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Significance of inflammation-related markers and histopathological features in mitral valve regurgitation

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Abstract

The lymphocyte-to-monocyte ratio (LMR), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), platelet-to-neutrophil ratio (PNR), C-reactive protein (CRP)-to-lymphocyte ratio (CLR) and fibrinogen-to-albumin ratio (FAR) are well-known indicators of the systemic inflammatory response (SIR). Less is known about the association of SIR with the echocardiographic parameters and the histopathological (HP) aspects of the mitral valve in patients who have undergone cardiac surgery to repair or replace the mitral valve. Information on serum parameters, transesophageal echocardiography findings, and HP results was obtained from 166 patients who had undergone cardiac surgery to address mitral valve regurgitation. Among these patients, 30 were diagnosed with mitral valve prolapse, with 15 cases showing mitral valve flail or *chordae* rupture. The possible association between SIR, echocardiographic aspects of mitral valve flail and the HP aspect was checked. Fibrosis, hyalinization and myxoid degeneration of the valve were scored under microscope. Hyalinization of the mitral valve had a significant positive association with LMR and PLR (p=0.041 and p=0.03, respectively) and with NLR (p=0.093). A higher fibrosis degree was present in the valves without flail compared with those with flail (p=0.000). The monocyte average values of the group without flail were statistically significantly higher than those in the flail group (p=0.029). An increase of one unit in the value of monocytes was found to decrease the chances of flail [odds ratio (OR) 0.017, p=0.068, significant at p<0.1 level]. SIR parameters can be used to appraise inflammation status in mitral valve disease and to establish the risk of *chordae* rupture/flail in the case of mitral valve prolapse.

Keywords: lymphocyte-to-monocyte ratio, C-reactive protein-to-lymphocyte ratio, neutrophile-to-lymphocyte ratio, platelet-to-lymphocyte ratio, fibrinogen-to-albumin ratio, platelet-to-neutrophil ratio.

Introduction

Certain markers of the systemic inflammatory response (SIR), including the lymphocyte-to-monocyte ratio (LMR), C-reactive protein (CRP)-to-lymphocyte ratio (CLR), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), platelet-to-neutrophil ratio (PNR), and fibrinogen-to-albumin ratio (FAR), are recognized for their impact on the structure and function of the mitral valve. However, the exact mechanism is currently not understood [1–3].

Many studies have associated the presence of inflammatory factors such as CRP with an increased risk of heart disease [4]. Lymphocytes are crucial to the adaptive immune response, enabling the body to recognize and respond to specific threats. CLR is a well-known indicator of poor prognosis in malignant tumors associated with sustained inflammation [5–8], but recent works have proved that it has a predictive value in heart disease [9]. Inflammatory indicators such as NLR are known to be increased in rheumatic mitral valve disease (RMVD), which is the most feared complication of rheumatic fever. In RMVD, NLR is higher because of a decreased lymphocyte count [10, 11]. Monocytes, traditionally known for their role as phagocytes within the immune system, have recently been found to be involved in both valve development and related diseases, according to recent research [12]. Additionally, the LMR, NLR, and PNR have been shown to be affected in patients with mitral valve prolapse, with an increase in NLR and PLR and a decrease in LMR compared to the control group [3].

As far as we are aware, no studies exist on the relationship between SIR and the histopathological (HP) characteristics of the mitral valve. All six parameters which reflect SIR (LMR, NLR, PLR, PNR, CLR and FAR) were also checked in the presence or absence of flail. In patients with mitral valve prolapse, a possible connection between SIR and the echocardiographic and HP aspects of

the mitral valve might be useful to predict evolution and status of the valve. We also analyzed the importance of serum levels of monocytes in the clinical evolution of the patients.

Aim

The aim of the present study was to identify the possible association between SIR and HP changes identified in the mitral valves of patients who receive surgery for mitral regurgitation (MR). Establishing such a correlation might help us to estimate preoperatively, based on SIR parameters, the valvular status, and hemodynamic consequences of mitral valve replacement or valvuloplasty. The role of inflammatory markers in predicting the evolution of mitral valve disease in patients with mitral valve prolapse was also checked.

Patients, Materials and Methods

Data collection

We enrolled 166 individuals who underwent cardiac surgery for mitral valve regurgitation at the Emergency Institute for Cardiovascular Diseases and Transplantation, Târgu Mureş, Romania, between 2019 and 2022. For all patients, transesophageal echocardiography was conducted in the operating room prior to surgery. This assessment focused on the mitral valve's structure, left ventricular ejection fraction (LVEF), and heart chamber dimensions. Only those patients diagnosed with mitral valve prolapse as the cause of regurgitation were included in the study. Data were extracted from hospital records, which provided demographic details like age, gender, and coexisting conditions such as systemic hypertension, diabetes, pulmonary diseases, and stroke. Exclusion criteria included individuals over 80 years of age, those suffering from chronic inflammatory conditions (like rheumatoid arthritis, Crohn's disease, or systemic vasculitis), active infections – including acute endocarditis – or malignant tumors. HP analysis was performed on the mitral valve samples after partial or complete resection.

The study received ethical approval from the Ethics Committee of the Emergency County Clinical Hospital, Târgu Mureş (Approval No. 2380/09.06.2023). All patients provided written informed consent to participate in this research.

Laboratory data

The red blood cell (RBC) count, white blood cell (WBC) level, lymphocyte-, monocyte-, neutrophil-, platelet-, CRP-, fibrinogen- and albumin-serum levels were recorded preoperatively on day of admission after at least eight hours of fasting. The cardiac surgery intervention was scheduled for the next day. For the measurement of the WBC level, an automatic hematology analyzer with fluorescence flow cytometry was used (Sysmex XS-800; Sysmex). The CRP levels were analyzed using a clinical chemistry analyzer with fluorescence technology (Architect c4000; Abbott). The LMR, NLR, PLR, PNR, CLR and FAR were calculated. During cardiac surgery, the mitral valve was collected for further examination in the histopathology lab.

