

Brief Report

Treatment of Ulcerative Colitis: Impact on Platelet Aggregation

Sasa Peric ¹, Zeljko Todorovic ^{2,3,*} , Nebojsa Zdravkovic ⁴, Andjela Gogic ⁴, Stefan Simovic ^{2,5} ,
Vesna Grbovic ^{6,7}, Mladen Maksic ^{2,8} , Stefan Jakovljevic ^{9,10}, Olivera Milovanovic ¹¹ and Natasa Zdravkovic ^{2,8}

- ¹ Clinic of Gastroenterology and Hepatology, Military Medical Academy, Crnotravska Street 17, 11000 Belgrade, Serbia; pericsasa@gmail.com
- ² Department of Internal Medicine, Faculty of Medical Sciences, University of Kragujevac, Svetozar Markovic Street 69, 34000 Kragujevac, Serbia; simovicst@gmail.com (S.S.); asussonicmaster95@gmail.com (M.M.); natasasilvester@gmail.com (N.Z.)
- ³ Clinic for Hematology, University Clinical Center Kragujevac, Zmaj Jovina Street 30, 34000 Kragujevac, Serbia
- ⁴ Department of Medical Statistics and Informatics, Faculty of Medical Sciences, University of Kragujevac, Svetozar Markovic Street 69, 34000 Kragujevac, Serbia; nzdravkovic@medf.kg.ac.rs (N.Z.); andjelica97@hotmail.com (A.G.)
- ⁵ Clinic for Cardiology, University Clinical Center Kragujevac, Zmaj Jovina Street 30, 34000 Kragujevac, Serbia
- ⁶ Department of Physical Medicine and Rehabilitation, Faculty of Medical Sciences, University of Kragujevac, Svetozar Markovic Street 69, 34000 Kragujevac, Serbia; grbovicvesna72@gmail.com
- ⁷ Center for Physical Medicine and Rehabilitation, University Clinical Center Kragujevac, Zmaj Jovina Street 30, 34000 Kragujevac, Serbia
- ⁸ Clinic of Gastroenterology and Hepatology, University Clinical Center Kragujevac, Zmaj Jovina Street 30, 34000 Kragujevac, Serbia
- ⁹ Department of Surgery, Faculty of Medical Sciences, University of Kragujevac, Svetozar Markovic Street 69, 34000 Kragujevac, Serbia; stefan_jakov87@yahoo.com
- ¹⁰ Clinic for Surgery, University Clinical Center Kragujevac, Zmaj Jovina Street 30, 34000 Kragujevac, Serbia
- ¹¹ Department of Pharmacy, Faculty of Medical Sciences, University of Kragujevac, Svetozar Markovic Street 69, 34000 Kragujevac, Serbia; olivera.milovanovic09@gmail.com
- * Correspondence: todorovic_zeljko@hotmail.com; Tel.: +381-66339086



Citation: Peric, S.; Todorovic, Z.; Zdravkovic, N.; Gogic, A.; Simovic, S.; Grbovic, V.; Maksic, M.; Jakovljevic, S.; Milovanovic, O.; Zdravkovic, N. Treatment of Ulcerative Colitis: Impact on Platelet Aggregation. *Medicina* **2023**, *59*, 1615. <https://doi.org/10.3390/medicina59091615>

Academic Editors: Amosy E MKoma and Jan Bilski

Received: 15 July 2023

Revised: 14 August 2023

Accepted: 4 September 2023

Published: 7 September 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: *Background and Objectives:* Ulcerative colitis is chronic and/or progressive inflammation of the colorectal mucosa and submucosa and represents one of two major inflammatory bowel diseases. Ulcerative colitis has been associated with increased risk of arteriosus and venous thrombosis. There are numerous factors responsible for this; one of them is platelet activation and aggregation. The objective of our study was to determine if different treatment options for ulcerative colitis have an impact on platelet aggregation. *Materials and Methods:* This research was a prospective, observational study and included 94 newly diagnosed patients with UC divided into four treatment groups. For all patients, we measured platelet aggregability by using an impedance aggregometry method with a multiplate analyzer before and after treatment with infliximab, adalimumab, vedolizumab and azathioprine. A Paired Samples *t* test was performed in order to determine the difference in platelet aggregability before and after a certain therapy, since the data followed a normal distribution. Taking into account the impact of some clinical characteristics, multiple linear regression was conducted for the purpose of estimating the effect of therapy on the level of reduction in platelet aggregability. *Results:* All four drugs significantly reduced platelet aggregability. After we excluded the influence of clinical and endoscopic scores and disease localization on the results, we found that infliximab had the greatest anti-platelet activity. *Conclusions:* In addition to the well-known traditional risk factors for atherosclerosis, activation and aggregation of platelets play a significant role in the development of arterial thrombosis, and our results suggested that therapy use for the treatment of UC, especially infliximab, can have a great impact on cardiovascular morbidity and mortality by decreasing platelet aggregability.

