Polski Merkuriusz Lekarski

POLISH MEDICAL JOURNAL

ISSN 1426-9686

VOLUME LIV, ISSUE 2, MARCH-APRIL 2026

EDITORIAL BOARD

Polski Merkuriusz Lekarski cited by PUBMED/MEDLINE, SCOPUS, INDEX COPERNICUS, EBSCO, POLISH MEDICAL BIBLIOGRAPHY, Ministry of Science and Higer Education.

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ORIGINAL ARTICLE

Features of macrophage marker CD68 expression in the colonic mucosa of patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis

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ABSTRACT

Aim: The aim of the study was to determine the features of macrophage marker CD68 expression in the colonic mucosa of patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis.

Materials and Methods: The study material comprised colonic mucosal biopsies obtained from 12 healthy individuals (group 1), biopsies of the colonic mucosa from the diverticular orifice region of 34 patients with symptomatic uncomplicated diverticular disease (group 2), and 26 patients with acute uncomplicated diverticulitis (group 3). Immunohistochemical study was performed using a monoclonal antibody to CD68. CD68 expression was assessed by counting the absolute number of immunopositive cells in the field of view of the microscope ×400. In immunopositive cells, the degree of expression of the monoclonal antibody to CD68 was assessed by determining the brightness coefficient.

Results: Comparative analysis of the obtained results revealed an increased number of macrophages in the colonic mucosa of patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis, with the greatest increase observed in acute uncomplicated diverticulitis. The brightness coefficient decreased in patients with symptomatic uncomplicated diverticular disease and, more markedly, in acute uncomplicated diverticulitis, indicating increased expression of the studied monoclonal antibody by macrophages. In these patients, macrophages acquired elongated and irregular shapes and increased in size.

Conclusions: The mucous membrane of the colon in patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis is characterized by a higher content of macrophages, mainly in the lamina propria, which are characterized by increased morphofunctional activity. The latter manifests itself in increased expression of the monoclonal antibody to CD68 by macrophages, an increase in the size of $CD68^+$-cells, and their acquisition of an elongated or irregular shape. The quantitative and qualitative changes in macrophages in the colonic mucosa identified by the authors are more pronounced in patients with acute uncomplicated diverticulitis compared to patients with symptomatic uncomplicated diverticular disease.

KEYWORDS: macrophage, colonic mucosa, morphology, CD68, symptomatic uncomplicated diverticular disease, acute uncomplicated diverticulitis

Pol Merkur Lek,2026;54(2):218-223 doi: 10.36740/Merkur202602121

INTRODUCTION

Macrophages are the most abundant immune cells in the wall of the large intestine [1]. It has been shown that during the antenatal and intranatal periods, as well as immediately after birth, embryonic-derived macrophages are present in the large intestinal wall. Postnatally, with increasing age, embryonic-derived macrophages are gradually replaced by monocyte-derived macrophages [2].

Macrophages in the wall of the large intestine perform complex and diverse functions. They maintain tissue homeostasis by engulfing and destroying microorganisms that have penetrated the intestinal wall and by clearing it of dead cells. Macrophages can also capture and endocytose antigens and subsequently present them to T and B lymphocytes, thereby participating in the initiation of adaptive immune responses [3]. Macrophages produce numerous cytokines, chemokines, complement components, and enzymes that induce and regulate inflammatory processes [2, 3]. Macrophages also contribute to angiogenesis in the large intestinal wall, regulate intestinal secretion, and are involved in the repair of the damaged epithelial layer [1]. Macrophages influence colonic motility through interactions with smooth muscle cells and enteric neurons [4].

Macrophages are functionally plastic cells capable of changing their phenotype in response to microenvironmental conditions [2]. Generally, macrophages are classified into classically activated, proinflammatory, M1 macrophages and alternatively activated, anti-inflammatory, M2 macrophages [5-7]. Under physiological conditions, M1 macrophages exert bacteriostatic and cytostatic functions with antigen presentation, whereas M2 macrophages are responsible for tissue regeneration and repair. Changes in

the morphofunctional state of macrophages and disruption of the balance between their phenotypes may lead to the development of inflammation in the colon. Recent studies have demonstrated the significance of inflammation in the onset, progression, and outcomes of numerous colonic diseases, which constitute a substantial health burden for both women and men [8].

