

Comparison of early stage electrocardiographic, echocardiographic and histopathologic results of partial pericardiectomy and thoracoscopic pericardial window techniques in an animal model

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ABSTRACT. This study aimed to compare early stage electrocardiographic (ECG), echocardiographic (ECHO) and histopathologic results of the pericardial surgery techniques. Partial pericardiectomy by lateral thoracotomy and thoracoscopic pericardial window techniques was performed under general anesthesia in goats (n=6), which were separated into two groups; partial pericardiectomy was performed in group I (GRI) (n=3) and thoracoscopic pericardial window was performed in group II (GRII) (n=3). ECG and ECHO examinations were performed pre- and postoperatively on days 1, 7 and 30. All experimental animals were sacrificed at the end of day 30 and macroscopic investigations were performed. Histopathological examinations were performed on the lung, visceral pleura, epicardium and myocardial tissues. ECG findings included sinus tachycardia, small complex QRS and T wave peak on day 1 in both groups. The left atrial and ventricular diameters as well as the stroke volume were lower on the 1st postoperative day in both groups. The stroke volume was lower on postoperative days 1 and 30 in GRII. The observed ejection fraction was lower in GRII and higher in GRI on postoperative day 30 compared with their baselines. Macroscopic and histopathological findings of the lung and heart tissues were more severe in GRI, but there was no meaningful variation in the epicardium or visceral pleura. There was no significant difference in the histopathological results between the groups. Although the thoracoscopic pericardial window technique seem less traumatic and better tolerated than partial pericardiectomy, our ECHO and histopathologic results indicate that both techniques can safely be performed by surgeons according to the pericardial disease indication.

Keywords: cardiology, pathology, partial pericardiectomy, thoracoscopic pericardial window

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INTRODUCTION

Pericardiectomy techniques are performed in both humans and animals to treat neoplastic or non-neoplastic pericardial diseases (Kerstetter et al, 1997; Busch et al, 2015), especially pericardial effusions (Kerstetter et al, 1997; Jackson et al, 1999). The presenting malignant, infectious or idiopathic etiologies may indicate the need for pericardiectomy in small animals (Dupre et al, 2001; Case, 2016). The objective of pericardiectomy is to excise sufficient pericardium to excise enough pericardium to eliminate tamponade and to obtain a histologic diagnosis of the patient's condition (Palsgaard et al, 2007; Case, 2016). For this purpose, partial and total pericardiectomy are described, and these techniques are performed via a lateral thoracotomy, median sternotomy or thoracoscopic approach (Hardy et al, 1992; Jackson et al, 1999; Dupre et al, 2001; Palsgaard et al, 2007; Vistarini et al, 2015; Case, 2016). Partial pericardiectomy is a valuable treatment for cases with constrictive pericarditis because echocardiographic symptoms of this constriction (progressive fluid overload, reduced cardiac output and right ventricular pressure disturbance) are only terminated by pericardial surgery (Hardy et al, 1992; Bicer et al, 2015; Busch et al, 2015; Vistarini et al, 2015). Thoracoscopic surgery, compared to the open thoracic approach, decreases surgical stress, pain and postoperative complications; as a result, it is recommended as a minimally invasive technique for treating some pericardial diseases in veterinary practice (Dupre et al, 2016; Palsgaard et al, 2007; Case, 2016). In humans, partial pericardiectomy increases the survival time, relieves the clinical symptoms and prevents a second disease from worsening the health status of the patient (Vistarini et al, 2015).

Because no postoperative study compares different therapeutic strategies for pericardial disease, pericardiectomy performed through thoracotomy is still advocated by surgeons (Schofield et al, 2004). Previous studies have only reported postoperative morbidity and mortality results after pericardiectomy in clinical cases (McCaughan et al, 1985; Hardy et al, 1992; Wood et al, 2002; Schofield et al, 2004; Nath et al, 2014; Bicer et al, 2015; Busch et al, 2015; Vistarini et al, 2015). The question about histopathologic formation of the adjacent surfaces between lung

and heart tissues and its effect on heart functions has not been clearly investigated yet. In addition, no experimental study has compared the pericardial techniques with the cardiologic and pathological results. As a result, this study aimed to compare early stage postoperative electrocardiography (ECG) and echocardiography (ECHO) findings as well as to demonstrate the histopathological results of partial pericardiectomy and thoracoscopic pericardial window techniques in an animal model.

