Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology
Volume 31 (1), 2024: 67–74.
www.Ejimmunology.org

Significance of inflammatory markers in primary Fibromyalgia syndrome and their relation in assessing the disease severity

Esraa A. El-Sawy, Mervat M. Abdul Hakim, Abeer El-Zohiery, and Sherihan M. Salama

Department of Physical Medicine, Rheumatology & Rehabilitation, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Corresponding author: Sherihan M. Salama, Department of Physical Medicine, Rheumatology & Rehabilitation, Faculty of Medicine, Ain Shams University, Cairo, Egypt.
Email: shrehan.mahdy@med.asu.edu.eg.

Abstract

Fibromyalgia syndrome (FMS) is a musculoskeletal disorder characterized by diffuse chronic musculoskeletal pain associated with various other symptoms. Although the etiology and pathogenesis of FMS are still unclear, it was reported to have a possible inflammatory basis. No laboratory marker is currently available to diagnose the disease. This study aimed to search for biomarkers useful in diagnosis of FMS. We assessed blood erythrocyte sedimentation rate (ESR), neutrophil lymphocyte ratio (NLR), the mean platelet volume (MPV) and platelet distribution width (PDW), and serum levels of C-reactive protein (CRP), as inflammatory markers in primary FMS patients and their relationship with disease severity and depression scores. The study included 30 FMS patients, diagnosed according to the 2010 ACR (American Colleague of Rheumatology) criteria and 30 normal volunteers as a control group. FMS patients filled out the Revised Fibromyalgia Impact Questionnaire (FIQR) and Montgomery Asberg Depression Score (MADRS) as well. There was a significant difference in the studied parameters including ESR, CRP, NLR, MPV between study patients and control groups ($p<0.05$ for all). However, PDW did not differ between the two study groups. Based on our study findings, we can conclude that serum levels of the tested inflammatory markers including ESR, CRP, NLR, and MPV were higher in patients than in controls which makes them of good diagnostic value in patients with fibromyalgia. Meanwhile, some of these markers, mainly the acute phase reactants, have a positive relation with disease severity and depression scores, which in turn affect the quality of daily living.

Keywords: Fibromyalgia, Inflammation, Depression, Severity, FIQR, NLR, MPV, PDW, CRP, ESR.

Date received: 11 June 2023; **accepted:** 25 November 2023

Introduction

Fibromyalgia syndrome (FMS) is a musculoskeletal condition that has been known to be of unknown etiology and is more common in females than males with a ratio of 7:1. In addition to chronic widespread pain, comorbidities might include fatigue, anxiety, depression, irritable bowel syndrome, sleep disorders, and memory problems.[1]

FMS cannot be diagnosed solely by laboratory tests or specific radiology techniques. Clinical findings can be the only way to make the FMS diagnosis. In FMS, it is important to exclude other diseases in which these symptoms can be common. Therefore, laboratory support is very important in FMS diagnosis.[2]

In FMS, the etiology and pathogenesis are still unclear, chronic inflammatory processes are thought to play an important role in the pathogenesis. Certain inflammatory protein levels were reported to be higher in the plasma and cerebrospinal fluid of FMS patients signifying systemic and neuro-inflammation.[3]

Being one of the acute phase reaction proteins, C-reactive protein (CRP) is synthesized by the liver, moreover, it increases in response to inflammatory reactions. Elevated erythrocyte sedimentation rate (ESR) is another marker of inflammatory reactions. Due to their ability to estimate the presence and severity of inflammatory diseases, both blood examinations are widely requested by physicians to detect and follow up of many inflammatory and infectious conditions.[4]

Many studies have shown an increase in pro-inflammatory cytokines like interleukin (IL)-8, and IL-6 in FMS patients. Although these biomarkers cannot be used in clinical practice due to the high price of their commercial kits, their subsequent changes and effect on blood indices could be detected. In recent years, neutrophil-lymphocyte ratio (NLR), the mean platelet volume (MPV), and platelet distribution width (PDW) were identified as important systemic inflammatory markers.[2]

Being with high sensitivity and lower specificity, NLR is introduced as a cheap, simple, and easily available parameter. It is a dynamic parameter with a quick response to insults and is considered as an index of systemic inflammation.[5]