Diverticular disease is a significant global burden on healthcare systems, which is characterized by the formation of solitary or multiple hernia-like protrusions of the mucosal and submucosal layers through defects in the muscular layer of the colon [9, 10].

Diverticular disease is characterized by a range of clinical manifestations from uncomplicated symptoms to acute complications requiring surgery [11]. This pathology remains asymptomatic in the majority of people. About 20-25% of those people will develop symptoms without complications, experiencing the so-called «symptomatic uncomplicated diverticular disease», while about 5% of them will develop diverticulitis (uncomplicated or complicated) [12, 13]. The mechanisms underlying the development of diverticular disease, its progression, and the occurrence of complications remain the subject of extensive research. Most authors emphasize the importance of the traumatic, ischemic, and inflammatory concepts in the pathogenesis of diverticular disease [12]. Macrophages may play a significant role in the development of inflammation in the colonic wall in diverticular disease, as they are known to regulate the functional and metabolic activity of this typical pathological process. However, the available literature on the role of macrophages in the pathogenesis of inflammation in diverticular disease remains controversial. Moreover, there is a lack of published data regarding the evaluation of macrophages within the inflammatory cellular infiltrate of the colon in patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis.

AIM

The aim of the study was to determine the features of macrophage marker CD68 expression in the colonic mucosa of patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis.

MATERIALS AND METHODS

The study material comprised colonic mucosal biopsies obtained from 12 healthy individuals (group 1), biopsies of the colonic mucosa from the diverticular orifice region of 34 patients with symptomatic uncomplicated diverticular disease (group 2), and 26 patients with acute uncomplicated diverticulitis (group 3). In patients of group 3, biopsies were obtained 8 weeks after an episode of acute diverticulitis. Patients in groups 2 and 3 were treated at the Center of Gastroenterology and Endocrinology of Feofaniya Clinical Hospital of the State Administration of Affairs during the period of 2019-2022.

The collection of material for group 1 was carried out during autopsies at the Department of Pathology of the Municipal Non-Profit Enterprise of the Kyiv Regional Council «Kyiv Regional Clinical Hospital». Autopsy findings and histological examination of the autopsy material revealed no pathology of the gastrointestinal tract.

Colonic mucosal biopsies were fixed in 10% neutral buffered formalin (pH 7.4) for 24-48 hours. After fixation, the materials were processed using a standard protocol in an Excelsior AS apparatus (Thermo Fisher Scientific, UK) and subsequently embedded in paraffin blocks using a HistoStar embedding system (Thermo Fisher Scientific, UK). Serial histological sections 2-3 μm thick were cut from the paraffin blocks on a rotary microtome (HM 325, Thermo Scientific Scientific, UK). Sections were stained with hematoxylin and eosin.

Immunohistochemical study was performed on Super Frost Plus adhesive slides (Menzel, Germany). The Master Polymer Plus Detection system (Peroxidase, chromogenic DAB) (Master Diagnostica, Spain) was used, citrate buffer (pH 6.0) and EDTA buffer (pH 8.0) were used for high-temperature processing of antigen epitopes. Immunohistochemical study was performed using a monoclonal antibody to CD68 (clone KP1, Master Diagnostica, Spain) (a marker of macrophage cells). Microscopic examinations were performed using Carl Zeiss PrimoStar microscope with a built-in color digital camera Axiocam 208 color (Carl Zeiss, Germany).

CD68 expression in the colonic mucosa was assessed by counting the absolute number of immunopositive cells in the field of view of the microscope ×400. In immunopositive cells, the degree of expression of the monoclonal antibody to CD68 was assessed by determining the brightness coefficient in the Lab color model using the computer program «Analysis of color properties of raster images» [14].

The data in groups 1-3 was statistically processed using the PAST program (version 4.15, Natural History Museum, University of Oslo, Norway). The mean values of the indicators in the groups were compared using Student's t-test and Mann-Whitney U-test. Differences in indicators were considered significant at $p<0.05$. A scatter plot was used to illustrate the results obtained. An approximation model was constructed for the obtained indicators using Microsoft Excel 2024.