MATERIALS and METHODS

Study design and animals

The local ethics committee of Uludag University approved the number of experiments in the study and gave permission to carrying out the study plan (decision no: 2010-05/05). A total of 6 mixed-breed (n=6), male, healthy goats (lacked cardiac and systemic disease based on the clinical examinations and bloodwork) were experiments of the study. Experiments were randomly separated into two groups: group I (GRI) (n=3) and group II (GRII) (n=3). The partial pericardiectomy and thoracoscopic pericardial window techniques were conducted in GRI and GRII, respectively.

Pre- and postoperative cardiologic examinations

Preoperatively, routine clinical examinations and thoracic auscultations were completed to evaluate the heart rhythms, lung sounds, and baseline ECG (P200®, Esoate, Italy) and ECHO (CarisPlus®, Esoate, Italy) examinations. The acquired data were compared with the postoperative data. All experiments were checked with routine clinical examinations; also, ECG and ECHO studies were repeated on postoperative days 1, 7 and 30. ECHO protocols were made in the same room and environmental conditions by the same investigator. The experimental subjects were not sedated during the ECG and ECHO examinations.

ECG examinations were performed in the right lateral recumbency position of the experiments with three standard bipolar (I, II, and III) and unipolar extremity derivations (aVR, aVL, and aVF). The durations (P, PR, QRS, and QT) and amplitudes (P,

R and T waves), heart rhythm, the mean electrical axis (MEA) and heart rate were measured in lead II. The configurations of the P wave, QRS complex and T wave were also determined.

ECHO examinations were completed in standing positions for the experiments, and the cardiac structures, dimensions and contraction indices were documented (Caris Plus[®], Esoate, Italy). Two-dimensional (2D) ECHO, color flow imaging and spectral Doppler examinations were performed using a 3.5-5 MHz phased-array transducer. The M-mode was performed from the right parasternal long axis view of the left ventricle (LV), at the level of the chordae tendineae, to measure the LV-related parameters (end-diastolic and -systolic dimensions of LV [LVDD and LVDS], interventricular septum [IVSDD and IVSSD], and LV-free wall [LVFWDD and LVFWSD]. Additionally, M-mode measurements provided numerical data of stroke volume, fractional shortening (FS) and ejection fractions (EF), all of which were estimated automatically with an ECHO machine. For these measurements, a cursor was placed perpendicular to the septum and left ventricular wall between the tips of the mitral valve leaflets and papillary muscles over the chordae tendineae. The left atrium (LA), aorta (Ao) and pulmonary artery (PA) dimensions were measured by 2-D ECHO from the right parasternal short-axis view. In this position, the M line cursor was placed on the mid-lumen of PA to characterize its Doppler flow. The PA maximum velocity (PAV_{max}) was measured. For all measurements, a total of 5 cardiac cycles were saved and measured for the correct measurements.

Surgical procedures

General anesthesia in the experiments was maintained with a 2% concentration of isoflurane and mechanical ventilation after induction with ketamine (4 mg/kg, im) and diazepam (0.3 mg/kg, im) as well as intratracheal intubation. The vital parameters (capnography, SPO₂, ECG, etc.) of the experiments were monitored during anesthesia.

The same surgical team performed all operative procedures. The experimental subjects were laid in the right recumbent position on the operation table,

and their left thoracic walls were shaved and prepared for aseptic surgery.

In the GRI, partial pericardiectomy (subphrenic-subtotal) was performed after the 4th lateral thoracotomy with a routine technique (Monnet, 2013). In GRII, a thoracoscopic pericardial window was performed by the “triangulation” technique described by Monnet, 2013. The technique entailed initial placement of a 3 mm cannula at the fifth intercostal space and dorsal to the costochondral junction of the left thoracic wall with localization to the left pericardium. A pneumothorax was established, and a 2.7 mm, 30° telescope (Karl Storz[®], Germany) was placed through this cannula to view the thoracic cavity. The 6 mm cannulas from the fourth and sixth intercostal spaces were established ventral to the costochondral junction for instrumentation. After visualization of the pericardial surface, a grasping forceps was employed and approximately 3 cm x 3 cm of pericardium was excised.

In both groups, a 32 Fr thoracostomy tube was inserted in the pleural space, and Heimlich valve drainage was applied at 12 and 2-4 hours in GRI and GRII, respectively. Parenteral antibiotic (cefazolin Na, 20 mg/kg, bid) and analgesic (flunixin meglumine, 1.1 mg/kg, bid) drugs were administered for 5 days postoperatively. All experiments were closely monitored during the study period, and care was taken to comply with the ethical rules.

