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The Role of Neutrophil-Lymphocyte Ratio and Mean Platelet Volume on the Prognosis of Cardiac Masses

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Abstract

Objectives: Neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and mean platelet volume (MPV) are hematological markers used as prognostic markers in cancer and thrombotic diseases. These markers' association with intracardiac masses is unknown. Our goal is to determine the value of NLR, PLR, and MPV as prognostic markers in patients undergoing surgery for intracardiac masses.

Materials and Methods: The study included primary and secondary heart tumors, intracardiac thrombi, and infectious diseases that cause a mass effect in the heart. The pathological examination, location, complications, and mortality are compared with the patients' preoperative characteristics in the heart. The pathological examination, location, complications, and mortality are compared with the patients' preoperative characteristics (NLR, PLR, and MPV).

Results: The surviving patients were followed for 41 (20-75; minimum: 11-maximum: 120) months. NLR was found to be significantly higher in patients undergoing surgery for intracardiac thrombus, pulmonary embolism (PE), or impaired cardiac function (CF) (p=0.031, p=0.021, and p=0.046, respectively). Patients with masses in the left heart chambers and those with postoperative atrial fibrillation had significantly higher MPV values (p=0.001). The expected survival in the

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impaired CF group was significantly lower than in the normal CF group (35.91±13.00 months vs. 109.7±76.92 months, p=0.001).

Conclusion: There was a link between CF impairment and the NLR. The presence or absence of PE, as well as significant differences in NLR between groups of impaired and normal CF, tumors, and thrombus, is thought to be influenced by the patients' clinical condition. The preoperative relationship between the localization of the mass in the left heart and the development of postoperative atrial fibrillation and MPV is remarkable.

Keywords: Neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, mean platelet volume, intracardiac masses, cardiac tumor, cardiac thrombus

Introduction

Intracardiac masses are uncommon but can cause significant morbidity and mortality. The prevalence of intracardiac tumors ranged between 0.001% and 0.03%[1]. Only 6.4% of patients have their intracardiac thrombi differentiated from tumors during surgery. It has been reported that 15.4% of patients who underwent surgery for an intracardiac tumor had thrombus[2,3].

The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are inflammatory markers used in tumor monitoring, and their relationship with survival has been demonstrated in various tumor types[4,5]. Increased mean platelet volume (MPV) has been linked to hemostatically active platelets and a proclivity to thrombosis. In the presence of atrial fibrillation, it is also linked to stasis, thrombus formation in the heart, and stroke[5,6]. However, there are conflicting findings regarding the role of MPV in thrombotic events[7,8]. The relationship between NLR and PLR with various adverse events has been demonstrated[9-12]. The purpose of this study was to determine the value of prognosticative NLR, PLR, and MPV in predicting the type of mass (thrombi or tumor), the location of the mass, and role in the prediction of complications and mortality in patients who underwent open heart surgery for intracardiac masses. The outcomes of cardiac surgery and their complications are demonstrated.

Materials and Methods

Patients who were operated on with the diagnosis of an intracardiac mass in our hospital's cardiovascular surgery clinic between 2010 and 2020 were analyzed retrospectively after approval from the Non-Interventional Clinical Research Ethics Committee of Kocaeli University with the number of 2021/03.17; 2021/20; 4/02/2021. Patients were divided into three groups based on their etiology: tumor, thrombus, and infection. The patients' demographic information, laboratory results, echocardiography and radiological examinations, and hospital records were all examined.

NLR, PLR, and MPV values were calculated using data from a preoperative blood test. Complications and mortality in the postoperative period were investigated using follow-up records. Cerebrovascular accident (CVA), acute kidney injury (AKI), atrial fibrillation, and the need for inotropic support for more than 12 h were identified as complications (inotrope group). AKI was determined using the Kidney Disease Improving Global Outcomes classification by examining postoperative blood tests[13,14].

