Systemic Inflammatory Markers among Ulcerative Colitis Patients

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Abstract

Background: Ulcerative colitis is an idiopathic inflammatory condition of the colon which results in diffuse friability and superficial erosions on the colonic wall associated with bleeding. It is the most common form of inflammatory bowel disease worldwide. It characteristically involves inflammation restricted to the mucosa and submucosa of the colon. Typically, the disease starts in the rectum and extends proximally in a continuous manner. Inflammation is a complex dynamic protective response to cell injury, infection via microbes, trauma, or toxins in the vascularized tissues. The causative agent is diluted, destroyed, or isolated and a sequential cascade of molecular events is set that leads to repairing, healing, and reconstituting the damaged tissue. It is thoroughly characterized by the reaction in tissues and its microcirculation as clinically reflected by redness (erythema), heat (hyperemia), swelling (exudation), pain (through nerves and chemical mediators), and loss of function. The combined vascular and cellular inflammatory responses are triggered by inflammatory stimulus and mediated through chemical factors derived from some cells or blood plasma. Even the injured or dead tissues release mediators.

Keywords: Systemic Inflammatory Markers

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Introduction

Ulcerative colitis is an idiopathic inflammatory condition of the colon which results in diffuse friability and superficial erosions on the colonic wall associated with bleeding. It is the most common form of inflammatory bowel disease worldwide. It characteristically involves inflammation restricted to the mucosa and submucosa of the colon. Typically, the disease starts in the rectum and extends proximally in a continuous manner **(1).**

Epidemiology

In the United States, the disease accounts for a quarter-million provider visits annually, and medical costs directly related to the disease are estimated to exceed four billion dollars annually. Prevalence of UC raised in Egypt in the last years. it was the 4th pathologic diagnosis after internal hemorrhoids, non-specific colitis and cancer colon constituting about 11.5%. Ulcerative has no cure and is a lifelong disorder with a significant impact on both physical and mental health **(2).**

Worldwide, the highest incidence and prevalence of inflammatory bowel diseases are seen in Northern Europe and North America. Inflammatory bowel disease is closely linked to a westernized environment and lifestyle. Ulcerative colitis has an incidence of 9 to 20 cases per 100,000 persons per year. Its prevalence is 156 to 291 cases per 100,000 persons per year. Compared to Crohn disease, ulcerative colitis has a greater prevalence in adults. When considering the pediatric population; however, ulcerative colitis is less prevalent than Crohn disease**.** Ulcerative colitis has a bimodal pattern of incidence. The main onset peaks between the age of 15 and 30 years. A second, and the smaller peak of incidence occurs between the age of 50 and 70 years. Though some studies show a slight predilection for men, most studies note no preference regarding sex **(3).**

Etiology and risk factors

The specific cause of inflammatory bowel disease is not known. There seems to be a primary genetic component since the most important independent risk factor is a family history of the disease (8% to 14% of patients). A first-degree relative of a patient with ulcerative colitis has a four times higher risk of developing the disease. Additionally, ulcerative colitis has a higher incidence in Jewish populations than other ethnicities. Many genome studies have identified approximately 200 risk loci for inflammatory bowel disease. Examples of loci associated with increased ulcerative colitis susceptibility include human leukocyte antigen and genes associated with barrier function, such as *HNF4A* and *CDH1*. However, genetics only explain 7.5% of disease variance, have little predictive capacity for phenotype, and currently are of limited clinical use **(3).**

Inflammation is a complex dynamic protective response to cell injury, infection *via* microbes, trauma, or toxins in the vascularized tissues. The causative agent is diluted, destroyed, or isolated and a sequential cascade of molecular events is set that leads to repairing, healing, and reconstituting the damaged tissue. It is thoroughly characterized by the reaction in tissues and its microcirculation as clinically reflected by redness (erythema), heat (hyperemia), swelling (exudation), pain (through nerves and chemical mediators), and loss of function**.** The combined vascular and cellular inflammatory responses are triggered by inflammatory stimulus and mediated through chemical factors derived from some cells or blood plasma. Even the injured or dead tissues release mediators **(2).**