RESULTS

In group 1, on survey microscopy of the specimens, a focal-diffuse moderately expressed cellular infiltration was observed in the lamina propria of the mucous membrane between connective tissue fibers, in the lamina muscularis mucosae between connective and muscle fibers. The infiltration predominated in the lamina propria of the mucous membrane and was represented by fibroblasts, lymphocytes, plasma cells, eosinophils, and $CD68^+$-macrophages. The absolute number of $CD68^+$-macrophages was 21.5±0.73. Macrophages were characterized by round and elongated shapes, small size, and light-brown cytoplasmic staining (Fig. 1). The brightness coefficient in $CD68^+$-cells was 0.62±0.012. The immune cells identified within the cellular infiltrate are known to mediate immune defense mechanisms.

In group 2, focal cellular infiltration was detected in the epithelial layer of the colonic mucosa, whereas

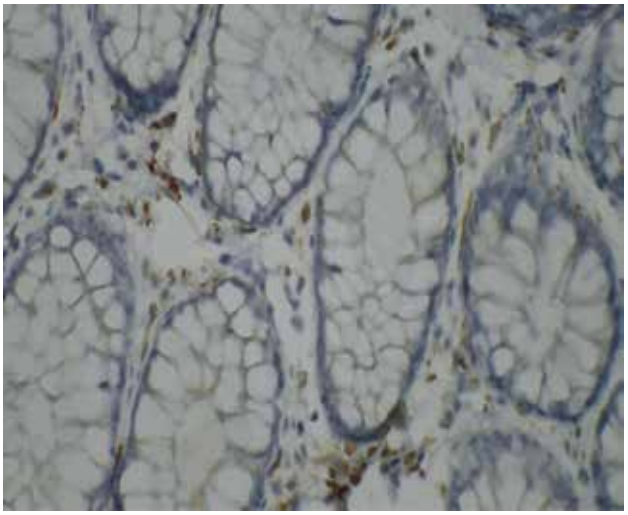

Fig. 1. Group 1. Macrophages in the lamina propria of the colonic mucosa. Immunohistochemical reaction with monoclonal antibody to CD68, × 400.
Source: Own materials

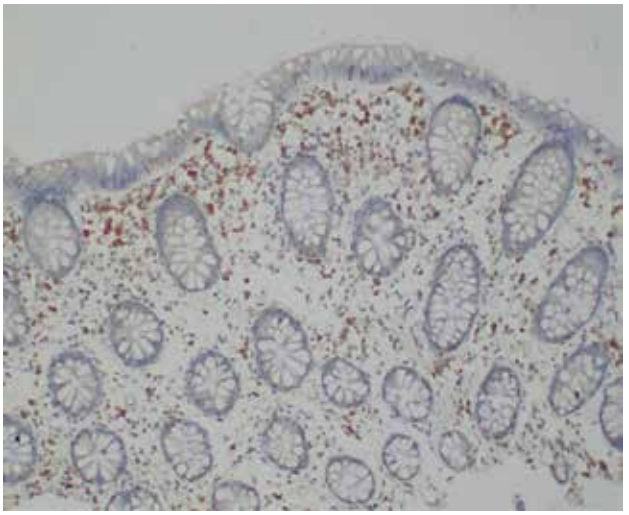

Fig. 2. Group 2. Macrophages as a part of the polymorphic cellular infiltration in the colonic mucosa. Immunohistochemical reaction with monoclonal antibody to CD68, × 400.
Source: Own materials

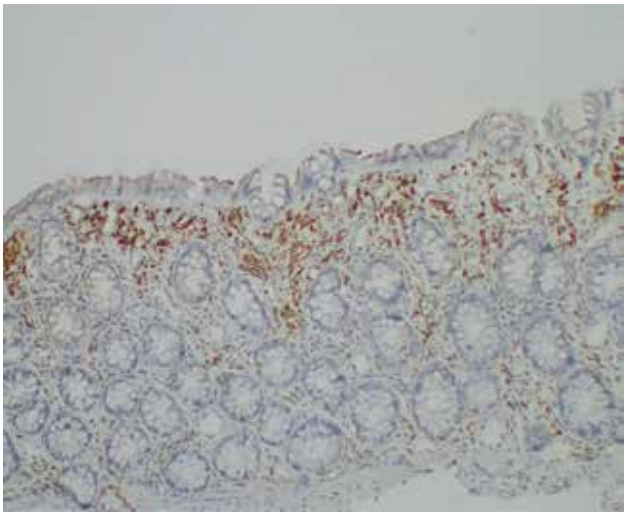

Fig. 3. Group 3. CD68+-cells in the mucous membrane of the colon. Immunohistochemical reaction with monoclonal antibody to CD68, × 400.
Source: Own materials

