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ORIGINAL RESEARCH

Cardiac Magnetic Resonance Studies in a Large Animal Model That Simulates the Cardiac Abnormalities of Human Septic Shock

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BACKGROUND: Septic shock is associated with increases in end-diastolic volume (EDV) and decreases in ejection fraction that reverse within 10 days. Nonsurvivors do not develop EDV increases. The mechanism is unknown.

METHODS AND RESULTS: Purpose-bred beagles (n=33) were randomized to receive intrabronchial *Staphylococcus aureus* or saline. Over 96 hours, cardiac magnetic resonance imaging and echocardiograms were performed. Tissue was obtained at 66 hours. From 0 to 96 hours after bacterial challenge, septic animals versus controls had significantly increased left ventricular wall edema (6%) and wall thinning with loss of mass (15%). On histology, the major finding was nonocclusive microvascular injury with edema in myocytes, the interstitium, and endothelial cells. Edema was associated with significant worsening of biventricular ejection fractions, ventricular-arterial coupling, and circumferential strain. Early during sepsis, (0–24 hours), the EDV decreased; significantly more in nonsurvivors (ie, greater diastolic dysfunction). From 24 to 48 hours, septic animals' biventricular chamber sizes increased; in survivors significantly greater than baseline and nonsurvivors, whose EDVs were not different from baseline. Preload, afterload, or heart rate differences did not explain these differential changes.

CONCLUSIONS: The cardiac dysfunction of sepsis is associated with wall edema. In nonsurvivors, at 0 to 24 hours, sepsis induces a more severe diastolic dysfunction, further decreasing chamber size. The loss of left ventricular mass with wall thinning in septic survivors may, in part, explain the EDV increases from 24 to 48 hours because of a potentially reparative process removing damaged wall tissue. Septic cardiomyopathy is most consistent with a nonocclusive microvascular injury resulting in edema causing reversible systolic and diastolic dysfunction with more severe diastolic dysfunction being associated with a decreased EDV and death.

Key Words: cardiac dysfunction ■ edema ■ sepsis

Understanding cardiac function in sepsis remains constrained by unexplained findings. Historically, sepsis was thought of as a "high output" state; yet, in a substantial number of patients, cardiac output was found to be depressed.[1–3] In the 1980s, serial measurements of left ventricular ejection fraction

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CLINICAL PERSPECTIVE

What Is New?

- This is the first controlled sepsis study employing serial cardiac magnetic resonance imaging combined with transthoracic echo and invasive hemodynamics demonstrating that the cardiac dysfunction of sepsis has "injury" and "reparative" phases and ventricular wall edema is a fundamental aspect of sepsis pathophysiology and dry mass loss a reparative mechanism.
- The "injury" phase (0–24 hours after bacterial challenge) of this sepsis induced cardiomyopathy has end-diastolic volume decreases more profound in nonsurvivors that are associated with worse ventricular compliance, myocardial edema, and diastolic dysfunction whereas the "recovery" phase (24–96 hours) of this cardiac injury has left ventricular mass loss consistent with a reparative process removing damaged tissue with wall thinning in survivors that explains the end-diastolic volumeincreases.

What Are the Clinical Implications?

- These findings yield new understanding into the pathophysiology of the septic cardiomyopathy and allow for novel phenotyping and prognostication of the syndrome with ventricular compliance and end-diastolic volumes and our findings also raise questions about the critical clinical therapeutic importance attached to fluid resuscitation as the major factor for cardiac functioning and survival during sepsis, because, despite optimizing filling pressures, the cardiac changes in ventricular wall structure and function associated with survival and nonsurvival in sepsis still occurred.

Nonstandard Abbreviations and Acronyms

EDV	end-diastolic volume
PAOP	pulmonary artery occlusion pressure
RVEDV	right ventricular end-diastolic volume
TTE	transthoracic echocardiogram

(LVEF) via cardiac radionucleotide cineangiograms coupled with data from indwelling thermodilution pulmonary artery catheters allowed detailed assessments of myocardial function during septic shock.[4–6] Human and animal data demonstrated in survivors a decrease in LVEF over 2 days after the onset of shock (humans) or bacterial challenge (animal models) with recovery to near normal in 7 to 10 days.[4–6] Simultaneous use of radionucleotide cine angiocardiogram and pulmonary artery catheter measurements provided a calculated left ventricular end-diastolic volume (LVEDV) equivalent to stroke volume/LVEF. In survivors, the LV chamber size markedly increased during the first 2 days after the onset of shock and normalized as the LVEF recovered over 7 to 10 days.[4–6] What remains unexplained in humans and animal survivors is the mechanism of this transient, rapidly reversible cardiac dysfunction and why human[4,5] and animal[7] nonsurvivors with sepsis do not undergo a similar degree of LV dilation.

This pattern of transient LVEF decreases and LVEDV increases in septic animal survivors, which occurs over 7 to 10 days, remains immutable despite altering the type,[8] viability,[8] dose,[7] site of bacterial inoculation,[6,9,10] administration of intravenous proinflammatory mediator challenges,[11] or increasing doses of mediator challenge.[12] This suggests a common pathway of cardiac injury for disparate inflammatory insults to the host. Yet, in these animals, at day 2 when the LVEF falls and LV dilation is most profound, light, and electron microscopy (EM) do not show evidence of myocardial inflammatory cell infiltrates. However, there is interstitial, myocyte, and endothelial cell edema consistent with a nonocclusive diffuse microvascular injury with fibrin deposition. Although mild focal myofilament dropout is observed, gross abnormities in myofilament structure or frank myocyte loss are not seen.[13] Since the 1980s, various observational transthoracic echocardiogram (TTE) studies in humans with sepsis have mostly confirmed these cardiac LVEF and LVEDV findings.[14–16] Despite the prognostic and potential therapeutic implications of these myocardial ventricular volume changes, the underlying pathophysiology has been largely unexplained.

We used serial cardiac magnetic resonance (CMR) imaging in a septic animal model to obtain precise direct measurements of both right ventricular EDV (RVEDV) and LVEDV at crucial time points. Simultaneously, we obtained invasive hemodynamic data to examine if sepsis-induced changes in preload, afterload, or heart rate contributed to any differential changes found in cardiac chamber size related to survival. To explore the contribution of changes within the cardiac walls to alterations in ventricular volumes, serial CMR measures of edema and LV mass were obtained. Tissue samples for light and EM were acquired to look for a cause of the cardiac dysfunction and to correlate the changes we observed on imaging with morphological changes on pathology.

METHODS

Thirty-three purpose-bred beagles (9–15 kg, 18–30 months. old, male, Marshall Farms) were studied. On

day 1 (baseline), 27 tracheostomized, sedated, and mechanically ventilated animals received an intrabronchial challenge of *S. aureus* ($0.5–1.0 \times 10^9$ colony forming units/kg) and 6 animals received an equivalent PBS challenge. The *S. aureus* was prepared and administrated as previously described.[10] The data that support the findings of this study are available from the corresponding author upon reasonable request.