MPV is considered a marker and indicator of platelet function represented as an accurate measure of platelet size. There is a correlation between MPV and active inflammatory diseases.[6] PDW, which measures the variation in platelet size as it increases during platelet activation, is thought to provide a more reliable indicator of platelet reactivity than the MPV. This is because PDW does not show affection caused by single platelet distention that occurs due to platelet swelling. PDW can identify parts of larger platelets that are more metabolically and enzymatically active.[7]

This study was designed to search for biomarkers useful in the diagnosis of FMS. Consequently, we evaluated blood levels of ESR, NLR, MPV and PDW and serum levels of CRP in primary FMS patients and their relation to each of the disease severity and depression scores.

Subjects and Methods

This case-control study included 30 FMS patients, diagnosed according to the 2010 ACR Criteria,[8] who were presented to our clinic, and 30 normal individuals matched for age and sex as a control group. The study was performed from November 2021 to June 2022.

The exclusion criteria included all patients with any other rheumatological diseases, endocrinal diseases, anemias, viral, neurological diseases, suspected malignancy, and any sort of infection or inflammation in the body.

All the study groups were subjected to full medical history taking and thorough clinical examination to exclude any arthritis, autoimmune, rheumatological diseases, associated malignancies, and neurological deficits.

Laboratory evaluation was done to exclude other rheumatological diseases and to compare inflammatory indices between patients and controls. A venous blood sample (5 ml) was withdrawn from each subject of both groups under complete aseptic conditions. The blood sample was divided as follows, two ml collected in ethylenediaminetetraacetic acid tripotassium (EDTA K3) tubes for complete blood count (CBC) testing, two ml in anti-coagulated tubes with citrate 1:4 for CRP testing and the last one ml for ESR testing.

CBC was done using an automated hematology analyzer (Sysmex XN1000, Siemens Diagnostics- Germany), according to the manufacturer's instructions. CBC parameters including NLR, MPV and PDW were calculated.

ESR was done by the conventional Westergreen method.

CRP was done using a latex agglutination method where the fresh serum samples were centrifuged before testing. Then according to the semi-quantitative method, 50 µL was added next to the samples to be tested. After 2 minutes, the approximate CRP concentration in the patient sample was calculated as follows 6 x CRP Titer = mg/L.

In order to assess the patients' conditions and quality of life, FMS patients were subjected to the Revised Fibromyalgia Impact Questionnaire (FIQR) to determine their disability score. It consisted of 21 questions, all questions were based on an 11-point rating scale from 0 to 10, where 10 is the 'worst'. All questions were framed in the context of the past 7 days. The FIQR was divided into three linked sets of domains, function, overall impact, and symptoms.[9]

The scoring of the FIQR is the sum of the three domain scores and was calculated as follows: the summed score for function (range 0 to 90) divided by 3, then the summed score for overall impact (range 0 to 20) is not changed and lastly, the summed score for symptoms (range 0 to 100) was divided by 2. The total maximal score of the FIQR is 100.[9]

The Montgomery Asberg Depression Rating Scale (MADRS) was used for the assessment of the severity of depression. It includes a 10-item diagnostic questionnaire [10]. In our study, for clarification, it was used in Arabic, the patient's own language [11]. The scoring of MADRS is as follows, normal (0–8), mild (9–17), moderate (18–34), and severe (≥35).[12]

Statistical Analysis

Data were collected and revised for completeness and consistency. The collected data were coded, tabulated, introduced to a personal computer, and then analyzed using the Statistical Package for Social Sciences (SPSS) program for Windows Version 22. Qualitative data were presented using the frequency and its related percentage. Quantitative data were presented using mean and standard deviation. The Chi-square test was used to examine the relationship between two qualitative variables but when the expected count is less than 5 in more than 20% of the cells, the Fisher's exact test was used. The comparison between two groups with quantitative data and parametric distribution was done by using an independent sample t-test. Pearson correlation was used to examine the relation between two quantitative variables. A probability (*p*-value) <0.05 was considered significant.