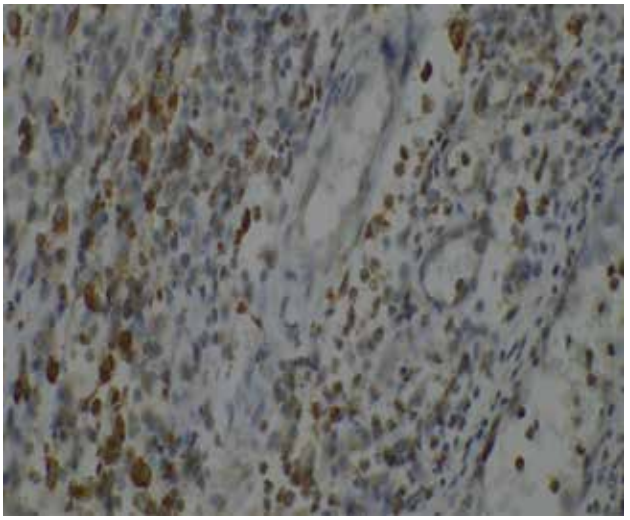

Fig. 4. Group 3. CD68+-cells in granulation tissue. Immunohistochemical reaction with monoclonal antibody to CD68, × 400.
Source: Own materials

diffuse cellular infiltration was observed in the lamina propria of the mucous membrane and lamina muscularis mucosae. The degree of cellular infiltration was maximal in the lamina propria compared with other layers of the mucosa. Compared with group 1, the cellular infiltration was more pronounced and consisted of similar cell types. In this group, macrophages expressing the monoclonal antibody to CD68 were larger than in group 1 and exhibited round, elongated, or irregular shapes with dark-brown cytoplasmic staining (Fig. 2). Compared with group 1, the absolute number of macrophages (48.9±1.17) increased ($p<0.05$), whereas the brightness coefficient in $CD68^+$-cells (0.53±0.010) decreased ($p<0.05$).

In group 3, polymorphic cellular infiltration of the colonic mucosa was observed in the epithelial layer, lamina propria of the mucous membrane and lamina muscularis mucosae. The infiltration was focal in the epithelium, diffuse in the lamina propria and muscularis mucosae, with the greatest severity in the lamina propria. The cellular infiltrate consisted of neutrophils, eosinophils, plasma cells, lymphocytes, fibroblasts, and macrophages. The degree of cellular infiltration was markedly greater than in groups 1 and 2.

In three cases of this group, granulation tissue of varying maturity was observed in some visual fields, filling erosive-ulcerative defects and characterized by different proportions of fibrous, vascular, and cellular components. The latter consisted of neutrophils, eosinophils, plasma cells, lymphocytes, fibroblasts and macrophages. Macrophages localized in the layers of the mucous membrane (Fig. 3) and in the granulation tissue (Fig. 4) were enlarged,

exhibited elongated and irregular shapes, and expressed the monoclonal antibody to CD68, as evidenced by intense dark-brown cytoplasmic staining.

The absolute number of macrophages was increased ($p<0.05$) compared to groups 1 and 2 and amounted to (68.4±1.33). The brightness coefficient in $CD68^+$-cells was (0.45±0.010), which indicated its decrease ($p<0.05$) compared to groups 1 and 2.

DISCUSSION

The study conducted by the authors revealed the peculiarities of the expression of the macrophage cell marker – monoclonal antibody to CD68 in the mucous membrane of the colon of patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis during examination microscopy, by counting the absolute number of $CD68^+$-cells and determining their brightness coefficient.

Comparative analysis of the obtained results revealed an increased number of macrophages in the colonic mucosa of patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis, with the greatest increase observed in acute uncomplicated diverticulitis. The brightness coefficient decreased in patients with symptomatic uncomplicated diverticular disease and, more markedly, in acute uncomplicated diverticulitis, indicating increased expression of the studied monoclonal antibody by macrophages. In these patients, macrophages acquired elongated and irregular shapes and increased in size. The observed decrease in the brightness coefficient of $CD68^+$-cells, together with changes in cell shape and enlargement, indicates activation of the morphofunctional state of macrophages in diverticular disease.

Subsequently, the results obtained in the groups allowed us to construct a scatter plot with a trend line, which is shown in Figure 5. A linear approximation model was constructed for the results in groups 1-3.