Animals were monitored around the clock for 96 hours to simulate care in a medical as well as an animal hospital intensive care unit as previously described.[9,10] Ceftriaxone (50 mg/kg IV) starting 4 hours after intrabronchial challenge was administered q24 hours. Catecholamine infusions were not administered to any animal as this could alter the sepsis-induced cardiac dysfunction (which is the purpose of the study) differentially compared with controls, who would usually require no catecholamine support. Maintenance fluid (2 mL/kg/h of Normasol-M with 5% dextrose supplemented with potassium chloride [27 mEq/L]) were infused 0 to 96 hours. Additionally, a pulmonary artery catheter was placed before intrabronchial challenges, and the pulmonary artery occlusion pressure (PAOP) was measured q4 hours throughout. Whenever the PAOP fell below 10 mm Hg, a 20 mL/kg bolus of plasmalyte (Vetivex) was given over 20 minutes and repeated until achieving a PAOP >10 mm Hg. At 62 hours, 6 of the septic animals were euthanized and tissue was obtained. At 96 hours, animals that did not succumb to sepsis were deemed survivors and euthanized.[9,10] Throughout, during mechanical ventilation, animals had their fractional inspired oxygen levels, positive end expiratory pressure levels, tidal volumes, and ventilatory rates adjusted to maintain normal arterial oxygen and carbon dioxide levels as per protocol.[9,10] Animals throughout also received sedation titrated to physiological end points, stress ulcer and venous thromboembolism prophylaxis, and their position changed at set intervals to avoid stasis ulcers.[9,10] All animals were treated equally, except for the type of intrabronchial challenge. The study protocol was reviewed and approved by the National Institutes of Health, Critical Care Institutional Animal Care and User Committee (CCM19-04).

Femoral lines, pulmonary artery catheters, and a tracheostomy were placed under anesthesia and baseline TTEs, CMR exams, and blood work were obtained before intrabronchial challenges.[10] TTEs were done daily, and CMR exams were performed at 2 and 4 days after challenges. At multiple time points, laboratory parameters were obtained using arterial blood for arterial blood gases, complete blood counts, and serum chemistries (Heska). Cytokines and chemokines were measured using commercially available ELISA kits (ProcartaPlex, Invitrogen, ThermoFisher, Waltham, MA). Troponin I and BNP (brain natriuretic peptide) was determined using a multidetector microplate reader (Synergy HT, BioTek Instruments, Winooski, VT).

Statistical Analysis

Please see Data S1 for details on TTE, CMR, literature searches, histology, animal inclusion criteria, and statistical analysis.

RESULTS

Table S1 provides the number of animals in each treatment group and their respective survival times. At 48 and 96 hours after challenge, as measured by CMR, septic animals had significant worsening from baseline of multiple measures of biventricular function and significant alterations in biventricular chamber size. Specifically, significant deterioration in mean right ventricular ejection fraction (RVEF), RV–pulmonary artery coupling, and circumferential strain (Figure 1A through 1C, respectively), as well as significant increases in mean RV end-systolic volume and RVEDV (Figure 1D and 1E respectively) and decreases in mean RV stroke volume (48 hours only, Figure 1F). Nonseptic controls had no significant changes in these CMR parameters throughout. The changes from baseline in mean RVEF, RV end-systolic volume, RV stroke volume, and RV–pulmonary artery coupling of septic animals on CMR were significantly more profound than in controls. The corresponding left heart parameters on CMR had remarkably similar but somewhat less pronounced abnormalities (Figure 1G–1L) which are potentially related to the significantly reduced afterload on the left *versus* right-sided circulation. Septic animals versus controls from 0 to 96 hours developed significantly higher mean pulmonary artery pressures and pulmonary vascular resistances and lower mean MAPs and systemic vascular resistances (Figure S1A–D). The quantity of fluids 0 to 96 hours received and cardiac filling pressures (PAOP) were similar comparing septic animals versus controls (all, $P>0.05$) (Figure S1E–H). Consequently, there were no preload differences to explain the cardiac dysfunction observed in septic animals versus controls. Furthermore, the right- and left-sided circulations demonstrated opposite hemodynamic changes from baseline in afterload during septic shock, that is, significant increases and decreases, respectfully. These increases could worsen right-sided but improve the left-sided cardiac function. These opposite afterload changes means that increases in afterload cannot easily explain the relatively similar directional biventricular decrease in cardiac function we found in septic animals (Figure S1A–D and Figure 1A, and 1G).

The importance of these CMR findings to outcome from sepsis was next investigated (Figure 2). Surprisingly, the only finding on CMR that was clearly associated

