The Importance of ECG, Cardiac Troponin T and High Sensitive C Reactive Protein in Predicting the Extent of Experimental Myocardial Infarction in Rats

Ratlarda Deneysel Miyokard İnfarktüsünün Yaygınlığını Öngörmede EKG, Kardiyak Troponin T ve Yüksek Duyarlıklı C Reaktif Proteinin Önemi

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ABSTRACT Objective: To analyze the predictive value of ECG variables, cardiac troponin T (cTnT) and high-sensitive C reactive protein (hsCRP) levels in estimating the degree of experimental myocardial infarction in the living rats before euthanasia. **Material and Methods:** Permanent ligation of the left anterior descending coronary artery was performed to develop acute myocardial infarction in 4-month-old male Wistar rats (n=18). Saline (n=5), low-dose erythropoietin (5 000 U.kg–1, n=6) and high-dose erythropoietin (10 000 U.kg–1, n=7) were administered intraperitoneally after the ligation to obtain varying degree of infarction. ECG records were taken before (phase 1), at the end of (phase 2), and at 6 hours after the ligation (phase 3). cTnT and hsCRP levels were measured in phase 3. The size of infarct area was calculated with planimetry on a single midventricular slice stained with triphenyltetrazolium chloride. **Results:** The height of ST segment in phase 2, the Q/R and ST/R ratios measured in phase 3, and the Q/R ratio difference between phases 2 and 3 were found to be associated with the large infarct area. The cutoff values estimated for these variables were capable of predicting the presence of an infarct area ≥40%. cTnT and hsCRP levels were unsuccessful in estimating the size of infarction. **Conclusion:** The variables of a 3-lead ECG may have a predictive value in estimating the presence of large infarction before euthanasia. ST segment elevation may be significantly different as early as the first-half hour of coronary ligation. The levels of cTnT and hsCRP are not associated with the size of infarction, and do not have a predictive value.

Key Words: Myocardial infarction; erythropoietin; troponin t; c-reactive protein;
electrocardiography

ÖZET Amaç: EKG değişkenleri, kardiyak troponin T (cTnT) ve yüksek duyarlıklı C reaktif protein (hsCRP) seviyelerinin, ratlarda oluşturulan deneysel miyokard infarktüsü derecesini ötenazi öncesinde canlı hayvanda belirlenmesi için kullanılabilirliğinin araştırılmasıdır. **Gereç veYöntemler:** Dört aylık erkek Wistar ratlarda (n=18) akut miyokard infarktüsü oluşturmak için sol ön inen koroner artere kalıcı ligasyon uygulandı. Değişen büyüklüklerde infarktüs alanı elde etmek için ligasyon sonrası salin (n=5), düşük doz eritropoietin (5000 U.kg–1, n=6) ve yüksek doz eritropoietin (10000 U.kg–1, n=7) periton içine uygulandı. Ligasyon öncesinde (faz 1), ligasyon işlemi sonunda (faz 2) ve ligasyondan 6 saat sonra (faz 3) 3 elektrotlu EKG kayıtları alındı. cTnT ve hsCRP seviyeleri faz 3'de ölçüldü. İnfarktüs alanı, ventrikül ortasından alınan ve trifeniltetrazolyum klorür ile boyanan tek kesitte planimetri yöntemiyle hesaplandı. **Bulgular:** Faz 2'de ST segment yüksekliği, faz 3'de Q/R ve ST/R oranları ile faz 2 ve faz 3 arasında Q/R oranları farkı, büyük infarktüs alanı varlığı ile ilişkili bulundu. Bu değişkenler için belirlenen kestirim değerleri ≥%40 infarktüs alanı varlığını uygun duyarlılık ve özgüllük değerleriyle öngörebiliyordu. cTnT ve hsCRP seviyeleri infarktüs büyüklüğünü tahmin etmede başarısızdı. **Sonuç:** Üç elektrotlu EKG değişkenleri (ST2, Q/R3, ST/R3, and ΔQ/R2-3) ratlarda deneysel olarak oluşturulan büyük infarktüs varlığını ötenazi öncesinde öngörebilir. ST segment yüksekliği, koroner ligasyon sonrası ilk yarım saatte anlamlı farklılık gösterebilir. cTnT ve hsCRP infarktüs büyüklüğü ile ilişkili değildir ve infarktüs varlığını öngörme değeri yoktur.

Anahtar Kelimeler: Miyokardiyal infarktüs; eritropoetin; troponin t; c-reaktif protein;
elektrokardiyografi

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Coronary artery ligation (CAL) to induce myocardial infarction in rats is a well-established experimental model of which results are very similar to clinical outcomes of ischemic heart diseases.[1-3] This model has frequently been performed in several studies examining ischemic heart disease and its complications including acute myocardial infarction, congestive heart failure and myocardial protection. Preclinical researches of some drugs including antihypertensives have been carried out using CAL models.[4,5]

CAL models described in the literature have been reported to produce the infarct sizes varied widely from 8% to 65%.[6] Postmortem histopathologic examinations, the most common method of measuring the size of infarct area (SIA), have disadvantages such as no remaining tissue for further analysis, and being not suitable for conducting longitudinal studies due to the need for separate groups of animals for each time point.[7] To overcome these disadvantages, placing the suture in a particular position along the coronary artery to obtain a certain size of infarction has been proposed.[3,6,8,9] But each of them has suggested a different ligation site, and the success of the methods in inducing infarction at a desired size is still controversial.[8] Alternatively, predicting the size of resulting infarction using noninvasive methods and selecting the subjects with a certain SIA before euthanasia has been discussed in several studies. These methods are serum markers, echocardiography, magnetic resonance imaging (MRI), computed tomography, single photon emission computed tomography, and positron emission tomography.[3,7,10-12] But they have disadvantages including low throughput, high costs, technical difficulties, low availability of these tests, and the need for profession and experience of performing them.[7,12] Therefore a reliable, achievable, and non-invasive alternative is highly desirable.[7] Following the report of Normann et al. describing electrocardiographic alterations with myocardial infarction in rats, 9-lead and 12-lead electrocardiography (ECG) recordings, a cheap and easy-to-perform test, have been used to predict the SIA induced by CAL before sacrificing the animal.[4,13,14] It has been reported that several ECG variables are accurate in predicting the SIA in rats, and vice versa.[15] None of these variables has widely been regarded as a single noninvasive predictor of the extent of myocardial infarction. Also, the importance of serum markers of myocardial damage for this purpose has not been examined sufficiently.