Documented postoperative *de novo* atrial fibrillation was described as an AF. All cases in the CVA, AKI, AF, and inotrope groups were analyzed as a single group called the "overall complications" group. The preoperative cardiac function (CF) of the patient is assessed. Patients with an ejection fraction less than 60%, right ventricular failure,

or the need of positive inotropic support for cardiogenic shock were described as having "impaired CF". Patients with normal cardiac function were classified as having "normal CF". Additionally, the patients were grouped according to the presence or absence of preoperative pulmonary embolism (PE), whether the mass was located in the right or left heart, and whether it was a tumor or thrombus. The resulting mortality and its causes were determined and categorized as 1st month mortality, 1st year mortality, and mortality at the end of the follow-up period.

The study included patients who underwent excision surgery for a mass in the myocardium, a heart cavity, or a heart valve. Patients with intra-pericardial tumors that invade the heart, tumors that only invade the intrathoracic great vessels, patients who have had PE without the presence of an intra-cardiac thrombus, patients with concomitant intra-cardiac thrombus with surgical indication for valvular heart disease, intra-cardiac thrombus secondary to a known arrhythmia or ventricular aneurysm, and patients with vegetation or abscess due to infective endocarditis were excluded.

Statistical Analysis

All statistical analyses were performed using IBM SPSS for Windows, version 20.0 (SPSS, Chicago, IL, USA). Kolmogorov-Smirnov and Shapiro-Wilk's tests were used to assess the assumption of normality. Numeric variables were presented with a mean, standard deviation, and median (25th-75th percentile). Categorical variables are summarized as counts (percentages). Numeric variable comparisons between groups were performed using independent sample t-tests or Mann-Whitney U tests, as appropriate. The association between two categorical variables was examined by chi-square and Fisher's exact test, if needed. The Kaplan-Meier method was used for survival analysis. All statistical analyses were carried out with 5% significance, and a two-sided p-value of 0.05 was considered statistically significant.

Results

Thirty-five patients (16 women and 19 men) were included in the study. Tumor (n=26), tromus (n=7), and infection (n=2) were found to be the causes of the masses. Due to its small population, the infection group could not be assessed in a statistical analysis as a single group. The patients were followed up for 37.5 (13.7-73) months. The mean follow-up period was 35 (12.75-73.00) months in the tumor group and 24 (0-60) months in the thrombus group. Two cases of hydatid cysts were followed for 42 and 99 months, respectively. The survivors were followed for an average of 41 (20-75; minimum: 11-maximum: 120) months. During the follow-up period, no recurring masses were discovered.

Preoperative Features

The baseline characteristics of all intracardiac masses (tumor and thrombus groups) and the comparison of tumor and thrombus groups are presented in Table 1. The infection group consisted of two men, ages 10 and 58, with hydatid cysts located in the left ventricular wall. A patient with atrial fibrillation and thrombus in the left atrial appendage who was operated on for papillary fibroelastoma was included in the tumor group.

In the tumor group, two patients (one with right atrial leiomyomatosis and one with myxoma in the right atrium) had PE without cardiac shock. Four cases in the thrombus group had massive PE hemodynamic instability and were not candidates for thrombolytic therapy. When the tumor and thrombus groups were compared, the risk of developing PE was found to be higher in the presence of intracardiac thrombus (p=0.011) (Table 1).

Positron emission tomography was performed in three patients with tumors before surgery (Figure 1).

Three patients in the tumor group were referred to cardiac surgery for metastasis in the heart (one for a testicular tumor with a diagnosis of mature cystic teratoma, one with a previous diagnosis of liposarcoma, and one

Table 1. The baseline characteristics of all intracardiac masses, tumor and thrombus groups and, the comparison of tumor and trombus groups