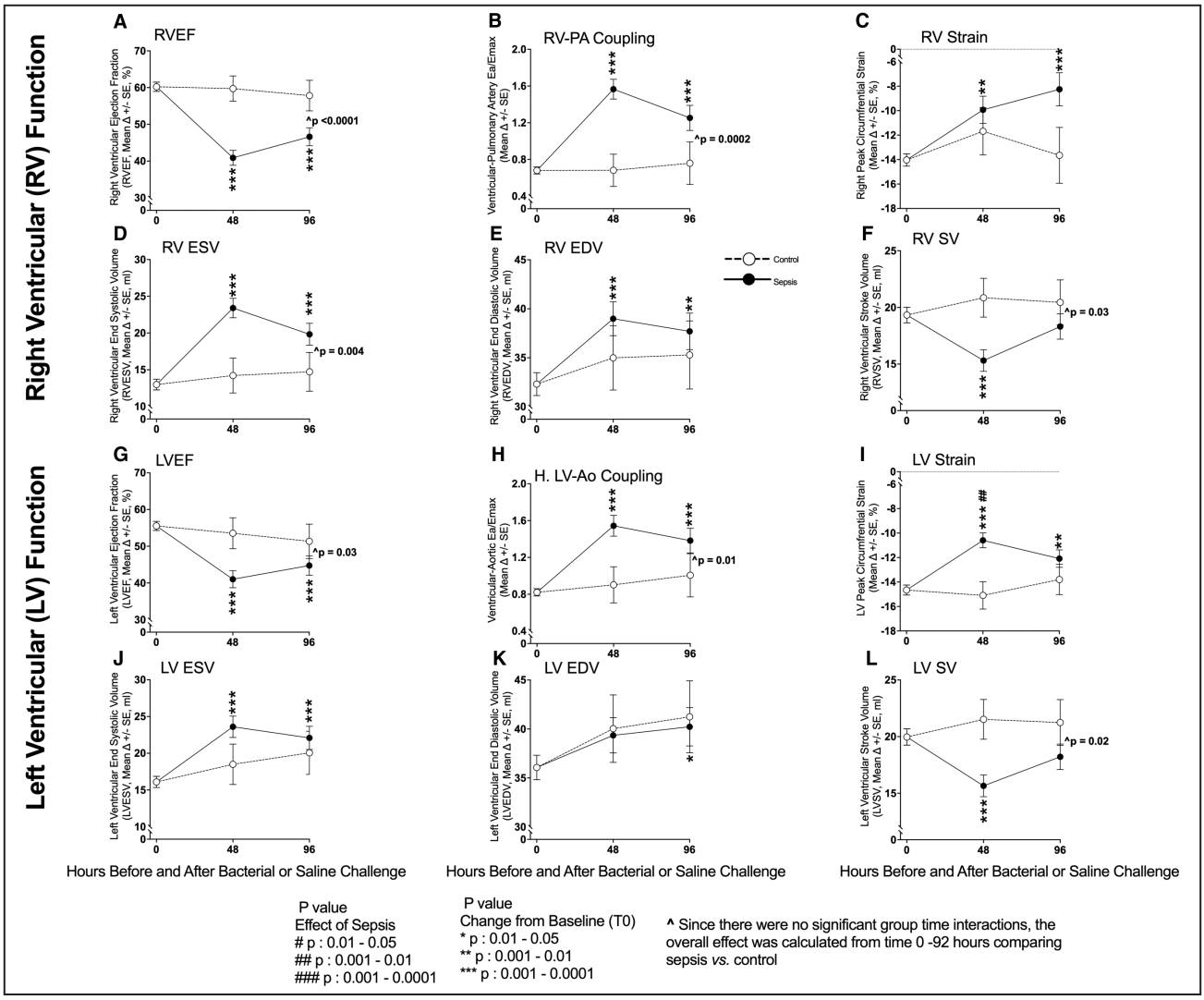

Figure 1. Serial cardiac magnetic resonance imaging during experimental septic shock.
Serial cardiac changes from baseline to 96 hours plotted from a common origin of the mean values of all animals at time 0 during sepsis (filled circles) vs control (open circles) in the right (**A–F**) and left (**G–L**) ventricles as ascertained by cardiac magnetic resonance imaging. EDV indicates end-diastolic volume; ESV, end-systolic volume; RV, right ventricular; RVEF, right ventricular ejection fraction; and RV–PA, right ventricular pulmonary artery.

with survival from septic shock was increases in biventricular volumes. In septic survivors, the mean RVEDV (Figure 2A) and ESV (2B) significantly increased from baseline to 48 hours compared with nonsurvivors, who had no significant changes from baseline. In contrast, the CMR measures of cardiac systolic performance were overall not significantly different comparing survivors and nonsurvivors as follows: The mean RV stroke volume (Figure 2C) in both survivors and nonsurvivors at 48 hours similarly significantly decreased. In both survivors and nonsurvivors at 48 hours, there was similar significant worsening in mean RVEF (Figure 2D). Mean RV circumferential strain diminished significantly in survivors at 48 hours but there was no significant change from baseline in nonsurvivors (Figure 2E). In both survivors and nonsurvivors at 48 hours, there was similar significant worsening in mean RV–pulmonary artery coupling (Figure 2F). Similar findings were observed in the left-sided circulation except for LV strain where there was less of a difference between survivors and nonsurvivors (Figure 2G–L). In summary, the CMR parameters that were most closely associated with survival were not differences in cardiac systolic function but rather increases in biventricular volumes. To directly compare the changes from baseline for the CMR parameters in controls to septic survivors and nonsurvivors up to 48 hours, please see Table S2.

We next examined if differences in loading conditions could explain the differential changes in chamber sizes at 48 hours in septic survivors and nonsurvivors (Figure 3). There were no significant differences in mean cardiac filling pressures between septic survivors

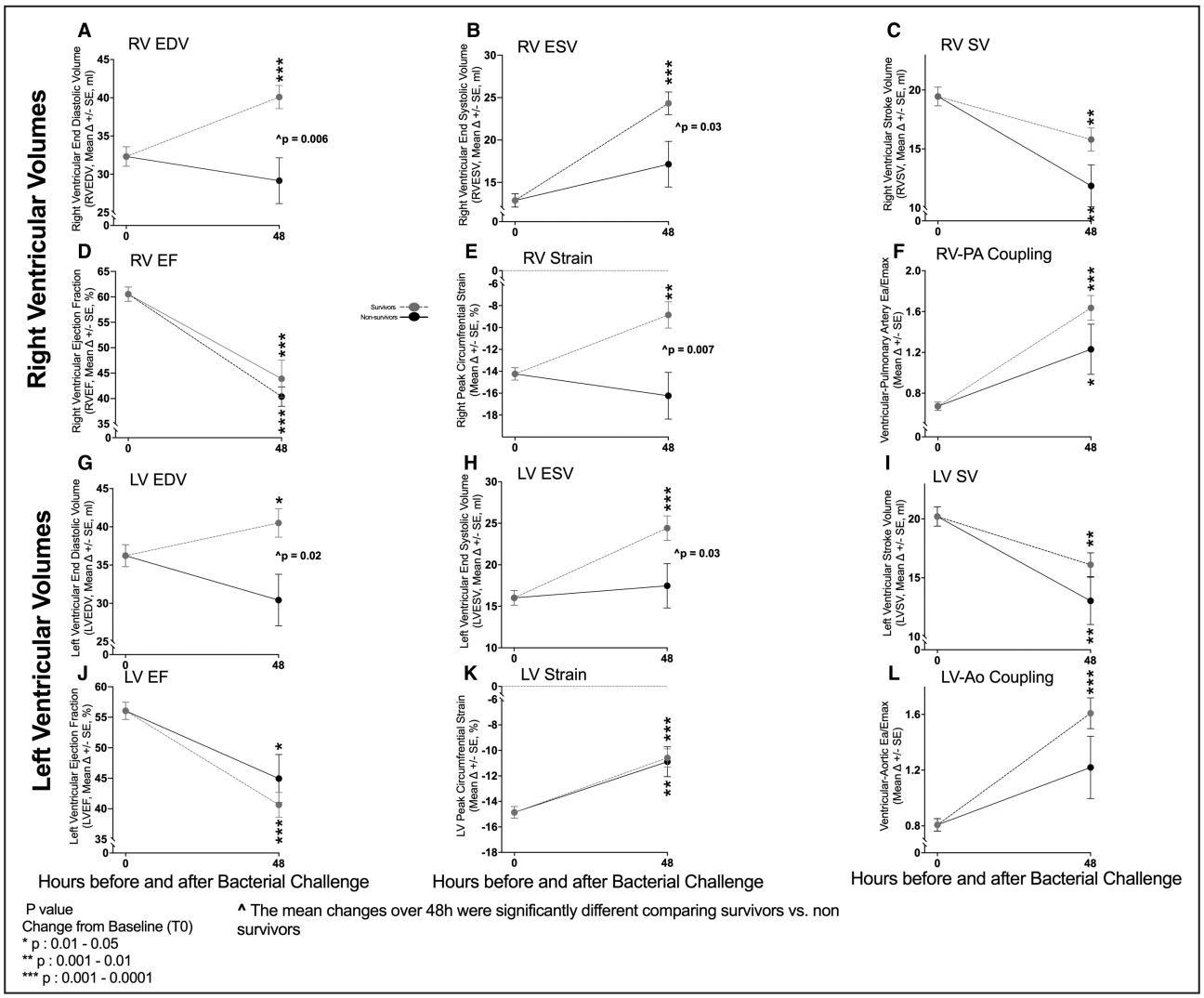

Figure 2. Serial cardiac magnetic resonance imaging comparing septic survivors and nonsurvivors.
At 48 hours, changes from baseline in the heart ascertained by cardiac magnetic resonance imaging plotted from a common origin of mean values of septic animals at time 0 in septic survivors (gray circles) vs septic nonsurvivors (filled circles) in the right (**A–F**) and left (**G–L**) ventricles. LV indicates left ventricular; LV–Ao, left ventricular-aortic; and SV, stroke volume.