Keywords: ulcerative colitis; platelet aggregation; infliximab; adalimumab; vedolizumab; azathioprine

1. Introduction

Ulcerative colitis (UC) is one of two major inflammatory bowel diseases (IBD). Ulcerative colitis is chronic and/or progressive inflammation limited to the colorectal mucosa and submucosa. Etiopathogenesis of UC has not been entirely defined, but it is known that major factors are genetic predisposition, environmental factors and inadequate immune response to these factors [1,2]. Worldwide, the incidence of UC is on the rise, ranging from 0.6 to 24.3 per 100,000 person-years in Europe and from 8.8 to 23.1 per 100,000 person-years in North America [3]. Incidence of UC is rising in many newly industrialized countries, which strongly implicates the role of environmental risk factors in the development of the disease [3].

For induction of UC remission, 5-aminosalicylic acid (5-ASA) drugs represent the standard therapy for patients with mild to moderate disease activity and corticosteroids for patients with moderate to severe UC or those who are unresponsive to standard therapy [4]. Thiopurines, such as azathioprine and mercaptopurine, were the first drugs used in maintenance of remission [4]. Today, there are many biological agents used in UC treatment. First, anti-tumor necrosis factor (TNF) drugs were introduced, including infliximab and adalimumab, and now, there are many agents like the anti-adhesion molecule vedolizumab, the interleukin inhibitor ustekinumab, the Janus kinase (JAK) inhibitor tofacitinib and many other therapeutic targets being explored in the treatment of UC in various clinical phases [1,5].

Ulcerative colitis has been associated with increased risk of arteriosus and venous thrombosis [6–8]. There are many factors contributing to thromboembolic events such as high levels of inflammatory cytokines [9] and, due to the systemic inflammatory response, increased factor VIII activity, elevated fibrinogen levels and accelerated thrombin generation [10]. Also, UC is associated with thrombocytosis due to the high plasma level of thrombopoietin and interleukin (IL)-6 [11]. Some studies found a correlation between thrombocytosis and atherosclerosis [12,13]. Aside from high platelet count, patients have also been found to have increased platelet activation and aggregation, which also increases the tendency for thrombosis in patients with UC [14,15]. There is evidence that treatment with adalimumab can also contribute to thromboembolic events [16].

The objective of our study was to determine if different treatment options for UC have an impact on platelet aggregation. As far as we know, this is the first study to show how different treatment options for UC impacts platelet aggregation.

2. Material and Methods

2.1. Patients and Treatment

A prospective, observational study was performed at the University Clinical Center Kragujevac, Center for Gastroenterohepatology and the Faculty of Medical Sciences, University of Kragujevac. All research procedures were sent to the Principle of Good Clinical Practice, and ethical approvals were obtained from relevant ethics committees.

A total of 94 newly diagnosed patients with UC were included in the trial. The patients were divided into four groups. All patients received induction treatment with only one drug, and platelet aggregability was measured before and 7 days after the induction treatment was finished. The first group had 27 patients, and they were treated with infliximab. Patients received infliximab intravenously with a dose of 5 mg/kg on the first day and the same dose after two weeks and after six weeks according to the induction protocol. The second group included 24 patients treated with adalimumab. Patients received 160 mg of adalimumab subcutaneously on the first day, 80 mg after two weeks and 40 mg after four weeks according to the induction protocol. The third group consisted of 19 patients who received vedolizumab intravenously, 300 mg on the first day and the same dose after two weeks and after six weeks. Lastly, the fourth group had 24 patients treated with azathioprine with a dose of 2–2.5 mg/kg administrated orally; after 14 weeks of treatment, we measured platelet aggregability in this group. Drug levels were measured after 7 days of the therapy and after the induction treatment was finished. All patients in

this study had drug levels within the reference range, and no antibodies were detected. Patients with inadequate drug levels and the presence of antibodies were excluded from this study.

