Evaluation of changes in cardiac biomarker concentrations and enzyme activities in serum after short- and long-duration transcutaneous cardiac pacing in dogs

Seungkeun Lee, DVM, PhD; Son-Il Pak, DVM, PhD; Changbaig Hyun, DVM, MVetClinStud, PhD

Objective—To evaluate serum cardiac biomarker concentrations and selected enzyme activities in dogs with experimentally induced bradyarrhythmias after short- (1-hour) and long- (3-hour) duration transcutaneous cardiac pacing (TCP).

Animals—10 healthy Beagles.

Procedures—In each dog, anesthesia was induced with propofol (5 mg/kg, IV) and maintained via inhalation of isoflurane in oxygen. To induce bradyarrhythmia, diltiazem was administered IV (20 to 50 mg/dog). Transcutaneous cardiac pacing was performed for 1 hour (5 dogs) or 3 hours (5 dogs) by use of an automated external cardiac pulse generator and a transdermal electrode. Serum concentrations of creatine kinase-MB fraction and cardiac troponin I and activities of aspartate transaminase, creatine kinase, and lactate dehydrogenase were evaluated the day before (baseline) and at intervals until 7 days after TCP.

Results—Increases (from baseline) in serum cardiac biomarker concentrations and enzyme activities were detected in the long-duration TCP group; changes in the short-duration TCP group were more minor and largely not significant. Although severity of myocardial and skeletal muscular injuries was apparently greater with greater duration of TCP, the injuries were not persistent; most variables were within reference range within 3 days after TCP.

Conclusions and Clinical Relevance—Results indicated that application of TCP for > 1 hour in dogs may cause myocardial and skeletal muscular injuries. Serum concentrations of creatine kinase-MB fraction and cardiac troponin I and activities of aspartate transaminase, creatine kinase, and lactate dehydrogenase should be more carefully monitored after TCP of > 1 hour's duration to evaluate potential myocardial damages. (*Am J Vet Res* 2009;70:599–603)

Transcutaneous cardiac pacing is a noninvasive temporary method for restoring normal heart contractions in humans and animals with abnormally slow heart rates. Transcutaneous cardiac pacing is relatively easy to perform and only requires minimal training; therefore, the procedure is commonly used for the emergency treatment of humans with a high risk of bradycardic rhythm disturbances (eg, high-grade heart block, sick sinus syndrome, or vasovagal syncope).[1]

The safety of prolonged use of TCP has always been a concern. However, in several studies[2–5] in dogs and humans, no enzymatic, ECG, or microscopic evidence of myocardial damage after pacing for as long as 30 minutes has been detected. In addition, in dogs with experimentally induced anoxia, TCP stimulation did not result in ventricular fibrillation or ventricular tachycardia during the vulnerable period.[6] Only 1 of the 32 human patients who received TCP in another study[7] developed ventricular fibrillation; however, life-threatening ventricular arrhythmias were induced in dogs in which the capture energy used was 10 times as great as the pacing threshold.[8]

Despite the fact that application of TCP for a period of 30 minutes appears not to induce serious myocardial injuries in dogs and humans,[2,3,5] the safety assessment of TCP of longer duration (ie, periods > 1 hour) may be more appropriate because most patients generally require treatment with TCP for long periods in clinical situations. However, the long-term use of TCP in either humans or dogs has never been investigated to our knowledge.

Abbreviations

AST	Aspartate transaminase
CK	Creatine kinase
CK-MB	Creatine kinase-MB fraction
cTnI	Cardiac troponin I
LDH	Lactate dehydrogenase
TCP	Transcutaneous cardiac pacing

Received May 20, 2008.
Accepted August 20, 2008.
From Korea Animal Hospital, 574-10 Sajik-dong, Cheongju 361-101, Republic of Korea (Lee); and the Sections of Veterinary Clinical Pathology and Epidemiology (Pak) and Small Animal Internal Medicine (Hyun), School of Veterinary Medicine, Kangwon National University, Chuncheon 201-100, Republic of Korea.
This study was supported by the Korean Ministry of Commerce and Energy (10027557) and CU-Medical Systems(Korea).
Address correspondence to Dr. Hyun.