C-reactive protein

C-reactive protein (CRP) was discovered by Tillett and Francis in 1930. The name CRP arose because it was first identified as a substance in the serum of patients with acute inflammation that reacted with the "c" carbohydrate antigen of the capsule of pneumococcus**.** CRP is a pentameric protein synthesized by the liver, whose level rises in response to inflammation. CRP is an acute-phase reactant protein that is primarily induced by the IL-6 action on the gene responsible for the transcription of CRP during the acute phase of an inflammatory/infectious process. It has been demonstrated to have some protective properties in animal studies on lung tissue in alveolitis by reducing neutrophil-mediated damage to the alveoli and protein leakage into the lung **(4).**

CRP has both proinflammatory and anti-inflammatory properties. It plays a role in the recognition and clearance of foreign pathogens and damaged cells by binding to phosphocholine, phospholipids, histone, chromatin, and fibronectin. It can activate the classic complement pathway and also activate phagocytic cells via Fc receptors to expedite the removal of cellular debris and damaged or apoptotic cells and foreign pathogens. As compared to the erythrocyte sedimentation rate, which is an indirect test for inflammation, the levels of CRP rise and fall rapidly with the onset and removal of the inflammatory stimulus, respectively. Persistently elevated CRP levels can be seen in chronic inflammatory conditions such as chronic infections or inflammatory arthritis such as rheumatoid arthritis **(4).**

There are numerous causes of an elevated C-reactive protein. These include acute and chronic conditions, and these can be infectious or non-infectious in etiology. However, markedly elevated levels of CRP are most often associated with an infectious cause (an example of pathogen-associated molecular pattern recognition). Trauma can also cause elevations in CRP (alarmin response). More modest elevations tend to be associated with a broader spectrum of etiologies, ranging from sleep disturbances to periodontal disease. Immunoassays and laser nephelometry are the methods to quantify CRP levels and are cheap, accurate, and fast. This is performed when the physician suspects acute or chronic inflammation (e.g., SLE or rheumatoid

arthritis [RA]) or infection To detect lower levels of CRP (0.3 to 1.0 mg/L), high-sensitivity CRP methods are recommended as the usual CRP detection tests are less precise. High-sensitivity CRP only denotes the assay process used, allowing for detection of lower levels of CRP and not a different, or more specific, differential diagnosis **(3).**

Interpretation of CRP levels:

Less than 0.3 mg/dL: Normal (level seen in most healthy adults).

0.3 to 1.0 mg/dL: Normal or minor elevation (can be seen in obesity, pregnancy, depression, diabetes, common cold, gingivitis, periodontitis, sedentary lifestyle, cigarette smoking, and genetic polymorphisms).

1.0 to 10.0 mg/dL: Moderate elevation (Systemic inflammation such as RA, SLE, or other autoimmune diseases, malignancies, myocardial infarction, pancreatitis, bronchitis).

More than 10.0 mg/dL: Marked elevation (Acute bacterial infections, viral infections, systemic vasculitis, major trauma).

More than 50.0 mg/dL: Severe elevation (Acute bacterial infections)

Interfering Factors

Certain medications, such as non-steroidal anti-inflammatory drugs (NSAIDs), will falsely decrease CRP levels. Statins, as well, have been known to reduce CRP levels falsely. Recent injury or illness can falsely elevate levels, particularly when using this test for cardiac risk stratification. Magnesium supplementation also can decrease CRP levels **(4).**

Limitations:

Given the highly variable causality of elevated CRP, marginal elevations in the CRP can be difficult to interpret and should not be used as an isolated test result interpreted as appropriate for the clinical picture. It is useful in suggesting infection versus inflammation if the levels are extremely high, but levels between 1 mg/dL and 10 mg/dL can be difficult to interpret accurately. Chronic conditions, such as inflammatory arthritis or SLE, can make these levels elevated chronically, making it harder to determine if there is any significance to an elevated hs-CRP level when using it as a predictive marker for cardiovascular disease **(5).**

CRP and ulcerative colitis

A systematic review and meta-analysis was reported that the probability of having IBD in the normal range of CRP levels was less than 1% **(5).**