2.2. Inclusion and Exclusion Criteria

Participants had to meet the following inclusion and exclusion criteria.

2.2.1. Inclusion Criteria

1. Diagnosis of UC made based on the endoscopic examination of the colon and the pathohistological findings of biopsies taken during the endoscopic examination in accordance with the criteria of the Third European Evidence-based Consensus on Diagnosis and Management of UC from 2017 [17];
2. Signed voluntary consent to participate in this study.

2.2.2. Exclusion Criteria

1. Persons under 18 years of age, pregnant women, nursing mothers and persons with limited legal responsibility and reduced cognitive abilities;
2. Persons with chronic and malignant diseases and/or therapy that may affect the investigated parameters: antiplatelet, immunosuppressive, immunomodulatory and corticosteroid therapy;
3. Infections and infectious syndromes two months before and at the time of the research;
4. Patients positive to *Helicobacter pylori*, *Cytomegalovirus*, Hepatis B and C, and human immunodeficiency virus;
5. Patients with inadequate drug levels and presence of antibodies.

2.3. Platelet Aggregability

Heparinized whole blood samples collected before and 7 days after the last treatment were used to assess platelet aggregability. For platelet aggregability measurement, we used the impedance aggregometry method with a multiplate analyzer (Dynabyte, Munchen, Germany). This method is based on measuring the impedance between the two electrodes immersed into the whole blood sample after the addition of an aggregation agonist such as adenosine phosphate (ADP test), arachidonic acid (ASPI test) and thrombin receptor-activating protein (TRAP test) [18].

2.4. Patient's Clinical and Endoscopic Characteristics

The clinical activity of the UC was determined according to the Truelove and Witts index [19], and the endoscopic disease activity was evaluated with the Mayo endoscopic subscore [20]. The Montreal classification of the extent of UC was used to classify patients into three subgroups [21].

2.5. Statistical Analysis

Statistical analysis was performed using IBM SPSS program version 22 (IBM SPSS Statistics 22. Armonk, NY, USA). Distribution of categorical variables was shown as absolute (N) and relative (%) frequency. The normality of the distribution was determined using the Shapiro–Wilk test. Since the data followed a normal distribution, in order to determine the difference in the level of reduction in aggregation before and after a certain therapy, a Paired Samples t test was performed. For the purpose of estimating the effect of therapy on the level of reduction in platelet aggregability (ADP, ASPI and TRAP), taking into account the impact of some clinical characteristics, multiple linear regression was conducted.