Potentially, continuous stimulation from cardiac pacing energy will damage cardiac and skeletal muscle cells and may result in the release of cellular leakage enzymes. Therefore, evaluation of changes in concentrations of cardiac biomarkers and activities of enzymes in serum as indicators of myocardial or skeletal damage may be clinically important because the use of TCP is gaining popularity in the treatment of bradyarrhythmias in small animals. The purpose of the study reported here was to evaluate cardiac biomarker concentrations and enzyme activities in serum in dogs with experimentally induced bradyarrhythmias after short- (1-hour) and long-duration (3-hour) TCP.

Materials and Methods

Animals—Ten healthy Beagles that had no evident abnormalities in thoracic cavity conformation were selected for the study. The mean ± SD weight of the dogs was 9.24 ± 0.71 kg; there were 6 females and 4 males (all of which were sexually intact). Each dog was considered healthy on the basis of results of a CBC[a] and serum biochemical analyses[b] (including assessment of variables indicative of hepatic and cardiac function). Each dog was examined for preexisting cardiac diseases via 12-lead ECG, thoracic radiography, and 2-dimensional (with M-mode) echocardiography.[c] Dogs with cardiomegaly or echocardiographic abnormalities (eg, cardiac dimensions or valvular competency that exceeded reference values) were excluded from study participation. The study was approved by the animal ethics committee of Kangwon National University and was in strict compliance with the guidelines set forth by the National Research Council of Korea (including animal care and euthanasia). Five dogs were each assigned to undergo short- or long-duration TCP.

Anesthesia and artificial ventilation—Anesthesia was induced in each dog via IV administration of propofol[d] (5 mg/kg). After tracheal intubation, anesthesia was then maintained via inhalation of isoflurane[e] in oxygen. The dog was mechanically ventilated at a rate of 20 to 30 breaths/min by use of a volume-cycled respirator.[f] End-tidal P_{CO_2} values and oxygen saturation as measured via pulse oximetry were monitored throughout the experiment by use of a patient monitor.[g]

Induction of bradyarrhythmia—To induce bradyarrhythmia, diltiazem[h] (20 to 50 mg) was administered via a cephalic vein in each dog. Bradyarrhythmia (identified by heart rate, < 30 beats/min and ECG detection of sinus node exit block, P-R interval prolongation, and atrial standstill) or cardiac arrest was confirmed by use of a continuous digital ECG monitor.[i]

Protocol for evaluating the safety of short- and long-duration TCP—An automated external cardiac pulse generator[j] and a transdermal electrode[k] were used for TCP. Five dogs underwent TCP for 1 hour (short-duration treatment), and 5 dogs underwent TCP for 3 hours (long-duration treatment). For each dog, TCP involved application of 5 mA of pacing current/kg of body weight (with 20 milliseconds of pulse duration) via transdermal electrodes (surface area, 40 cm^2) in the left apex–right apex positions (ie, electrode placement over the costochondral junctions of the fourth to seventh ribs on the left and right sides, respectively) at a heart rate of 120 beats/min; this rate was selected because the mean ± SD heart rate among the study dogs was 114.4 ± 12.4 beats/min. The success of the TCP was confirmed by the presence of spike impulses detected via ECG, the continuous formation of pressure waveforms confirmed via aortic pressure measurements, and left ventricular contraction detected via M-mode echocardiography. To evaluate detrimental effects associated with short- and long-duration TCP, serum concentrations of cardiac biomarkers (cTnI and CK-MB) and activities of AST, CK, and LDH were measured on the day prior to TCP (baseline; day –1), immediately after TCP (day 0), and at 1, 2, 3, 5, and 7 days after TCP.