In this study, we aimed to analyze the value of three non-invasive, simple, inexpensive, and achievable methods in predicting the SIA before sacrificing the animal. These methods were the variables of 3-lead ECG records, cardiac troponin T (cTnT) and high-sensitive C reactive protein (hsCRP) levels obtained during early period of CAL.

MATERIAL AND METHODS

The study was approved by Gazi University Animal Experiments Local Ethics Committee (approval date: 22.10.2013 and number: 66332047-604.01.02/179-23309), and conformed to the Guide for the Care and Use of Laboratory Animals (www.nap.edu/catalog/5140.html). To reduce the number of killed rats according to the 3Rs principles described by Russell and Burch, we performed the ECG recordings on the rats enrolled in our previous study, and used their data.[16,17] Although both studies were simultaneously carried on the same rats, only animals that were sacrificed at six hours after CAL in the previous study were enrolled into the current study. The rats with an inadequate ECG trace in any of measurement phases and their data were excluded. All procedures on the animals were performed in Gazi University Laboratory Animals Care and Experimental Research Center, Ankara, Turkey. The animals were 4-month-old male Wistar rats (n=18). They were housed on a 12-hour light-dark cycle, and fed ad libitum.

MYOCARDIAL INFARCTION MODEL AND EPO ADMINISTRATION

Permanent ligation of the left anterior descending artery (LAD) was performed to develop acute myocardial infarction in rats. The rats were anesthetized with intraperitoneal 45 mg.kg^{-1} ketamine (Alfamine %10, Alfasan International BV, Woerden, The Netherland) and 5 mg.kg^{-1} xylazine (Al-

fazyne %2, Alfasan International BV, Woerden, The Netherland). Before CAL, basal ECG was taken (phase 1). Tracheotomy was performed through a midline cervical skin incision, and the rats were intubated and ventilated with room air using a volume controlled rodent ventilator (Inspira ASV, Harvard Apparatus, Holliston, MA, USA). Thoracotomy through fourth intercostal space was performed to expose the heart. CAL was obtained by placement of a 7-0 polypropilene suture around the space between the pulmonary artery and the left auricle. Following CAL, saline in group 1 (n=5), low-dose erythropoietin (EPO) (5000 U.kg^{-1}, Eprex 4000 IU/0.4 mL pre-filled syringe, Janssen-Cilag

AG, Schaffhausen, Switzerland) in group 2 (n=6) and high-dose erythropoietin (10 000 U.kg^{-1}) in group 3 (n=7) was administered intraperitoneally (erythropoietin groups). Thoracotomy was closed without residual pneumothorax, and tracheotomy was repaired after weaning from the ventilator. ECG was recorded at the end of the procedure corresponding 15-25 minutes following occlusion of the LAD (phase 2). The rats were allowed to awake in a warm and oxygen rich compartment until they were fully active, and then they were transferred to their cages. At 6 hours after CAL, they were fully anesthetized again, and ECG was taken (phase 3). They were re-intubated via the previous tra-
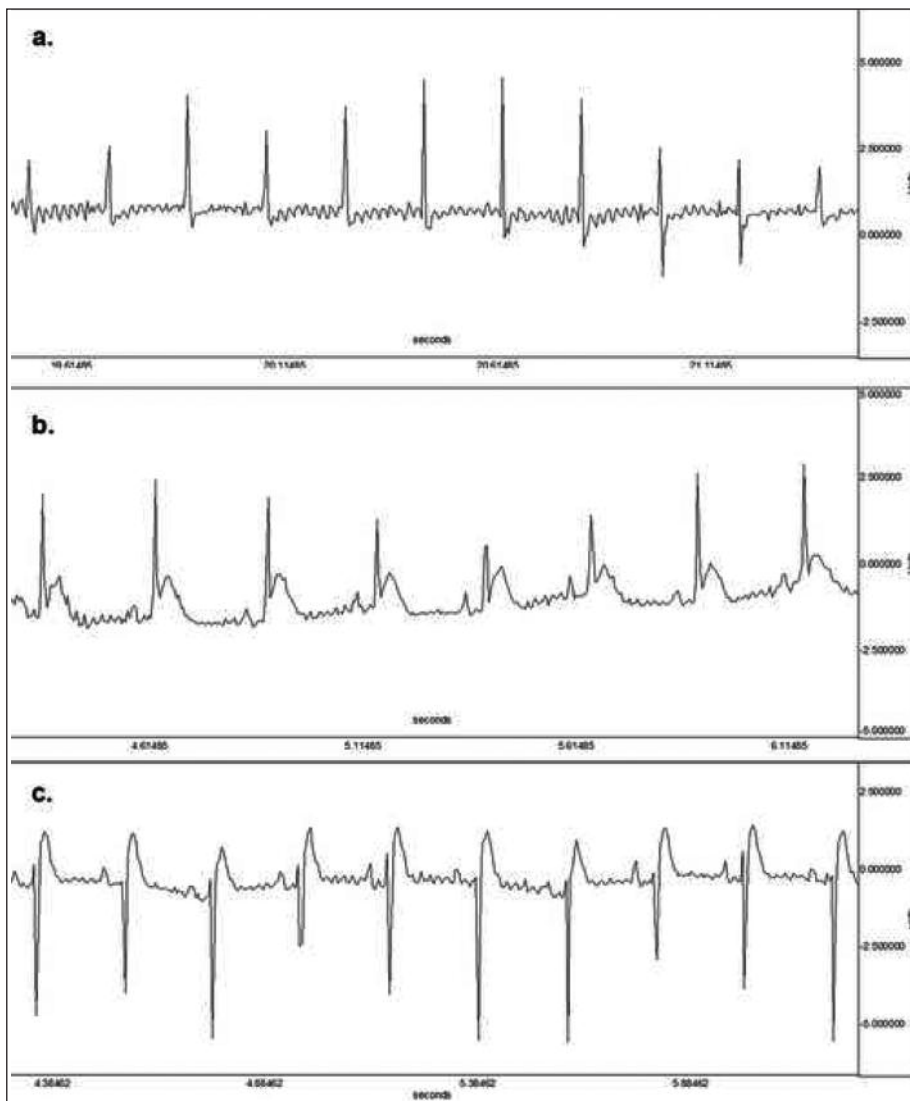