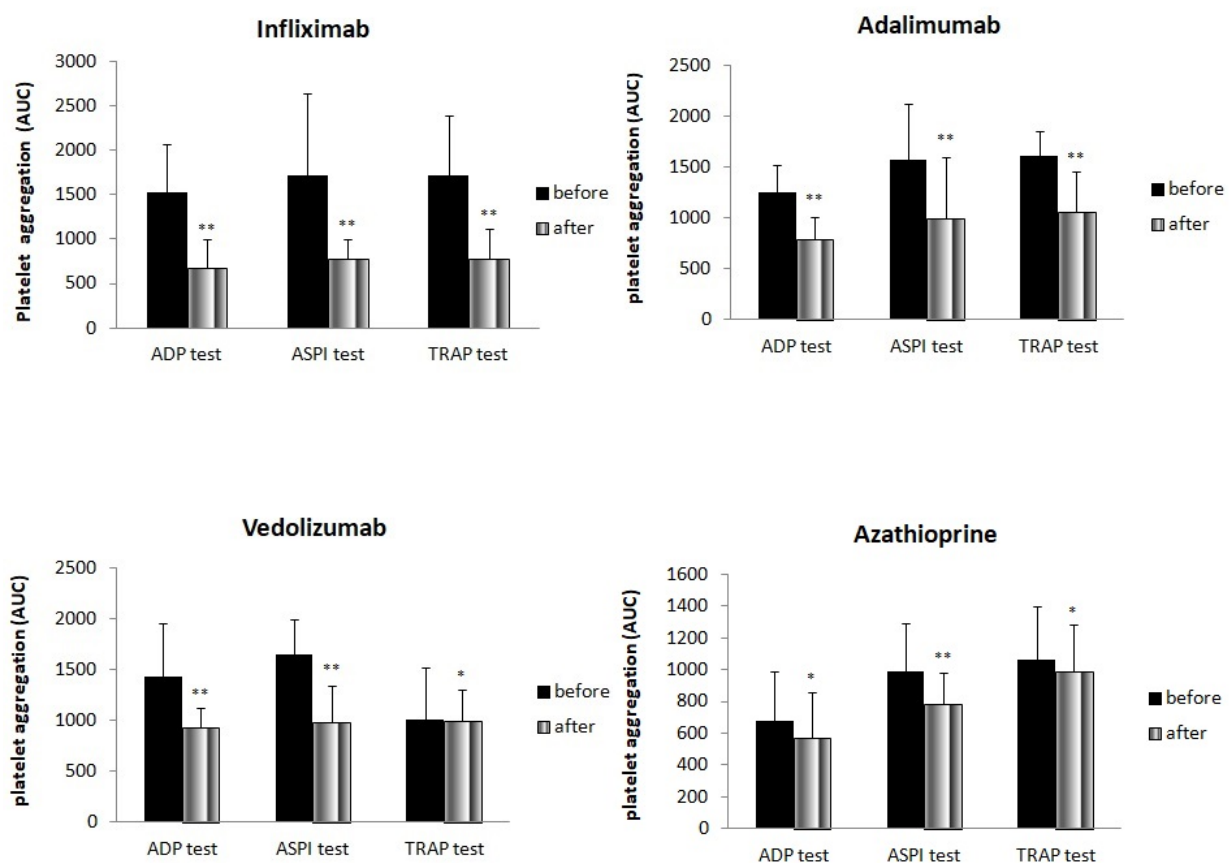
3. Results

This study included 94 patients, divided into four groups based on the applied therapy. Their clinical and endoscopic characteristics are shown in Table 1.

Table 1. Patient's clinical and endoscopic characteristics.

Patient Characteristics		Infliximab		Adalimumab		Vedolizumab		Azathioprine	
		N	%	N	%	N	%	N	%
clinical score	mild	0	0.0	1	4.2	0	0.0	10	41.7
	moderate	13	48.1	11	45.8	15	78.9	10	41.7
	severe	14	51.9	12	50.0	4	21.1	4	16.7
endoscopic score	normal	0	0.0	0	0.0	0	0.0	0	0.0
	mild	0	0.0	0	0.0	0	0.0	9	37.5
	moderate	15	55.6	13	54.2	16	84.2	12	50.0
	severe	12	44.4	11	45.8	3	15.8	3	12.5
disease localization	proctitis	0	0.0	0	0.0	1	5.3	0	0.0
	left-sided colitis	8	29.6	12	50.0	12	63.2	18	75.0
	pancolitis	19	70.4	12	50.0	6	31.6	6	25.0

Using the impedance aggregometry method, we analyzed the impact of the four drugs on platelet aggregability. As it is shown in Figure 1, treatment with infliximab significantly reduced aggregability measured with all three tests, ADP, ASPI and TRAP. Similar results were found after treatment with adalimumab. Vedolizumab also showed a significant reduction in platelet aggregability using all three tests, with the smallest difference observed using the TRAP test. Azathioprine treatment also significantly reduced platelet aggregability measured with all three tests. However, the ASPI test showed the greatest reduction (Figure 1).

**Figure 1.** Platelet aggregability (ADP, ASPI and TRAP) before and after treatment (* $p < 0.05$, ** $p < 0.01$).

Multiple linear regression was performed to evaluate the effect of therapy on the reduction in platelet aggregability. Considering the heterogeneity of the groups in terms of clinical characteristics (clinical and endoscopic scores and disease localization), the model was adjusted for the aforementioned characteristics. The results showed that all four treatments significantly reduced platelet aggregation measured with the ADP and ASPI tests but not with the TRAP test. The greatest reduction in platelet aggregation was observed in the group with infliximab, while the lowest level of reduction was detected in the group treated with azathioprine (Table 2).

Table 2. Effect of therapy on the level of reduction in platelet aggregability (ADP, ASPI and TRAP), adjusted for clinical and endoscopic scores and disease localization (* $p < 0.05$, ** $p < 0.01$).