Results

This study included 30 FMS patients, who were all females (100%). Their age ranged from 21 to 55 years with mean ± SD of 33.47 ± 7.3 years. Their disease duration ranged from 0.42-19 years with mean ± SD of 4.8 ± 4.3. The control group included 30 apparently healthy individuals, who were all females (100%). Their age ranged from 31 – 56 years with the mean ± SD of 31.4 ± 7.6 years.

Among our 30 FMS patients, according to FIQR score,[9] one patient (3.33%) had mild disease severity, 10 patients (33.33%) with moderate disease severity, and nine patients (30%) with severe disease severity while 10 patients (33.33%) with extreme disease severity. FIQR assessment ranged from 37.1 - 96.1 with a mean ± SD of 67.5 ± 13.2.

As for MADRS scores,[12] among our 30 FMS patients two patients (6.67%) had normal score with no signs of depression, five patients (16.67) with mild depression, and 19 patients (63.33%) with moderate depression while four patients (13.33%) with severe depression and suicidal thoughts. Those four cases were referred to psychiatric consultation. MADRS assessment ranged from 10-44 with a mean ± SD of 24.8 ± 8.8.

Laboratory data were compared between 30 FMS patients and 30 controls which revealed that ESR, CRP, NLR and MPV were statistically significantly higher among cases than controls (*p*=0.001, *p*=0.02, *p*=0.048 and *p*=0.004, respectively). PDW was not different between the two study groups (*p*>0.05), as shown in (Table 1).

Table 1. Comparison of the studied laboratory parameters between cases and controls.

	Cases		Controls		*p* value
	Range	Mean ± SD	Range	Mean ± SD	
MPV (fl)	7 - 17.8	9.6 ± 1.9	6.3 - 11.5	8.3 ± 1.3	0.004
PDW (%)	10.2 - 22.4	14.8 ± 3.1	12.7 - 18.4	14.6 ± 1.5	NS
NLR	0.7 - 2.9	1.5 ± 0.6	0.75 - 1.77	1.2 ± 0.3	0.048
ESR (mm/h)	6 - 72	29.2 ± 20.7	2 - 18	8.4 ± 4.8	0.001
CRP (mg/dl)	0.3 - 22	4.6 ± 5.7	0.1 - 8	2.03 ± 1.6	0.02

P > 0.05 is not significant (NS). (An independent sample T-test was used), ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, MPV: Mean platelet volume, PDW: Platelet distribution width, NLR: Neutrophil lymphocyte ratio.

In the 30 FMS patient group, the correlation between the laboratory markers and FIQR revealed a statistically significant positive correlation between FIQR and each of ESR (p=0.027) and CRP (p=0.021). However, no correlation was found between FIQR and neither MPV nor PDW (p>0.05). A marginal significance was detected with NLR (p= 0.06) (Table 2, Figure 1-2).

Table 2. Correlation between Revised Fibromyalgia Impact Questionnaire (FIQR) and laboratory parameters among the fibromyalgia syndrome (FMS) patients' group.

Correlations		
Parameters	FIQR	
	Pearson correlation (r)	*p* value
ESR (mm/h)	0.4	0.027
CRP (mg/dl)	0.42	0.021
NLR	0.34	NS
MPV (fl)	-0.29	NS
PDW (%)	-0.19	NS

P > 0.05 is not significant (NS), (Pearson correlation was used), FIQR: Revised Fibromyalgia Impact Questionnaire, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, MPV: Mean platelet volume, PDW: Platelet distribution width, NLR: Neutrophil lymphocyte ratio.

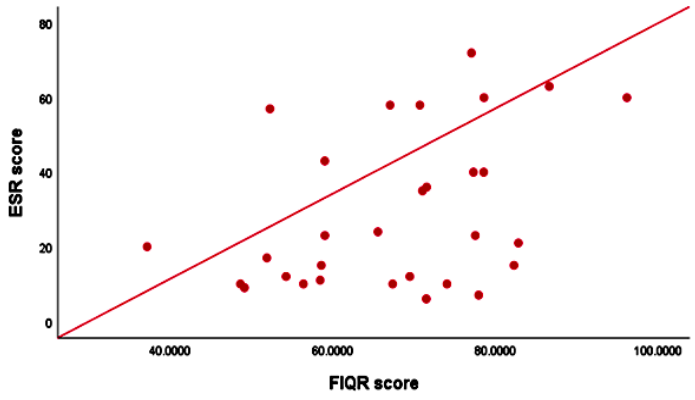

Figure 1. Scatter plot between Revised Fibromyalgia Impact Questionnaire (FIQR) score and Erythrocyte sedimentation rate (ESR) among the fibromyalgia syndrome (FMS) patients' group.