Pathological examinations

The experiments were sacrificed on postoperative day 30; then the lung and heart tissues were removed from the affected hemithorax. After macroscopic investigations, all tissues were fixed in 10% formalin solution and embedded in paraffin. Sections with a thickness of approximately 5 microns were prepared from the tissues and stained with Hematoxylin & Eosin (H&E); then, they were examined under a light microscope. Investigations focused on four histologic areas (epicardium, myocardium, visceral pleura and lung), and histopathological results were categorized as absent, mild, mildly moderate, moderate, moderately severe and severe. In addition, microscopic findings were scored with consideration for the

microscopic findings (inflammation, hemorrhage, fibroblastic proliferation, neovascularization, accumulation of hemosiderin and mesothelial hyperplasia) and mean values of the scores in the groups were analyzed according to the histologic areas.

Statistical analysis

ECHO measurement results are expressed as the mean \pm standard deviation. ECHO data were evaluated by one or two ways analysis of variance (ANOVA) by repeated measures, which were followed by the Tukey test for pairwise comparisons (IBM®, SPSS Statistics, USA). The microscopic finding scores were evaluated by the Chi Square test (IBM®, SPSS Statistics, USA) as previously reported (Tiruvoipati et al, 2003). P values less than 0.05 were considered significant.

RESULTS

Pre- and postoperative cardiologic findings

ECG: Preoperatively, all experiments had a sinus rhythm. Although ECG revealed sinus tachycardia (preoperatively 75 ± 10 bpm and postoperatively 112 ± 12 bpm in GRI, and preoperatively 77 ± 11 bpm and postoperatively 114 ± 9 bpm in GRII), small complexes of QRS (preoperatively 0.4 ± 0.0 mV and

postoperatively 0.2 ± 0.0 mV in GRI, preoperatively 0.4 ± 0.0 mV and postoperatively 0.3 ± 0.0 mV in GRII) and peak T waves (preoperatively 0.3 ± 0.1 mV and postoperatively 0.2 ± 0.1 mV in GRI, preoperatively 0.3 ± 0.1 mV, postoperatively 0.25 ± 0.1 mV in GRII) on postoperative day 1, there were no noticeable ECG abnormalities, except respiratory sinus arrhythmias on days 7 and 30 in the two groups.

ECHO: Compared to the baseline, the left ventricular diameters at diastole and systole as well as the stroke volume (SV) were lower ($P<0.05 - 0.01$) on postoperative day 1 in both groups. However, these results (except SV) returned to baseline values on postoperative days 7 and 30 (Table 1). SV observed on postoperative days 1 and 30 in GRI were lower ($P<0.05 - 0.01$) than the baseline and GRII's values. The ejection fraction (EF %) was significantly lower ($P<0.05$) on day 30 in GRI compared to baseline values, but it was higher ($P<0.05$) in GRII than that of GRI on postoperative day 30 (Table 1).

Macroscopic findings

The hemithoraxes in both groups only revealed pleural adhesions in the effected sites. Additionally, there was no abnormal view in the necropsy of the heart tissues. In the experiments of GRI, there were