	All masses (n=35)	Tumor (n=26)	Thrombus (n=7)	Tumor vs thrombus
Age	56.74±15.46	56.54±14.02	64.00±10.45	p>0.05
Female	16 (45.71%)	12 (46.15%)	4 (57.14%)	p>0.05
Male	19 (54.28%)	14 (53.84%)	3 (42.85%)	p>0.05
Impaired CF	8 (22.85%)	4 (15.38%)	4 (57.14%)	**0.042**
CAD requering CABG				p>0.05
Three vessel disease	2 (5.71%)	2 (7.69%)	-	
LAD stenosis	1 (2.85%)	1 (3.84%)	-	
RCA stenosis	1 (2.85%)	-	1 (14.28%)	
Hypertension	19 (54.28%)	13 (50.00%)	5 (71.42%)	p>0.05
Type II diabetes mellitus	9 (25.71%)	7 (26.92%)	2 (28.57%)	p>0.05
CKD	2 (5.71%)	1 (3.84%)	1 (14.28%)	-
PE	6 (17.14%)	2 (7.69%)*	4 (57.14%)**	**0.011**
Pulmonary hypertension	10 (25.57%)	6 (23.07%)	4 (57.14%)	p>0.05
COPD	2 (5.71%)	2 (7.69%)	-	-
CVA	5 (24.28%)	3 (11.53%)***	2 (28.57%)	p>0.05
PAD	2 (5.71%)	1 (3.84%)	1 (14.28%)	-
Anemia	14 (40.00%)	12 (46.15%)	1 (14.28%)	p>0.05
Female	4 (25%)	3 (25.00%)	1 (25.00%)	-
Male	10 (52.63%)	9 (64.28%)	-	-
Hypotiroidism	2 (5.71%)	2 (7.69%)	-	-
Creatinin (mg/dL)	0.79 (0.62-0.99)	0.74 (0.62-0.89)	1.04 (0.62-1.44)	p>0.05
Neutrophyl count (10^3/μL)	4.40 (3.48-5.80)	4.3 (3.32-5.17)	8.3 (5.10-17.90)	**0.001**
Lymphocyte count (10^3/μL)	1.81 (1.41-2.20)	1.75 (1.47-2.20)	1.81 (1.30-2.50)	p>0.05
Hemoglobin (mg/dL)	12.71±1.55	12.44±1.46	13.58±1.61	p>0.05
Hemotocrit (%)	37.97±4.52	37.09±4.18	41.17±4.50	**0.031**
Platelet count (10^3/μL)	234.14±70.51	234.92±66.94	217.57±91.54	p>0.05
MPV (fL)	8.58±1.31	8.78±1.40	8.06±0.86	p>0.05
NLR	2.37 (1.85-3.42)	2.20 (1.84-2.90)	3.81 (3.07-10.00)	**0.021**
PLR	132.44±43.91	132.75±42.61	133.63±57.37	p>0.05

**: One case with leiomyomatosis,and one case with right atrial myxoma*
***: Right vetricular thrombus with acute massive PE with cardiogenic shock and contraindication to systemic thrombolysis*
****: Three cases with left atrial myxoma were presented with CVA*
CABG: Coronary artery bypass grafting, CAD: Coronary arterial disease, CF: Cardiac function, COPD: Chronic obstructive pulmonary disease, CKD: Chronic kidney disease, CVA: Cerebrovascular accident, LAD: Left anterior descending artery, MPV: Mean platelet volume, NLR: Neutrophyl to lymphocyte ratio, PAD: Peripheral arterial disease, PE: Pulmonary embolism, PLR: Platelet to lymphocyte ratio, RCA: Right coronary artery

with a history of intra-abdominal leiomyoma) (Figure 2). Two patients in the thrombus group had organized thrombus in the left atrium and a history of cancer (breast and prostate).

NLR, PLR, and MPV are compared among preoperative CF, tumor localization, preoperative PE and AF, overall complications, and the 30-day, one-year, and total mortality groups (Table 2).