versus nonsurvivors from 0 to 48 hours associated with these differences in volumes either in the left-sided circulation (PAOP) pre- (Figure S2A) or postcompletion of the fluid boluses algorithm (Figure 3A) or the right-sided (central venous pressure) circulation (Figure 3B). Ventricular compliance plots showing mean changes over time in ventricular filling pressures versus volumes are shown in Figure 3C for the LV and in Figure 3D for the RV. These indicate that marked biventricular compliance differences can explain the ventricular volume differences between survivors and nonsurvivors. A significant shift from baseline to 48 hours to the right and upward occurred in survivors with increases in both mean PAOP (LV filling pressure) and central venous pressure (RV filling pressure) and concomitant increases in both mean LVEDV (Figure 3C) and RVEDV (Figure 3D), respectively. A similar shift to the right with increases in ventricular filling pressures (both mean PAOP and central venous pressure) occurred in nonsurvivors but no concomitant significant shift upward took place (ie, no increases in biventricular volumes). As opposed to the group mean differences described previously, we next examined comparisons of correlations using individual animal data. In both survivors and nonsurvivors, there was no relationship between changes in filling pressures (PAOP [Figure 3E] and central venous pressure [Figure 3F]) from baseline to 48 hours and changes in LVEDV and RVEDV. Thus, in survivors and nonsurvivors alike, the differential changes in ventricular volumes were not associated with corresponding alterations in filling pressures. Importantly, because the end-diastolic pressure was held constant as part of the study design, the changes in EDV as a direct consequence are directly related to

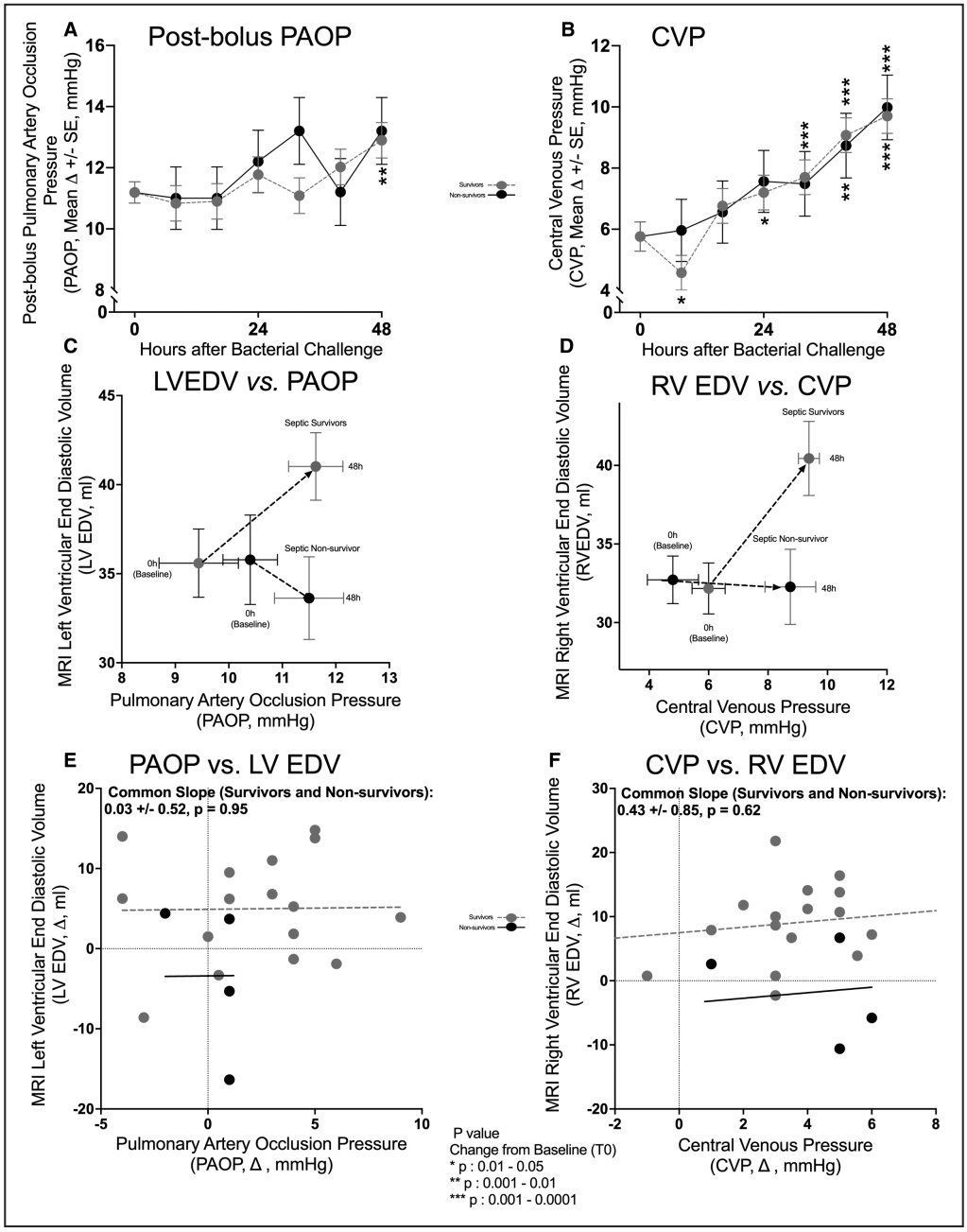

Figure 3. Loading conditions in septic survivors versus nonsurvivors.
The format is similar to Figure 2 except now serial mean postfluid bolus pulmonary artery occlusion pressures are shown in (**A**) and serial mean central venous pressure in (**B**). Ventricular compliance plots of pressure vs volume are shown for the left-sided circulation (mean pulmonary artery occlusion pressures vs left ventricular end-diastolic volume) in (**C**) and the right-sided circulation (mean central venous pressure vs mean right ventricular end-diastolic volume) in (**D**). Correlations of changes in pulmonary artery occlusion pressures vs left ventricular end-diastolic volume in (**E**) and changes in central venous pressure vs mean right ventricular end-diastolic volume in (**F**). CVP indicates central venous pressure; MRI, magnetic resonance imaging; LVEDV, left ventricular end-diastolic volume; PAOP, pulmonary artery occlusion pressure; and RVEDV, right ventricular end-diastolic volume.

ventricular compliance. Thus, the differential changes between volume but not pressure in survivors and nonsurvivors strongly support that changes in ventricular size are not simply the result of fluid administration and sepsis-induced alterations in vascular tone. Rather, in nonsurvivors, the failure to increase ventricular volumes appears intrinsic to the walls themselves. Decreases in wall compliance and impaired ventricular relaxation restrict chamber filling and size in nonsurvivors, despite similar or potentially greater (see next

paragraph) increases in ventricular filling pressures compared with survivors.

Because all animals received similar maintenance fluids, the number of fluid boluses received was examined next (Figure 4A). Septic survivors compared with nonsurvivors received significantly more fluid boluses in the first 24 hours (Figure 4B). Accordingly, the PAOP fell below 10 mm Hg significantly more frequently in survivors than nonsurvivors, triggering the fluid bolus algorithm more and therefore increasing the number of boluses. From 26 to 42 hours, the number of fluid boluses received comparing septic survivors and nonsurvivors were not significantly different. More important, overall fluid balance (fluid in minus out) did not differ significantly between septic survivors and nonsurvivors from 0 to 48 hours (Figure 4A).