Evaluation of Disease Activity by CRP

CRP is more sensitive in CD compared to that in UC when assessing disease activity. In CD, the presence of active lesions is strongly suspected when CRP is positive due to its high specificity. Moreover, **Denis et al. (6)** reported that 92.9% of patients with CD with clinical symptoms had laboratory data that showed normal CRP, but the majority of their revealed lesions had mild inflammation. In this regard, they described that it is possible to rule out severe endoscopic lesions in patients with a clinically active CD if CRP is negative **(6).**

However, It was reported that a poor association between disease phenotype and CRP in patients with CD. It was reported that CRP had a sensitivity range of 50.5–53.3% and a specificity range of 85.1–87.2%, and ESR had a sensitivity range of 68.7–71.3% and a specificity range of 63.4–66.4% for the detection of endoscopic remission using some endoscopic indices. Endoscopic activity was better correlated with CRP than with ESR in patients with UC.

A review by **Mosli et al. (7)** reported the ability to detect the endoscopic activity of IBD by CRP value, with a sensitivity of 0.49 (95% confidence interval [CI] 0.34–0.64) and a specificity of 0.92 (95% CI 0.72–0.96). Therefore, low CRP levels do not necessarily reflect that there is no endoscopic activity. **Ishida et al. (3)** evaluated the association between endoscopic scores of colonic inflammations and FCP, FIT, and CRP in patients with UC. FCP and CRP tended to correlate more strongly with the sum of Mayo Endoscopic Subscore (S-MES) and Ulcerative Colitis Colonoscopic Index of Severity (UCCIS) than with maximum Mayo Endoscopic Subscore (M-MES) and Ulcerative Colitis Endoscopic Index of Severity (UCEIS). In the M-MES ≤ 1, FC and FIT showed a strong correlation with S-MES and UCCIS compared to CRP. On the other hand, in the M-MES ≥ 2, only CRP was significantly correlated with S-MES and UCCIS

Prediction of Mucosal Healing by CRP

CRP has not been able to provide sufficient accuracy to replace endoscopy as an independent biomarker for MH. **Krzystek-Korpacka et al. (8)** investigated the role of CRP in the detection of MH in a review of 30 studies. CRP ranged from 0.4 to 28 mg/L, with large variations in the optimal cutoff value selected. The median sensitivity of CRP performance as an MH marker was 79.5% and the median specificity was 61% for CD, and the sensitivity was superior to the specificity. Regarding UC, the median sensitivity was 66% and the median specificity was 82%, and the specificity was superior to the sensitivity.

Prediction of Therapeutic Effect by CRP

It was reported that in 226 patients with CD, anti-TNF-α agents were more effective in those with higher pretreatment CRP. **Reinisch et al. (9)** valuated CRP levels at baseline and 14 weeks after infliximab (IFX) induction as predictors for maintained response or remission. CRP normalization 14 weeks after the induction of IFX increased the likelihood of maintained response or remission for 1 year **(9).**

Magro et al. (10) reported that CRP levels 14 weeks after IFX induction in patients with CD were associated with a sustained response, independent of baseline CRP serum levels. However, unlike previous reports, high baseline CRP values correlated with worse responses. The contradictory results are thought to be due to differences in CRP cutoff values and low albumin levels. A retrospective study of 1189 patients with CD by Tanaka et al. also found that high baseline CRP was associated with inadequate retention of adalimumab (ADA) treatment over a 4-year follow-up period **(10).**

Reinisch et al. (9) evaluated the remission rate of patients with moderate to severe active UC treated with ADA. In this multicenter, randomized, double-blind, placebo-controlled trial, a high baseline of high-sensitivity CRP (hsCRP) was associated with a reduced remission rate. In a study of 72 patients with UC by Iwasa et al., improvement of clinical symptoms and reduction in CRP 2 weeks after IFX induction therapy were associated with subsequent prognosis**.** Oxford criteria state that the risk of in-hospital colectomy is 85% if CRP exceeds 45 mg/L or if there are more than eight bowel movements in 24 h on the third day of intravenous corticosteroids **(10).**

Prediction of Recurrence by CRP

Regarding disease monitoring, there are several reports that CRP predicts clinical recurrence with CD. **Consigny et al. (11)** measured CRP every 6 weeks in 71 patients with CD and reported that CRP predicted recurrence. It was reported that higher CRP was a predictor of relapse by measuring CRP every 3 months in patients with CD. **Roblin et al. (12)** conducted a prospective observational cohort study enrolling patients with IBD in clinical remission 14 weeks after the introduction of IFX therapy. It was reported that CRP > 5 mg/L 22 weeks after the introduction of IFX therapy predicted loss of response in patients with CD **(12).**