The trend line (regression) equation, as shown in Figure 5, was as follows: $y = -0.0037 \times x + 0.7015$, where «y» is the brightness coefficient and «x» is the absolute number of macrophages. As shown in the figure, with increasing absolute macrophage number, the brightness coefficient decreased; the coefficient of determination (R^2) was 0.9962. The coefficient of determination ($0 \leq R^2 \leq 1$), as is well known, characterizes the degree of agreement between experimental data and data obtained from the approximation model. The coefficient of determination equals the proportion of observed outcomes explained by the model equation. In our study, this corresponded to 99.62% of all observations.

The increase in the number of macrophages and the activation of their morphofunctional state in the mucous membrane of the colon in patients with symptomatic uncomplicated diverticular disease and, especially, acute uncomplicated diverticulitis, in our opinion, has not only physiological but also pathological significance. Macrophages in the colon wall in diverticular disease not only perform a protective function, contributing to the restoration of damaged structures, but also induce inflammation, causing the onset and progression of sclerosis. Sclerotic changes in the colon wall lead to degenerative changes and atrophy of muscle fibers in the lamina propria and muscularis. The qualitative and quantitative changes detected in the connective tissue cause the development of colonic diverticula [15, 16]. Sclerosis of the vascular walls also occurs, leading to impaired trophic support of all layers of the colonic wall, which may underlie the development of diverticular disease [17].

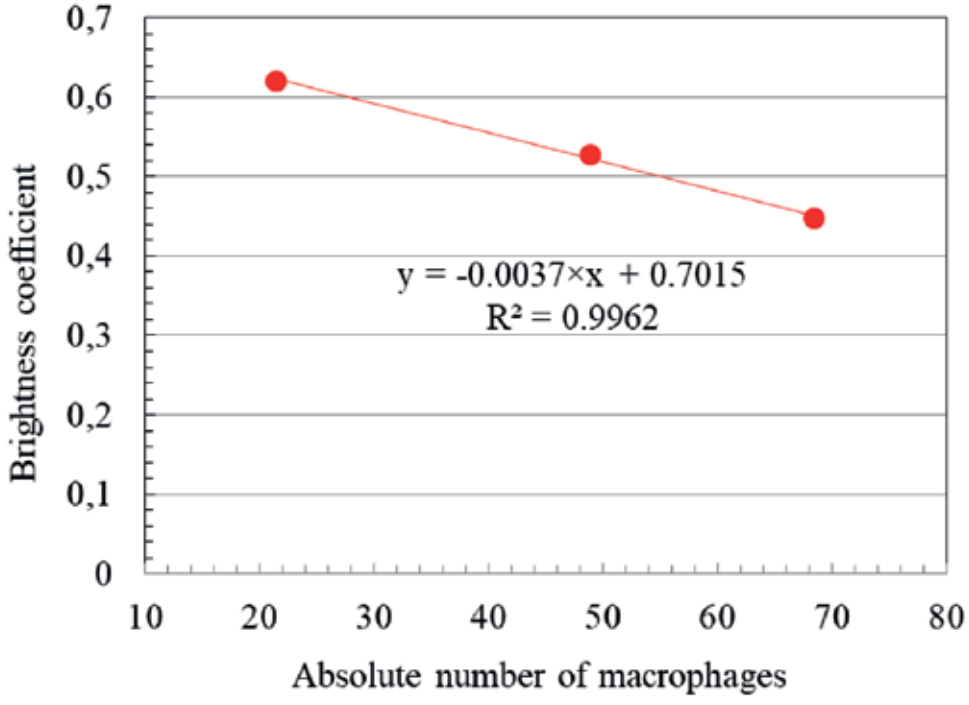

Fig. 5. Graph showing the dispersion of the brightness coefficient and the absolute number of macrophages with a trend line.

Source: Own materials

Macrophage dysfunction and imbalance between their phenotypes cause colonic motility disorders, increased intraintestinal pressure, muscle fiber divergence, and diverticulum formation [4]. Numerous studies have shown that impaired colon motility is the basis for the formation of diverticula [18, 19].

CONCLUSIONS

The mucous membrane of the colon in patients with symptomatic uncomplicated diverticular disease and acute uncomplicated diverticulitis is characterized by a higher content of macrophages, mainly in the lamina propria, which are characterized by increased morphofunctional activity. The latter manifests itself in increased expression of the monoclonal antibody to CD68 by macrophages, an increase in the size of $CD68^+$-cells, and their acquisition of an elongated or irregular shape. The quantitative and qualitative changes in macrophages in the colonic mucosa identified by the authors are more pronounced in patients with acute uncomplicated diverticulitis compared to patients with symptomatic uncomplicated diverticular disease.