Biochemical assays for cardiac cell damage—From each dog, blood samples (2 mL each) were collected via a jugular vein on day –1, on day 0 (immediately after short- or long-duration TCP), and on days 1, 2, 3, 5, and 7. Samples were immediately placed in a serum

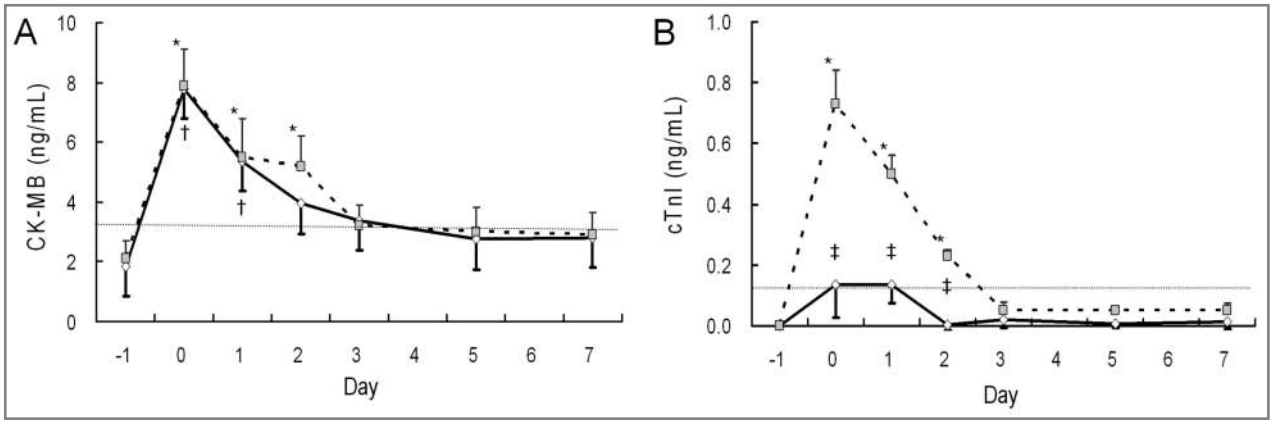

Figure 1—Mean ± SD serum concentrations of CK-MB (A) and cTnI (B) in 10 dogs before and after short- (1-hour; white diamonds; n = 5) or long (3-hour; gray squares; n = 5) duration TCP. Data were collected 1 day prior to TCP (day –1), immediately after TCP (day 0), and at 1, 2, 3, 5, and 7 days after TCP. The horizontal line indicates the upper limit of the reference range for the variable. *At this time point, the value differs significantly ($P < 0.05$) from the baseline value in the long-duration TCP group. †At this time point, the value differs significantly ($P < 0.05$) from the baseline value in the short-duration TCP group. ‡At this time point, the value in the short-duration TCP group differs significantly ($P < 0.05$) from the value in the long-duration TCP group.

separation tube[l] and centrifuged for the separation of serum. All experiments were done with freshly collected samples. Serum activities of AST, CK, and LDH were measured by use of an automated chemical analyzer.[b] Serum concentration of CK-MB was measured by use of an automated enzyme immunoassay system.[m] Serum cTnI concentration was measured by use of a second-generation cTnl assay.[9,10,n]

Statistical analysis—Statistical analysis was performed by use of a 2-way ANOVA with treatment group as a between-animal factor and time as a within-animal repeated-measures factor to determine whether significant differences existed in serum enzyme activities and cardiac biomarker concentrations over time between groups. When appropriate, a post hoc multiple comparison was applied between or within groups by use of the Bonferroni correction to ensure an experimental error rate at $\alpha < 0.05$. Within- and between-group comparisons were performed for the baseline values (day –1) and for data at each subsequent time point, respectively. Data for serum CK and LDH activities were logarithmically transformed prior to analysis. All statistical analyses were performed by use of statistical computer software[o]; a value of $P < 0.05$ was considered significant.

Results

Immediately after short- and long-duration TCP, there was an approximately 3.5-fold increase in serum CK-MB concentration, compared with the baseline values (**Figure 1**). At subsequent time points, serum CK-MB concentrations in the dogs that underwent short- and long-duration TCP were highly similar, although a difference (albeit not significant) between groups was detected at day 2. Compared with the respective baseline value (ie, the marginal means of day –1), serum concentrations of CK-MB in each group were increased throughout the study period, although these changes were significant only at days 0 and 1 in the short-duration TCP group and at days 0, 1, and 2 in the long-duration TCP group.