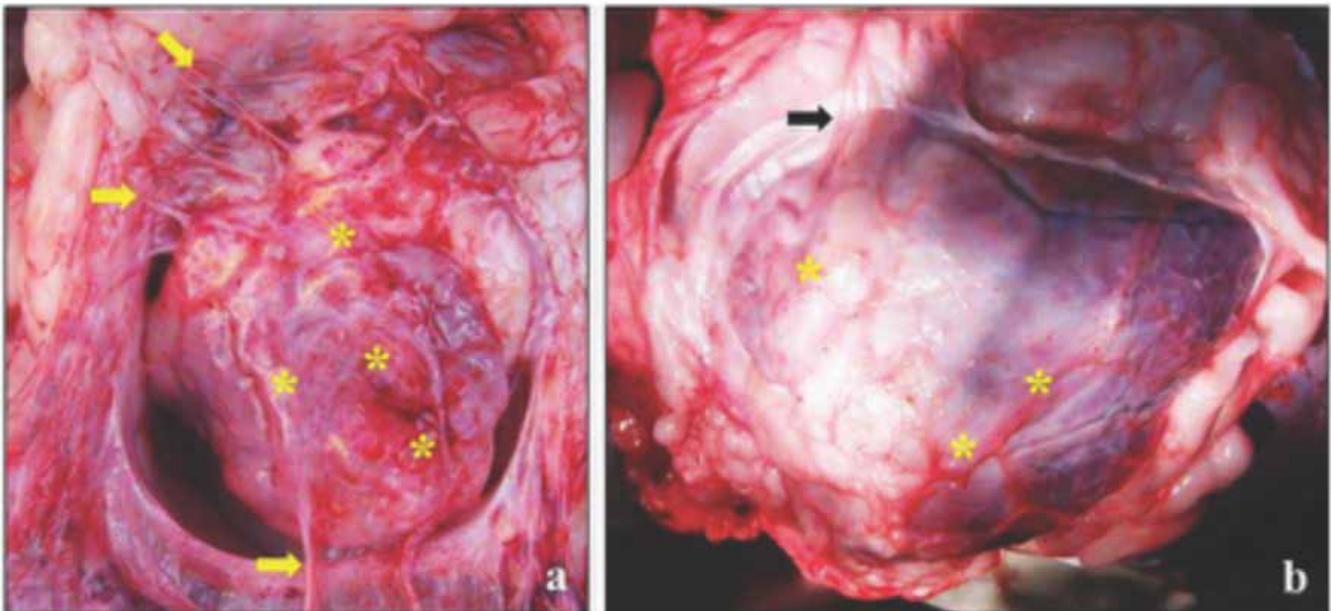


Fig. 1. Instances about the postmortem macroscopic results of the groups, a shows the fibrous tissues (asterisks) and fibers (arrows) in GRI and b shows views from GRII pointing out new fibrous formations on e icardium (asterisks) and some fibers (arrow).

new fibrous tissues and fibers between the epicardium and surface of the visceral pleura together with the lung tissue. The lung and myocardium tissues were also affected by the operative procedure (Fig. 1a). In GR II experiments, the hole generated by the pericardial window technique seemed to enlarge, and new fibrous formations on the epicardium and some fibers between the epicardial surface and visceral pleu-

ra were determined (Fig. 1b).

Histopathological findings

Microscopic examination of the myocardium, epicardium, visceral pleura and lung tissues is summarized below.

Epicardium: The obtained findings were mostly similar in both groups. All views had mild hemosid-

Table 1. Pre- and postoperative geometric, functional and Doppler measurements of the experiments in both groups (mean \pm standard deviation).

Parameters	Gr- oups	Preoperative values	Postoperative values		
			1st day	7th day	30th day
Ao (cm)	I*	3.08 \pm 0.13	2.75 \pm 0.13	2.91 \pm 0.13	2.57 \pm 0.13