Statistical analysis

We conducted a comprehensive statistical analysis using Stata 18 MP-4 Statistical Package 2023 (StataCorp LLC, College Station, TX, USA). To examine the association between the response variables and indicators, we employed multinomial logistic regression, reporting the relative risk ratio (RRR), logistic regression, the odds ratio (OR) and linear regression. The validation parameters for assessing the significance of the models and the predictors included a p-value of <0.05.

Echocardiography

The parameters followed in echocardiography were leaflet morphology and motion, sub-valvular involvement, annulus size and aspect, left ventricle (LV) and left atrium (LA) size and function, LVEF and severity of MR. For the echocardiographic description of the mitral valve aspect, we used the Carpentier classification of MR: type I – valves with normal mobility (dilation of the mitral annulus), type II – valves with excessive mobility (prolapse, rupture of cords) and type III – valves with restricted movement [13]. The mitral valve "prolapse" is defined as a systolic displacement of the mitral leaflet at least 2–3 mm above the annular plane and is best diagnosed in long axis, parasternal view. If the free edge of the leaflet is observed in the LA, then we diagnosed a "flail" leaflet. In prolapse and flail leaflets (type II), the regurgitation jet, seen with a color Doppler, is oriented towards the opposite wall of the LA [14]. In our study, the valve with *chordae* rupture is referred to as the flail valve. If the movement of one of the mitral leaflets is restricted, the regurgitation jet, seen with a color Doppler, is oriented towards the same side wall of the LA [15]. For the severity of the mitral valve regurgitation, we evaluated the effective regurgitant orifice area (EROA), regurgitant volume (RVol) and regurgitant fraction (RF), which is the percentage of MR volume relative to total LV stroke volume [15–17]. All patients had severe MR, which was the main reason for cardiac surgery.

Microscopic assessment

All cases were microscopically analyzed using sections stained with both Hematoxylin–Eosin (HE), which allowed the assessment of the overall morphology, and with Masson's trichrome (MT), for the assessment of the degree of fibrosis/hyalinization. Fibrosis and hyalinization were counted on classical HE and using the MT stains, based on the degree of fibrosa layer expansion and *spongiosa* layer collapse, as follows (Figure 1, A–F; Figure 2, A–D): grade 1 – mild fibrosis/hyalinization/myxoid degeneration, with discrete degenerative changes, seen at intermediate (100×) or high magnification (200×); grade 2 – moderate fibrosis/hyalinization/myxoid degeneration seen at intermediate magnification (100×); grade 3 – severe fibrosis/hyalinization/myxoid degeneration seen at low magnification (40×). Based on these aspects, we considered all cases with grade 1 fibrosis/hyalinization/myxoid degeneration as cases with low degenerative changes and all cases with grade 2 and 3 fibrosis/hyalinization/myxoid degeneration as cases with high degenerative changes. These degenerative changes were graded individually by three pathologists with experience in cardiovascular pathology (IJ, CBS, SG), with a concordance of 90%. The two divergent assessments were reanalyzed for a final, mutually accepted diagnosis.