FIGURE 1: Figures represent ECG traces of a rat obtained by the software of the data acquisition system during phase 1 (a), phase 2 (b) and phase 3 (c).

cheotomy site and ventilated. The heart was exposed through a midline sternotomy and excised quickly after the blood sample was withdrawn from the right atrium.

ECG RECORDING

ECG records were taken using the module of a data acquisition system (MP 150 Data Acquisition System, BIOPAC Systems Inc, Goleta, CA, USA). Subcutaneous ECG leads were inserted into the upper paws and the left lower paw of the rats. A single ECG trace in the lead I position was recorded according to the manufacturer's instructions. A minimum 20-second stable trace was obtained for each ECG. All ECG records were stored in a digital medium (Figure 1), and analyzed at the end of the experiments by a single researcher. All measurements on ECG records were done using the software of the data acquisition system (AcqKnowledge version 3.8.1, BIOPAC Systems Inc, Goleta, CA, USA). The averages of measurements obtained from 10 consecutive QRS complex without interference were calculated for each variable. These variables were heart rate and the amplitude of R waves in phase 1 (HR_1 and R_1), phase 2 (HR_2 and R_2), and phase 3 (HR_3 and R_3), and the depth of Q waves and the height of ST segments in phase 2 (Q_2 and ST_2) and phase 3 (Q_3 and ST_3). The ratio of the height of ST segment to the amplitude of R wave and the ratio of the depth of Q wave to the amplitude of R wave were calculated for each ECG in phase 2 (ST/R_2 and Q/R_2) and phase 3 (ST/R_3 and Q/R_3). The differences in heart rate and the amplitude of R waves between phases 1 and 2 (ΔHR_{1-2} and ΔR_{1-2}), phases 1 and 3 (ΔHR_{1-3} and ΔR_{1-3}), phases 2 and 3 (ΔHR_{2-3} and ΔR_{2-3}), and the differences in the depth of Q waves (ΔQ), the height of ST segments (ΔST), the ST/R ($\Delta ST/R$) and Q/R ($\Delta Q/R$) ratios between phases 2 and 3 were calculated.

BIOCHEMISTRY

Rat hs-CRP and rat cTnT levels in the sera samples were measured using the commercially available enzyme-linked immunosorbent assay (ELISA) kits (Cusabio Biotech Co Ltd, Newark, DE, USA, Catalog no: CSB-E08618r and CSB-E11305r respectively) on Model 680 microplate reader (Bio-Rad Laboratories Inc, CA, USA) according to the manufacturer's instructions. The detection limits of hs-CRP and cTnT in rat serum were 0.16 ng.mL^{-1} and 15.6 pg.mL^{-1}, respectively.

MEASUREMENT OF MYOCARDIAL INFARCTION AREA

The hearts were stained with 2,3,5-triphenyltetrazolium chloride (TTC) for the delineation of myocardial infarction using the method described previously.[18] The transverse heart slices at the midventricular level were obtained freehand, and incubated in a 1% solution of TTC in phosphate buffer for 5 minutes at 37°C, pH 7.4. The TTC-incubated slices were photographed in a macroscope (Leica M125 Stereoscope, Leica Microsystems GmbH, Wetzlar, Germany). Photographs were processed in a graphics editing software (Photoshop CS4 Extended, Adobe Systems, San Jose, CA, USA) to calculate the planimetric SIA, which was expressed as a fraction of the total cross-sectional area of the left ventricle in midventricular slices. Calculations of SIAs were done separately for each
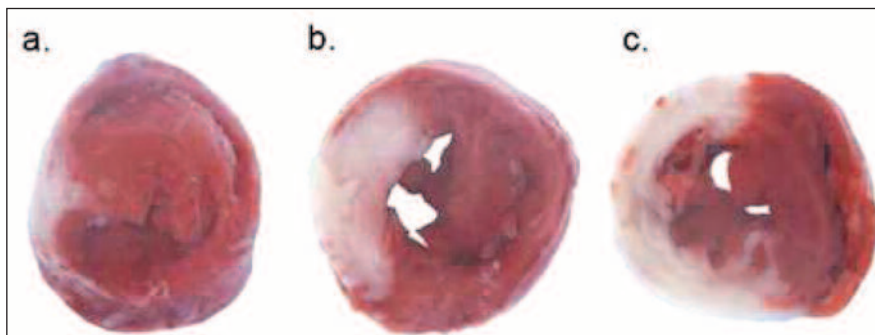

FIGURE 2: The images represent small (a), moderate (b) and large (c) myocardial infarction areas delineated by 2,3,5-triphenyltetrazolium chloride (TTC) staining.