Conversely, contradictory data have been published regarding the correlation between CRP and postoperative recurrence in patients with CD. A study of 24 postoperative patients with CD showed no consistent association between endoscopic scores and CRP 54 weeks after surgery. Another study indicated a weak but statistically significant difference in hsCRP between patients with postoperative recurrence and those with endoscopic remission within 18 months (median 7 months) of resection **(11).**

Hypoalbuminemia and ulcerative colitis

Albumin functions as a negative acute phase reactant; levels drop during inflammation (due to both decreased synthesis and increased catabolism). Levels also decrease in response to conditions such as malnutrition and malabsorption, so that in UC it might reflect these downstream effects of inadequately controlled disease. Several studies have demonstrated that low levels of albumin, especially in patients presenting with acute severe colitis, are associated with refractoriness to corticosteroid therapy and with the need for surgery **(8).**

Ho et al. (13) proposed the use of a risk score incorporating hypoalbuminemia to identify patients who should have more aggressive approaches including earlier addition of second-line medical therapy or surgery. More recently, studies showed greater risk of needing any steroids, multiple courses of steroids, or second-line medical therapies in patients with hypoalbuminemia at diagnosis, as well as a trend toward increased need for colectomy. While albumin may be effective as a component of a more comprehensive risk score, it is unlikely to be useful as an independent measure indicating need for colectomy **(13).**

CRP/Albumin Ratio

Knowing that both CRP and albumin levels are associated with need for colectomy in patients with UC, **Gibson, et al. (14)** proposed that the combination might have additional predictive capacity. They found that an elevated CRP/albumin ratio on day 3 of IV corticosteroids was a more accurate marker of risk for colectomy within 30 days than was day 3 CRP or albumin alone. They also concluded that a CRP/albumin ratio greater than 0.85 optimally predicted need for colectomy within three years (50% of patients with day 3 CRP/albumin ratio above 0.85 required colectomy vs. 20% with a lower day 3 CRP/albumin ratio) **(14).**

Similarly, **Choy, et al. (15)** sought to identify factors that might predict treatment failure and need for colectomy in a recent study. While they were unable to confirm the early predictive value of CRP/albumin ratio, their analysis did show that a CRP/albumin ratio >0.37 at the time of discharge after treatment with infliximab (regardless of whether accelerated or standard induction was used) was significantly predictive of 12-month colectomy rates **(15).**

Erythrocyte sedimentation rate

The erythrocyte sedimentation rate (ESR) is a common hematology test that may indicate and monitor an increase in inflammatory activity within the body caused by one or more conditions such as autoimmune disease, infections or tumors. The ESR is not specific for any one disease but is used in combination with other tests to determine the presence of increased inflammatory activity. The ESR has long been used as a "sickness indicator" due to its reproducibility and low cost. Over many decades, several methods have evolved to perform the test. However, the reference method for measuring the ESR proposed by the International Committee for Standardization in Hematology (ICSH) is based on the findings described by Westergren a century ago. Newer automated systems using closed blood collection tubes and automatic readers have been introduced into laboratories to decrease the biohazardous risk to operators and to decrease the time that it takes to perform the ESR **(12).**

The Westergren method measures the distance (in millimeters) at which red blood cells in anticoagulated whole blood fall to the bottom of a standardized, upright, elongated tube over one hour due to the influence of gravity. The tube used for the test is called the Westergren tube. Today, these tubes are made of either glass or plastic, with an internal diameter of 2.5 mm and lengths of 190 to 300 mm long. Perhaps the first to notice a change in the sedimentation of blood due to illness was a British surgeon John Hunter. A Polish physician, Edmund Faustyn Biernacki, later refined the clinical use of the ESR near the end of the 19 century. Biernacki detailed his findings and he developed his test for measurements. Because of his work, the ESR is occasionally referred to as the Biernacki Reaction world-wide. The applied use of ESR in clinical diagnostics by Biernacki was furthered refined by Dr. Robert Fahraeus in 1918 and by Dr. Alf Vilhelm Albertsson Westergren in 1921. Dr. Westergren defined the standard measurement of the ESR that is still in use today. Together, Robert Fahraeus and Alf Vilhelm Albertsson Westergren are often remembered for the test, historically called the Fahraeus-Westergren test (FW test or Westergren test), which uses a standardized tube and sodium citrate anticoagulated blood **(12).**