Treatment	Difference in ADP before and after Treatment		Difference in ASPI before and after Treatment		Difference in TRAP before and after Treatment	
	B	95% CI	B	95% CI	B	95% CI
Infliximab	854.289 **	656.56–1052.02	517.303 **	276.45–758.16	166.934	−82.88–416.75
Adalimumab	351.064 *	146.30–555.83	308.267 *	58.84–557.69	71.881	−186.83–330.59
Vedolizumab	311.067 *	118.92–503.21	327.070 *	93.01–561.13	136.894	−379.66–105.87
Azathioprine	178.365 *	85.25–268.75	212.214 *	32.51–452.37	59.856	−153.98–254.40

4. Discussion

Ulcerative colitis diseases can exhibit a wide range of extraintestinal manifestations, including cardiovascular ones [22]. The frequency of arterial and venous thromboembolism is 1.7–5.5 times higher than that in the general population [23]. Arterial thromboembolic events are most common in the active phase of UC. The most common manifestations are acute myocardial infarction, stroke and mesenteric ischemia [24].

In addition to the well-known traditional risk factors for atherosclerosis, activation and aggregation of platelets play a significant role in the development of arterial thrombosis. Under pathological conditions, platelets are able to adhere to the endothelium in the absence of endothelial denudation [25]. After initial contact, platelets contribute to the initiation and progression of atherosclerotic lesions via the release of granule proteins and microparticles, interaction with oxidized low-density lipoprotein (oxLDL) and crosstalk with immune and endothelial cell [26]. In animal models, aspirin and clopidogrel showed inhibitory effects on the progression of atherosclerotic lesions accompanied by a reduction in vascular inflammation, decreased serum levels of inflammatory markers, reduced NF κ B activity and a more stable plaque phenotype [27,28]. These data suggested that anti-platelet drugs have anti-inflammatory properties and inhibit atherosclerosis.

In our research, we used anti-TNF drugs infliximab and adalimumab; the anti-adhesion drug vedolizumab; and one immunosuppressive drug, azathioprine. By using the impedance aggregometry method with a multiplate analyzer (ADP, ASPI and TRAP tests) we measured the impact of these drugs on platelet aggregation. In the ADP test, platelets were activated by ADP, which triggers several receptors on the platelet surface. clopidogrel and related drugs blocked the P2Y₁₂ receptor, which is believed to be the most important receptor for ADP on the platelet surface. In the ASPI test, platelets are activated by arachidonic acid. Further on, platelet cyclooxygenase enzyme (COX-1) converts the arachidonic acid to the potent platelet agonist thromboxane A₂. Arachidonic acid alone is not a platelet agonist and is the physiological substrate of the platelet COX-1. Therefore, platelet activation in the ASPI test allows very sensitive and specific detection of acetylsalicylic acid action. In the TRAP test, platelets are activated by TRAP-6, a peptide that mimics the activation of platelets via the action of thrombin. Thrombin is the most potent platelet activator. The action of thrombin is not blocked by the antiplatelet agents—acetylsalicylic acid or clopidogrel. However, thrombin (and TRAP-6)-activated platelets are blocked by the action of GPIIb/IIIa antagonists [18]. These tests are widely used to evaluate non-

responders to acetylsalicylic acid and clopidogrel therapy with high accuracy [29,30]. Our investigated drugs are not blockers of P2Y₁₂ receptor, nor of COX-1 and GPIIb/IIIa, so all three tests were equal in assessing platelet aggregation.

All four drugs showed significant anti-platelet activity, and platelet aggregation measured with the ADP, ASPI and TRAP tests was reduced significantly. Compared with each other, infliximab was the drug with the greatest anti-platelet activity and azatioprin showed the least anti-platelet activity. Because our treatment groups were heterogeneous in terms of clinical and endoscopic scores and disease localization and all these factors could influence platelet aggregability, we excluded the influence of these factors on results using regression analyses. We found that all four treatments significantly reduced platelet aggregation measured with the ADP and ASPI tests but not with the TRAP test, but infliximab still showed the greatest reduction in platelet aggregability and azatioprin showed the least reduction.

These results correlate with a previous study that showed that platelet activity was reduced in patients with rheumatoid arthritis treated with TNF α inhibitors. Also, previous studies have shown that TNF α antagonists are associated with risk reduction in cardiovascular events among patients with rheumatoid arthritis, psoriasis and psoriatic arthritis [31–33]. Tumor necrosis factor α contributes to the risk of cardiovascular disease by promoting platelet hyperreactivity due to reprogramming of the inflammatory, mitochondrial and metabolic pathways in megakaryocytes [34]. Perhaps the better results obtained with the use of infliximab compared to adalimumab are due to its intravenous administration, but this remains a topic for additional research.