r = 0.4 Pearson correlation is positive with medium strength

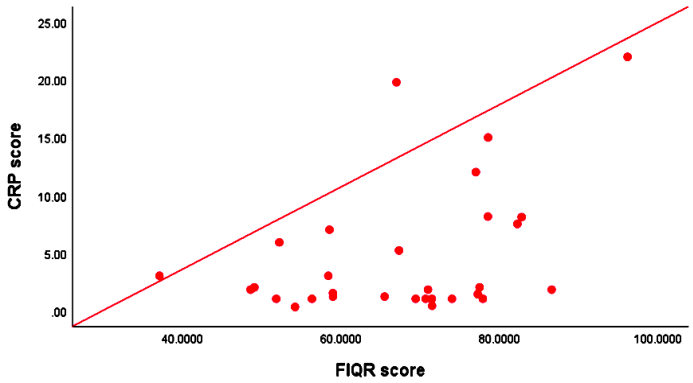

Figure 2. Scatter plot between Revised Fibromyalgia Impact Questionnaire (FIQR) score and C-reactive protein (CRP) among the fibromyalgia syndrome (FMS) patients' group.
r = 0.42 Pearson correlation is positive with medium strength

In the 30 FMS patient group, the correlation between the laboratory markers and MADRS revealed a statistically significant positive correlation between MSADRS and CRP (p=0.01). However, no correlation was found between MADRS with each of ESR, NLR, MPV and PDW (p>0.05) (Table 3, Figure 3).

Table 3. Correlation between Montgomery Asberg Depression Rating Scale (MADRS) and laboratory parameters among fibromyalgia syndrome (FMS) patients' group.

Correlations		
Parameters	MADRS	
	Pearson correlation (r)	*p* value
ESR (mm/h)	0.34	NS
CRP (mg/dl)	0.46	0.01
NLR	0.013	NS
MPV (fl)	0.042	NS
PDW (%)	-0.29	NS

P > 0.05 is not significant (NS)., (Pearson correlation was used), MADRS: Montgomery Asberg Depression Rating Scale, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, MPV: Mean platelet volume, PDW: Platelet distribution width, NLR: Neutrophil lymphocyte ratio

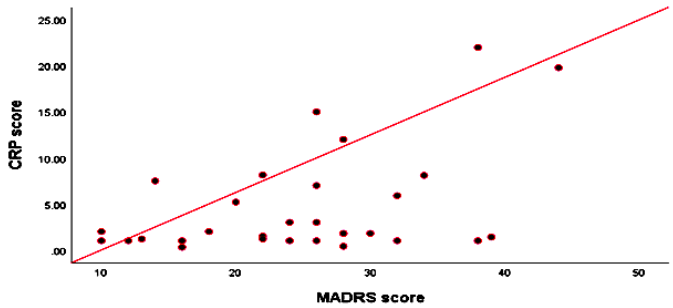

Figure 3. Scatter plot between Montgomery Asberg Depression Rating Scale (MADRS) score and C-reactive protein (CRP) among the fibromyalgia syndrome (FMS) patients' group
r = 0.46 Pearson correlation is positive with medium strength

Discussion

This study was designed to search for biomarkers useful in diagnosis of FMS among blood levels of ESR, CRP, NLR, MPV and PDW in primary FMS patients and determine their possible relation to the disease severity and depression scores.

Among our 30 FMS patients, 18 (60%) had elevated serum levels of ESR, with a mean ± SD of 29.2 ± 20.7. As ESR depends on concentration of acute-phase proteins circulating in the blood, particularly fibrinogen, ESR is a good indicator of the overall inflammation.[13] Fibrinogen, a soluble protein, is a driver of chronic low-grade inflammation, as it plays a key role in the acute phase response and results in increased hepatic expression and circulating protein.[14] Such information may explain the presence of elevated ESR among our patients and point to the inflammatory origin of the disease.