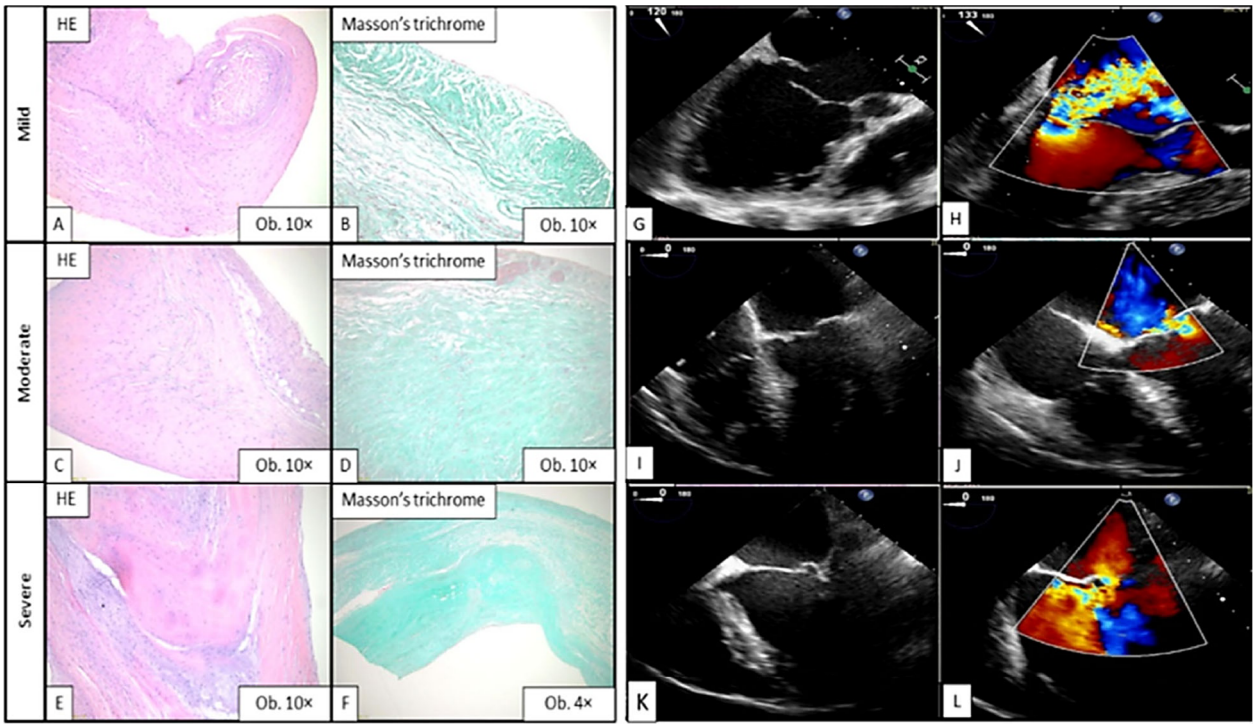

Figure 1 – *Valvular fibrosis. Left: Mitral valve showing mild (A and B), moderate (C and D) and severe (E and F) degenerative changes, mainly due to fibrosis, with fibrous layer expansion and spongiotic layer collapse. Right: Transesophageal echocardiography (2D and color Doppler) of the mitral valve of the same patients showing flail of the mitral valve (G and H) and prolapsed mitral valve without flail (I–L). 2D: Two-dimensional; HE: Hematoxylin–Eosin.*

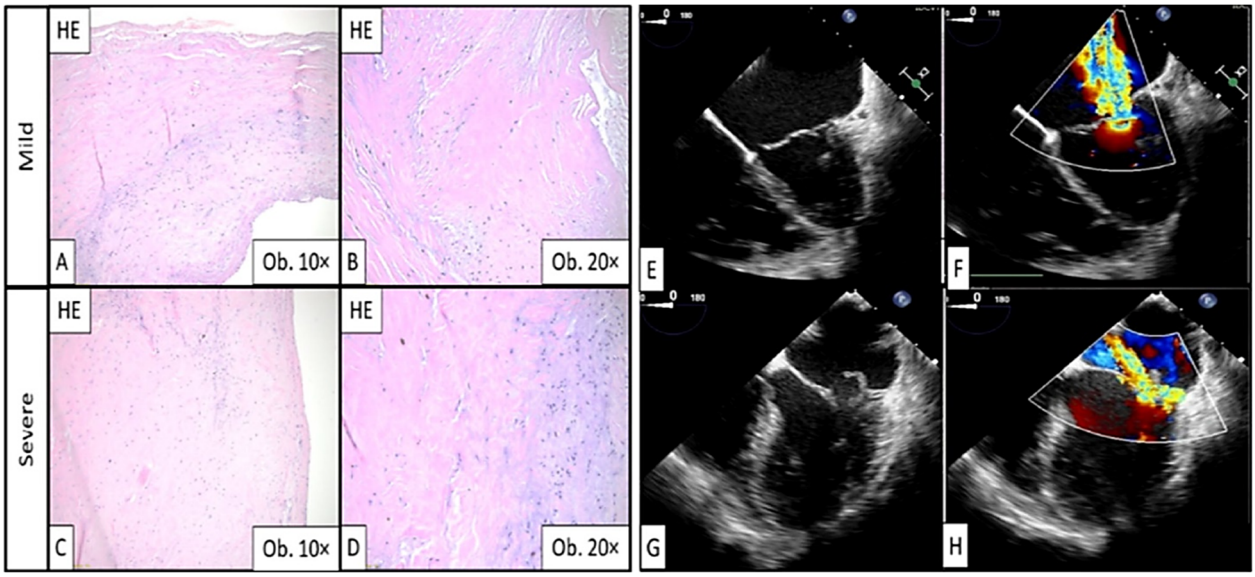

Figure 2 – *Hyalinization of the valves. Left: Mitral valve showing mild (A and B) and severe (C and D) degenerative changes. Both examples show spongiotic layer collapse and fibrous layer expansion from different degrees of hyalinization. Right: Transesophageal echocardiography of the mitral valve of the same patients showing prolapsed mitral valve without flail (E and F) and with flail (G and H). HE: Hematoxylin–Eosin.*

Results

Clinicopathological features associated with mitral regurgitation

Data from 166 patients with mitral valve regurgitation were analyzed. The majority of patients were male (63.5%) and over the age of 60 (63.86%), with the overall cohort's ages ranging from 20 to 76 years. HP changes of the mitral valve were available in 113/166 patients.

In 110 (66.27%) patients, HP analysis revealed fibrosis [24 (14.46%) with low-degree fibrosis and 86 (51.81%) with high-degree fibrosis], in 104 (62.65%) cases hyalinization [35 (21.08%) with low-degree hyalinization and 69 (41.57%) with high-degree hyalinization], and in 107 (64.46%) patients myxoid degeneration [35 (21.08%) with low-degree myxoid degeneration and 72 (43.37%) with high-degree myxoid degeneration].

The relationship between inflammatory markers (LMR, CLR, NLR, PLR, PNR and FAR) and HP changes (fibrosis, hyalinization and myxoid) was further analyzed and presented

in Table 1. In the case of fibrosis severity analysis, a higher CLR value decreased the chances of severe fibrosis (OR 0.026, p=0.066). One unit increase in CLR value reduced the odds of high severity fibrosis of the mitral valve. For the severity of myxoid degeneration analysis, an increased value of PLR indicates increased chances for severe myxoid degeneration (OR 1.08, p=0.07, significant at p<0.1 level). An increased value of PNR indicates lower chances for severe myxoid degeneration (OR 0.90, p=0.095, significant at p<0.1 level). An increased value of FAR indicates low chances of severe myxoid degeneration (OR 0.95, p=0.058).