An approximately 7.3-fold increase in serum concentration of cTnI was evident immediately after TCP (day 0) in the long-duration TCP group, compared with the baseline value; in the short-duration TCP group, there was only a 1-fold increase (from baseline) in this variable (Figure 1). In the long-duration TCP group, serum cTnI concentration was significantly ($P < 0.05$) increased from baseline at days 0, 1, and 2; by day 3, values were within reference limits and did not differ from baseline. From day 0 through day 2, serum cTnI concentrations differed significantly ($P < 0.05$) between groups. Although serum cTnI concentration was increased slightly at days 0 and 1 in the short-duration TCP group, these values were not significantly different from the baseline value and were within reference limits.

In the long-duration TCP group, there was an 8.6-fold increase in serum CK activity at day 0, compared with the baseline value (**Figure 2**). From day 0 through day 3, CK activity in this group was significantly ($P < 0.05$) increased from baseline and values differed significantly ($P < 0.05$) from values in the short-duration TCP group. At days 5 and 7, serum CK activity in the long-duration TCP group was within reference limits and similar to the baseline value. In contrast, changes in serum CK activity in the short-duration TCP group were not significant at any time point, compared with the baseline value, and values remained within reference limits throughout the study period.

In dogs that underwent long-duration TCP, serum AST activity increased considerably from the baseline value at day 0; a peak value was detected at day 1

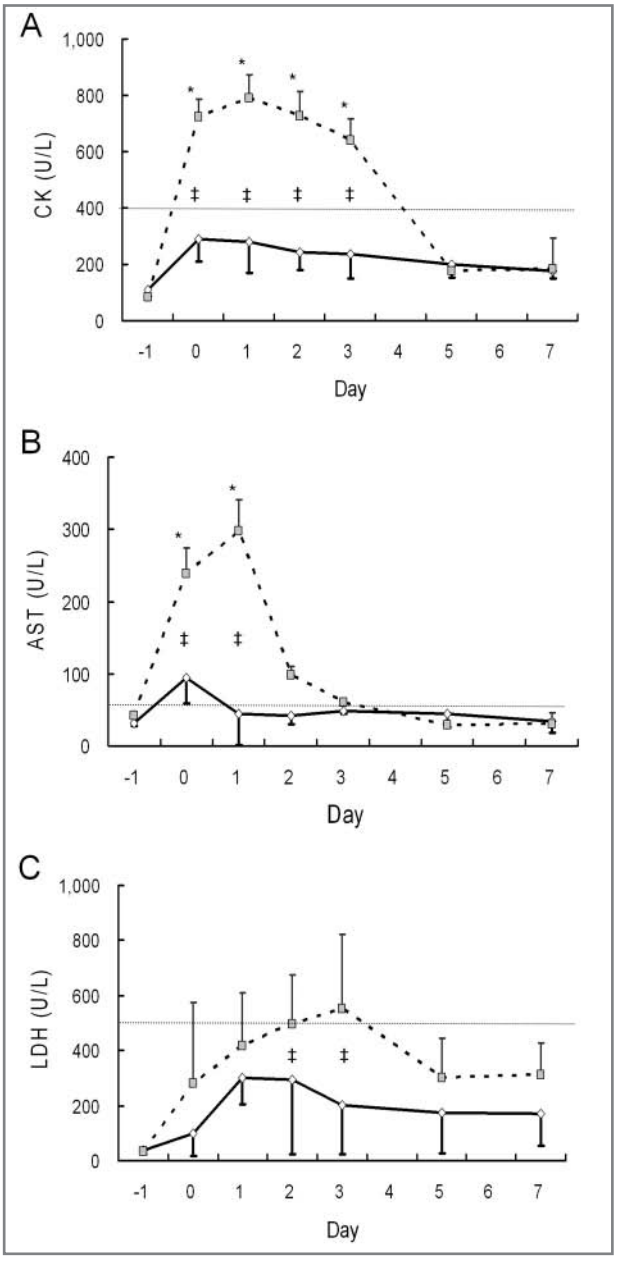

Figure 2—Mean ± SD serum activities of CK (A), AST (B), and LDH (C) in 10 dogs before and after short- (1-hour; white diamonds; n = 5) or long- (3-hour; gray squares; 5) duration TCP. Data were collected 1 day prior to TCP (day –1), immediately after TCP (day 0), and at 1, 2, 3, 5, and 7 days after TCP. The horizontal line indicates the upper limit of the reference range for the variable. *See* Figure 1 for remainder of key.