In our study, CRP was elevated in 8 of our patients (26.67 %). CRP is a homopentameric protein, regarded as an inflammatory biomarker and is used to assess the presence and severity of inflammation.[15] It is one of the proteins secreted by the liver in response to the cytokines released due to fibrinogen signaling. Playing a crucial role in the inflammatory process, CRP activates neutrophils and monocytes, promotes phagocytosis, and causes activation of the complement system which in turn helps in maintaining the inflammatory process.[16]

In our study, blood levels of NLR were higher among cases and showed low inflammatory status in 6 (20%) patients. NLR is calculated by dividing the absolute neutrophil count by absolute lymphocyte count from peripheral blood cells. It is a low-cost, basic, dynamic parameter that responds quickly to fluctuations and accurately measures the level of neuroendocrine stress and immune-inflammatory response. As circulating leukocytes' physiological reactions to stress, injury, trauma, major surgery, bacteremia, systemic inflammation, and sepsis are reflected by both neutrophilia and lymphocytopenia.[5]

Although the results of MPV were at normal rates, they were higher in patients than in controls. PDW results were within the normal range as well. Arterial and venous thrombus formation can be enhanced by inflammation, as inflammation is well recognized to be a regulator of coagulation and fibrinolytic system activity and shifts the hemostatic balance toward a prothrombotic and antifibrinolytic state.[14] The discharge of neutrophil extracellular traps (NETs) results in thrombus formation, which with the help of thrombin and other agonists plays a role in the activation of platelets. The activation of platelets is indicated by its indices such as PDW and MPV, which increases with the activation.[17] Some studies observed a prothrombotic state in FMS patients.[18]

In 2020, Karataş & Gündüz[19] reported an increase in MPV and PDW in the presence of inflammation. Megakaryocytes in the bone marrow act as the progenitor cells that determine MPV, under the effect of cytokines, IL-6 and IL-3, that regulate megakaryocyte ploidy as thrombopoietin. Increased MPV is indicative of an increased platelet diameter which can be a reflective marker of the production rate and activation of the platelets.[20]

Recent reports indicated that megakaryocytic maturation and platelet production and size are regulated and maintained by various cytokines, such as IL-6, granulocytes colony-stimulating factor (G-CSF), and macrophage colony-stimulating factor (MCSF). PDW is a direct measure of the platelet size variability and a marker of its activation.[21]

These previous scientific data combined with our results draw attention to the presence of chronic low-grade inflammation in FMS pathogenesis and that the determination of these parameters may be an uncomplicated, inexpensive means of improving the diagnosis of FMS. We further analyzed our obtained data in a trial to find out the relation between these inflammatory markers with each of the disease severity and depression levels.

The disease severity of our patients reflected by FIQR ranged from 37.1 to 96.1 with a mean ± SD of 67.5 ± 13.2. It was positively correlated with each of ESR (p=0.027) and CRP (p=0.021), as well as a marginal significance with NLR (p=

0.06). On the other hand, no correlation was found between FIQR with either MPV or PDW. The wholesome idea about the quality of life and assessment of physical function in FMS patients can be obtained using the FIQR. Hence, these results may indicate that inflammation can cause reduction in the quality of life that may in turn be exacerbated by psychological distress creating a vicious cycle over time [22]. It was also shown that FMS symptoms and increased disease severity often lead to reduction in the quality of life.[23]

Regarding the correlation between inflammatory markers and MADRS scores for depression, the data showed a statistically significant positive correlation between MADRS and CRP (p=0.01), while no correlation was found between MADRS with each of ESR, NLR, MPV and PDW. Accordingly, this indicates the concept that depression has a relationship with elevated circulating levels of inflammatory immune markers such as CRP.[24] It has been reported that CRP promotes the secretion of IL-6, IL-1b and tumor necrosis factor (TNF)- α 16. The most studied cytokines in the context of psychoneuroimmunology are IL-6, TNF, IL-1b, and interferons on the inflammatory side and IL-10 on the resolving side. Moreover, excess, or prolonged inflammatory cytokine activity perturbs multiple neuronal functions including impairment of neurotransmitter signaling, disruption of the synthesis, reuptake, and release of neurotransmitters. This, in turn, affects neuro-circuit function including that implicated in mood and cognition.[25]