Histopathological studies found mesenteric vascular microthrombi to be the first finding in the mucosa of UC patients [35]. Those microthrombi contribute to ischemia so our study suggested that all four drugs could reduce severity of UC by reducing thrombosis events in colon mucosa.

Our study was unique because, for the first time, it showed how different treatments for UC influence platelet aggregability. These results suggested that therapy for the treatment of UC, especially infliximab, not only reduces the activity of the disease but also can have a great impact on cardiovascular morbidity and mortality.

5. Conclusions

In addition to the well-known traditional risk factors for atherosclerosis, activation and aggregation of platelets play a significant role in the development of arterial thrombosis and our results suggested that therapy for treatment of UC can decrease platelet aggregability.

Author Contributions: Conceptualization, S.P. and N.Z. (Natasa Zdravkovic); methodology, N.Z. (Nebojsa Zdravkovic) and N.Z. (Natasa Zdravkovic); formal analysis, N.Z. (Nebojsa Zdravkovic) and A.G.; investigation, S.S., M.M. and V.G.; resources, O.M., M.M. and S.J.; data curation, S.P. and Z.T.; writing—original draft preparation, S.P. and Z.T.; writing—review and editing, N.Z. (Natasa Zdravkovic) and Z.T.; visualization, S.S., A.G. and V.G.; supervision, N.Z. (Nebojsa Zdravkovic), N.Z. (Natasa Zdravkovic) and Z.T.; project administration, N.Z. (Natasa Zdravkovic). All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of University Clinical Center Kragujevac (protocol code 01-7012, 2 July 2015).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available from the corresponding author upon request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Segal, J.P.; LeBlanc, J.F.; Hart, A.L. Ulcerative colitis: An update. *Clin. Med.* **2021**, *21*, 135–139. [\[CrossRef\]](#)
2. Du, L.; Ha, C. Epidemiology and Pathogenesis of Ulcerative Colitis. *Gastroenterol. Clin. N. Am.* **2020**, *49*, 643–654. [\[CrossRef\]](#)
3. Ng, S.C.; Shi, H.Y.; Hamidi, N.; Underwood, F.E.; Tang, W.; Benchimol, E.I.; Panaccione, R.; Ghosh, S.; Wu, J.C.Y.; Chan, F.K.L.; et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: A systematic review of population-based studies. *Lancet* **2017**, *390*, 2769–2778. [\[CrossRef\]](#)
4. Lamb, C.A.; Kennedy, N.A.; Raine, T.; Hendy, P.A.; Smith, P.J.; Limdi, J.K.; Hayee, B.; Lomer, M.C.E.; Parkes, G.C.; Selinger, C.; et al. British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. *Gut* **2019**, *68*, s1–s106. [\[CrossRef\]](#)
5. Zurba, Y.; Gros, B.; Shehab, M. Exploring the Pipeline of Novel Therapies for Inflammatory Bowel Disease; State of the Art Review. *Biomedicines* **2023**, *11*, 747. [\[CrossRef\]](#)
6. Nelson, A.D.; Fluxá, D.; Caldera, F.; Farraye, F.A.; Hashash, J.G.; Kröner, P.T. Thromboembolic Events in Hospitalized Patients with Inflammatory Bowel Disease. *Dig. Dis. Sci.* **2023**. *epub ahead of print*. [\[CrossRef\]](#)
7. Novacek, G.; Weltermann, A.; Sobala, A.; Tilg, H.; Petritsch, W.; Reinisch, W.; Mayer, A.; Haas, T.; Kaser, A.; Feichtenschlager, T.; et al. Inflammatory bowel disease is a risk factor for recurrent venous thromboembolism. *Gastroenterology* **2010**, *139*, 779–787.e1. [\[CrossRef\]](#)
8. Giannotta, M.; Tapete, G.; Emmi, G.; Silvestri, E.; Milla, M. Thrombosis in Inflammatory Bowel Diseases: What's the Link? *Thromb. J.* **2015**, *13*, 14. [\[CrossRef\]](#)
9. Esmon, C.T. The interactions between inflammation and coagulation. *Br. J. Haematol.* **2005**, *131*, 417–430. [\[CrossRef\]](#)
10. Lee, L.C.; Spittell, J.A.; Sauer, W.G.; Owen, C.A.; Thompson, J.H. Hypercoagulability associated with chronic ulcerative colitis: Changes in blood coagulation factors. *Gastroenterology* **1968**, *54*, 76–85. [\[CrossRef\]](#)