TABLE 1: Rat weights, ECG variables, cTnT and hsCRP levels according to the presence of an infarct area equal to or greater than 20% or 40%.

| | Infarct size groups | | | | |
	Small (n=9)	Moderate (n=4)	Large (n=5)	Total (n=18)	p
Rat weight (g)	253,89±75,96	290,5±97,37	233,8±31,58	256,44±71,06	0,721
HR_1 (beat/min)	314,79±105,21	233,19±69,36	336,24±53,67	302,61±91	0,218
R_1 (mV)	2,39±1,58	1,94±0,39	2,95±0,83	2,44±1,23	0,229
HR_2 (beat/min)	244,42±56,27	209,29±41,58	234,55±15,31	233,87±45,29	0,648
Q_2 (mV)	1,04±1,13	0,39±0,45	0,44±0,72	0,73±0,93	0,473
R_2 (mV)	3,1±1,69	1,67±0,79	3,52±2,25	2,9±1,77	0,184
ST_2 (mV)	1,2±1,02	0,74±0,72	2,13±1,1	1,36±1,07	0,089
Q/R_2	0,35±0,32	0,17±0,2	0,07±0,11	0,23±0,27	0,181
ST/R_2	0,42±0,28	0,47±0,39	0,79±0,47	0,53±0,38	0,245
HR_3 (beat/min)	299,21±79,43	287,53±65,3	316,88±35,96	301,52±64,38	0,768
Q_3 (mV)	1,55±1,86	1,21±1,33	2,3±0,74	1,68±1,5	0,222
R_3 (mV)	1,88±0,55	2,19±1,33	1,66±0,85	1,89±0,81	0,747
ST_3 (mV)	1,15±0,78	1,71±1,65	2,01±1,02	1,51±1,08	0,359
Q/R_3	0,84±0,87	0,48±0,25	1,82±1,14	1,03±0,97	0,127
ST/R_3	0,6±0,41	0,61±0,5	1,23±0,4	0,78±0,5	0,067
ΔHR_{1-2} (beat/min)	70,37±109,46	23,9±96,39	101,69±62,95	68,74±94,89	0,695
ΔHR_{1-3} (beat/min)	15,58±124,72	-54,34±100,31	19,36±83,93	1,09±108,1	0,443
ΔHR_{2-3} (beat/min)	54,79±110,01	78,23±79,24	82,33±41,14	67,65±85,9	0,855
ΔQ_{2-3} (mV)	0,51±1,56	0,82±1,16	1,85±1,09	0,95±1,42	0,149
ΔR_{1-2} (mV)	-0,71±2,15	0,27±1,11	-0,57±1,66	-0,45±1,79	0,505
ΔR_{1-3} (mV)	0,51±1,53	-0,25±1,64	1,29±1,17	0,56±1,49	0,229
ΔR_{2-3} (mV)	-1,22±1,8	0,52±1,38	-1,86±1,92	-1,01±1,87	0,121
ΔST_{2-3} (mV)	-0,06±0,7	0,98±1,68	-0,12±1,74	0,15±1,28	0,530
$\Delta Q/R_{2-3}$	0,49±0,85	0,3±0,14	1,75±1,19a,b	0,8±1,03	0,033
$\Delta ST/R_{2-3}$	0,19±0,39	0,14±0,71	0,44±0,44	0,25±0,47	0,474
cTnT (pg.mL^{-1})	384,76±317,1	182,87±87,12	464,93±467,32	362,16±333,14	0,579
hsCRP (ng.mL^{-1})	0,06±0,03	0,05±0,03	0,07±0,03	0,06±0,03	0,501

HR: Heart rate, R: The amplitude of R wave, Q: The depth of Q wave, ST: The height of ST segment, Q/R: The ratio of the depth of Q wave to the amplitude of R wave, ST/R: The ratio of the height of ST segment to the amplitude of R wave, numbers given in subscript are the measurement phases of the relevant variable (for the explanation of the measurement phases please refer to Material and Methods), Δ: The difference in the variable between the phases given in subscript, cTnT: Cardiac troponin T, hsCRP: high-sensitive C reactive protein.

rat by two independent researchers. The rats with a SIA equal to or smaller than 4.99% were accepted as non-infarcted, and the rats with a SIA greater than 4.99% were classified into three groups (infarct size groups) as described previously[4]: small for 5% to 19.99%, moderate for 20% to 39.99%, and large for 40% or more (Figure 2).

STATISTICS

Statistical analyses were performed using IBM SPSS Statistics version 19 (IBM Corporation, NY, USA).

Rat weight, the SIA in TTC staining, levels of biochemical markers, and ECG variables were expressed as mean±SD. Inter- and intra-observer reliability analysis for the SIA were done with intra-class correlation coefficient. Test-retest for intra-observer reliability was performed in one-month interval. Heart rates and the amplitude of R waves were compared between phases using the Friedman test. The Wilcoxon signed ranks test with Bonferroni correction was performed for multiple comparisons. The comparison of other ECG

TABLE 2: ECG variables according to the measurement phases.			
	Measurement phases		
	Phase 1	Phase 2	Phase 3	p
HR (beat/min)	302,61±91	233,87±45,29a,b	301,52±64,38	0,001
R (mV)	2,44±1,23	2,9±1,77	1,89±0,81	0,249
Q (mV)	-	0,73±0,93	1,68±1,5	0,007
Q/R	-	0,23±0,27	1,03±0,97	0,004
ST (mV)	-	1,36±1,07	1,51±1,08	0,486
ST/R	-	0,53±0,38	0,78±0,5	0,071

HR: Heart rate, R: The amplitude of R wave, Q: The depth of Q wave, Q/R: The ratio of the depth of Q wave to the amplitude of R wave, ST: The height of ST segment, ST/R: The ratio of the height of ST segment to the amplitude of R wave.
ap=0.018 for phase 1 vs. phase 2, bp=0.006 for phase 2 vs. phase 3.

variables between phases was done using the Wilcoxon signed ranks test. The comparison of the variables between both the erythropoietin groups and the infarct size groups was carried out using the Kruskal-Wallis test. The Mann-Whitney U test with Bonferroni correction was done for multiple comparisons. The presence and the degree of any correlation between the SIA and the results of ECG variables, cTnT, and hsCRP measurements were analyzed with the Spearman's rank correlation test. ROC analysis was performed to evaluate the predictive value of the ECG variables, the levels of cTnT and hsCRP in estimating the presence of a SIA equal to or more than both 20% (moderate and large infarction) and 40% (large infarction). The cutoff points for the variables with a significant area under curve (AUC) value were estimated, and the specificity/sensitivity pairs for these cutoff points were obtained. p<0.05 was considered to be statistically significant difference in all analyses.