We compared the mean value of inflammatory parameters in cases of low and high degree of HP changes. Statistically significant results are presented in Table 2. An increased LMR value decreased the chances of severe fibrosis [OR 0.68 (0.44–1.02), p=0.068]. A t-test showed that the average LMR was higher in the group with less severe fibrosis than in the group with severe fibrosis (p=0.0315). A significant value in the univariate analysis was found only in the case of monocytes compared with the severity of hyalinization. An increased value of monocytes significantly increased the chances of severe hyalinization [OR 7.80 (0.93–65.22), p=0.058]. A t-test showed that the mean value of monocytes in the group with less severe hyalinization was significantly lower than in the group with severe hyalinization (p=0.026). From the indicators of SIR studied, age appeared to be a significant indicator only for LMR. Increasing the age by one unit (*i.e.*, one year) showed a decrease in LMR value by approximately 0.018 (p=0.036). Participants aged 60 and above displayed a decrease in LMR value by 0.54 (p=0.004). The values of the other of the monitored indicators of SIR seemed to be resilient to age and their variation was not associated with age.

Table 1 – ***Logistic regression analysis to investigate the influence of inflammatory markers on the histopathological exam results expressed as high severity***

Fibrosis – severity: High	**Adj-OR**	***p>z***	**95% CI**	**Crude-OR**	***p>z***	**95% CI**
LMR	1.236124	0.838	0.161885–9.438813	0.6796823	0.068	0.4490486–1.028771
CLR	0.026167	0.066	0.000541–1.264589	0.8172535	0.712	0.2799948–2.385413
NLR	4.381537	0.381	0.160431–119.6647	1.3009280	0.208	0.8634719–1.960011
PLR	0.984444	0.672	0.915579–1.058487	1.0065160	0.214	0.9962574–1.01688
PNR	1.132023	0.250	0.916463–1.398286	0.9909183	0.483	0.9659877–1.016492
FAR	1.006982	0.798	0.954760–1.062060	1.0080150	0.559	0.9813881–1.035365
Monocytes	6.526683	0.620	0.003922–10861.89	3.7384040	0.244	0.4058579–34.43487
Hyalinization – severity: High	**Adj-OR**	***p>z***	**95% CI**	**Crude-OR**	***p>z***	**95% CI**
LMR	2.134951	0.326	0.469471–9.708839	1.143941	0.494	0.777912–1.682198
CLR	0.340239	0.446	0.021304–5.433774	1.692366	0.440	0.445682–6.426336
NLR	1.628378	0.575	0.295874–8.961975	0.7927842	0.121	0.591215–1.063077
PLR	0.998302	0.936	0.957624–1.040708	0.9946308	0.179	0.986854–1.002469
PNR	1.008470	0.822	0.937181–1.085182	1.005633	0.628	0.983039–1.028746
FAR	1.004738	0.825	0.963584–1.047649	1.008866	0.476	0.984669–1.033657
Monocytes	270.3985	0.134	0.177626–411625.5	7.794882	0.058	0.931615–65.22032
Myxomatous degeneration – severity: High	**Adj-OR**	***p>z***	**95% CI**	**Crude-OR**	***p>z***	**95% CI**
LMR	3.530233	0.232	0.446664–27.90136	1.29767	0.197	0.873739–1.927290
CLR	119.7388	0.184	0.103176–138960.0	5.478831	0.261	0.281635–106.5832
NLR	0.188885	0.183	0.016251–2.195454	0.885941	0.407	0.665292–1.179769
PLR	1.080728	0.070	0.993659–1.175427	1.000058	0.988	0.992253–1.007924
PNR	0.903903	0.095	0.802699–1.017867	0.998614	0.898	0.977572–1.020108
FAR	0.956957	0.058	0.9144–1.001494	0.991838	0.480	0.969523–1.014667
Monocytes	18.611570	0.434	0.012212–28365.6	0.382518	0.285	0.065661–2.228427

Adj-OR: Adjusted odds ratio; CI: Confidence interval; CLR: C-reactive protein (CRP)-to-lymphocyte ratio; FAR: Fibrinogen-to-albumin ratio; LMR: Lymphocyte-to-monocyte ratio; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; PNR: Platelet-to-neutrophil ratio; OR: Odds ratio.

Table 2 – ***Relationship between the inflammatory markers and histopathological changes of the mitral valve using two-sample t-tests with equal variances (statistically significant results)***

						Hypothesis		
	Severity	***Mean***	***SE***	***SD***	***95% CI***	***Ha: diff<0***	***Ha: diff !=0***	***Ha: diff > 0***
Fibrosis						Pr(T<*t*)	Pr(T<*t*)	Pr(T<*t*)
LMR	Low	3.376958	0.236226	1.157265	2.888288–3.865628	0.9685	0.0631	0.0315
	High	2.905133	0.116591	1.062194	2.673196–3.137069			
Hyalinization						Pr(T<*t*)	Pr(T<*t*)	Pr(T<*t*)
NLR	Low	2.908971	0.294861	1.744418	2.309743–3.50820	0.9468	0.1064	0.0532
	High	2.422545	0.151380	1.229813	2.12022–2.724871			
PLR	Low	130.0442	10.60365	61.82935	108.4709–151.6175	0.9146	0.1708	0.0854
	High	114.7960	5.756533	47.11925	103.3027–126.2893			
Monocytes	Low	0.609429	0.022027	0.130315	0.564664–0.654193	0.026	0.052	0.974
	High	0.704242	0.033040	0.268420	0.638257–0.770228			

						Hypothesis		
	Severity	**Mean**	**SE**	**SD**	**95% CI**	**Ha: diff<0**	**Ha: diff !=0**	**Ha: diff > 0**
Myxomatous degeneration						Pr(T<*t*)	Pr(T<*t*)	Pr(T<*t*)
LMR	Low	2.82980	0.177624	1.050838	2.468825–3.190775	0.0982	0.1964	0.9018
	High	3.122928	0.132861	1.103630	2.857807–3.388048			
CLR	Low	0.14940	0.025571	0.114355	0.095880–0.202920	0.0879	0.1757	0.9121
	High	0.417385	0.138779	0.866675	0.136441–0.698328			

CI: Confidence interval; CLR: C-reactive protein (CRP)-to-lymphocyte ratio; LMR: Lymphocyte-to-monocyte ratio; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SD: Standard deviation; SE: Standard error. Mean (SD): *p*-value from a pooled *t*-test. Frequency (%): *p*-value from Pearson's test.