Pathophysiology

The ESR test measures the rate at which the red blood cells (RBCs), or erythrocytes, in a sample of whole blood, fall to the bottom of the Westergren tube. This process of "falling" is called sedimentation. RBCs typically fall at a faster rate in people with inflammatory conditions such as infections, cancer, or autoimmune conditions. These conditions lead to an increase in the number of proteins in the blood. This increase causes red blood cells to stick together (clump) and settle at a faster rate. A group of RBCs that are clumped together will form a stack (similar to a stack of coins) called a rouleau. Rouleaux formation is possible because of the particular discoid shape of RBCs. The flat surfaces of the RBCs allow them to make contact with other RBCs and stick together **(16).**

Normally, RBCs have negative charges on the outside of the cells, which cause them to repel each other. Many plasma proteins have positive charges and can effectively neutralize the negative surface charges of the RBCs, which allows for the formation of the rouleaux. Therefore, an increase in plasma proteins (present in inflammatory conditions) will propagate an increase in rouleaux formations, which settle more readily than single red blood cells The settling of the rouleaux aggregates in the Westergren tube occurs at a constant rate.

The formation of rouleaux allows the RBCs to settle at a faster rate, thus increasing the ESR. Therefore, the ESR is not the measure of a single marker but a physical process **(4).**

Rouleaux formation (and thus the ESR) is affected by the amounts of immunoglobulins and acute phase proteins (prothrombin, plasminogen, fibrinogen, C-reactive protein, alpha-1 antitrypsin, haptoglobin, complement proteins) that are present in several inflammatory conditions. "Acute-phase proteins" (APP) is the name given to a class of approximately 30 distinct, chemically unrelated plasma proteins that are innately regulated in response to infection and inflammation. APP's are produced by the liver and are functionally controlled by the body in response to several forms of tissue damage or insult. These proteins act as inhibitors or mediators of the inflammatory response **(4).**

Although the Westergren method is commonly used for determining the ESR, it is time-consuming, and there is room for error. In an attempt to find faster and more reliable means of obtaining the ESR, newer methods have evolved. Some methods utilize a centrifuge and automated machines and can produce results in as quickly as 5 minutes. The micro ESR is a method of obtaining the ESR using capillary tubes and quicker testing times. This method uses 4 drops of capillary blood drawn from a finger poke that is then mixed in a 4:1 ratio on a slide with a 3.8 percent sodium citrate solution. The sample is then drawn into a 7.5-centimeter heparin-free microhematocrit capillary tube. The results are measured at just 20 minutes and then adjusted to predict conventional ESR values from the micro ESR value**.** Several new automated and semi-automated techniques have become available for determining the ESR that are safer and faster with a higher level of accuracy **(16).**

Clinical Significance

Several factors may influence the ESR. Females tend to have slightly increased erythrocyte sedimentation rates compared to males. Pregnancy and aging may also increase the ESR. Anemia, RBC abnormalities, technical factors such as tilted ESR tubes, increased temperature of the specimen, and dilution errors may increase the ESR. The ESR is neither sensitive nor specific as a general screening test. Because an elevated ESR may occur in multiple clinical settings, it is meaningless as a stand-alone laboratory value. Furthermore, some patients who have malignant lesions, serious infections, or significant inflammatory disorders may have normal ESR values. An ESR level that is elevated should heighten the practitioner's index of suspicion of the potential for underlying illness **(16).**