Because there was a significantly increased number of fluid boluses from baseline to 24 hours in survivors compared with nonsurvivors we next determined whether these early increases in fluid boluses could explain the differential changes over time in ventricular volumes. As CMRs at 24 hours were not obtained,

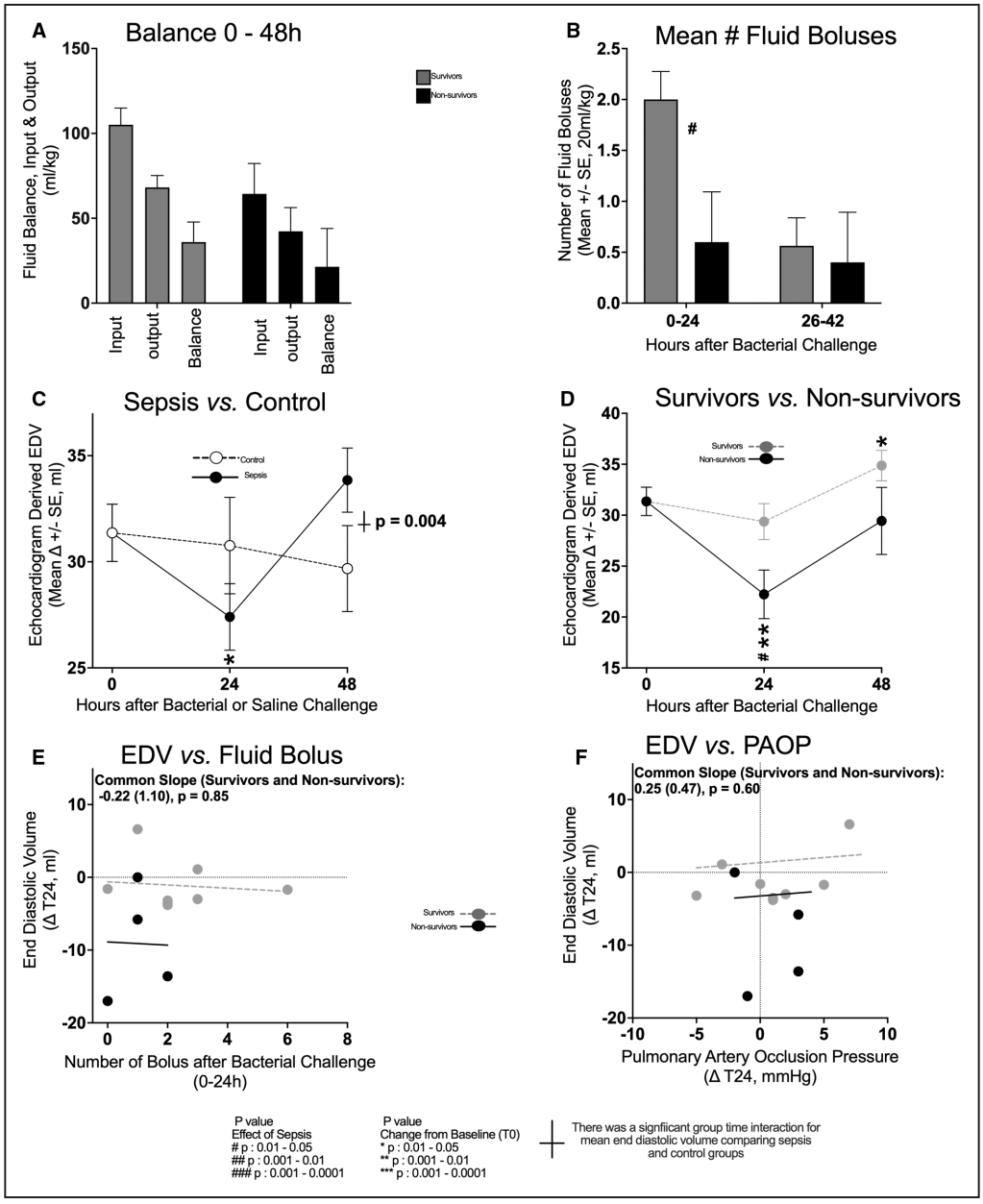

Figure 4. Loading conditions day 1 of sepsis.
The format for **A, B, D to F** is similar to Figures 2 and 3. The format for **C** is similar to Figure 1. End-diastolic values are ascertained by transthoracic echocardiogram. EDV indicates end-diastolic volume; and PAOP, pulmonary artery occlusion pressure.

we instead evaluated TTE changes. Serial TTE-derived mean LVEDVs varied significantly more in septic animals from 0 to 24 to 24 to 48 hours compared with nonseptic controls (Figure 4C, group/time interaction, P=0.004). In nonseptic controls, there were no significant changes from baseline in mean LVEDV throughout (Figure 4C). Because the EDV increased compared with baseline in survivors at 48 hours, it was unexpected in the crucial first 24 hours after challenge and surprising that the mean LVEDV in septic animals significantly decreased from baseline to 24 hours (Figure 4C) and this decrease was significantly greater in nonsurvivors (Figure 4D).

However, overall there were no hemodynamic changes from baseline to 24 hours associated with increased fluid administration in septic survivors and nonsurvivors to explain the 0 to 24 hours differential decrease in mean EDV. More specifically, there were no significant mean decreases from baseline or differences comparing groups for mean PAOP from 0 to 24 hours (Figure 3A and Figure S2A); no significant relationship in either group between the decreases in LVEDV and the number of fluids boluses received 0 to 24 hours (Figure 4E) or the changes in PAOP (Figure 4F). For explanations why fluid boluses in the first 24 hours altering heart rate or afterload also cannot easily explain the differential changes over time in biventricular volumes in survivors and nonsurvivors, please see Data S2 and Figure S2D–I. Thus, the differential loss of ventricular volume in the first 24 hours is not attributable to decreases in filling pressures or changes in afterload and is most consistent with a sepsis-induced "restrictive-like" cardiac physiology.

Because preload differences could not easily explain the differential changes in ventricular volumes, we next examined different aspects of LV wall structure using CMR to better understand the physiologic underpinnings of cardiac dysfunction and ventricular volume changes during sepsis. As seen in Figure S3A (48 hours) and Figure 5A (96 hours), most of the changes in endocardial volume (ie, LVEDV) versus the epicardium volume at 48 hours and 96 hours fall below the identity line. This indicates that there are greater increases in the endocardial surface relative to the epicardial surface or that the LVEDV is changing more than the epicardial volume. Therefore, the distance between the 2 walls is getting smaller indicating that the LV wall is thinning. In controls, the volumes derived from tracing the epicardial and endocardial walls have values that appear closer to the identity line, indicating the LV wall is not thinning (Figure S3B). This wall thinning in septic animals was associated with significant decreases in wall mass from 0 to 96 hours (Figure S3C), which was likewise not seen in controls. The decreased wall mass comparing septic to control animals approached statistical significance (P=0.055).