The limitation of our study was the small sample size of patients in comparison to the growing number of cases. Also, the variability in treatment modalities that were provided to the patients could have affected their clinical and psychological outcome. In light of the study findings, we can conclude that the serum levels of the tested inflammatory markers can be considered as good indicators of ongoing inflammatory process among FMS patients reflecting the severity of the disease.

Author Contributions

EAE, AE, SMS examined the patients. EAE, collected samples and made the statistical analysis. EAE, SMS, writing the paper. MAH, AE, SMS ,reviewed the paper.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee, Faculty of Medicine, Ain Shams University (FMASU MS 635/2021).

Informed consent

A written informed consent was taken from each study participant before being included in the study.

References

1. Akaltun, M. S., Altindag, O., Turan, N., et al., (2019). Can blood parameters be guiding in fibromyalgia syndrome? *Annals of Medical Research* DOI: 10.5455/annalsmedres.2019.05.273.

2. Tezel, N., & Gültuna, S. (2020). Comparisons of neutrophil, monocyte, eosinophil, basophil and lymphocyte ratios among the fibromyalgia syndrome and healthy individuals: Pro-inflammatory blood cell markers in fibromyalgia. *Med. Sc. & Dis.*, 7(4), 455–458.

3. Al-Nimer MSM, Mohammad TAM (2018). Correlation of hematological indices and ratios derived from them with FIQR scores in fibromyalgia. *Pakistan J. Med. Sc.* 34(5). https://doi.org/10.12669/pjms.345.15169.

4. Zareifar, S., Farahmand Far, M. R., Golfeshan, F., et al., (2014). Changes in Platelet Count and Mean Platelet Volume during Infectious and Inflammatory Disease and Their Correlation with ESR and CRP: Platelet Count and MPV in Infectious and Inflammatory Diseases. *J. Clin. Lab. Analy.* 28(3), 245–248. https://doi.org/10.1002 /jcla.21673.

5. Zahorec R, (2021). Neutrophil-to-lymphocyte ratio, past, present and future perspectives. *Bratisl Lek Listy.*; 122(7):474-488. doi: 10.4149/BLL_2021_078. PMID: 34161115.

6. Peng YF, Huang YX, Wei YS, (2016). Altered mean platelet volume in patients with polymyositis and its

association with disease severity. *Braz. J. Med. & Biol. Rese.* 49(6): e5168. DOI: 10.1590/1414-431x20165168. PMID: 27191605; PMCID: PMC4869824.

7. Wang WM, Wu C, Gao YM, et al. (2021). Neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, and other hematological parameters in psoriasis patients. *BMC Immunol*, Sep 26; 22(1):64. doi: 10.1186/s12865-021-00454-4. PMID: 34565327; PMCID: PMC8474773.

8. Wolfe, F., Clauw, D. J., Fitzcharles, M.-A., et al., (2010). The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. *Arth. Care & Res.*, 62(5), 600–610.

9. Bennett, R. M., Friend, R., Jones, K. D., et al., (2009). The revised fibromyalgia impact questionnaire (FIQR): Validation and psychometric properties. *Arth. Res. & Ther.* 11(4), 1–14.

10. Williams, J. B. W., & Kobak, K. A. (2008). Development and reliability of a structured interview guide for the Montgomery-Åsberg Depression Rating Scale (SIGMA). *British J. Psychiatry,* 192(1), 52–58. https://doi.org/10.1192/bjp.bp.106.032532.

11. Hallit, S., Obeid, S., El Hage, W., et al. (2019). Validation of the Arabic version of the MADRS scale among Lebanese patients with depression. L'Encéphale, 45(3), 195–199. https://doi.org/ 10.1016/j.encep.2018.05.004.