(Figure 2). In that group, AST activity was decreased markedly (value not significantly different from baseline) at day 2 and was within reference limits at days 3 through 7. In the short-duration TCP group, a slight (albeit not significant) increase from baseline in serum AST activity was detected only at day 0. With the exception of the mildly high value at day 0, serum AST activity was within reference limits at all other time points in the short-duration TCP group. Between the 2 groups, values differed significantly only at days 0 and 1.

In both the short- and long-duration TCP groups, serum LDH activity was not increased significantly from baseline at any time point (Figure 2). With respect to the reference limits, values were mildly high at days 2 and 3 in the long-duration TCP group; in the short-duration TCP group, values remained within reference limits throughout the study period. Between the 2 groups, serum LDH activities differed significantly ($P < 0.05$) only at days 2 and 3.

Discussion

The electrical energy applied during TCP has the potential to induce myocardial injury. In 1 study[2] to evaluate the risk of tissue damage associated with TCP, cardiac tissue samples were collected for histologic examination from dogs that had undergone TCP (100 mA; 20-millisecond pulse duration; 80 stimuli/min) for 30 minutes. Pathologic lesions consistent with electrically induced myocardial damage were detected in all dogs. The lesions included myocardial pallor and focal myofibril coagulation necrosis in the right ventricular outflow tract and perivascular microinfarcts in the posterior left ventricular myocardium; however, these lesions were not extensive, involving < 5% of the right ventricular free wall and < 1% of the left posterior ventricular wall.[2] Electrocardiography and measurements of serum CK-MB concentration have also been performed in dogs undergoing similar TCP procedures, but major ECG abnormalities and increases in serum CK-MB activity were not detected.[5] In another study[3] in humans, myocardial injuries were assessed after TCP of 30 minutes' duration and serum myoglobin and CK-MB concentrations and CK and LDH activities were measured at intervals during a 24-hour period after TCP; the procedure caused no significant changes in those variables, compared with findings before TCP. Hence, those researchers concluded that noninvasive TCP applied for a period of 30 minutes causes no muscular or myocardial injury in humans.[3]

Creatine kinase-MB fraction is an enzyme in myocardial cells that is released into the bloodstream as the result of myocardial injury, such as in myocardial infarction. Although some investigators who assessed serum CK-MB concentrations as a means of evaluating myocardial injuries in dogs and humans found it to be useful,[3,5] the popularity of such assessments for detection of myocardial injuries in dogs is decreasing because CK-MB is present in low concentrations in the myocardium (4% to 15% of CK), concentrations of serum CK-MB increase in association with chronic renal diseases, and assessment of serum CK-MB concentration has low specificity and sensitivity for diagnosis of myocardial injury in the presence of skeletal muscular injury (especially among large-breed dogs).[11] However, a recent study[4] has revealed that multiple marker analysis—assessments of serum concentrations of CK-MB along with concentrations of myoglobin and troponins—has good prognostic value for myocardial injuries and could overcome the limitations of a single marker (CK-MB) assay.