Simultaneously, water per unit area of LV wall (ie, edema) from 0 to 48 hours (≈2–3%) and 0 to 96 hours

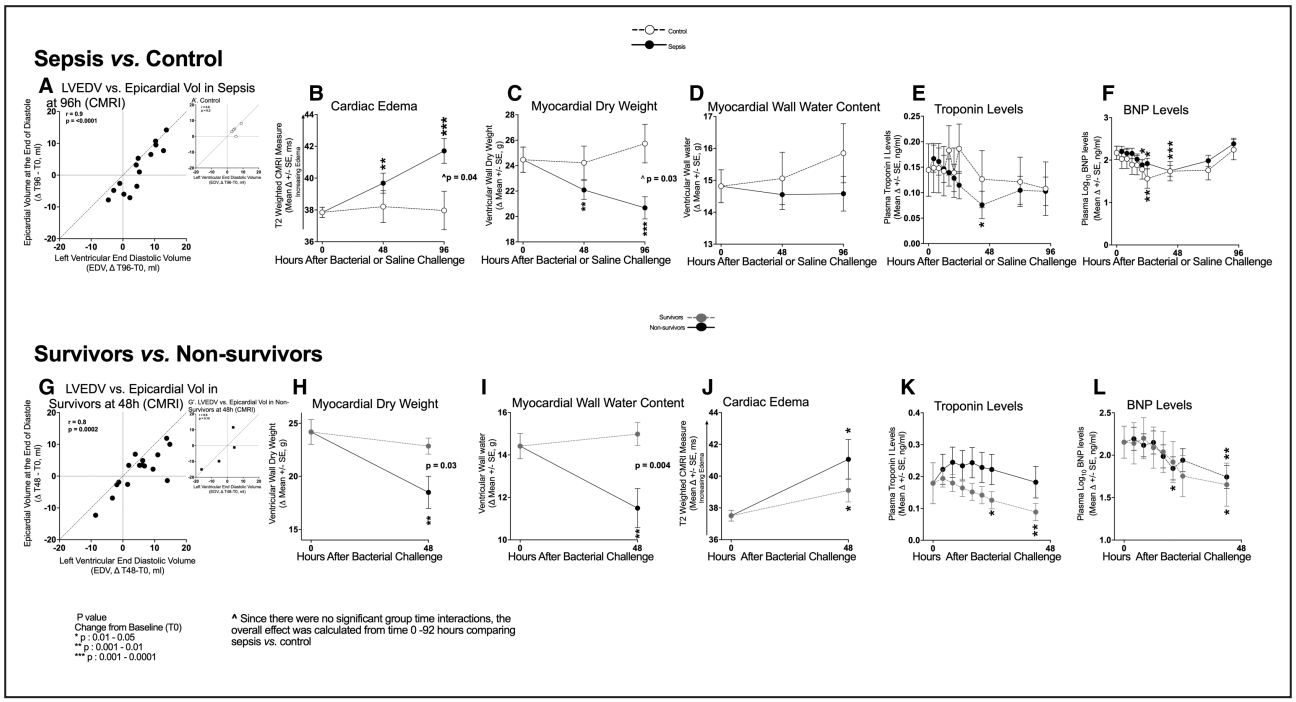

Figure 5. Serial changes in ventricular walls during sepsis.
The format for **A–F** is similar to Figure 1. The format for **G–L** is similar to Figures 2 and 3. BNP indicates brain natriuretic peptide; CMRI, cardiac magnetic resonance imaging; EDV, end-diastolic volume; and LVEDV, left ventricular end-diastolic volume.

(≈4–6%) significantly increased in septic animals compared with controls (Figure 5B). Using these CMR measurements, LV dry (Figure 5C) and wet weight (myocardial water content, Figure 5D) were each calculated (see Data S1). In septic animals, from 0 to 96 hours, there was a significant loss of LV dry weight (P=0.03) but not water content. Pertinent blood work showed that troponin I and BNP levels were not significantly elevated nor different from controls throughout (Figure 5E and 5F, respectively). Thus, there was no evidence of fluid overload causing atrial stretch or LV dilatation in survivors nor was there evidence of ischemia causing myocyte loss during sepsis to explain the decrease in LV wall mass.

The changes in endocardial versus the epicardial volumes at 48 hours in septic survivors also fell below the identity line, consistent with wall thinning (Figure 5G). Associated with this wall thinning, ventricular wall mass decreased significantly more in nonsurvivors compared with survivors at 48 hours (Figure S3 panel D). Nonsurvivors also lost more LV (dry mass) tissue (–5.69±1.52 g versus –1.33±0.76 g, P=0.03; Figure 5H), more water content (–2.92±0.92 g versus +0.56±0.54 g P=0.004) (Figure 5I), and had nominally greater increases in edema in the LV wall (Figure 6J). In nonsurvivors, the mean troponin I (Figure 6K) and BNP (Figure 6L) levels were also not significantly elevated from baseline nor significantly different than survivors throughout.

Lastly, as ventricular wall water content increased in the septic survivors (Figure 6A and 6B) and nonsurvivors (Figure 6B and D), RVEDV and LVEDV also increased. No such relationship was seen among nonseptic controls (Figure 6E and 6F). Thus, as the LV wall thins, due predominantly to tissue loss rather than water, the LV chamber size and overall total percentage of water content increases. Sepsis is defined by initial ventricular volume decreases followed by chamber dilation, LV wall thinning with tissue loss, and a relative increase in wall edema.

The morphological analysis of ventricular tissue collected at 62 hours from 6 septic animals confirmed previous cardiac histopathology and EM results.[13] On histology, the LV had focal interstitial edema (Figure 7A) but no findings consistent with inflammation, microvascular occlusion, nor myocyte necrosis. EM of myocytes showed mild myofilament loss and intracellular edema (Figure 7B) but no necrosis or other degeneration. The main EM findings were of nonocclusive capillary endothelial cell injury as evidenced by increased edema. Specifically, the endothelial cells making up the capillaries showed a mix of findings with mild to moderate edema and no changes in many endothelial cells (Figure 7C). Although these results are qualitatively like prior histological and EM findings at 48 hours after bacterial challenge in a similar model[13]; quantitatively, the endothelial and myocyte changes and edema appeared milder at this later closer to recovery time point (62 hours).

There were isolated significant findings comparing mean serial values for septic animals versus nonseptic controls and septic survivors versus nonsurvivors for serum cytokines, chemistries, complete blood count, electrolytes, and arterial blood gas parameters that do not explain the observed cardiac findings or changes in EDV associated with outcome during sepsis. These results (Figures S1–S13) and times of deaths of nonsurvivors (Table S1) are available in Data S2.

DISCUSSION

During sepsis and into a relatively rapid recovery phase, there is an increase in edema and loss of dry tissue mass within the LV wall. During the first 24 hours of sepsis, there are decreases in ventricular volumes consistent with injury associated with the development of wall edema and diastolic dysfunction. Later, between 24 and 48 hours, ventricular volumes increase and dry mass decreases along with ventricular wall thinning. The extension and the acceleration of these changes in survivors out to 96 hours into the early recovery period suggest that they may represent the physiologic manifestations of sepsis resolution and cardiac repair.

These same ventricular volumetric chamber changes have been reported previously in this model.[6,7] However, this was thought to be the sequela of an inflammatory response that caused vasodilation and low filling pressures requiring fluid resuscitation and potent vasopressors administration that subsequently affected ventricular volumes. This study challenges those assumptions. Throughout, loading conditions were tightly controlled, exogenous catecholamines were not used, and cardiac filling pressures were optimized using invasive hemodynamic monitoring. Despite these measures, the same previously documented changes in ventricular chamber size and differences in survival were again seen. Here, preload, afterload, or heart rate did not explain the evolution of ventricular volume changes that occur during septic shock. This indicates that septic shock must directly alter the structure and function of ventricular walls to produce these sepsis-induced changes in RV and LV chamber size.