11. Heits, F.; Stahl, M.; Ludwig, D.; Stange, E.F.; Jelkmann, W. Elevated Serum Thrombopoietin and Interleukin-6 Concentrations in Thrombocytosis Associated with Inflammatory Bowel Disease. *J. Interferon Cytokine Res.* **1999**, *19*, 757–760. [\[CrossRef\]](#)
12. Liu, K.; Xu, J.; Tao, L.; Yang, K.; Sun, Y.; Guo, X. Platelet Counts Are Associated with Arterial Stiffness in Chinese Han Population: A Longitudinal Study. *BMC Cardiovasc. Disord.* **2020**, *20*, 353. [\[CrossRef\]](#)
13. Lee, M.K.S.; Kraakman, M.J.; Dragoljevic, D.; Hanssen, N.M.J.; Flynn, M.C.; Al-Sharea, A.; Sreejit, G.; Bertuzzo-Veiga, C.; Cooney, O.D.; Baig, F.; et al. Apoptotic Ablation of Platelets Reduces Atherosclerosis in Mice with Diabetes. *Arterioscler. Thromb. Vasc. Biol.* **2021**, *41*, 1167–1178. [\[CrossRef\]](#)
14. Lugonja, S.I.; Pantic, I.L.; Milovanovic, T.M.; Grbovic, V.M.; Djokovic, B.M.; Todorovic, Ž.D.; Simovic, S.M.; Medovic, R.H.; Zdravkovic, N.D.; Zdravkovic, N.D. Atherosclerotic Cardiovascular Disease in Inflammatory Bowel Disease: The Role of Chronic Inflammation and Platelet Aggregation. *Medicina* **2023**, *59*, 554. [\[CrossRef\]](#)
15. Webberley, M.J.; Hart, M.T.; Melikian, V. Thromboembolism in inflammatory bowel disease: Role of platelets. *Gut* **1993**, *34*, 247–251. [\[CrossRef\]](#)
16. Schaardenburg, D.; Wijbrandts, C.A.; Tak, P.P.; Lems, W.F.; Dijkmans, B.A.; van Vugt, R.M.; Wolbink, G.J. Venous and arterial thromboembolic events in adalimumab-treated patients with antiadalimumab antibodies: A case series and cohort study. *Arthritis Rheum.* **2011**, *63*, 877–883.
17. Magro, F.; Gionchetti, P.; Eliakim, R.; Ardizzone, S.; Armuzzi, A.; Barreiro-de Acosta, M.; Burisch, J.; Gecse, K.B.; Hart, A.L.; Hindryckx, P.; et al. Third European Evidence-based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 1: Definitions, Diagnosis, Extra-intestinal Manifestations, Pregnancy, Cancer Surveillance, Surgery, and Ileo-anal Pouch Disorders. *J. Crohn's Colitis* **2017**, *11*, 649–670. [\[CrossRef\]](#)
18. Practical-haemostasis.com. Platelet Function Testing: Impedance Platelet Aggregometry. Available online: https://www.practical-haemostasis.com/Platelets/platelet_function_testing_impedance_platelet_aggregometry.html (accessed on 1 July 2023).
19. Truelove, S.C.; Witts, L.J. Cortisone in ulcerative colitis; preliminary report on a therapeutic trial. *Br. Med. J.* **1954**, *2*, 375–378. [\[CrossRef\]](#)
20. Schroeder, K.W.; Tremaine, W.J.; Ilstrup, D.M. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. *N. Engl. J. Med.* **1987**, *317*, 1625–1629. [\[CrossRef\]](#)
21. Satsangi, J.; Silverberg, M.S.; Vermeire, S.; Colombel, J.F. The Montreal classification of inflammatory bowel disease: Controversies, consensus, and implications. *Gut* **2006**, *55*, 749–753. [\[CrossRef\]](#)
22. Olpin, J.D.; Sjoberg, B.P.; Stilwill, S.E.; Jensen, L.E.; Rezvani, M.; Shaaban, A.M. Beyond the Bowel: Extraintestinal Manifestations of Inflammatory Bowel Disease. *Radiographics* **2017**, *37*, 1135–1160. [\[CrossRef\]](#)
23. Harbord, M.; Annesse, V.; Vavricka, S.R.; Allez, M.; Barreiro-de Acosta, M.; Boberg, K.M.; Burisch, J.; De Vos, M.; De Vries, A.M.; Dick, A.D.; et al. European Crohn's and Colitis Organisation. The First European Evidence-based Consensus on Extra-intestinal Manifestations in Inflammatory Bowel Disease. *J. Crohn's Colitis* **2016**, *10*, 239–254. [\[CrossRef\]](#)
24. Bigeh, A.; Sanchez, A.; Maestas, C.; Gulati, M. Inflammatory bowel disease and the risk for cardiovascular disease: Does all inflammation lead to heart disease? *Trends Cardiovasc. Med.* **2020**, *30*, 463–469. [\[CrossRef\]](#)
25. Massberg, S.; Brand, K.; Grüner, S.; Page, S.; Müller, E.; Müller, I.; Bergmeier, W.; Richter, T.; Lorenz, M.; Konrad, I.; et al. A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. *J. Exp. Med.* **2002**, *196*, 887–896. [\[CrossRef\]](#)
26. Nording, H.M.; Seizer, P.; Langer, H.F. Platelets in inflammation and atherogenesis. *Front. Immunol.* **2015**, *6*, 98. [\[CrossRef\]](#)