Correlation between the inflammatory markers and the mitral valve prolapse

Of the 166 patients, 30 presented with mitral valve prolapse, most of whom (22; 73.33%) were male. Of these patients, 15 presented with flail of the mitral valve (two females and 13 males). The HP exam was available for 21 patients and showed different degrees of fibrosis, hyalinization and myxoid degeneration, which we classified as low (1+) and high (2+, 3+) degree (Table 3). Contingency tables and the Fisher's exact test were applied to examine if a significant relationship existed between the prolapse group and the severity determined by HP analysis. Results showed that there was a dependent relationship between the two variables, with the Fisher's exact score for fibrosis (p=0.017), hyalinization (p=0.000) and myxoid degeneration (p=0.040) being statistically significant. We then applied logistic regression analysis to investigate the connection between the mitral valve prolapse observed in echocardiography and the aspect of the valve revealed after the HP exam. The following results were obtained: fibrosis [OR 0.27 (0.09–0.75), p=0.013], hyalinization [OR 0.02 (0.005–0.119), p=0.00] and myxoid degeneration [OR 0.35 (0.13–0.93), p=0.036]. In all cases, the prolapse indicated lower chances for increased severity of the observed condition. Thus, the presence of prolapse indicated low chances of severe HP changes.

The association between the inflammatory markers and the presence of mitral valve flail

We analyzed the association between the inflammatory markers and the presence of mitral valve flail in echocardiography using a two-sample *t*-test with equal variances. For the group without flail, the mean of LMR preoperatory value was approximately 2.67, with a standard deviation (SD) of 0.85. For the group with flail, the mean of LMR preoperatory value was approximately 3.29, with a SD of 0.99. The combined mean for both groups was 2.98. For the two-tailed *t*-test, a *p*-value of 0.0797 indicated that the difference in means was marginally significant (p<0.1). For the one-tailed *t*-test, a *p*-value of 0.0399 suggested that the mean for the group without flail was statistically significantly lower than for the group with flail (p<0.05). The mean value of LMR for the group without flail was lower than the group with flail, which was statistically significant at the 0.05 level in a one-tailed test (assuming our hypothesis was that the mean for group without flail would be lower).

Table 3 – ***Clinicopathological parameters of patients with mitral valve prolapse***

Clinicopathological parameters		**Flail**		**Test**
		No	***Yes***	
n (%)		15 (50.0%)	15 (50.0%)	
Age, mean (SD) [years]		57.667 (15.041)	54.467 (11.376)	0.516
Gender, n (%)				
▪ Female		6 (40.0%)	2 (13.3%)	0.099
▪ Male		9 (60.0%)	13 (86.7%)	
Blood tests, mean (SD)				
▪ Lymphocytes [×10^3/μL]		1.731 (0.424)	1.796 (0.467)	0.694
▪ Monocytes [×10^3/μL]		0.701 (0.243)	0.563 (0.118)	0.058
▪ Neutrophils [×10^3/μL]		5.010 (2.594)	4.337 (1.125)	0.364
▪ Platelets [×10^3/μL]		214.400 (68.312)	195.067 (49.992)	0.384
▪ CRP [mg/dL]		1.183 (2.705)	0.136 (0.073)	0.262
▪ Fibrinogen [mg/dL]		382.736 (161.913)	309.607 (71.362)	0.131
▪ Albumin [g/dL]		4.250 (0.384)	4.389 (0.454)	0.480
Inflammatory markers, mean (SD)				
▪ LMR		2.675 (0.858)	3.290 (0.992)	0.080
▪ NLR		1.731 (0.424)	1.796 (0.467)	0.694
▪ PLR		125.826 (33.245)	112.943 (29.278)	0.270
▪ PNR		50.382 (23.650)	46.429 (13.574)	0.579
▪ CLR		0.826 (2.021)	0.095 (0.099)	0.293
▪ FAR		79.695 (25.526)	76.525 (12.823)	0.744
Fibrosis degree, n (%)				
1+	Low	0 (0.0%)	8 (80.0%)	<0.001
2+	High	1 (9.1%)	0 (0.0%)	
3+		10 (90.9%)	2 (20.0%)	
Hyalinization degree, n (%)				
1+	Low	7 (63.6%)	3 (30.0%)	0.159
2+	High	4 (36.4%)	5 (50.0%)	
3+		0 (0.0%)	2 (20.0%)	
Myxoid degeneration degree, n (%)				
1+	Low	1 (9.1%)	0 (0.0%)	0.416
2+	High	4 (36.4%)	6 (60.0%)	
3+		6 (54.5%)	4 (40.0%)	

CLR: C-reactive protein (CRP)-to-lymphocyte ratio; FAR: Fibrinogen-to-albumin ratio; LMR: Lymphocyte-to-monocyte ratio; *n*: No. of cases; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; PNR: Platelet-to-neutrophil ratio; SD: Standard deviation. Mean (SD): *p*-value from a pooled *t*-test. Frequency (%): *p*-value from Pearson's test.

