen in direct association with the wall of a lymphatic vessel. However, he stressed the fact that granulomas only adhered to the vessels, but did not invade their walls. In a lower percentage of cases – 10% (range, 2.4%–25.8%), granulomas are seen in contact with blood vessel walls. Based on these observations, Mooney concluded that in the pathogenesis of Leśniowski-Crohn's disease, the presence of granulomas associated with lymphatic vessels is of importance, while the presence of granulomas in the direct vicinity of blood vessels is a secondary phenomenon. On the other hand, Matson did not find such correlations between granulomas and a specific type of vessels.

Confirmation of the observations made by the above quoted authors in the presently described material would be difficult. We often found granulomas in the vicinity of vessels, but only in a single case were they in a direct contact with the adventitia. Granulomas were present in the vicinity of both lymphatic and blood vessels.

With respect to vascular changes involving the remaining intestinal wall layers, we observed small and non-significant differences between the two experimental groups. In both groups, numerous distended vessels (capillaries, veins and lymphatic vessels) were seen in the mucosa. Often they formed clusters resembling an angiomatous growth. Distended vessels were also noted in the submucosa in the controls, yet, in such cases, the vessels were isolated rather than clustered.

In our opinion, the most significant observation is associated with the presence of perivascular inflammatory infiltrations involving all the intestinal wall layers, seen not only in patients with Leśniowski-Crohn's disease, but also predominantly observed in ulcerative colitis. We may thus adopt the view that the role of vessels in ulcerative colitis is significant and – although the most intense changes occur in the mucosa and possibly submucosal membrane – the remaining layers of the intestinal walls are also involved by the disease process. The presence of leukocyte clusters within the vessels should be also emphasized. Although such vessels were not numerous, they were seen in both experimental groups and involved all the intestinal wall layers. They were generally seen outside the segments demonstrating inflammatory changes. In general, they were not accompanied by perivascular infiltrates. We may thus conclude that the phenomenon represents the earliest stage of inflammatory change development.

The participation of vessels in these processes is also indicated by immunohistochemical tests.

The majority of reports on immunohistochemical assessment of the vascular status in the two discussed diseases concentrate on adhesion molecules. The investigators focus predominantly on the studies on the presence of adhesion molecules in the serum and on white blood cells, especially on monocytes, in the circulating blood. Blood is an easily available material, and numerous authors associated observations of changes in blood concentrations of the investigated molecules with an opportunity for monitoring the course of the disease [4, 10, 20, 22]. They demonstrated that in the acute phase of the disease, the concentration of adhesion molecules increased (similarly as it happens in the case of other inflammatory markers), while the value decreased in the course of remission. The majority of such reports concentrated on ICAM-1. The prevalent opinion is that an increased serum concentration of this molecule occurs in Leśniowski-Crohn's disease [1, 14, 19, 20]. Liu et al. [6] achieved similar results when they evaluated the expression of various adhesion molecules, including ICAM-1, on peripheral blood monocytes. Jones [4], who confirmed the view on the increased serum ICAM-1 concentration levels in Leśniowski-Crohn's disease, observed also an elevated level of VCAM-1 in ulcerative colitis. Nevertheless, some authors, such as for example Nielsen et al. [10], found no differences in concentrations of these molecules in both diseases. They associated increased concentration levels with Leśniowski-Crohn's disease and ulcerative colitis alike. The opinion is shared by Niederau [9], who believed that an increase in concentration levels of serum adhesion molecules was associated with an acute stage of the disease in both entities.

As it follows from the above quoted data, to-date, opinions on this subject expressed by various authors are not entirely in accord.

Tissue expression of adhesion molecules is determined much less frequently. This subject was investigated by Bernstein et al. [1], Souza et al. [16] and Vainer et al. [19]. The investigators observed a similar increase in ICAM-1 expression in endothelial cells and in cells originating from inflammatory infiltrate both in ulcerative colitis and in Leśniowski-Crohn's disease as compared to the controls.

In contrast to the results obtained by Bernstein, Souza and Vainer, in our material, differences in ICAM-1 expression were distinctly seen in both disease entities. The expression was considerably higher in ulcerative colitis.