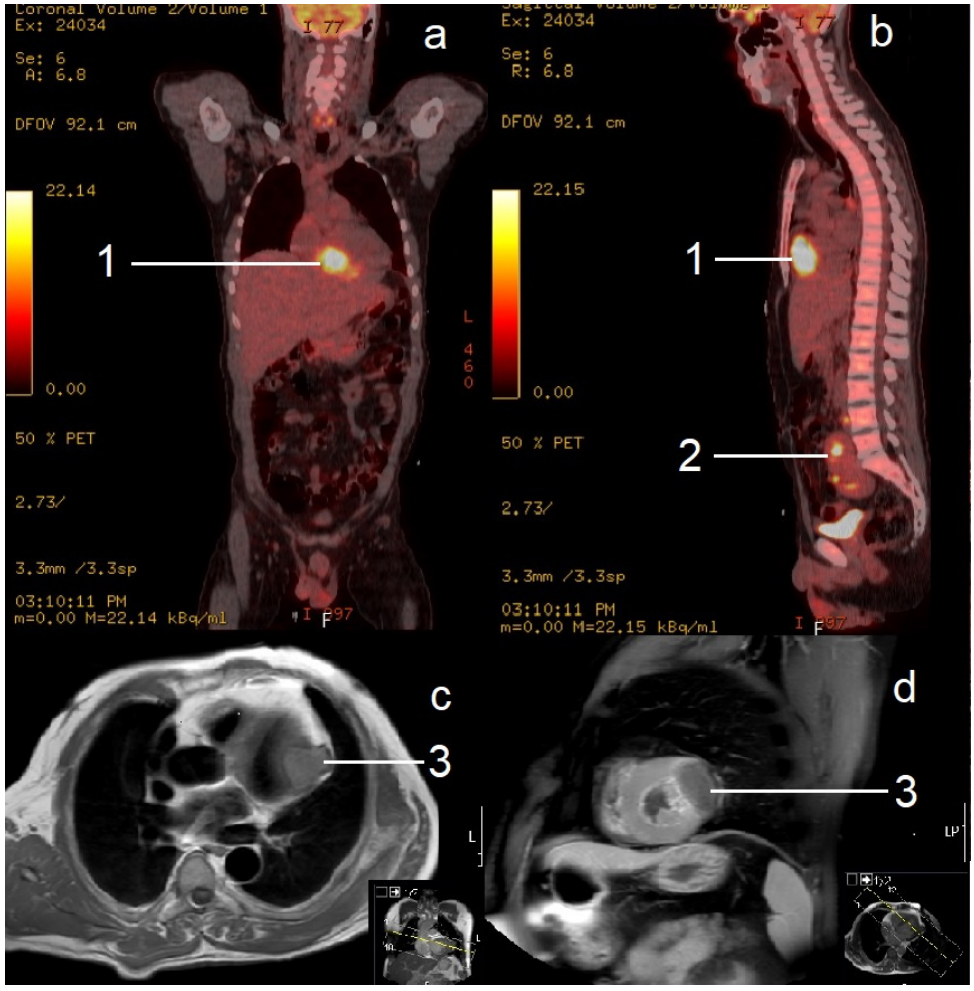

Figure 1. Coronal (a) and sagittal (b) positron emission tomography images of a patient with cardiac (1) and retroperitoneal (2) spread of a testicular tumor. Axial and sagittal magnetic resonance imaging of cardiac cysts (3)

The distribution of the masses according to their location in the heart is given in Table 3.

Postoperative Features

AF developed in 6 (23%) patients in the tumor group and in 1 (14.2%) patient in the thrombus group; AF was not observed in patients operated on for hydatid cyst. AKI developed in 2 (7.6%) patients in the tumor group, and none of the patients required postoperative dialysis. In the tumor group, 1 (3.8%) patient developed postoperative CVA. Eighteen (54.25%) patients were in the inotrope group. The inotrope group consisted of patients in the tumor group (n=13; 50%), in the thrombus group (n=5; 71.4%), and in the infection group (n=1; 50%). There was no significant difference between the tumor and thrombus groups in terms of the inotropic group and overall complications ($p>0.05$). When all complications were examined, no significant difference was found between the tumor and thrombus groups ($p>0.05$).

Thirty-day mortality occurred in a case of left atrial myxoma and postoperative acute respiratory distress syndrome despite the use of veno-venous extracorporeal membrane oxygenation in the tumor group and in two patients in the thrombus group who presented with massive PE and impaired CF. Mortality between 30 days and 1 year was noted to be the result of ventricular fibrillation in one patient operated on for myxoma.