12. Müller, M. J., Szegedi, A., Wetzel, H., et al. (2000). Moderate and severe depression. *J. Affect. Dis.* 60(2), 137–140. https://doi.org/10.1016/S0165-0327 (99)00162-7.

13. Alende-Castro V, Alonso-Sampedro M, Vazquez-Temprano N, et al., (2019). Factors influencing erythrocyte sedimentation rate in adults: New evidence for an old test. *Medicine (Baltimore).* Aug; 98(34): e16816. doi: 10.1097/MD.0000000000016816. PMID: 31441853; PMCID: PMC6716712.

14. Luyendyk JP, Schoenecker JG, Flick MJ, (2019). The multifaceted role of fibrinogen in tissue injury and inflammation. *Blood*. Feb 7; 133(6):511-520. doi: 10.1182/blood-2018-07-818211. Epub 2018 Dec 6. PMID: 30523120; PMCID: PMC6367649.

15. Del Giudice, Marco, and Steven W. Gangestad. (2018). "Rethinking IL-6 and CRP: Why They Are More than Inflammatory Biomarkers, and Why It Matters." *Brain, Behavior, and Immunity* 70:61–75.

16. Sproston, Nicola R., and Jason J. Ashworth. (2018). "Role of C-Reactive Protein at Sites of Inflammation and Infection." *Frontiers in Immunol.* 9:754.

17. Pogorzelska K, Krętowska A, Krawczuk-Rybak M, et al., (2020). Characteristics of platelet indices and their prognostic significance in selected medical condition - a systematic review. *Adv Med Sci*. Sep; n 65(2):310-315. doi: 10.1016/j.advms.2020.05.002. Epub 2020 Jun 4. PMID: 32505856.

18. Molina, Francisco, María Luisa Del Moral, Mercedes La Rubia, et al., (2019). "Are Patients with Fibromyalgia in a Prothrombotic State?" *Biol. Res. for Nursing* 21(2):224–30.

19. Karataş, G., & Gündüz, R. (2020). The Significance of inflammation markers in complete blood count in patients with fibromyalgia. *Med. Sc. & Dis.* 7(1), 364–367.

20. Budak, Yasemin Ustundag, Murat Polat, et al., (2016). "The Use of Platelet Indices, Plateletcrit, Mean Platelet Volume and Platelet Distribution Width in Emergency Non-Traumatic Abdominal Surgery: A Systematic Review." *Biochemia Medica* 26(2):178–93.

21. Li B, Lu J, Peng DZ et al., (2020). Elevated platelet distribution width predicts poor prognosis in hilar cholangiocarcinoma. *Medicine (Baltimore).* Mar; 99(12): e19400. doi: 10.1097/MD.0000000000019400. PMID: 32195935; PMCID: PMC7220385.

22. Nowakowski AC, (2014). Chronic inflammation and quality of life in older adults: a cross-sectional study using biomarkers to predict emotional and relational outcomes. *Health Qual Life Outcomes.* Sep 28; 12:141. doi: 10.1186/s12955-014-0141-0. PMID: 25260501; PMCID: PMC4189208.

23. Collado-Mateo D, Dominguez-Muñoz FJ, Adsuar JC, et al., (2017). Effects of Exergames on Quality of Life, Pain, and Disease Effect in Women With Fibromyalgia: A Randomized Controlled Trial. *Arch Phys Med Rehabil*. 2017 Sep;98(9):1725-1731. doi: 10.1016/j.apmr.2017.02.011. Epub 2017 Mar 18. PMID: 28322760.

24. Euteneuer F, Neuert M, Salzmann S, et al., (2022). Does psychological treatment of major depression reduce cardiac risk biomarkers? An exploratory randomized controlled trial. *Psychol Med*. Mar 2:1-15. doi: 10.1017/S0033291722000447. Epub ahead of print. PMID: 35232509.

25. Beurel E, Toups M, Nemeroff CB, (2020). The Bidirectional Relationship of Depression and Inflammation: Double Trouble. *Neuron*. Jul 22; 107(2):234-256. doi: 10.1016/j.neuron.2020.06.002. Epub 2020 Jun 17. PMID: 32553197; PMCID: PMC7381373.