Any process that elevates fibrinogen (e.g., pregnancy, infection, diabetes mellitus, end-stage renal failure, heart disease, malignancy) may also elevate the ESR. An extremely high ESR value (>100 mm/hr) may indicate the presence of infection, multiple myeloma, Waldenstrom macroglobulinemia, temporal arteritis, polymyalgia rheumatica, or hypersensitivity vasculitis. One study reported that the average ESR was over 90 mm per hour in patients who had temporal arteritis, with values over 30 mm per hour in 99% of the patients. The extremely high elevation of the ESR (>100 mm per hour) is associated with a low false-positive rate for a significant underlying illness. Infection is likely the cause of an extreme elevation, followed by collagen vascular disease and metastatic tumors. In oncology, a high ESR tends to correlate with a poor prognosis for various types of cancers**.** An elevated ESR may be an important adjunct in detecting coronary artery disease. This is possibly linked to the inflammatory condition of coronary disease. There is a relationship between the ESR in ischemic stroke and the amount of local brain injury, atherosclerosis, and short term outcomes **(16).**

Regular alcohol use is negatively associated with ESR. Alcohol drinkers of low, moderate, and high quantities of alcohol will show a lower ESR compared to abstainers and occasional drinkers. Moderate and high regular physical exercise were associated with lower-than-expected erythrocyte sedimentation rates. The test must be performed using blood that was drawn within two hours of testing. In standing blood, erythrocytes tend to become spherical, which impedes rouleaux formation. Anisocytosis and poikilocytosis also interfere with the stacking of erythrocytes, thus decreasing the ESR**.** Certain medications (valproic acid, statins, non-steroidal anti-inflammatory drugs) may lower the ESR value **(12).**

Erythrocyte Sedimentation Rate and ulcerative colitis

Despite the widespread use of ESR and CRP alone and together in clinical practice, their utility has seldom been compared, nor has the value of assessing both parameters been examined. Previous studies have reported

only on the independent prevalence of normal and elevated ESR or CRP values in children with varying UC activity. Other studies have assessed blood markers to differentiate disease activity in UC. The combination of serum orosomucoid and ESR was found to be complementary in assessing disease activity in IBD children, while CRP was of no additional value. ESR correlated with physicians' global assessment of disease activity in 77 UC and CD patients. ESR, CRP, albumin, WBC, and platelets were significantly different between endoscopically quiescent or active UC, but CRP had the best combination of sensitivity (74%) and specificity (70%). CRP was correlated with endoscopic score in 21 UC patients, although less closely than did serum albumin and fecal alpha-1-antitrypsin. In contrast, Gomes et al. found no correlation between colonoscopic appearance and any laboratory values, including CRP and ESR **(17).**

Furthermore, CRP (at a cutoff of 5 mg/L) and ESR (15 mm/hr) had poor sensitivity (69% for both) and specificity (62% and 65%, respectively) to differentiate UC patients in remission from those with active disease judged by the Mayo score **(.** CRP and ESR were found to be good predictors of response to therapy in severe UC in some, but not in all studies. CRP and ESR have been studied long enough to become established in IBD diagnosis. While both tests lack the specificity and accuracy to be considered a gold-standard diagnosis, CRP has some advantages over ESR. For example, the CRP concentration changes faster than the ESR value upon a change in disease activity, CRP has a broader range of abnormal values than ESR, and (unlike ESR) CRP does not show age-related variation It was reported that "fair" correlation of ESR and CRP with colitis in 78 children with UC **(17).**

Blood Platelets and Inflammatory Response

The involvement of blood platelets in an inflammatory response is associated with the release of cytokines and chemokines that attract leukocytes and facilitate adhesion to endothelium at the site of damage. During the inflammatory process, blood platelets may interact with leukocytes by forming platelet-leukocyte aggregates. These bindings are possible through adhesion proteins expressed on the cell surface during activation. Moreover, platelets support leukocytes to combat bacterial infections via direct contact, encapsulation of bacteria, and release of reactive oxygen species and platelet microbicidal proteins (PMP). Platelet growth factors, such as TGF-beta, PDGF, or VEGF, are also engaged in wound healing **(4).**

Recent research has shown the involvement of blood platelets in the development of neoplastic disease. It is suggested that interactions of cancer cells with thrombocytes allow their migration from the primary tumor and formation of metastases. Encapsulation of transformed cells by blood platelets protects them from recognition by the host immune system and enables their binding with adhesion proteins on endothelial surface. The involvement of blood platelets in these processes is associated with changes in their count and morphology. During coagulation, the count may decrease due to platelet wear, whereas the activation of megakaryocytes by proinflammatory cytokines may lead to a considerable increase in the production and release of thrombocytes. In some diseases, specific alterations are noted in platelet parameters, which can be thus used as diagnostic markers of these conditions **(4).**