RESULTS

The mean weight of the rats was 256.44±71.06 g (range 163-420 g). It was 238.2±43.84 g in group 1, 287.5±106.43 g in group 2, and 242.86±46.15 g in group 3. The difference between the groups was insignificant (p:0.822).

THE SIZE OF MYOCARDIAL INFARCT AREA (SIA)

Intra-class correlation coefficients were 0.934 (p<0.001) and 0.995 (p<0.001) for inter- and intra-observer reliability for the SIA, respectively.

There was a SIA greater than 5% in all rats. Again in all rats, infarction involved the whole apex of the heart, while the basis and the interventricular septum remained intact. The mean SIA was 28.09±17.59% (range 5.88-61.4%). Five rats (27.8%) had a large infarct area, 4 rats (22.2%) had a moderate infarct area, and 9 rats (50%) had a small infarct area. There was no correlation between the SIA and the rat weight (ρ:-0.173 and p:0.483). Also, rat weights were similar in the infarct size groups (Table 1).

In the erythropoietin groups, the mean SIA was 41.96±22.15% in group 1, 21.49±10.58% in group 2, and 23.84±15.23% in group 3. Although the mean SIA was higher in group 1, the difference between erythropoietin groups was statistically insignificant (p:0.266). Three rats in group 1 (60%), 0 rat in group 2 (0%), and 2 rat in group 3 (28.6%) had a large infarct area (p:0.064).

ECG VARIABLES

The results of ECG variables are represented in Table 2. There was a significant decrease in heart rate just after CAL (phase 2). But it was similar in phases 1 and 3. The amplitude of R waves, the height of ST segments and the ST/R ratio remained similar through the phases, but there was a statistically significant increase in Q_3 and Q/R_3.

Table 1 shows the results of ECG variables according to the infarct size groups. There was a significant difference in $\Delta Q/R_{2-3}$ between the infarct size groups. Multiple comparisons showed that $\Delta Q/R_{2-3}$ were significantly higher in the rats with the large infarct area than those with both the small and moderate infarct sizes. Also, ST/R_3 was insignificantly higher in the rats with the large infarct area. Table 3 represents the results of ECG variables according to the presence of an infarct area ≥20% or 40%. None of the ECG variables were associated with the presence of a SIA ≥20%. But ST_2, Q/R_3, ST/R_3, and $\Delta Q/R_{2-3}$ were significantly higher in the rats with a SIA ≥40%.

Table 4 represents the correlation between the SIA and ECG variables. There was a positive correlation between the SIA and ST/R_2, ST/R_3, ΔQ_{2-3}, and $\Delta Q/R_{2-3}$.

TABLE 3: Rat weights, ECG variables, cTnT and hsCRP levels according to the presence of an infarct area equal to or greater than 20% or 40%.

	Presence of an infarct area equal to or greater than 20%			Presence of an infarct area equal to or greater than 40%		
	No (n=9)	Yes (n=9)	p	No (n=13)	Yes (n=5)	p
Rat weight (g)	253,89±75,96	259±70,33	0,931	265,15±80,78	233,8±31,58	0,633
HR_1 (beat/min)	314,79±105,21	290,44±78,7	0,605	289,68±100,59	336,24±53,67	0,387
R_1 (mV)	2,39±1,58	2,5±0,83	0,489	2,25±1,33	2,95±0,83	0,095
HR_2 (beat/min)	244,42±56,27	223,32±30,7	0,931	233,61±53,18	234,55±15,31	0,566
Q_2 (mV)	1,04±1,13	0,42±0,58	0,258	0,84±1	0,44±0,72	0,387
R_2 (mV)	3,1±1,69	2,7±1,93	0,546	2,66±1,59	3,52±2,25	0,387
ST_2 (mV)	1,2±1,02	1,51±1,15	0,489	1,06±0,93	2,13±1,1	0,035
Q/R_2	0,35±0,32	0,12±0,15	0,094	0,29±0,29	0,07±0,11	0,143
ST/R_2	0,42±0,28	0,65±0,44	0,190	0,43±0,3	0,79±0,47	0,117
HR_3 (beat/min)	299,21±79,43	303,83±49,85	1,000	295,62±72,83	316,88±35,96	0,633
Q_3 (mV)	1,55±1,86	1,81±1,12	0,387	1,44±1,66	2,3±0,74	0,095
R_3 (mV)	1,88±0,55	1,89±1,05	0,796	1,97±0,81	1,66±0,85	0,503
ST_3 (mV)	1,15±0,78	1,88±1,25	0,222	1,32±1,08	2,01±1,02	0,208
Q/R_3	0,84±0,87	1,22±1,08	0,297	0,72±0,74	1,82±1,14	0,046
ST/R_3	0,6±0,41	0,95±0,53	0,113	0,61±0,42	1,23±0,4	0,019
ΔHR_{1-2} (beat/min)	70,37±109,46	67,12±84,54	1,000	56,07±103,96	101,69±62,95	0,566
ΔHR_{1-3} (beat/min)	15,58±124,72	-13,39±93,83	0,489	-5,94±118,38	19,36±83,93	0,703
ΔHR_{2-3} (beat/min)	54,79±110,01	80,51±56,61	0,931	62±98,81	82,33±41,14	0,775
ΔQ_{2-3} (mV)	0,51±1,56	1,39±1,18	0,161	0,6±1,41	1,85±1,09	0,059
ΔR_{1-2} (mV)	-0,71±2,15	-0,2±1,42	0,546	-0,41±1,9	-0,57±1,66	0,775
ΔR_{1-3} (mV)	0,51±1,53	0,61±1,53	0,605	0,28±1,54	1,29±1,17	0,117
ΔR_{2-3} (mV)	-1,22±1,8	-0,8±2,03	0,489	-0,68±1,82	-1,86±1,92	0,336
ΔST_{2-3} (mV)	-0,06±0,7	0,36±1,7	0,340	0,26±1,13	-0,12±1,74	0,849
$\Delta Q/R_{2-3}$	0,49±0,85	1,11±1,14	0,113	0,43±0,7	1,75±1,19	0,007
$\Delta ST/R_{2-3}$	0,19±0,39	0,31±0,56	0,931	0,17±0,48	0,44±0,44	0,336
cTnT (pg.mL^{-1})	384,76±317,1	339,57±366,25	0,796	322,64±279,89	464,93±467,32	0,566
hsCRP (ng.mL^{-1})	0,06±0,03	0,06±0,03	1,000	0,06±0,03	0,07±0,03	0,387