We then explored the influence of the components on the LMR ratio. Lymphocytes did not have a significant influence on flail, but monocytes did. The average monocyte value in the flail group was 0.563, compared to 0.7 in the group without flail, which was statistically significant (Ha: diff !=0, p=0.0579), one-tailed test (Ha: diff>0, p=0.029). This result was expected, as the variation of LMR is directly proportionate to the value of lymphocytes and inversely proportionate to the value of monocytes. Using logistic regression to further analyze this relationship, we observed that an increase of one unit in the value of monocytes decreased the chances of flail [OR 0.017 (1.83e–1.605), p=0.068, significant at p<0.1 level].

The influence of NLR, PLR, PNR, CLR and FAR on the presence of flail was also analyzed and did not show any significant results (Table 4).

Table 4 – ***Inflammatory markers in patients with mitral valve prolapse, with or without flail using logistic regression***

Variables	Univariate analysis		
	p-value	*Crude-OR*	*95% CI*
LMR	0.091	2.140	0.886–5.207
NLR	0.683	1.410	0.266–7.523
PLR	0.264	0.980	0.961–1.010
PNR	0.566	0.990	0.950–1.028
CLR	0.697	0.160	–
FAR	0.726	0.990	0.944–1.040
Monocytes	0.068	0.002	0–1.605

CI: Confidence interval; CLR: C-reactive protein (CRP)-to-lymphocyte ratio; FAR: Fibrinogen-to-albumin ratio; LMR: Lymphocyte-to-monocyte ratio; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; PNR: Platelet-to-neutrophil ratio; OR: Odds ratio.

Correlation between the presence of mitral valve flail evaluated in echocardiography and the histopathological data

As there were only 21 cases, we used the Fisher's exact test for small groups, followed by logistic regression. The p-value from the Fisher's exact test was 0.000, indicating a statistically significant association between flail and fibrosis at the conventional 0.05 level. Specifically, individuals with mitral valve flail seemed more likely to have a low degree of fibrosis (first degree), while those without flail seemed more likely to have a high degree of fibrosis (third degree) (Figure 1, G–L). We then checked the association using logistic regression. For an increase of one unit in the value recorded for fibrosis, the chances of flail decreased [OR 0.08 (0.130–0.532), p=0.009]. The HP aspect of fibrosis and the echocardiographic aspect of the mitral valve in the same patients are presented in Figure 1 (A–L).

We also used logistic regression to investigate the association between hyalinization and flail. However, at the p<0.1 level, the logistic regression indicates that an increase by one unit of the degree of hyalinization increases the chances of flail [OR 4.2 (0.849–20.823), p=0.078]. The HP aspect of hyalinization and the echocardiographic aspect of the mitral valve in the same patients are presented in Figure 2 (A–H). There was no statistically significant association between flail and myxoid degeneration.

In the above cases, we reported crude ORs following the 1:1 association (flail with each, separately). As patients can have several of these conditions simultaneously, it is also interesting to see their combined influence on flail. We ran the logistic regression again using all three variables as predictors (Table 5). Among the predictors in the full model, only fibrosis showed a statistically significant relationship with flail. Specifically, increased values of fibrosis (severity) were associated with decreased odds of flail being present [OR 0.08 (0.096–0.711), p=0.023]. Hyalinization and myxoid degeneration did not show significant relationships with flail at the conventional 0.05 level.

The relationship between the inflammatory markers and the HP aspect of the mitral valve was analyzed using linear regression (Table 6). Hyalinization showed a significant positive association with LMR. For every unit increase in the degree of hyalinization, the expected value of LMR increased by approximately 0.5606 units (p=0.041). The model explained about 20.15% of the variability in LMR (model fit p=0.04, R^2 0.2015).

Table 5 – ***Correlation between mitral flail and presence of fibrosis, hyalinization and myxoid degeneration in histopathological exam using logistic regression***

Flail	OR	SE	*z*	*p>|z|*	95% CI
Fibrosis	0.082952	0.0909641	-2.27	0.023	0.0096697–0.7116117
Hyalinization	3.383246	4.96651	0.83	0.406	0.1904557–60.09984
Myxoid degeneration	0.6333681	0.7661184	-0.38	0.706	0.0591627–6.780546

CI: Confidence interval; OR: Odds ratio; SE: Standard error.

Table 6 – ***The relationship between LMR, NLR, PLR, PNR, CLR and FAR, and the presence of fibrosis, hyalinization and myxoid degeneration in histopathological exam of the mitral valve using linear regression***

Parameter	Coefficient	SE	*t*	*p>t*	95% CI
LMR					
Fibrosis	-0.2717698	0.1852234	-1.47	0.159	-0.65945–0.115907
Hyalinization	0.5605957	0.2560044	2.19	0.041	0.024772–1.096419
Myxoid degeneration	-0.15330	0.3188025	-0.48	0.636	-0.82056–0.513961
NLR					
Fibrosis	-0.0403713	0.0977935	-0.41	0.684	-0.24506–0.164313
Hyalinization	0.2360638	0.1334274	1.77	0.093	-0.0432–0.515331
Myxoid degeneration	-0.14280	0.1578469	-0.9	0.377	-0.47318–0.187577