The first cardiac abnormality observed after bacterial challenge was a deterioration in ventricular compliance where nonsurvivors developed a restrictive ventricular physiology. This “restrictive-like” cardiomyopathy was associated with decreasing chamber size without an associated decrease in cardiac filling pressures. Loading conditions alone were insufficient to explain this as all animals were fluid resuscitated to

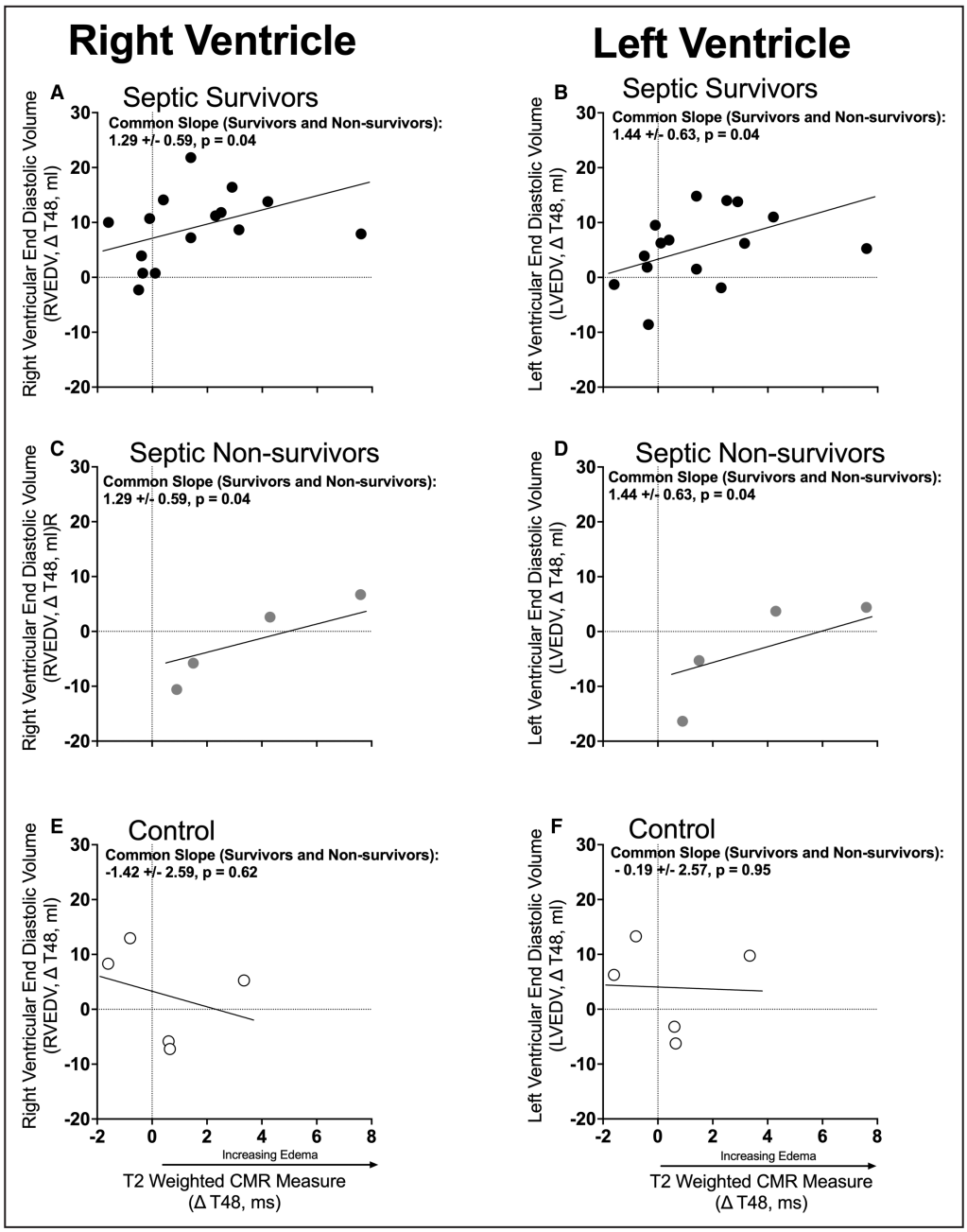

Figure 6. Association between magnetic resonance imaging derived cardiac edema and left and right end-diastolic volumes.
The format is for **A–F** is similar to Figures 2 and 3. CMR indicates cardiac magnetic resonance.

appropriate filling pressures. In fact, nonsurvivors required less fluid resuscitation during the first 24 hours after bacterial challenge. Nonsurvivor PAOPs fell below 10 mm Hg less frequently and required fewer fluid boluses. Therefore, in the first 24 hours, nonsurvivors have smaller ventricular chambers with fewer fluid boluses than survivors. As such, nonsurvivors compared with survivors had serially higher filling pressures and smaller chamber sizes, a pattern consistent with decreased ventricular compliance. Diastolic dysfunction with inadequate ventricular relaxation appeared to limit cardiac filling and impair forward flow particularly among nonsurvivors. Biventricular systolic dysfunction alone is not associated with a poor prognosis but when coupled with more severe diastolic dysfunction during sepsis is associated with a very poor outcome in our study. This should not be a surprise because systolic dysfunction coupled with severe diastolic dysfunction is a well-recognized entity in patients with heart failure and has been associated with a very poor prognosis.[17,18]

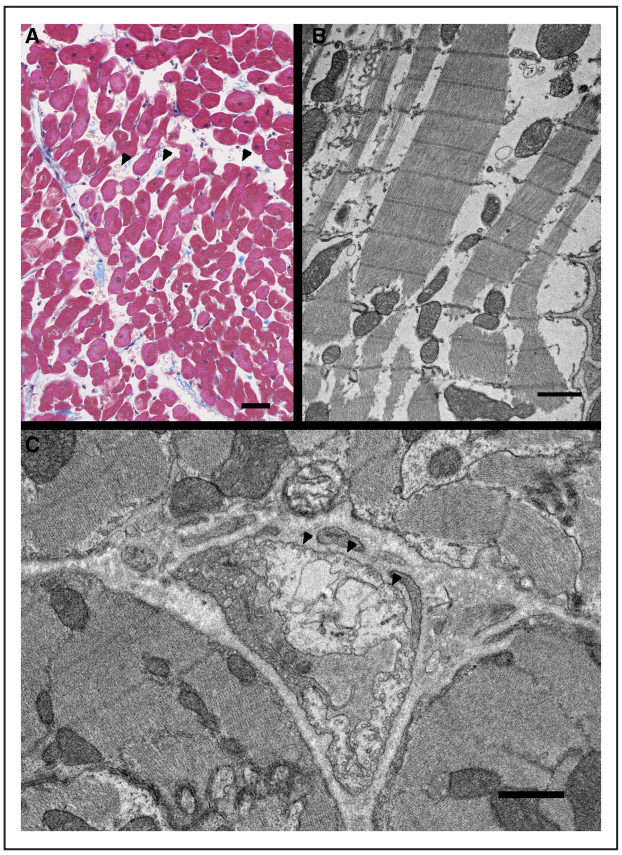

Figure 7. Representative images of histology and electron microscopy.
A, Micrograph of left ventricular wall stained with Masson trichrome, shows myocardial interstitial edema (arrow heads). Bar, 25 μm. **B**, Myocyte with myofilament loss and intracellular edema. **C**, Partially damaged capillary endothelial cells. Note that the endothelial cell right side clearly shows edema (arrowheads), whereas the endothelial cell on the left side shows normal density. Bars, 1 μm.

