

Impact of Left Ventricular Hypertrophy on Troponin Release During Acute Myocardial Infarction: New Insights From a Comprehensive Translational Study

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Background—Biomarkers are frequently used to estimate infarct size (IS) as an endpoint in experimental and clinical studies. Here, we prospectively studied the impact of left ventricular (LV) hypertrophy (LVH) on biomarker release in clinical and experimental myocardial infarction (MI).

Methods and Results—ST-segment elevation myocardial infarction (STEMI) patients (n=140) were monitored for total creatine kinase (CK) and cardiac troponin I (cTnI) over 72 hours postinfarction and were examined by cardiac magnetic resonance (CMR) at 1 week and 6 months postinfarction. MI was generated in pigs with induced LVH (n=10) and in sham-operated pigs (n=8), and serial total CK and cTnI measurements were performed and CMR scans conducted at 7 days postinfarction. Regression analysis was used to study the influence of LVH on total CK and cTnI release and IS estimated by CMR (gold standard). Receiver operating characteristic (ROC) curve analysis was performed to study the discriminatory capacity of the area under the curve (AUC) of cTnI and total CK in predicting LV dysfunction. Cardiomyocyte cTnI expression was quantified in myocardial sections from LVH and sham-operated pigs. In both the clinical and experimental studies, LVH was associated with significantly higher peak and AUC of cTnI, but not with differences in total CK. ROC curves showed that the discriminatory capacity of AUC of cTnI to predict LV dysfunction was significantly worse for patients with LVH. LVH did not affect the capacity of total CK to estimate IS or LV dysfunction. Immunofluorescence analysis revealed significantly higher cTnI content in hypertrophic cardiomyocytes.

Conclusions—Peak and AUC of cTnI both significantly overestimate IS in the presence of LVH, owing to the higher troponin content per cardiomyocyte. In the setting of LVH, cTnI release during STEMI poorly predicts postinfarction LV dysfunction. LV mass should be taken into consideration when IS or LV function are estimated by troponin release. (*J Am Heart Assoc.* 2015;4:e001218 doi: 10.1161/JAHA.114.001218)

Key Words: creatine kinase • hypertrophy • magnetic resonance imaging • myocardial infarction • troponin

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Systemic release of cardiac biomarkers is commonly used to quantify the extent of cardiac damage after an acute myocardial infarction (AMI). Peak and area under the curve (AUC) of total creatine kinase (CK) and cardiac troponin (cTn) have been consistently shown to correlate with infarct size (IS) measured by reference standards: cardiac magnetic resonance (CMR),^{1–3} single-photon emission computed tomography (SPECT),^{4,5} and postmortem analysis.⁶ Accurate quantification of IS is of value given that it correlates closely with long-term left ventricular (LV) performance and, more important, with clinical outcomes.⁷ However, reference standard techniques for IS quantification (CMR or SPECT) are not widely available. Infarct size is therefore commonly estimated from the levels of cardiac biomarkers in peripheral blood, especially in clinical trials in which IS is used as an endpoint.^{8,9}

We recently reported on a retrospective observational analysis showing that patients with LV hypertrophy (LVH) who suffer an ST-segment elevation myocardial infarction (STEMI) can have disproportional blood concentrations of cardiac troponin I (cTnI)/total CK, compared with STEMI patients with no LVH.¹⁰ Given the high prevalence of LVH in the general population^{11,12} and the importance of accurate IS quantification, unequivocal demonstration of the influence of LVH on biomarker release is of clinical and research value.

In the present study, we conducted a prospective analysis to determine whether LV mass influences cardiac biomarker release after STEMI. Biomarker estimates of IS were compared with state-of-the-art CMR, a gold standard for IS quantification, in STEMI patients from a prospective clinical trial, and a similar analysis was conducted in a controlled experimental pig STEMI model (with/without LVH) to gain insight into the underlying mechanisms. The main aims of the present study were to (1) analyze the influence of LVH on the cTnI/total CK release pattern after STEMI, (2) study the impact of LVH on IS quantification and LV ejection fraction (LVEF) prediction by these biomarkers, and (3) study the effect of LVH on cTnI expression in myocardial tissue samples from LVH and control pigs.

Methods

Clinical Study

Patients with first anterior STEMI presenting early (<6 hours) and undergoing primary angioplasty were recruited within the METOCARD-CNIC trial.^{13,14} A prespecified analysis within this trial was the study of the association between cTnI/total CK and CMR-measured LVH, IS, and LVEF. Inclusion/exclusion criteria can be found elsewhere.¹⁵ Serial cTnI and total CK measurements were taken in 140 patients, and data from these patients were used for the current analysis. All patients underwent CMR studies at 5 to 7 days (1 week)¹³ and 6 months¹⁴ after STEMI. This study was approved by the ethics committee, and patients signed informed consent.