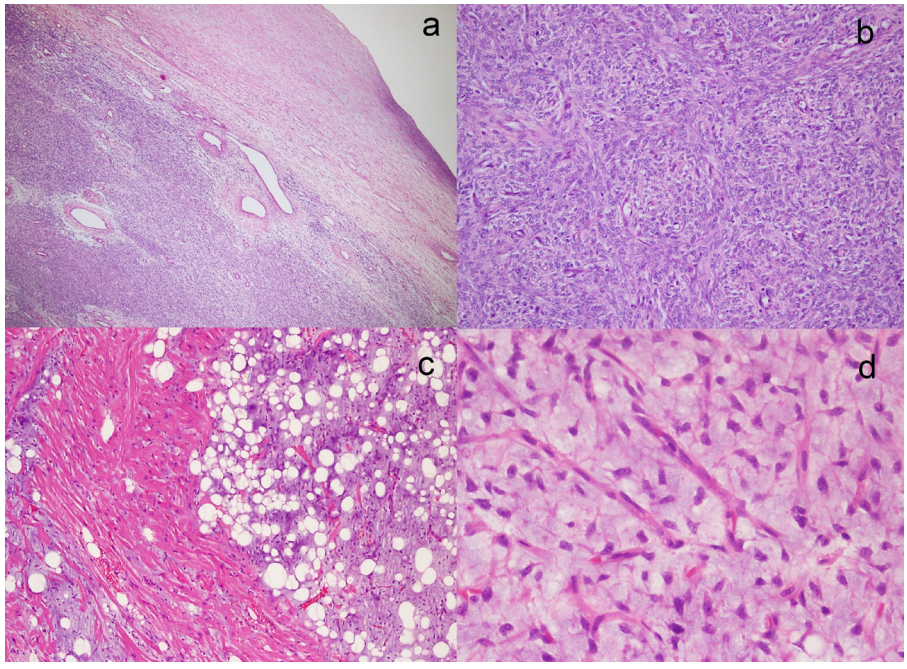

Figure 2. Histological image of intracardiac leiomyoma metastasis (a, b): Cells forming spindle-shaped, intersecting fascicles with indistinct borders are observed. Tumor-forming cells consist of eosinophilic cytoplasm, cigar-shaped nuclei (with tapering ends), and small nucleoli. Atypical mitosis, pleomorphism, and necrosis are not observed in the cells.
Histological image of cardiac liposarcoma metastasis (c, d): lipoblasts with monotonous nuclei, with signet ring cell morphology in places, in a myxoid matrix with a prominent plexiform vasculature between muscle fibers, showing an infiltrative growth pattern. Lipoblasts with indistinct, monotonous nuclei and indistinct cell borders are found in various areas of the tumor within the plexiform vessel framework

Table 2. Compairson of NLR, PLR, MPV among preoperative features and outcomes

	MPV (fL)	p-value	NLR	p-value	PLR	p-value
All mases (n=35)	8.58±1.31	-	2.37 (1.85-3.42)	-	132.44±43.91	-
Impaired CF (n=27)	8.5±1.36	p>0.05	2.18 (1.8-3.07)	**0.046**	136.01±42.98	p>0.05
Normal CF (n=8)	8.88±1.13		3.38 (2.13-9.73)		120.38±47.83	
Preoperative PE (n=6)	8.05±0.94	p>0.05	3.56 (2.38-14.78)	**0.031**	127.54±47.27	p>0.05
Without PE (n=29)	8.69±1.36		2.18 (1.80-3.23)		133.45±44.00	
Right heart (n=12)	7.75±1.00	**0.006**	2.10 (1.80-3.07)	p>0.05	135.99±51.35	p>0.05
Left heart (n=23)	9.01±1.26		2.92 (2.01-7.66)		130.58±40.63	
Postoperative AF (n=7)	9.66±1.44	**0.022**	2.45 (1.54-4.17)	p>0.05	127.41±60.28	p>0.05
Without AF (n=28)	8.31±1.15		2.30 (1.86-3.36)		133.66±40.16	
All complications (n=24)	8.83±1.36	p>0.05	2.44 (1.86-3.44)	p>0.05	134.01±49.05	P>0.005
No complications (n=11)	8.04±1.04		2.18 (1.80-3.38)		129.00±31.67	
30 days mortality (n= 3)	8.02±1.07	p>0.05	3.32 (1.86-29.13)	p>0.05	119.58±51.42	p>0.05
30 days survival (n=32)	8.64±1.33		2.30 (1.82-3.41)		133.64±43.89	
One year mortality (n=4)	8.34±1.08	p>0.05	3.38 (2.22-22.70)	p>0.05	130.82±47.62	p>0.05
One year survival (n=31)	8.61±1.35		2.23 (1.80-3.38)		132.65±44.25	
Overall mortality (n=8)	8.27±1.27	p>0.05	3.00 (1.90-7.57)	p>0.05	135.88±48.51	p>0.05
Overall survival (n=27)	8.68±1.33		2.23 (1.80-3.38)		131.42±43.4	