Platelet Morphological Parameters

Basic platelet parameters are assessed during a routine blood morphology test providing valuable information on blood platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT). Modern hematological analyzers enable the assessment of the percentage of large platelets with MPV > 15 fl (P-LCR), the number of giant platelets with MPV > 20 fl (LP), the number of reticulated platelets (PLRET), microplatelets (PDMP), and the mean platelet component (MPC). The latest research has shown that the platelet parameters can contribute to the diagnosis of patient's general condition and have a prognostic value in some diseases. Although the routine assessment of the platelet parameters has been available for many years now, their clinical significance has not been fully elucidated, and their diagnostic usage has been limited **(12).**

The Mean Platelet Volume (MPV)

The mean platelet volume (MPV) is a precise measurement of their dimension, calculated by hematological analyzers on the basis of volume distribution during routine blood morphology test. MPV ranges between 7.5 and 12.0 fl, whereas the percentage of large platelets should amount to 0.2-5.0% of the whole platelet population. In physiological conditions, MPV is inversely proportional to the platelet count, which is

associated with hemostasis maintenance and preservation of constant platelet mass. This means that the increased production of platelets is accompanied by a reduction in their mean volume. In various diseases, this physiological proportion is disturbed. Markedly enhanced or abnormal thrombocytopoiesis, increased wear, or the effect of activating factors on blood platelets may lead to changes in the proportions between MPV and PLT **(4).**

Therefore, possible application of these parameters to the diagnosis of certain diseases has been suggested. Moreover, MPV correlates with platelet activity and is thus considered a marker of platelet activity. Blood platelets are not a homogenous population. Those with increased MPV (>15 fl) are often younger and characterized by higher reactivity than those with normal MPV. Their generation is associated with marked activation of megakaryocytes by cytokines, which increases the ploidy of these cells and enhances the release of larger platelets **(18).**

It is also suggested that large thrombocytes show a greater content of cell granules, display higher expression of adhesion molecules, and undergo faster activation, which results in platelet hyperactivity and increased risk of clot formation. Elevated MPV correlates with increased platelet aggregation, enhanced synthesis, and release of thromboxane TXA2 and *β*-thromboglobulin **(4).**

MPV in Inflammation

In healthy individuals, the increased platelet count, via feedback, leads to considerable inhibition of thrompopiotein synthesis by the liver and in consequence causes platelet release by megakaryocytes, which is to maintain constant platelet mass. However, in patients with ongoing inflammation, the increasing concentration of proinflammatory cytokines, mainly IL-6, can lead to platelet release. This is associated with the stimulation of thrombopoietin generation by IL-6 and with a direct effect of this cytokine on megakaryocytes. IL-6 causes an increase in the ploidy of megakaryocytic nuclei and an increase in cytoplasm volume, which in consequence leads to the production of a large number of blood platelets **(18).**

The course of an inflammatory condition is also associated with increased percentage of large platelets, probably due to intracellular synthesis of procoagulatory and proinflammatory factors, degranulation of granules, and initiation of the platelet pool stored in the spleen. Simultaneously, these cells rapidly migrate to the site of inflammation where they undergo activation and wear. This seems to explain the drop in MPV in patients with ongoing inflammation **(18).**

Platelet count and mean platelets volume in ulcerative colitis

The reason of reduced MPV in IBD is unclear. Some authors speculated that the reduced MPV could be due to the consumption or sequestration of the large activated platelets in the intestinal vasculature. Other mechanism for decreased MPV may be presence of a defect in the regulation of thrombopoiesis in IBD. Studies show increased levels of TNF-, IFN-g, IL-1, and IL-6 in UC and Crohn's disease. Some authors put forward that IL-6 among these mediators is primarily responsible cytokine in secondary thrombocytosis **(19).** IL-6 induces many biological effects, such as the stimulation of blood platelets activation. The activation of blood platelets causes the appearance of P-selectin on their surface which, as a receptor protein, contributes to the pathogenesis of inflammation and thrombosis**.** A part of P-selectin peels from the surface of blood platelets and occurs in plasma in a soluble form. It was found that the concentration of sP-selectin in subjects with ulcerative colitis was higher than in the control group, which was statistically significant ($p < 0.01$). Such increases in sP-selectin concentrations prove the activation of blood platelets and their part in the inflammatory process. Studies showed a positive correlations between concentrations of IL-6 and sP-selectin were found in subjects with ulcerative colitis, but this was not statistically significant. An increase in the number of blood platelets has been observed in the course of chronic inflammatory bowel disease **(20).**