HR: Heart rate, R: The amplitude of R wave, Q: The depth of Q wave, ST: The height of ST segment, Q/R: The ratio of the depth of Q wave to the amplitude of R wave, ST/R: The ratio of the height of ST segment to the amplitude of R wave, numbers given in subscript are the measurement phases of the relevant variable (for the explanation of the measurement phases please refer to Material and Methods), Δ: The difference in the variable between the phases given in subscript, cTnT: Cardiac troponin T, hsCRP: high-sensitive C reactive protein.

Table 5 summarizes the results of ROC analysis of ECG variables. None of the AUC values of ECG variables calculated according to the presence of a SIA ≥20% were significant. But ST_2, Q/R_3, ST/R_3, and $\Delta Q/R_{2-3}$ had significant AUC values according to the presence of a SIA ≥40%. To predict the occurrence of a SIA ≥40%, the estimated cutoff points were 1.37 mV for ST_2 with a sensitivity of 0.8 and a specificity of 0.692; 0.76 for Q/R_3 with a sensitivity of 0.8 and a specificity of 0.692; 1.14 for ST/R_3 with a sensitivity of 0.8 and a specificity of 0.846; and 0.48 for $\Delta Q/R_{2-3}$ with a sensitivity of 1 and a specificity of 0.796.

BIOCHEMICAL MARKERS

The mean cTnT level was 362.16±333.14 pg.mL^{-1} (range 53.29-1206.04 pg.mL^{-1}), and the mean hsCRP level was 0.06±0.03 ng.mL^{-1} (range 0.01-0.12 ng.mL^{-1}). The levels of these biochemical markers were similar in the infarct size groups (Ta-

TABLO 4: The correlation between the size of infarct area and the analyzed variables

	Infarct size	
	ρ	p
Rat weight (g)	-0,173	0,493
HR_1 (beat/min)	-0,036	0,887
R_1 (mV)	0,273	0,274
HR_2 (beat/min)	0,205	0,414
Q_2 (mV)	-0,196	0,437
R_2 (mV)	0,018	0,945
ST_2 (mV)	0,345	0,161
Q/R_2	-0,322	0,193
ST/R_2	0,484	0,042
HR_3 (beat/min)	-0,044	0,861
Q_3 (mV)	0,381	0,119
R_3 (mV)	-0,084	0,742
ST_3 (mV)	0,358	0,144
Q/R_3	0,437	0,070
ST/R_3	0,506	0,032
ΔHR_{1-2} (beat/min)	-0,125	0,622
ΔHR_{1-3} (beat/min)	-0,073	0,773
ΔHR_{2-3} (beat/min)	-0,115	0,651
ΔQ_{2-3} (mV)	0,484	0,042
ΔR_{1-2} (mV)	0,065	0,798
ΔR_{1-3} (mV)	0,253	0,311
ΔR_{2-3} (mV)	0,030	0,906
ΔST_{2-3} (mV)	0,164	0,515
$\Delta Q/R_{2-3}$	0,529	0,024
$\Delta ST/R_{2-3}$	0,102	0,687
cTnT ($pg.mL^{-1}$)	0,024	0,925
hsCRP ($ng.mL^{-1}$)	0,187	0,457

HR: Heart rate, R: The amplitude of R wave, Q: The depth of Q wave, ST: The height of ST segment, Q/R: The ratio of the depth of Q wave to the amplitude of R wave, ST/R: The ratio of the height of ST segment to the amplitude of R wave, numbers given in subscript are the measurement phases of the relevant variable (for the explanation of the measurement phases please refer to Material and Methods), Δ: The difference in the variable between the phases given in subscript, cTnT: Cardiac troponin T, hsCRP: high-sensitive C reactive protein.

bles 1 and 3). There was no correlation between the SIA and them (Table 4). They had insignificant AUC values in the ROC analysis (Table 5).

DISCUSSION

The results of this study could be summarized as follows: (i) standard CAL with erythropoietin injection in rats induced the occurrence of infarction in an acceptable distribution; (ii) some variables in

3-lead ECG taken after CAL including ST_2, Q/R_3, ST/R_3, and $\Delta Q/R_{2-3}$ were found to be associated with the occurrence of a large infarct area; (iii) the estimated cutoff values of these variables predicted the presence of a SIA ≥40% with satisfactory sensitivity and specificity pairs, but (iv) none of the ECG variables predicted the presence of a SIA ≥20%; (v) the presence of a SIA ≥40% was predicted as early as the first-half hour of CAL by the height of ST segment; and (vi) cTnT and hsCRP levels measured six hours after CAL were unsuccessful in estimating the SIA.