AF: Atrial fibrillation, CF: Cardiac function, MPV: Mean platelet volume, NLR: Neutrophyl to lymphocyte ratio, PLR: Platelet to lymphocyte ratio

Table 3. Localisation of the masses

	Myxoma	Thrombus	Other tumors	PFE	Hydatid cyst
Aortic valve	-	-	-	3	-
Right atrium	2	5*	1**	-	-
Right ventricle	-	-	1***	-	-
Left atrium	17	1	-	-	-
Left ventricle	-	-	-	-	2†††
Right atrium and VCI	-	1	1†	-	-
Interatrial septum compressing VCI	-	-	1††	-	-

**: One with extension on interartrial septum and patent foramen ovale, one with extension on VCI, one with extension on right ventricle*
***: Rosai Dorfmann disease (Sinus histiositosis)*
****: Fibrin and exudate in a patient with testis tumor*
†: Intravascular leiomyoma
††: Metastasis of myxoid liposarkoma
†††: One of the cases was treated with ventriculoplasty (Dor procedure)
PFE: Papillary fibroelastoma, VCI: Vena cava inferior

Table 4. Relation of localisation, cardiac function and mass type with postoperative outcomes

	n, (%)	AF n, (%)	All complications n, (%)	30 days mortality n, (%)	One year mortality n, (%)	Overall mortality n, (%)
Left heart	23, (65.7%)	6, (26.1%)	15, (65.2%)	1, (4.3%)	2, (8.7%)	3, (13%)
Right heart	12, (34.3%)	1, (8.3%)	9, (75%)	2, (16.7%)	2, (16.7%)	5, (41.7%)
Impaired CF	8, (22.9%)	1, (12.5%)	6, (25%)	2, (25%)	**3, (37.5%)***	**6, (75%)****
Normal CF	27	6, (22.2%)	18, (66.7%)	1, (3.7%)	**1, (3.7%)***	**2, (7.4%)****
Tumor	7 (21.2%)	6, (23.1%)	19, (73.1%)	1, (3.8%)	2, (7.7%)	5, (19.2%)
Thrombus	26 (78.8)	1, (14.3%)	5, (71.4%)	2, (28.6%)	2 (28.6%)	3, (42.9%)

Involvement of the left heart and right; impaired and normal CF, tumor and trombus are compared with the prescence or abscence of AF, overall complications, 30 days mortality, one year mortality, and overall mortality. All of the comparisons had a p>0.05, excepting 1 year mortality and overall mortality between CF groups
**: p=0.030*
***: p<0.001*
AF: Atrial fibrillation, CF: Cardiac function

After 1 year, mortality occurred in patients operated on the right atrial thrombus (at 14 months), Rosai Dorfmann disease (at 42 months), left atrial myxoma and CABG (at 48 months), and leiomyomatosis (at 77 months) due to non-cardiac causes. At the end of the whole follow-up period, mortality was observed in 8 cases (22.8%). The relationship between complications and mortality among the groups of localization, type, complications, and CF of the masses is presented in Table 4.

In the Kaplan-Meier survival analysis, the 1-month survival of the 35 patients examined in the study was 91.4±0.47%. The 12-month survival was 88±0.54%, and the 60-month survival was 73.8±0.09%.