	II*	2.38 \pm 0.19	2.28 \pm 0.19	2.33 \pm 0.13	2.63 \pm 0.13
LA (cm)	I	3.1 \pm 0.3	2.3 \pm 0.3	2.9 \pm 0.3	2.9 \pm 0.2
	II	3.4 \pm 0.5	2.6 \pm 0.5	3.7 \pm 0.3	2.9 \pm 0.3
PA (cm)	I	1.96 \pm 0.26	1.89 \pm 0.26	2.22 \pm 0.26	1.69 \pm 0.26
	II	2.66 \pm 0.37	1.90 \pm 0.37	1.91 \pm 0.26	2.02 \pm 0.26
EF (%)	I	82.0 \pm 4.6	80.0 \pm 4.8	73.0 \pm 5.2	67.0 \pm 5.4*
	II	78.5 \pm 5.7	76.0 \pm 8.4	89.0 \pm 4.3	81.5 \pm 5.3#
SV (ml)	I	41.2 \pm 4.4	15.6 \pm 6.2***	42.05 \pm 3.4*	23.6 \pm 5.4**
	II	37.1 \pm 6.4	21.9 \pm 9.0**	43.45 \pm 6.5*	35.6 \pm 6.6#
IVSD (cm)	I	1.48 \pm 0.18	1.20 \pm 0.18	1.19 \pm 0.18	1.50 \pm 0.18
	II	1.12 \pm 0.18	1.23 \pm 0.25	1.10 \pm 0.18	1.35 \pm 0.18
PWD (cm)	I	1.09 \pm 0.20	1.55 \pm 0.20	1.31 \pm 0.20	1.70 \pm 0.20
	II	1.31 \pm 0.20	1.10 \pm 0.28	1.36 \pm 0.20	1.46 \pm 0.20
LVDd (cm)	I	4.36 \pm 0.29*	3.71 \pm 0.29*	4.29 \pm 0.29*	4.50 \pm 0.29
	II	4.55 \pm 0.29*	3.05 \pm 0.41*	4.71 \pm 0.29*	4.02 \pm 0.29
LVDs (cm)	I	3.03 \pm 0.24	2.50 \pm 0.24	2.90 \pm 0.24	2.80 \pm 0.24
	II	2.94 \pm 0.24	2.22 \pm 0.24	2.23 \pm 0.24	2.58 \pm 0.24
FS (%)	I*	30.00 \pm 4.81	29.50 \pm 4.81	32.50 \pm 4.81	38.00 \pm 4.81
	II*	38.5 \pm 4.81	47.00 \pm 6.80	52.50 \pm 4.81	36.00 \pm 4.81
PA-V max (m/s)	I	1.04 \pm 0.05	1.09 \pm 0.05	1.07 \pm 0.07	1.09 \pm 0.05
	II	1.23 \pm 0.07	0.96 \pm 0.07	1.21 \pm 0.05	1.15 \pm 0.07