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CONFLICT OF INTERESTS
The Authors declare no conflict of interest

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RECEIVED: 03.01.2026
ACCEPTED: 10.03.2026

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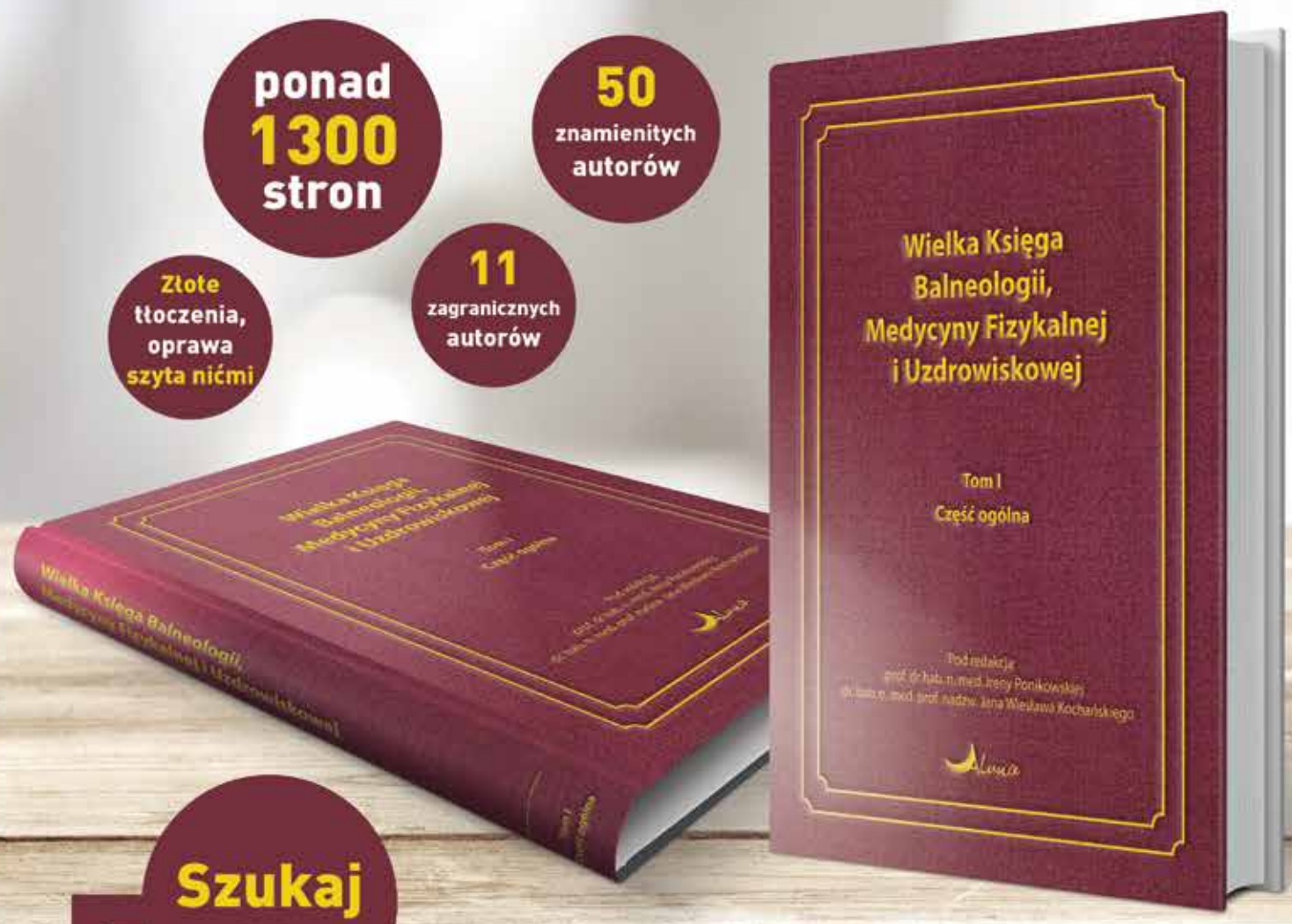

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