In the impaired CF group, 1-month survival was 75%, 12-month survival was 62.5%, and 60-month survival was 33.3%. In the normal CF group, 1-month survival was 96.3%, 12-month survival was 96.3%, and 60-month survival was 88.9%. The estimated survival in the impaired CF group was 35.91±13.00 months, and the estimated survival in the normal CF group was 109.77±6.92 months (0<0.001) (Figure 3).

In the tumor group, 1-month survival was 96.2±0.03%, 12-month survival was 92.3±0.52%, 60-month survival was 74.6±0.12%, and the estimated survival was 85.73±10.02 months. In the thrombus group, 1-month survival was 71.4±0.17%, 12-month survival was

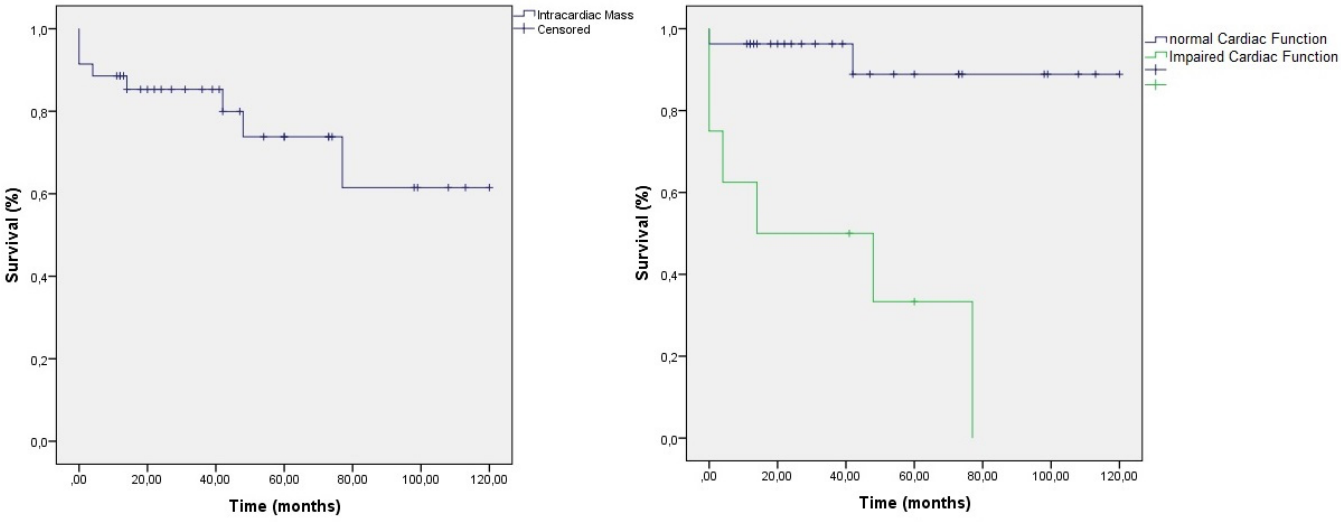

Figure 3. Kaplan-Meier survival analysis of all patients and the comparison of normal and impaired cardiac function

57.1±0.18%, 60-month survival was 57.1±0.18%, and the estimated survival was 70.51±21.63 months. The Kaplan-Meier estimates of overall survival after surgery did not differ significantly between the tumor and thrombus groups (p>0.05).

Discussion

Our study determined the prognostic importance of preoperative NLR, PLR, MPV, preoperative characteristics, and tumor types. The most important prognostic factor was preoperative CF. NLR was related to preoperative cardiac functional status but not to prognosis. MPV was related to postoperative atrial fibrillation and the localization of the mass.

Simple or complex excision, total artificial heart implantation, and cardiac transplantation are surgical treatment options[2]. In our cases, simple excision or patch reconstruction were the most commonly used methods.

The intravascular spread of pelvic or uterine leiomyomas has been described in this context[15,16]. In our series, one patient was operated on for leiomyoma spreading from the inferior vena cava to the heart.

Rarely, infective masses such as hydatid cysts settle in the heart and create a mass effect in the myocardium. The prevalence of Rosai-Dorfman disease (sinus histiocytosis) is also rare[17]. Since we examined cardiac masses, we included cases treated for hydatid cysts and Rosai-Dorfman disease in our study. Both the patients with hydatid cysts survived the follow-up period.