After bacterial challenge, the major abnormality found by both histology and CMR was ventricular wall edema. We found histologic and biochemical evidence that myocytes were relatively preserved. EMs demonstrated only minimal focal myofilament loss in myocytes and no obvious myofilament remodeling or significant structural derangements with mostly mild edema and no inflammatory cell infiltrates. Troponin I levels were not elevated throughout the study, arguing against cell death or tissue integrity loss due to ischemia. Plasma BNP levels were also not elevated, suggesting fluid overload did not induce myocyte stretch with resulting increases in biventricular EDV and ESV. We found no other obvious histopathological cardiac abnormalities that explained the observed increases in RVEDV and LVEDV or the reversible profound falls in LVEF after bacterial challenge.

Myocardial edema has been strongly associated with cardiac dysfunction in animal models of acute cardiac disease.[19] Small increases in myocardial edema was found to compromise cardiac function in both acute and chronic models of hypertension in canines,[20] with other models reporting a reduction of cardiac function by 40%.[19,21] In a canine coronary venous hypertension model, as myocardial edema increased, there were elevations in LV chamber stiffness and reductions in EDVs.[22] In septic mice, decreases in LVEF were associated with myocardial vascular injury and leakage,[23] resulting in increased edema and cardiac dysfunction.

Cardiac edema has been reported in cardiac human sepsis studies employing cardiac EM and CMR. This agrees with our cardiac EM and CMR data and previously reported preclinical cardiac EM data.[13] An observational study of 15 patients with sepsis showed increased myocardial T2-times and decreases in LVEF within 48 hours after intensive care unit admission.[24] Three patients with sepsis in another study showed increased T2-signal and histological evidence of "striking" interstitial edema.[25] An autopsy report described 2 subjects with sepsis with cardiac dysfunction and myocardial edema.[26] Therefore, edema is a plausible antecedent mechanism of cardiac dysfunction during sepsis given (1) the rapidity of cardiac injury (chamber size changes and drop in LVEF and RVEF) and speedy reversal over days; (2) the findings of edema on CMR and histology in septic humans and animals; (3) the degree of edema found and the consistency across the literature of its association with reversible cardiac dysfunction; and, (4) no other obvious major histopathological injury was found to explain the EDV and LVEF findings. As this controlled study shows a crucial link between cardiac edema and dysfunction during sepsis, a well-documented feature in the literature by CMR and EM, we suggest the following term: septic cardiac edema-related reversible injury.

Initially, decreased ventricular chamber size at 24 hours was followed by a marked increase from 24 to 48 hours in all septic animals. We also found a significant strong positive correlation during this time between increases in EDV and LV wall edema among septic animals. This correlation reinforces the concept that EDV increases are closely related to the loss of ventricular dry mass. LV wall edema increased because LV wall dry weight decreased without a loss of water, which therefore led to a relative increase in the water content of the LV wall. Simultaneously, as mass loss thinned the LV wall, a corresponding increase was observed in EDV. Collectively, these CMR-measured effects were reflected in the positive correlation between EDV and edema in both survivors and nonsurvivors. The loss of mass continued from the onset of infection throughout recovery of cardiac function, with an approximate 15% decrease from baseline in LV wall dry weight by 96 hours. Because mass loss continued during LVEF recovery, we speculate that this is part of a reparative process in survivors. Nonsurvivors

presumably have greater, nonrecoverable cardiac injury and were found to have significantly greater LV dry mass loss at an early time point (48 hours), just before death. If dry mass loss is a manifestation of a repair process, it started early and was stronger in sicker animals, but was not able to overcome an otherwise unrevivable episode of septic shock.

Surprisingly, we found that cardiac volumetric changes (increased LVEDV and RVEDV) rather than functional ones (ie, LVEF, strain, ventricular-arterial coupling) correlated with outcome. Because greater LV chamber dilation and larger LVEF decreases have been associated with better outcomes in human sepsis,[27] we evaluated the human cardiac sepsis literature to assess its compatibility with our results (Data S3, Figure S14 and Tables S3–5). Overall, our animal data reported here and the human literature agree: LV chamber dilation is associated with sepsis survival but not changes in LVEF.

Evidence for myocyte dropout, necrosis, or stretch was not found biochemically or histopathologically in our canine model of septic shock. Therefore, our finding of ventricular dry mass loss must have occurred due to loss of intracellular or extracellular constituents or destruction of some other abundant, nonmyocyte cardiac cell type. Cardiac endothelial cells constitute ≈65% of nonmyocyte heart cells, have an integral role in cardiac remodeling and regeneration, and have been implicated in the modulation of myocytes contractility.[28,29] Sepsis-induced dysfunction of this dynamic barrier through inflammatory injury with disruption of crucial intracellular signaling and cell–cell crosstalk can lead to increased vascular permeability and precipitate significant cardiac dysfunction.[30] Endothelial disruption during sepsis has been well characterized in other organs. Liu et al. showed in murine lungs that inflammatory injury by Lipopolysaccharide was associated with a loss of pulmonary endothelial cells which progressively recovered over the 7 days.[31] This is consistent with the time frame of dry weight loss and recovery of cardiac function that we observed in survivors. This disrupted vascular endothelium and potential loss of endothelial cells that improves over 5 to 7 days provides a potential mechanism for cardiac edema and loss of ventricular mass in sepsis observed in our study. During sepsis, we observed some focal myofilament degradation on EM. Autophagy is a normal quality control process where eukaryotic cells form autolysosomes to degrade and remove damaged molecules and organelles. In murine endotoxin models, the activation of autophagy has been shown to reduced cardiac dysfunction.[32,33] Notably, autophagy with removal and replacement of injured or dysfunctional cell components has been found to be protective in several models of acute cardiac injury.[34–36] The loss of extracellular matrix and matricellular proteins involved in injury and repair could also contribute to this tissue loss.[37]

There are limitations to this study. CMR scans were obtained only at baseline and 48 and 96 hours, leaving notable gaps in the timeline of sepsis-induced cardiac injury and dysfunction. However, TTEs were obtained daily, which bridged these gaps and allowed us to follow some aspects of cardiac function. Second, different types, doses, and sites of bacteria could potentially result in different findings; however, our well-established animal model has demonstrated that the pattern of changes in LVEDV and EF is independent of these factors. Third, although echocardiographic diastology measures were collected there are no well-validated guidelines in canines, and the values obtained differ substantially from the range of human echocardiographic reference standards. Lastly, our baselines studies were obtained after invasive surgical procedures. However, this was the same for all animals studied so any effect should cancel out when examining group differences.

CONCLUSIONS

This study provides evidence that changes in preload, afterload, or heart rate cannot explain changes in biventricular chamber size over time in septic survivors compared with nonsurvivors. In the absence of catecholamine administration and despite optimizing preload, nonsurvivors developed from 0 to 24 hours a worsening restrictive physiology with less dilation compared with survivors despite similar EFs and higher PAOPs. An increase in cardiac edema seen by CMR and histology was associated with cardiac injury and dysfunction. Furthermore, sepsis-induced loss of ventricular wall dry mass over 96 hours extended into the recovery of biventricular EFs, indicating that it may be a reparative process rather than ongoing injury. This loss of mass in part can explain sepsis-induced increases in EDV from 24 to 48 hours. The loss of endothelial cells, extracellular matrix, or the autophagy and replacement of damaged endothelial and myocyte molecules and organelles may explain this loss of ventricular mass and thereby account for wall thinning during septic shock. The findings are important in that such changes are associated with outcome and therefore warrant further investigation. Finally, this is the first controlled CMR sepsis study to demonstrate that ventricular wall edema is a critical element of sepsis pathophysiology and dry mass loss may be part of the reparative process.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Data S1

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