PLR					
Fibrosis	4.914057	6.610142	0.74	0.466	-8.92113–18.74924
Hyalinization	-20.35639	8.649690	-2.35	0.030	-38.4604 – -2.25238
Myxoid degeneration	15.0130	10.45193	1.44	0.167	-6.86315–36.88915
PNR					
Fibrosis	-0.0693614	4.075797	-0.02	0.987	-8.6001–8.461381
Hyalinization	-0.6057872	5.973242	-0.10	0.920	-13.1079–11.89635
Myxoid degeneration	-0.89864	6.685818	-0.13	0.894	-14.8922–13.09494
CLR					
Fibrosis	0.0551429	0.0450243	1.22	0.260	-0.05132–0.161609
Hyalinization	-0.03335	0.0820581	-0.41	0.697	-0.22739–0.160687
Myxoid degeneration	-0.03335	0.0820581	-0.41	0.697	-0.22739–0.160687
FAR					
Fibrosis	3.05380	6.293678	0.49	0.637	-10.7985–16.90609
Hyalinization	2.740044	9.721789	0.28	0.783	-18.6575–24.13756
Myxoid degeneration	-14.97305	11.56483	-1.29	0.222	-40.4271–10.48097

CI: Confidence interval; CLR: C-reactive protein (CRP)-to-lymphocyte ratio; FAR: Fibrinogen-to-albumin ratio; LMR: Lymphocyte-to-monocyte ratio; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; PNR: Platelet-to-neutrophil ratio; SE: Standard error.

Hyalinization also showed a significant positive association with NLR at the $p<0.1$ level. For every unit increase in the degree of hyalinization, the expected value of NLR increased by approximately 0.236 units ($p=0.093$). The model only explained about 14.14% of the variability in NLR (model fit $p=0.09$, R^2 0.1414).

A significant positive association was also shown between hyalinization and PLR. For every unit increase in the degree of hyalinization, the expected value of PLR decreased by approximately 20.35 units ($p=0.03$). The model explained about 22.5% of the variability in PLR (model fit $p=0.029$, R^2 0.2257). For PNR, CRL and FAR there were no significant associations.

Discussions

MR has different etiologies, and its mechanisms (primary or secondary) dictate the therapeutic approach. Primary MR is due to intrinsic valve pathology. Secondary mitral valve regurgitation occurs when the normal structure of the valve is altered due to dysfunction in the LV and the sub-valvular mitral apparatus. From a histological perspective, the mitral valve leaflets are made up of four distinct layers, arranged from the atrial side to the ventricular side. These layers include the *atrialis* (or *auricularis*), *spongiosa*, *fibrosa*, and *ventricularis*. The *atrialis*, located on the surface closest to the atrium, primarily consists of elastic fibers intertwined with collagen fibers and smooth muscle cells. Beneath this, the *spongiosa* forms a sparse cellular layer of loose connective tissue, containing scattered elastic and collagen fibers, and is rich in glycosaminoglycans (GAGs), such as chondroitin sulfates, heparin sulfates, and hyaluronic acid. The *atrialis* covers the *spongiosa* only at the proximal third of the valve near the fibrous ring (*annulus fibrosus*), where the *spongiosa* may contain myocytes and capillaries, possibly extending from the blood supply of the LA. Traditionally, it has been thought that the distal two-thirds of the mitral valve lack vascularization, but recent research suggests that capillaries may appear in the *spongiosa* layer, even in the absence of inflammation. The *fibrosa*, the most prominent layer, spans the full length of the leaflet from the *annulus fibrosus* to the free edge of the valve. This layer is composed of thick connective tissue arranged circumferentially, linking the fibrous ring to the papillary muscles. Due to its density and robust structure, the *fibrosa* provides structural support for the valve. While the *spongiosa* plays a role in cushioning the valve during the cardiac cycle, the *fibrosa* offers mechanical strength. The *ventricularis*, like the *atrialis*, only partially covers the valve, consisting mostly of elastic fibers mixed with loose collagen, and extends from the subendocardial layer of the LV [18–20].

The HP parameters observed in our study were hyalinization (with different aspects: nodules or plaques), fibrosis and myxoid degeneration. Various indicators of inflammation, including NLR, LMR, PNR, PLR, and FAR, are widely recognized as prognostic markers for conditions such as cancer, tuberculosis, and autoimmune disorders. Recently, attention has grown regarding their association with cardiovascular diseases [21–23]. Studies have revealed that immune cells account for 8% of the total cell population in remodeling heart valves [24].

In our study, the average LMR was higher in the group with less severe fibrosis than in the group with severe fibrosis, meaning that an increased LMR value decreases the chances of severe fibrosis. An increased value of PNR indicates reduced chances for severe myxoid degeneration and an increased value of PLR indicates increased chances for severe myxoid degeneration.

The role of inflammation in atherosclerosis has also been well described. CRP is a non-specific marker of inflammation that has been linked to the development and progression of vascular atherosclerosis. However, its levels can also be influenced by non-cardiac factors such as tissue damage, infections, and abnormal liver or kidney function. As an acute-phase protein, CRP plays a key role in the immune system by promoting complement activation, which enhances opsonization and phagocytosis. It is primarily produced by liver cells (hepatocytes), but recent research indicates that CRP can also be synthesized locally in atherosclerotic plaques by smooth muscle cells, macrophages, and endothelial cells in the aorta, as well as by neurons, kidneys, and alveolar macrophage [25, 26].

Lymphocytes are part of the immune system and have an important role in fighting bacteria, viruses, parasites and tumor cells. They play a central role in the adaptive immune response. CLR is a marker regarding the inflammatory and immune status of the body [9, 27]. However, when analyzing the CLR value in patients with mitral valve regurgitation, we observed that an increased CLR decreases the chances of severe fibrosis.

Monocytes and monocyte-derived macrophages are crucial in both the onset and progression of atherosclerosis. Lymphocytes, including T-cells, B-cells, and natural killer (NK) cells, are key components of the immune system. Lymphocytopenia has been associated with worse prognosis in patients suffering from heart failure with reduced ejection fraction [28]. Increased catecholamine and cortisol levels under stress may lead to excessive apoptosis of lymphocytes and lymphocytopenia [29–31].