*P<0.05, **P<0.01, ***P<0.001 compared to baseline value within groups.

P<0.05 compared between two groups.

Ao: Aorta; LA: left atrium; PA: pulmonary artery; EF: ejection fraction; SV: stroke volume; IVSD: Interventricular septum diastole; PWD: Post wall diastole; LVDd: left ventricular diastolic dimension; LVDs: left ventricular systolic dimension; FS: Fractional shortening; PA-Vmax: Pulmonary artery maximum velocity.

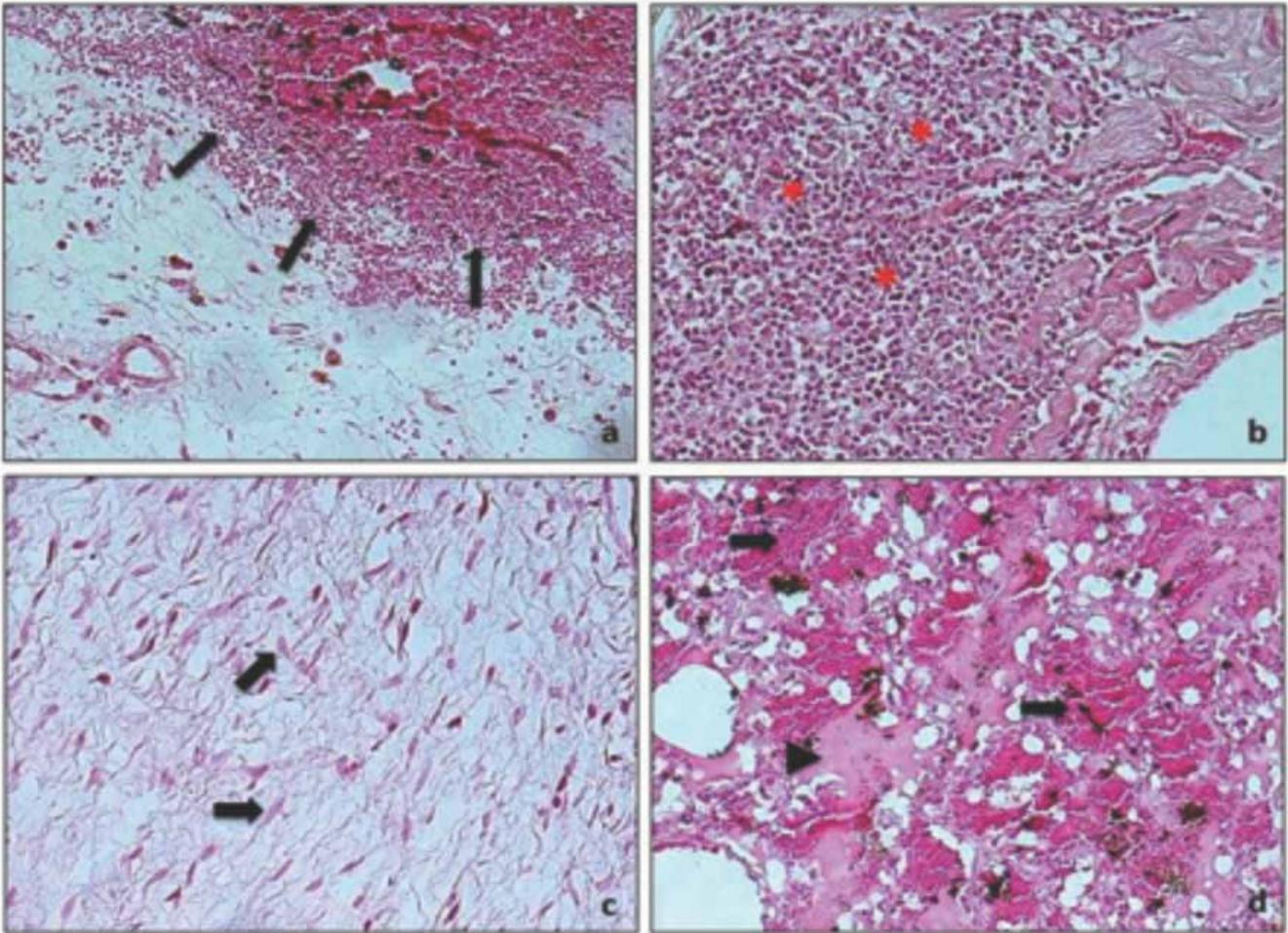


Fig. 2. Microscopic views from the cases of GRI, a: hemorrhage in epicardium (arrows), x400, H&E; b: mononuclear cell infiltration in myocardium (asterisks), x400, H&E; c: fibroblastic proliferation in visceral pleura (arrows), x400, H&E; and d: edema (arrow head) and hemorrhage (arrows) in lung tissue, x400, H&E.

erophages, mildly moderate inflammation (Fig. 3a), moderate hemorrhage (Fig. 2a) and moderately severe fibroblastic proliferation and neovascularization.

Myocardium: Hemorrhage was mild, and inflammation (Fig. 2b) and fibrosis were moderate in GRI. In GRII, hemorrhage was mildly moderate (Fig. 3b), and there was no inflammation or fibrosis.

Visceral pleura: Inflammation, hemorrhage, fibroblastic proliferation, neovascularization, hemosiderophages, mesothelial hyperplasia and fibrinous exudate were seen to varying degrees. Hemosiderophages and mesothelial hyperplasia were mild, inflammation was mildly moderate, hemorrhages and fibrinous exudate were moderate, and fibroblas-

tic proliferation (Fig. 2c) and neovascularization were moderately severe in GRI. In GRII, mesothelial hyperplasia was mild, inflammation was mildly moderate, and hemorrhage, hemosiderophages, fibrinous exudate, fibroblastic proliferation and neovascularization (Fig. 3c) were moderate.

Lungs: Inflammation, hemorrhage (Fig. 2d), hemosiderophages and edema (Fig. 3d) were observed to varying degrees. These lesions were moderate in GRI and mild in GRII.

In addition to these evaluations, statistical analysis of the histopathological results revealed that there was no significant difference between the groups ($P=0.336$).