Half of the left heart's myxomas present with thromboembolism due to thrombus formation on them, and echocardiography is often sufficient to differentiate between tumor thrombus and vegetation[18,19]. If needed, MRI can be used for thrombus and tumor differentiation[20]. In our series, left atrial myxoma was presented with CVA in three cases due to thromboembolism of its surface.

Right heart thrombi are usually caused by peripheral embolization[21]. 10% of cases with PE have a thrombus in the right heart[22]. It is known that the presence of a thrombus in the right heart and shock in acute PE are indicators of decreased survival and a poor prognosis[23,24]. In our study, six patients had PE. All of them had tumors or thrombus in the right heart. Four patients with impaired CF and PE and a right heart thrombus had 50% early mortality. A higher rate of PE was found in the thrombus group (p=0.11). Additionally, NLR and the presence of PE were associated (p=0.031). As 4 of 6 patients with PE had acutely developed CF, the patient's clinical picture may

be the reason for the increased NLR rate in the thrombus group.

It has been reported that high MPV is a marker related to a tendency to thrombosis and is associated with higher platelet-related inflammation and thrombotic events[25]. However, there are conflicting findings regarding the association of MPV with the development of left atrial thrombosis and venous thromboembolism[7,8].

In our study, when the masses were divided into right- and left heart involvement, a higher MPV was found with the left heart involvement (p=0.006). Although only three patients had systemic emboli in masses in the left heart, given the increase in MPV, occult systemic emboli in the left systemic mass are possible. Interestingly, despite not reaching statistical significance, when thrombus and tumors were compared, MPV was found to be higher in the tumor group (p>0.05). This can be explained by the difference in the duration of the formation of tumors and thrombi. The longer contact and activation of platelets on an irregular surface in the left-sided masses may have affected the MPV. Meanwhile, considering that 4 of the 7 cases in the thrombus group were presented with acute PE, the acute formation of thrombi and its embolism may not have changed MPV at the time of diagnosis. However, our findings still preclude establishing a relationship between MPV and increased thrombotic processes.

It was found that the increased MPV value in the preoperative period was associated with postoperative AF (p=0.022). As stated above, the MPV was also increased in the masses located in the left heart (p=0.006). It can be argued that the surgical technique needed to reach the masses for excision in the left system may also affect the occurrence of AF. However, no relationship between AF and localization (right or left) in the heart is found (Table 4).

When only complications and mortality were analyzed, the only factor associated with one-year mortality and overall mortality was impaired CF before surgery. Interestingly, the association of impaired CF with 30-day mortality has not been demonstrated. This result shows that although the preoperative cardiac reserve is low, the patient can survive the early postoperative period, but the long-term unfavorable course cannot be prevented.

Study Limitations

The main limitation of our study is the inherited nature of a retrospective study. The small study population can be counted among the limitations. The small number of cases made it difficult to group them according to their clinical status, complications, and preoperative characteristics and compare them during statistical analysis. In addition, the general clinical status and diseases of the patients included in the study were heterogeneous. This prevents us from drawing firm conclusions about the significance of these factors under consideration. However, considering that these diseases are seen rarely, it can be considered that the results obtained from the analysis of the available data are still important. Studies with larger patient numbers may provide additional information on this topic.

Conclusion

In our study, the only factor that predicted mortality at one year and at the end of follow-up was the adequacy of preoperative cardiac function. No relationship between preoperative NLR value and survival could be demonstrated. On the other hand, there was a correlation between poor ventricular function and NLR. The relationship between the localization of the mass in the left heart and the formation of postoperative atrial fibrillation and MPV in the preoperative period is remarkable. No prognostic significance of PLR in intracardiac masses has been demonstrated.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Non-Interventional Clinical Research Ethics Committee of Kocaeli University with the number of 2021/03.17; 2021/20; 4/02/2021.

Informed Consent: The study was designed as a retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Design: Data Collection and/or Processing: Analysis and/or Interpretation: Literature Search: Writing: All authors contributed equally.

Conflict of Interest: The authors declare no conflicts of interest concerning the authorship or publication of this article.

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