Cardiac troponin I is a cardiospecific biomarker, and changes in circulating concentration of this protein reflect various cardiac injuries. Assessment of serum cTnI concentration is widely used for detection of acute myocardial infarction in humans.[11] Because the concentration of cTnI in heart muscle is 1,000 times as great as that in the skeletal muscle, measurement of circulating cTnI concentration can help differentiate myocardial injuries from skeletal muscular injuries.[11] In humans with acute myocardial infarction, the plasma concentration of cTnI increases at 2 to 3 hours, peaks at 20 to 24 hours, and markedly decreases at 48 hours after myocardial injury.[12] In the present study, the serum concentration of CK-MB was increased from baseline in both groups at day 0 through day 7; however, the changes were significant at days 0, 1, and 2 in the long-duration TCP group and at days 0 and 1 in the short-duration TCP group. The serum cTnI concentration was markedly increased only in the long-duration TCP group at days 0, 1, and 2. The pattern of increase in circulating cTnI concentration in the dogs in the long-duration TCP group was similar to the pattern detected in humans with acute myocardial infarction. Because the organs injured by pacing current (energy) could be major skeletal muscles and the myocardium, an increase in circulating CK-MB might be indicative of both skeletal and myocardial injuries; however, an increase in circulating cTnI concentration after TCP is perhaps more specifically indicative of myocardial injuries. The findings of the present study clearly suggest that long-duration TCP can induce myocardial injuries and that the severity of myocardial damage might be correlated with the duration of TCP. Therefore, a reason for the failure of other researchers to detect increases in circulating concentrations of cardiac biomarkers (eg, CK-MB and cTnI) and enzyme activities (eg, CK and AST) in dogs and humans in previous studies[3,5] might be because the duration of TCP was short (30 minutes).

Assessments of serum AST, CK, and LDH are not sensitive for detection of myocardial injuries because activities of these enzymes can be increased as a result of any kind of muscular injury, including skeletal muscular injuries.[13–15] No remarkable increases in these enzyme activities following TCP in humans and dogs have been observed in previous studies.[3,5] Similarly, activities of serum AST, CK, and LDH were not remarkably increased in the dogs in the short-duration (1-hour) TCP group in the present study. However, considerable increases in those activities were detected in the long-duration (3-hour) TCP group. The findings of our study also suggest that the magnitude and duration of the increases in serum AST, CK, and LDH activities were directly linked to the duration of TCP, as determined for the changes in serum concentrations of cardiac biomarkers. Although the timing of the peak value, magnitude of change, and duration of increase

(from baseline) for each enzyme activity differed in our study, this might be attributable to biological characteristics of those enzymes.[13–15]

Although results of the present study clearly indicated that TCP may induce myocardial and skeletal muscular injuries depending on the duration of TCP, the injuries were not persistent; most values of serum enzyme activity and cardiac biomarker concentration were within reference range within 3 days after TCP. However, the myocardium may be more severely affected as the duration of TCP increases because the concentration of cTnI increased significantly from baseline in the long-duration TCP group, whereas the change was considerably less in the short-duration TCP group. Therefore, careful monitoring for myocardial injury is required for dogs that undergo longer periods of TCP treatment in clinical situations.

We believe that the causes of the increases in serum enzyme activities and cardiac biomarker concentrations after TCP might not be entirely attributable to myocardial and skeletal muscular injuries that are caused by the continuous pacing energy applied during the treatment. Preliminary studies performed by our group to evaluate hemodynamic and ECG changes after TCP revealed that there was profound reduction of blood pressure after TCP because of isoflurane anesthesia and loss of atrial kick (unpublished data). Inadequate blood flow and low blood pressure in the preliminary study population may have caused reduced perfusion of some vascular beds, leading to mild myocardial and skeletal muscular damages. Furthermore, increases in activities of AST, CK, and LDH in circulation in the study dogs might be associated with experimental protocol factors unrelated to TCP, such as organ specificity or systemic effects of diltiazem and restraint or immobilization, although these effects have never been reported. Therefore, evaluation of control dogs without TCP treatment is required for the clarification for this issue. However, it was almost impossible to ensure survival of control dogs without cardiac pacing once profound bradycardia or asystole had been induced. Although the difference in methods for cardiac biomarker assays might have influenced the results or the present study, the likelihood is thought to be low because serially diluted control samples provided by the manufacturer were tested concurrently with each assay for quality-control purposes.