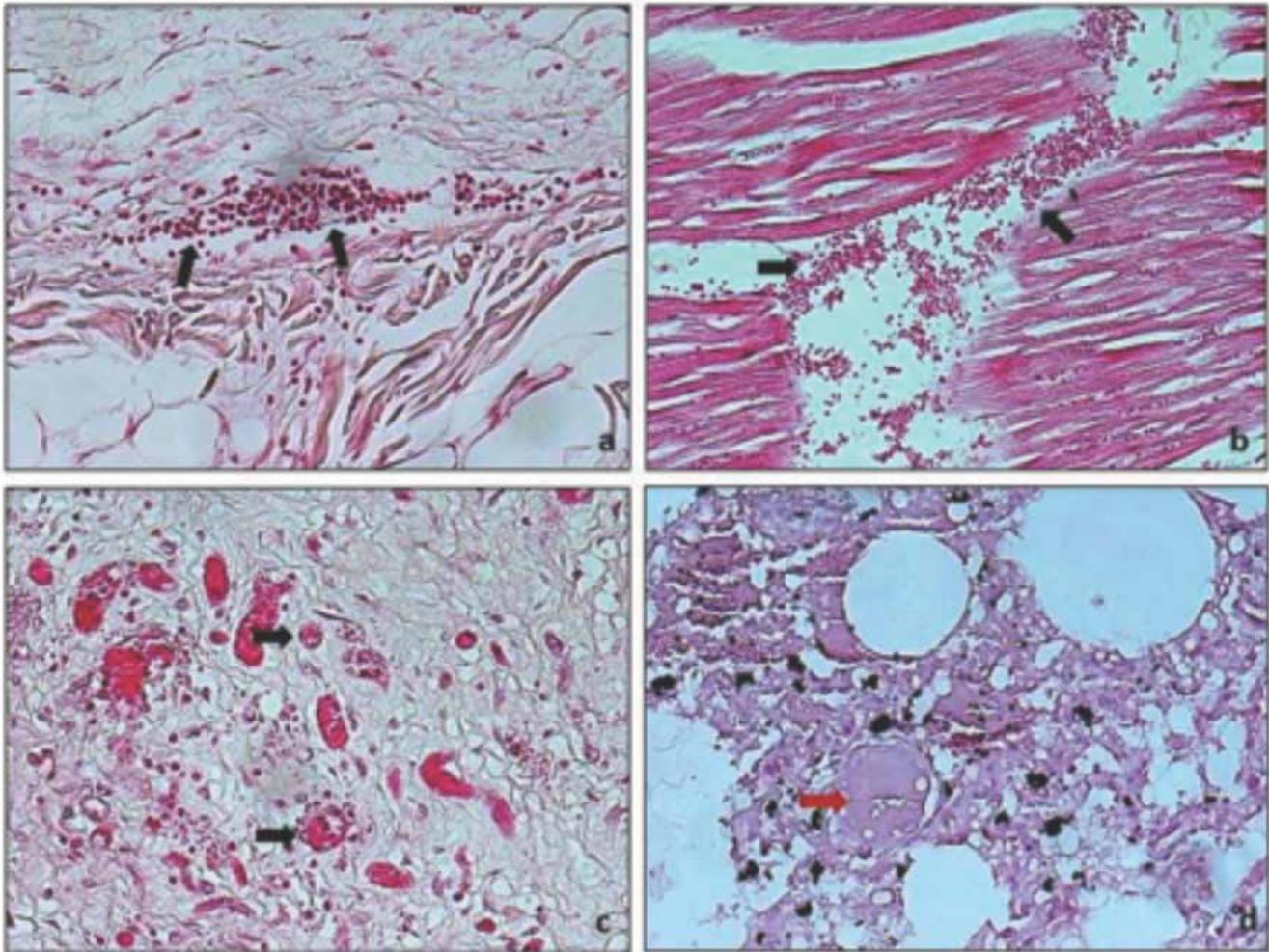


Fig. 3. Microscopic views from GR11 experiments, a: mononuclear cell infiltration in epicardium (arrows), x400 H&E; b: hemorrhage in myocardium (arrows), x400, H&E; c: neovascularization in the visceral pleura (arrows), x400 H&E; and d: edema in the alveolar space of the lung (arrow), x400 H&E.

DISCUSSION

Pericardiectomy is considered to be the definitive treatment for pericardial disease in humans and animals (Dupre et al, 2001; Vistarini et al, 2015). When pericardiectomy is considered for treatment, the surgeon must decide how much pericardium to excise (Case, 2016). As a result, open thoracic approaches for pericardiectomy are preferred because they provide better surgical exposure and incision (Dupre et al, 2001; Vistarini et al, 2015). These techniques are performed by median sternotomy, subxiphoid and left or right lateral thoracotomy (Hardy et al, 1992; Robles et al, 1997; Vistarini et al, 2015). Because thoracotomy has potential for substantial postoperative problems, including postoperative pain, hypoventilation, hypothermia, acid-base

disturbance, shock, oliguria and prolonged anesthesia time (Jackson et al, 1999) thoracoscopy is preferred to manage pericardial disease and effusions (Jackson et al, 1999; Palsgaard et al, 2007; Bicer et al, 2015). Considering this and previous postoperative clinical results, comparative investigation of the partial pericardiectomy through thoracotomy and thoracoscopic pericardial window techniques was planned in an experimental animal model.

Because pathologic pericardium disturbs the heart functions and hemodynamic problems causing clinical symptoms, a surgical approach to the pericardial sac is required (Bicer et al, 2015). In the presented study, the well-known pericardiectomy incisions were planned to show the encountered postopera-

tive heart function changes at the early stages. Some heart problems, such as ventricular dysfunction and low output syndrome, can occur following surgical removal of the pathologic pericardium (Wood et al, 2002; Bicer et al, 2015). Myocarditis is often inferred from ST segment and T wave abnormalities on the ECG (Wood et al, 2002). We observed sinus tachycardia, small complexes of QRS and peak T, which were sudden onset ECG abnormalities following pericardiectomy in both groups that were not observed at postoperative day 30. Early stage alterations in the ECG parameters may result from the adaptation of the heart in response to pericardial surgery. These spontaneous recovered abnormalities suggest that rhythms and morphological changes are reversible.