We used the percentage of infarct area calculated with a planimetric method on a single midventricular slice stained with TTC to evaluate the involvement of myocardial infarction. Postmortem TTC staining of heart samples in rats and mice is the most common method in measuring the SIA.[7] Additionally, it is cheap, high throughput, reliable, and reproducible. Calculation of the SIA on multiple slices obtained at defined intervals from the whole heart of sacrificed rats is accepted as the standard method.[7] But the major disadvantage of this method is lack of residual tissue enough to allow further analysis.[7] It was shown by dos Santos et al that the SIA calculated on a single midventricular slice produced similar results with those on multiple slices.[11] This method has been reported in the literature.[5] In our study, resultant infarction involved the whole apex while the basis of the heart and the interventricular septum remained intact in all rats. Such occurrence of infarction in rats has previously been reported.[19] Because calculating the SIA on the apical and basal parts was practically meaningless, the SIA calculated on the midventricular slices alone was used to represent the extent of infarction.

Although performing ligation at different levels along the coronary artery to induce infarction in a certain size has been reported in the literature, the methods described for this purpose and their results are still controversial.[3,6,8,9] These methods ensure neither the occurrence of the desired infarct size nor the adequate categorization of the rats into different groups in a balanced size according to their SIA. Also, ligation of the LAD at different lev-

TABLE 5: Results of ROC analysis of the variables according to the presence of moderate to large or only large infarction.					
	Presence of an infarct area equal to or greater than 20%			Presence of an infarct area equal to or greater than 40%		
	AUC	SE	p	AUC	SE	p
Rat weight (g)	0,519	0,144	0,895	0,415	0,132	0,588
HR_1 (beat/min)	0,420	0,144	0,566	0,646	0,130	0,349
R_1 (mV)	0,605	0,139	0,453	0,762	0,123	0,094
HR_2 (beat/min)	0,481	0,145	0,895	0,600	0,135	0,522
Q_2 (mV)	0,340	0,132	0,251	0,362	0,143	0,375
R_2 (mV)	0,407	0,140	0,508	0,646	0,161	0,349
ST_2 (mV)	0,599	0,139	0,480	0,823	0,104	0,038
Q/R_2	0,265	0,126	0,093	0,269	0,118	0,139
ST/R_2	0,685	0,132	0,185	0,754	0,121	0,104
HR_3 (beat/min)	0,494	0,144	0,965	0,585	0,138	0,588
Q_3 (mV)	0,630	0,137	0,354	0,769	0,113	0,085
R_3 (mV)	0,457	0,151	0,757	0,385	0,167	0,460
ST_3 (mV)	0,673	0,137	0,216	0,708	0,135	0,183
Q/R_3	0,654	0,135	0,270	0,815	0,107	0,043
ST/R_3	0,722	0,126	0,112	0,862	0,091	0,021
ΔHR_{1-2} (beat/min)	0,494	0,147	0,965	0,600	0,140	0,522
ΔHR_{1-3} (beat/min)	0,401	0,141	0,480	0,562	0,140	0,693
ΔHR_{2-3} (beat/min)	0,481	0,144	0,895	0,554	0,141	0,730
ΔQ_{2-3} (mV)	0,704	0,132	0,145	0,800	0,105	0,055
ΔR_{1-2} (mV)	0,593	0,143	0,508	0,446	0,146	0,730
ΔR_{1-3} (mV)	0,580	0,146	0,566	0,754	0,131	0,104
ΔR_{2-3} (mV)	0,605	0,140	0,453	0,338	0,141	0,301
ΔST_{2-3} (mV)	0,642	0,140	0,310	0,538	0,160	0,805
$\Delta Q/R_{2-3}$	0,728	0,123	0,102	0,908	0,073	0,009
$\Delta ST/R_{2-3}$	0,519	0,147	0,895	0,662	0,144	0,301
cTnT (pg.mL^{-1})	0,457	0,144	0,757	0,592	0,163	0,554
hsCRP (ng.mL^{-1})	0,494	0,142	0,965	0,638	0,138	0,375

AUC: Area under curve, SE: Standard error, HR: Heart rate, R: The amplitude of R wave, Q: The depth of Q wave, ST: The height of ST segment, Q/R: The ratio of the depth of Q wave to the amplitude of R wave, ST/R: The ratio of the height of ST segment to the amplitude of R wave, numbers given in subscript are the measurement phases of the relevant variable (for the explanation of the measurement phases please refer to Material and Methods), Δ: The difference in the variable between the phases given in subscript, cTnT: Cardiac troponin T, hsCRP: high-sensitive C reactive protein.

els may be related to high unsuccessful attempt rates and high mortality. It was reported by Degabriele et al that the mortality rate was higher in rats with a high suture level, and the size of resultant infarction was related to the position of ligation rather than the level of ligation.[6] In the study of Ahn et al, standard LAD ligation was reported to induce myocardial infarction in 100% of the rats, whereas the success rate of distal ligation performed to produce a smaller infarction was only 57.2%.[8] For that reason, we preferred performing a method consisting of CAL, described commonly in several studies with a high success rate, and erythropoietin administration in different doses to obtain infarction in different sizes. We hypothesized that administration of erythropoietin in different doses after CAL might reduce the resultant infarction producing a varied sized mean SIA which is enough to distribute in a wide range of spectrum in each group of the rats. We aimed to get a sufficient sample size for the rats with a smaller infarction area using the infarct size-limiting effect of erythropoietin as well as the rats with a large infarction area instead of placing the ligature at dif-

ferent levels along the coronary artery. When the rats without erythropoietin administration considered in our study, it was seen in group 1 that CAL alone induced a large infarct area in 60% of the rats, whereas the rest had a small or a moderate infarct area. We observed that erythropoietin administration following CAL reduced the infarct size in groups 2 and 3. Although the difference was statistically insignificant, erythropoietin administration provided the occurrence of smaller infarct areas and an acceptable distribution for the SIA.