With regard to the safety of TCP in dogs, the present study revealed that serum cardiac biomarker concentrations and enzyme activities increase markedly (albeit transiently) after TCP of 3 hours' duration, indicative of some degree of TCP-induced myocardial injury. Therefore, it appears that serum cardiac biomarker concentrations and enzyme activities should be carefully monitored for a week in dogs that undergo TCP for periods of > 1 hour to evaluate potential myocardial damage.

a. Hemavet 880, CDC Technologies, Oxford, Conn.
b. FUJI dry-chem 3500i, Fuji Film Corp, Tokyo, Japan.
c. SONOACE-8900, Medison, Seoul, Republic of Korea.
d. Sampoong, Seoul, Republic of Korea.
e. Forane, 2% concentration, Sampoong, Seoul, Republic of Korea.
f. MDS Matrix 3000, Hallowell EMC, Pittsfield, Mass.
g. VSM 7, Vet Specs, Norcross, Ga.
h. Sampoong, Seoul, Republic of Korea.
i. PH-1, CU Medical Systems, Wonju, Republic of Korea.
j. MX1, CU Medical Systems, Wonju, Republic of Korea.
k. Patch, MX1, CU Medical Systems, Wonju, Republic of Korea.
l. BD Vacutainer SST, Becton, Dickenson & Co, Franklin Lakes, NJ.
m. AIA-360, Tosoh Corp, Tokyo, Japan.
n. AIA-PACK cTnI 2G assay, Tosoh Corp, Tokyo, Japan.
o. SAS, version 9.1, SAS Institute Inc, Cary, NC.

References

1. Bocka JJ. External pacemakers. Available at: emedicine.medscape.com/article/780639-overview. Accessed Feb 5, 2008.
2. Kicklighter EJ, Syverud SA, Dalsey WC, et al. Pathological aspects of transcutaneous cardiac pacing. *Am J Emerg Med* 1985;3:108–113.
3. Madsen JK, Pedersen F, Grande P, et al. Normal myocardial enzymes and normal echocardiographic findings during noninvasive transcutaneous pacing. *Pacing Clin Electrophysiol* 1988;11:1188–1193.
4. Maisel AS, Bhalla V, Braunwald E. Cardiac biomarkers: a contemporary status report (Erratum published in *Nat Clin Pract Cardiovac Med* 2006;3:288). *Nat Clin Pract Cardiovasc Med* 2006;3:24–34.
5. Syverud SA, Dalsey WC, Hedges JR, et al. Transcutaneous cardiac pacing: determination of myocardial injury in a canine model. *Ann Emerg Med* 1983;12:745–748.
6. Zoll PM, Zoll RH, Belgard AH. External noninvasive electric stimulation of the heart. *Crit Care Med* 1981;9:393–394.
7. Morrison LJ, Long J, Vermeulen M, et al. A randomized controlled feasibility trial comparing safety and effectiveness of prehospital pacing versus conventional treatment: 'PrePACE'. *Resuscitation* 2008;76:341–349.
8. Voorhees WD III, Foster KS, Geddes LA, et al. Safety factor for precordial pacing: minimum current thresholds for pacing and for ventricular fibrillation by vulnerable-period stimulation. *Pacing Clin Electrophysiol* 1984;7:356–360.
9. Lee SG, Moon HS, Hyun C. The efficacy and safety of external biphasic defibrillation in toy breed dogs. *J Vet Emerg Crit Care* 2008;18:362–369.
10. Pagani F, Stefini F, Micca G, et al. Multicenter evaluation of the TOSOH AIA-pack second-generation cardiac troponin I assay. *Clin Chem* 2004;50:1707–1709.
11. Archer J. Cardiac biomarker: a review. *Comp Clin Pathol* 2003;12:121–128.
12. Sadony V, Korber M, Albes G, et al. Cardiac troponin I plasma levels for diagnosis and quantitation of perioperative myocardial damage in patients undergoing coronary artery bypass surgery. *Eur J Cardiothorac Surg* 1998;13:57–65.
13. Crowley LV. Creatine phosphokinase activity in myocardial infarction, heart failure, and following various diagnostic and therapeutic procedures. *Clin Chem* 1968;14:1185–1196.
14. Schober KE. Biochemical markers of cardiovascular disease. In: Ettinger SJ, Feldman EC, eds. *Textbook of veterinary internal medicine*. 6th ed. St Louis: Elsevier Saunders, 2005;940–948.
15. Willard MD, Twedt DC. Gastrointestinal, pancreatic, and hepatic disorders. In: Willard MD, Tvedten H, eds. *Small animal clinical diagnosis by laboratory methods*. 4th ed. St Louis: Saunders, 2004;208–246.