In some measure, the divergent results obtained by the present authors and by the above-mentioned investigators may be a consequence of the fact that Bernstein, Souza and Vainer studied considerably less numerous groups. Vainer investigated ten cases of ulcerative colitis and ten cases of Leśniowski-Crohn's disease. The studies carried out by Bernstein included 15 cases of Leśniowski-Crohn's disease and seven cases of ulcerative colitis and were performed in surgical specimens. The controls consisted of sections collected from ten non-inflammatory colons. Finally, Souza investigated ten cases of active ulcerative colitis and nine cases of Leśniowski-Crohn's disease in its active phase. The control material included intestinal sections originating from patients with the irritable colon syndrome. It should be stressed here that the entire material studied by Souza was collected endoscopically.

In addition to the fact that the results presented by the afore-mentioned authors were obtained in relatively small groups, the investigators failed to correlate associate their results with the degree of inflammatory change intensity, limiting their classification of changes to terming them "active". In our material, the intensity of inflammatory changes determined according to the scoring system developed by Sandborn was high in the majority of cases (grade III), being comparable in both groups. For this reason, we believe that the observation of differences in the expression of adhesion molecules is reliable.

In our material, statistically significant differences were also noted in the case of the second investigated molecule – VCAM-1. Its expression turned to be higher in the group with Leśniowski-Crohn's disease.

In our review of the literature on the subject, we have failed to find any information on tissue expression of VCAM-1 in ulcerative colitis and Leśniowski-Crohn's disease.

The correlation of ICAM-1 and VCAM-1 expression in each of the investigated groups – given the existing inter-group differences – is understandable. An increase of adhesion molecule expression is associated with intensification of inflammatory changes. This is most clearly seen in the mucosa and observable both in vascular expression and in expression within regions involved by inflammatory infiltrates.

The tissue expression of ICAM-1 and VCAM-1 determined in our material differs considerably from the results of studies on the serum concentration values of these molecules and their expression on monocytes that were reported by other authors. As it has been already mentioned, the majority of investigators who studied this problem noted higher ICAM-1 levels in patients with Leśniowski-Crohn's disease, while VCAM-1 demonstrated higher concentrations in individuals with ulcerative colitis. We have failed to find in any of the published reports a comparison of results of studies on adhesion molecule concentration in the serum or on their expression on circulating blood monocytes and in colonic wall cells. This is why we find it difficult to address these differences.

In our material, an increase in the expression of ICAM-1 and VCAM-1 was not always associated with an increased expression of the other investigated vascular markers. Here, distinct differences between the two experimental groups were noted. In cases of ulcerative colitis, significant correlations were observed in the vascular expression within the mucosa of such pairs of markers as VCAM-1 and UEA-1, CD34 and FVIII, as well as FVIII and UEA-1. No such correlations were detected in Leśniowski-Crohn's disease. In this case, correlations were demonstrated between the expression of ICAM-1 and CD34, as well as ICAM-1 and UEA-1, but these correlations were noted solely within inflammatory infiltrates.

Significant correlations between particular inflammatory markers in ulcerative colitis are understandable. The activation of endothelial cells is here associated with an increased number of capillaries. However, it is difficult to explain the differences in the observed correlations between the two experimental groups. Such differences should be borne in mind, since they may be helpful in differentiating between the two disease entities, especially in cases when no other signs that would clearly differentiate the two groups of patients are present, and which are termed ***"indeterminate colitis".*** Such a diagnosis is often proposed following the evaluation of materials collected in the course of endoscopy. The above-mentioned correlations – similarly as the majority of significant differences – are mostly observed in the mucosa. An attempt might thus be made at employing the determinations in question in endoscopic materials. Investigating all the markers and assessing the correlations in a limited in size endoscopic specimen is doubtlessly difficult, in addition to increasing the costs. Yet, the report by Sousa that is based solely on endoscopic materials indicates that such investigations may be carried out.

Apart from determining ICAM-1 expression, Souza et al. [16] performed numerous other reactions in their material (CD3, OX40, MAdCAM-1).

An additional comment is necessary with respect to CD34 and FVIII expression in inflammatory infiltrate cells.

CD34 is an antigen of the circulatory stem cells and endothelial cells. The expression of CD34 was also observed in the bone marrow interstitial cells and in some nervous system cells [5], as well as in cells originating from various tumors. In addition to neoplasms of a vascular origin, CD34 was reported to occur in some connective tissue tumors, interstitial tumors of the gastrointestinal tract, neuromas and neurofibromas, in *sarcoma epithelioides*, as well as in some lipomas [17, 21]. CD34 was also demonstrated in the fibroblasts and dendritic cells of normal skin [17].

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