Persistent cardiac dysfunction after pericardiectomy is described in ECHO measurements of diastolic left ventricular relaxation (Schofield et al, 2004). A pericardiectomy performed in constrictive pericarditis has a high rate of early mortality and morbidity due to the reduced left ventricular EF and right ventricular dilatation (Busch et al, 2015). After partial pericardiectomy through a median sternotomy, a low output syndrome can occur in the immediate postoperative period (Wood et al, 2002). Although our study was performed on the healthy experimental animals, we agree from these results that left ventricular diameters at diastole and systole as well as the SV were lower on postoperative day 1 in both groups compared to their baselines. On postoperative days 7 and 30, it was determined that left atrial and ventricular diameters at diastole and systole were at baseline values. Observed differences in SV and EF values on postoperative days compared to their baseline values suggest that cardiac remodeling might occur within day 1 after surgery, which may be followed by spontaneous normalization of geometric and functional parameters of the left heart.

In the constrictive pericarditis cases, there was a 28% rate of low output following complete pericardiectomy that has been reported within 30 days after operations, which relates to the preoperative degree of constriction and subsequent cardiomyopathy (McCaughan et al, 1985). The low output syndrome

may cause a form of myocarditis that is induced by operative trauma during visceral pericardiectomy (Wood et al, 2002). In addition, pericardiectomy by the thoracotomy approach and the extent of the pericardial resection may lead to low output syndrome; therefore, minimal invasive operations are more poorly tolerated (Nath et al, 2014). In the present study, compared to the baseline value, open thoracic partial pericardiectomy in the GRI had lower SV on postoperative days 1 and 30 as well as a lower EF on postoperative day 30, indicating that the open thoracic partial pericardiectomy technique might have been responsible for these side effects due to the intense invasive surgical procedure.

Underlying cardiac problems (low cardiac output) and pericardial surgery techniques are not the only factors affecting mortality; morphological changes of the cardiac structures and disturbance of the ventricles and myocardium can contribute to pericardial pathologies (effusion, etc.), which leads to myocardial fibrosis and adhesions (Nath et al, 2014). In this study, after surgical approaches to the normal pericardium, pleural adhesions, a new fibrous tissue and fibers between the epicardium and the lung tissue were macroscopically observed in both groups. It can be concluded that these results may cause cardiologic disturbances in the long-term period after pericardiectomy. In the case of constrictive pericarditis, mortality reported on postoperative day 30 results from progressive heart and circulatory failure, therapy resistant right heart failure, sepsis, pulmonary embolism, massive bleeding and multiple organ failure in humans (Busch et al, 2015). In the present study, there was no mortality in the early stage. Although our experiments had intact pericardial pathology, the myocardium and lung tissues had more severe histopathological changes in the GRI than GRII. Therefore, open thoracic approaches might be much less traumatic on myocardium and lung tissues than the thoracoscopic procedure, which can be cause long-term heart failure or further pathologies. However, statistical analysis of the histopathological results showed that there was no significant difference between the groups ($P=0.336$). Because there were few animals, long-term, postoperative investigations after pericardiectomy tech-

niques should be planned with a high number experiments to obtain valuable and exact results.

Pericardiectomy techniques may have the potential to improve mortality and morbidity, but they could also be selected depending on pericardial disease. Thoracoscopic approaches are minimally invasive and less traumatic to the myocardium and lungs, but they are also better tolerated. As a conclusion of the study, it is suggested, based on the presented early stage postoperative cardiological and histopathological results, that surgeons may effectively apply partial pericardiectomy and thoracoscopic pericardial window techniques in both small animals and humans.

Although there are no detailed studies investigating early and long-time pericardiectomy techniques in experimental models, this study has reached its aims with limitations. The results were valid in animals with normal pericardium, and the absence of

variability study and the fact that the histopathology results were rather subjective and that EF and SV were not measured with the more appropriate echocardiographic technique (Simpson's method). In this study, only six experimental animals were our materials, and our evaluations were based on the early stage results of the pericardiectomy techniques. The long-term evaluations with a high number of experiments should be performed with future studies to answer the questionable issues on ECG, ECHO and histopathology.

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Conflict of Interest

There are no conflicts of interest to report. ■

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