In mitral valve prolapse, HP analysis shows different degrees of fibrosis in the LV wall, especially at the infero-basal wall and in the papillary muscle [32, 33]. These are maladaptive structural changes where elevated mechanical stress on these areas, combined with disrupted mitral annular motion, results in fibrosis in valve-related regions. This occurs independently of the volume overload caused by MR. Myocardial fibrosis may cause LV dysfunction after surgery [34–36]. The "gold standard" of myocardial fibrosis diagnosis is myocardial biopsy, which in most cases is very difficult to obtain [37–39]. An alternative option is cardiac magnetic resonance imaging, which is an expensive but more accessible for predicting myocardial fibrosis.

LMR, NLR and PNR have been shown to be affected in patients with mitral valve prolapse. In a study with 461 patients by Yalim & Ersoy, NLR and PNR were significantly higher and LMR was significantly lower in patients with mitral valve prolapse compared to the control group [3]. The variation of LMR is directly proportionate to the value of lymphocytes and inversely proportionate to the value of monocytes. In our study, the lymphocyte value was constant and the LMR differed because of the monocyte value.

Monocytes are components of the immune system with a traditional role of phagocytes, but recent studies have demonstrated their implication in valve development and disease [21]. By producing both inflammatory and anti-inflammatory cytokines, the monocytes' role in tissue inflammation and hemostasis is observed in endothelial regulation [40–42]. They are classified as classical, non-classical and intermediate. Classical monocytes have an inflammatory role, represent 80–90% of the circulating monocytes [43, 44]. Non-classical or anti-inflammatory monocytes contribute to the regulation of matrix remodeling and exhibit antifibrotic properties [45]. It is well known that the vessels are patrolled by intraluminal non-classical monocytes and that the damaged endothelium is a trigger for patrolling monocytes. They scavenge and repair the damaged tissue in the microvascular system and the big arteries. They have an endothelial protective role, which is crucial for endothelial integrity. Non-classical monocytes patrol healthy vessels, and their depletion aggravates endothelial damage [42, 46]. Intermediate monocytes have low phagocytic activity, and their role is not yet well established. The mitral valve contains endothelial and smooth cells at its root but the primary cells of the *spongiosa* of the valve are interstitial cells [43]. In the interstitium of the atrioventricular valves, there is normal, age-related monocyte recruitment. Monocytes are continually recruited to the valve *via* a homeostatic process [47, 48]. In aortic valve disease, macrophage/monocyte function turnover results in aberrant macrophage presence, which has an important role in the progression of valve disease [49]. Some studies have demonstrated that macrophage mediated inflammation may promote valve disease by producing interstitial valve cell calcification; this was found for the first-time by Li *et al. via in vitro* studies looking at macrophage derived products driving disease programming [50]. These are base repair mechanisms that can be beneficial in certain situations but may become pro-degenerative, altering normal tissue. In diseased valves, the presence of monocytes differentiated into inflammatory macrophages is well established. The macrophages' inflammatory mediators can drive the degeneration mechanism into the interstitial valve cells [43]. Our study found that an increased value of monocytes significantly increased the chances of severe hyalinization and the mean value of monocytes in the group with less severe hyalinization was significantly lower than in the group with severe hyalinization.

The presence of fibrosis reduced the risk of flail of the mitral valve. We can therefore conclude that a low LMR or a high monocyte count reduces the chance of mitral valve *chordae* rupture. The correlation between multiple inflammatory markers and the HP aspect of the mitral valve demonstrated that hyalinization has a significant positive association with LMR, NLR and PLR. For every unit increase in the degree of hyalinization, the expected values of LMR increased by approximately 0.5606 units of NLR increased by approximately 0.236 units and of PLR decreased by approximately 20.35 units. For PNR, CRL and FAR and there were no significant observations.

Study limitations

Our study was conducted with careful sample selection and analysis, yet, like all research, our results are subject to the limitations of the sample used. To further validate and generalize these results, this study should be replicated with multiple samples of patients.

Conclusions

The indicators of the SIR can be utilized to evaluate the inflammatory status in mitral valve disorders and to assess the likelihood of *chordae* rupture in patients with mitral valve prolapse. There is a relationship between the fibrosis degree and the odds of mitral valve flail: the higher the fibrosis degree, the lower the odds of mitral valve flail. The mean LMR and CLR values are higher in the group with low fibrosis and the lower the fibrosis severity in mitral valve prolapse, the higher the risk of mitral valve *chordae* rupture and flail. LMR and PLR are inflammatory markers significantly associated with the presence of hyalinization in the mitral valve. An increased value of PNR and FAR indicates lower chances of severe myxoid degeneration and an increased value of PLR indicates higher chances of severe myxoid degeneration. Increased monocyte count increases the chance of hyalinization found in a HP exam of the mitral valve and reduces the chance of flail. The results of our study provide an important contribution to understanding the potential indicator of inflammatory

markers but should be considered as an initial step. We advocate for further research and replication of our study in order to solidify our understanding and derive more conclusive and robust evidence.

Conflict of interests

The authors declare no conflict of interests.

Institutional Review Board Statement

The study was approved by the Ethics Committee of Emergency County Clinical Hospital, Târgu Mureş, Romania (Approval No. 2380/09.06.2023).

Author contributions

Conceptualization, original draft preparation, ECO and SG; methodology, ECO, HS, AIP, SF, MMH, KASN and CIO; software, ECO and COP; validation, SG and IJ; review, editing and supervision, SG. All authors have read and agreed to the final version of the manuscript.

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