27. Cyrus, T.; Sung, S.; Zhao, L.; Funk, C.D.; Tang, S.; Praticò, D. Effect of low-dose aspirin on vascular inflammation, plaque stability, and atherogenesis in low-density lipoprotein receptor-deficient mice. *Circulation* **2002**, *106*, 1282–1287. [[CrossRef](#)]
28. Li, M.; Zhang, Y.; Ren, H.; Zhang, Y.; Zhu, X. Effect of clopidogrel on the inflammatory progression of early atherosclerosis in rabbits model. *Atherosclerosis* **2007**, *194*, 348–356. [[CrossRef](#)]
29. Heringer, S.; Kabelitz, L.; Kramer, M.; Nikoubashman, O.; Brockmann, M.A.; Kirschner, S.; Wiesmann, M. Platelet function testing in pigs using the Multiplate® Analyzer. *PLoS ONE* **2019**, *14*, e0222010. [[CrossRef](#)]
30. Lenk, E.; Spannagl, M. Platelet Function Testing-Guided Antiplatelet Therapy. *EJIFCC* **2013**, *24*, 90–96.
31. Greenberg, J.D.; Kremer, J.M.; Curtis, J.R.; Hochberg, M.C.; Reed, G.; Tsao, P.; Farkouh, M.E.; Nasir, A.; Setoguchi, S.; Solomon, D.H.; et al. Tumour necrosis factor antagonist use and associated risk reduction of cardiovascular events among patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **2011**, *70*, 576–582. [[CrossRef](#)]
32. Roubille, C.; Richer, V.; Starnino, T.; McCourt, C.; McFarlane, A.; Fleming, P.; Siu, S.; Kraft, J.; Lynde, C.; Pope, J.; et al. The effects of tumour necrosis factor inhibitors, methotrexate, non-steroidal anti-inflammatory drugs and corticosteroids on cardiovascular events in rheumatoid arthritis, psoriasis and psoriatic arthritis: A systematic review and meta-analysis. *Ann. Rheum. Dis.* **2015**, *74*, 480–489. [[CrossRef](#)] [[PubMed](#)]
33. Westlake, S.L.; Colebatch, A.N.; Baird, J.; Curzen, N.; Kiely, P.; Quinn, M.; Choy, E.; Ostor, A.J.; Edwards, C.J. Tumour necrosis factor antagonists and the risk of cardiovascular disease in patients with rheumatoid arthritis: A systematic literature review. *Rheumatology* **2011**, *50*, 518–531. [[CrossRef](#)] [[PubMed](#)]
34. Davizon-Castillo, P.; McMahon, B.; Aguila, S.; Bark, D.; Ashworth, K.; Allawzi, A.; Campbell, R.A.; Montenont, E.; Nemkov, T.; D'Alessandro, A.; et al. TNF- α -driven inflammation and mitochondrial dysfunction define the platelet hyperreactivity of aging. *Blood* **2019**, *134*, 727–740. [[CrossRef](#)] [[PubMed](#)]
35. Dhillon, A.P.; Anthony, A.; Sim, R.; Wakefield, A.J.; Sankey, E.A.; Hudson, M.; Allison, M.C.; Pounder, R.E. Mucosal capillary thrombi in rectal biopsies. *Histopathology* **1992**, *21*, 127–133. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.