In some studies, the rats were assigned to the different infarct size groups, or enrolled into the study assuming that they had an infarction greater than a certain size according to their post-ligation ECG findings.[4,5,14] They have reported an average SIA over 40% in the rats selected by the presence of pathological Q wave or the loss of R wave amplitude on the postligation ECG.[14,20] We analyzed a single derivation of 3-lead ECG for each phase to prevent an abundance of variables. 3- and 4-lead ECG have been used in previous studies.[1,3,21] In our study, ST_2, Q/R_3, ST/R_3, and $\Delta Q/R_{2-3}$ were found to be feasible in predicting the large infarct area. It has been accepted in clinical practice for decades that the presence of pathological Q wave reveals the occurrence of myocardial infarction; the depth of Q wave reflects the extent of infarction; and the derivations showing Q waves is used to estimate the localization of infarction.[22] We observed that the depth of Q wave neither in phase 2 nor in phase 3 were directly associated with the SIA. But the presence of Q/R_3 ≥0.76, which corresponds a Q wave deeper than three-fourth of the amplitude of adjoining R wave, was found to be a significant predictor of infarction involving 40% or more of the left ventricle. Additionally, 0.48 or more increase in the Q/R ratio difference between the ECGs taken at the end of and six hours after CAL was another predictor of the large infarction. But these variables were unsuccessful in predicting the presence of 20% or more SIA. Pathological Q wave is accepted as the most prominent marker of myocardial infarction in rats.[2,13] Bonilha et al. reported that the presence of deep Q waves in rats represents the occurrence of myocardial infarction, but it is insufficient to characterize the extent of infarction.[15] Normann et al. reported that pathological Q wave can occur as early as 2 hours after coronary artery occlusion in rats.[13] Pimentel et al reported that the presence of Q wave one day after CAL confirms the success of the procedure, and there is a significant linear correlation between the depth of Q wave and the extent of infarction.[21] It has been notified that Q wave may be absent in rats with small sized infarction or with small epicardial lesions not extending to endocardium.[13,21] We did not observe a relationship between the loss of R wave amplitude and the presence or the size of infarction. Similarly, Bonilha et al. reported that the loss of R wave amplitude did not predict the SIA ≥40%.[15]

We found that ST segment was elevated in the ECG taken at the end of CAL, and it was persisted with a similar level until six hours after the procedure. ST segment elevation was used in some studies to confirm the occurrence of myocardial infarction following CAL.[3,23,24] But none has clarified which changes in ST segment were used to estimate the SIA, or at least to confirm the presence of infarction equal to or greater than a certain size that is required to conduct the study. Also, ST segment changes were reported to be irrelevant to the diagnosis of myocardial infarction in rats.[13] We observed in our study that ST_2 and ST/R_3 may estimate the presence of a large infarct area. ST_2 with a cutoff value over 1.37 mV may predict the presence of infarction ≥40% with an acceptable sensitivity and specificity pair, and may be treated as an early marker of large myocardial infarction in rats. Additionally, ST/R_3 with a cutoff value over 1.14 may predict a large infarction. Such a relationship between early ST segment changes and the SIA has not previously been reported.

The importance of some biochemical markers including creatine phosphokinase, creatine phosphokinase-MB isoenzyme, lactate dehydrogenase, troponin I (cTnI) and T, α-hydroxybutyrate dehydrogenase, and brain natriuretic peptide (BNP) in estimating the infarct size in humans have been evaluated.[25-27] Serum cTnI was found to be correlated with the SIA measured with MRI in hu-

mans.[28] Jiang et al. reported a strong correlation between the serum levels of cTnT measured 24 hours after CAL and both the SIA on histopathologic examination and the left ventricular function on echocardiography.[29] Vietta et al. reported that although cTnI levels measured 2 hours after CAL were not correlated with the SIA, those measured 8 hours after the procedure were correlated with that, but these levels could not discriminate the small, moderate or large infarct sizes from each other.[12] Additionally, it was reported in the study of Li et al. that cTnI levels measured in the first four hours of CAL did not have a significant correlation with the SIA, but BNP levels had.[24] In the review of Csonka et al., it was concluded that troponin levels are not helpful in estimating the size of experimental infarction due to frequent false positive results.[7] To date, there is an absence of data addressing primarily the relationship between CRP levels and the SIA in rats. But Griselli et al. showed that the SIA was larger in the rats received human CRP than controls.[23] Elevated CRP levels were reported to be associated with a larger SIA.[30] We found that the levels of cTnT and hsCRP were similar in the infarct size groups, and were not correlated with the SIA. Also, ROC analysis showed that these biochemical markers did not have a predictive value in estimating the size of experimental myocardial infarction.

We did not evaluate whether erythropoietin injection have an effect on ECG, and it was the major limitation of the study. But to our knowledge, such an effect on the electrical activity of the heart has not been described previously in the literature.

In conclusions, ST_2, Q/R_3, ST/R_3, and $\Delta Q/R_{2-3}$ are associated with the size of experimental myocardial infarction in rats, and may have a predictive value in estimating the presence of infarction involving 40% or more of the left ventricle before euthanasia. ST segment elevation may predict that as early as the first-half hour of coronary ligation. The levels of cTnT and hsCRP are not associated with the size of resultant infarction, and do not

Conflict of Interest

Authors declared no conflict of interest or financial support.

Authorship Contributions

Conception: H. Fatih Aşgün, Aysel Güven Bağla, Ertuğrul Ercan; **Design:** H. Fatih Aşgün, Aysel Güven Bağla, Ertuğrul Ercan; **Supervision:** H. Fatih Aşgün, Aysel Güven Bağla, Ertuğrul Ercan; **Data acquisition and/or processing:** H. Fatih Aşgün, Aysel Güven Bağla, Ertuğrul Ercan; **Data analysis and/or interpretation:** H. Fatih Aşgün, Aysel Güven Bağla, Ertuğrul Ercan; **Literature search:** H. Fatih Aşgün, Aysel Güven Bağla, Ertuğrul Ercan; **Manuscript writing (Drafting the manuscript):** H. Fatih Aşgün, Aysel Güven Bağla, Ertuğrul Ercan; **Critical revision and final approval:** H. Fatih Aşgün, Aysel Güven Bağla, Ertuğrul Ercan.

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