Morphological parameters of platelets were also analyzed (MPV, LPLT), which may change in relation to their functional state and measurement of the parameters may indirectly indicate the degree of blood platelets activation. It was found that the PLT in subjects with ulcerative colitis was significantly higher compared to the PLT in the control group. Previous studies have shown that the blood platelet count was increased in patients with active ulcerative colitis compared to inactive ulcerative colitis or healthy subjects **(20).**

It was found that the number of metabolically active immature large blood platelets was determined. It was demonstrated that their number was slightly lower in subjects with ulcerative colitis compared to the control

group, and that this difference was not statistically significant. These results suggest the hypothesis that active large blood platelets are used up in the inflammatory process and that smaller platelets influence a decrease of MPV **(18).**

Other studies have also shown that the number of reticulated platelets was reduced in active ulcerative colitis patients compared to inactive ulcerative colitis patients and healthy subjects. It needs to be remembered that the decrease in MPV in subjects with active ulcerative colitis can be associated with the increase of blood platelets activation in these patients. In the present study, MPV was significantly lower in the ulcerative colitis group (B) compared to the control group (C), something which agreed with the results of other studies **(20).**

The aim of the study by **Yüksel et al. (21)** was to determine whether mean platelet volume would be a useful marker of ulcerative colitis, and to analyze its overall accuracy in evaluating disease activity in comparison with other inflammatory markers (leucocytosis, ESR, concentration of CRP). The study showed that MPV was reduced in ulcerative colitis compared to the control group, and that the reduction was statistically significant. MPV values in active ulcerative colitis (8.06 ± ± 1.19 fl) and inactive ulcerative colitis (8.45 ± 0.87 fl) were compared. The differences were shown to be statistically significant. In ulcerative colitis, MPV did not correlate with other markers: leucocytosis, ESR and CRP **(19).**

Moreover, It appears that decreased MPV may be an indicator for increased disease activity in patients with ulcerative colitis. Parallel dependencies between activity of ulcerative colitis and mean platelet volume were observed.

In 2001, **Kapsoritakis et al. (22)** studied two groups of patients with inflammatory bowel disease, comprising 93 subjects with ulcerative colitis, 66 with Crohn's disease (CD) and 38 healthy subjects. The activity of disease was defined using the Clinical Colitis Activity Index for patients with ulcerative colitis and the Crohn's Disease Activity Index for patients with Crohn's disease. In all groups, blood platelet count and their morphological parameters were measured. It was shown that the complete blood platelet count was significantly increased in patients with active UC and CD compared to patients with inactive UC and CD or healthy subjects. In the case of MPV, parallel dependencies were demonstrated **(22).**

MPV was significantly reduced in active inflammatory bowel diseases and correlated negatively with leucocytosis, ESR, concentration of CRP, markers of activation of blood platelets, such as plasma b-TG and PF4. The increase in the concentration of plasma b-TG and PF4 indicate platelets activation and the release of active biological substances, which are stored in the platelet's granules. They initiate and support the inflammatory process in the colon. The increase in the complete blood platelet count and the reduction in MPV reflected this process **(22).**

Neutrophil-to-Lymphocyte and Platelet-to-Lymphocyte Ratio

The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have been linked to outcomes in various disease processes. Several studies in the last decade have shown some promise for NLR and PLR in differentiating patients with active or more severe UC from quiescent UC and healthy controls, though sensitivity and specificity have been limited. They have also been shown to be able to predict disease response and relapse after medical therapies **(19).**

The optimal cutoff for these different applications has not been determined, but high NLR generally correlates with active disease, loss of response, and overall poor outcomes. These studies imply that NLR and PLR may be useful in predicting which patients will require surgical intervention, whether due to loss of response to medical therapy or more severe disease at presentation. However, optimal ranges and a direct correlation still need to be established before this can be used to predict colectomy in patients with UC **(19).**

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