CMR protocol

A detailed description of the CMR protocol and methods for imaging analysis has been reported elsewhere¹⁵ and is detailed below. A comprehensive CMR study was performed with dedicated sequences to evaluate cardiac function and myocardial necrosis. Scans were performed with a 3.0-T magnet (Achieva Tx; Philips Medical Systems, Best, the Netherlands), with vectorcardiographic gating and a dedicated cardiac 32-channel phased-array surface coil. All sequences were acquired during expiration breath-hold mode. After standard localizer scan, contiguous short-axis

slices were acquired to cover the whole LV. For functional cine imaging, we first ran a balanced turbo field echo steady-state free precession (SSFP) with a sensitivity encoding fast parallel imaging technique sequence. Typical parameters were voxel size 1.6×2 mm, slices 13, slice thickness 8 mm, gap 0 mm, cardiac phases 30, repetition time (TR) 3.5 ms, echo time (TE) 1.7 ms, flip angle (FA) 40°, sensitivity encoding 1.5, averages 1, and field of view (FOV) 360×360 mm. Delayed enhancement images to assess necrotic myocardium were acquired with a T1-weighted two-dimensional (2D) segmented inversion recovery turbo field echo (2D IR-TFE) sequence performed 10 to 15 minutes after intravenous administration of 0.20 mmol of gadopentate dimeglumine contrast agent (Magnevist; Schering AG, Berlin, Germany) per kg body weight.¹⁶ The delayed enhancement images were thus acquired in short-axis slices that matched function imaging and had the following parameters: voxel size 1.5×1.5 mm; slices 13; slice thickness 8 mm; gap 0; TR 6.1; TE 3.0; inversion time 250 to 350 (optimized to null normal myocardium); FA 25°; TFE factor 20; averages 1; and FOV 360×360 mm.

CMR data analysis

Blinded analyses were undertaken by the core laboratory at the Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC). Data were quantified with dedicated software (QMass MR 7.5; Medis, Leiden, the Netherlands). LV mass, LVEF, and extent of necrosis were determined. LV mass was normalized to body surface area according to Du Bois' formula.¹⁷ Myocardial necrosis (IS), expressed as a percentage of LV mass (the gold standard for IS quantification), was defined according to the extent of delayed gadolinium enhancement.¹⁸

Measurement of cTnI and total CK

Blood samples (3 mL) were taken from a peripheral vein into clot activator tubes for serum total CK analysis and into lithium-heparin tubes for analysis of plasma cTnI. Samples were prepared from blood by centrifugation following standard procedures. Total-CK was assessed with a standard enzymatic method on an Olympus AU2700 analyzer (Beckman Coulter Clinical Diagnostics, Nyon, Switzerland). cTnI was assessed by a chemoluminescence immunoassay on a Dimension Vista system (Siemens Healthcare Diagnostics, Deerfield, IL) or the AccuTnI paramagnetic chemoluminescence immunoassay on an Access-2 analyzer (Beckman Coulter Clinical Diagnostics) with reagents provided by the manufacturer. Blood samples for biomarkers were obtained on admission, every 4 hours during the first 12 hours post-STEMI, and then every 12 hours up to 72 hours post-STEMI.

Animal Study

Experimental procedures were performed in castrated male Large-White pigs. The study protocol was approved by the institutional animal research committee and conducted in accord with recommendations of the Guide for the Care and Use of Laboratory Animals.

Generation of pressure-overload LVH

Pressure-overload LVH was induced in 10 four-week-old Large-White pigs (10 to 14 kg) by surgical banding of the ascending aorta. Sedation was induced by intramuscular injection of ketamine (20 mg/kg), xylazine (2 mg/kg), and midazolam (0.5 mg/kg) and then maintained with sevoflurane. Continuous intravenous infusion of fentanyl served as an analgesic during surgery and a single dose of prophylactic antibiotic with cefuroxime was administered just before the procedure. Mechanical endotracheal ventilation was controlled by external respirator. A minimally invasive right lateral thoracotomy was performed in the fourth intercostal space. Through a small pericardiotomy, the ascending aorta was constricted ≈ 3 cm above the aortic valve with a rigid polyethylene band adjusted to 70% of the measured perimeter of the aorta. In all cases, the band was tied to fit snugly around the aorta, but did not create a palpable thrill. The small pericardiotomy was left open, the pneumothorax evacuated with a chest tube, and the chest wall and skin incisions were closed. After surgery and before the animals recovered from anesthesia, intramuscular (0.01 mg/kg) buprenorphine was administered as a postoperative analgesia. In 8 sham-operated animals, the same procedure was followed, but the band was not tightened. All animals were returned to the farm to grow for an average of 16 weeks before myocardial infarction (MI) procedure.

MI procedure

AMI was induced in all pigs 4 to 5 months after surgery when animals reached a weight of 60 to 70 kg. The protocol of AMI induction has been detailed elsewhere.^{19,20} In summary, anesthesia was induced in all animals as described above and maintained by continuous intravenous infusion of ketamine (2 mg/kg/h), xylazine (0.2 mg/kg/h), and midazolam (0.2 mg/kg/h). All animals were intubated and mechanically ventilated with oxygen (fraction of inspired O_2 : 28%). Central venous and arterial lines were placed and a single bolus of unfractionated heparin (300 mg/kg) was administered immediately before introduction of the catheter. A continuous infusion of amiodarone (300 mg/h) was maintained during the procedure in all pigs to prevent malignant ventricular arrhythmias. The left anterior descending coronary artery immediately distal to the origin of the first diagonal branch

was occluded for 60 minutes with an angioplasty balloon introduced with a catheter inserted by the percutaneous femoral route. Balloon location and state of inflation were monitored angiographically. After balloon deflation, a coronary angiogram was recorded to confirm patency of the coronary artery. In cases of ventricular fibrillation, a biphasic defibrillator was used to deliver nonsynchronized shocks. During their recuperation, animals were cared for by dedicated veterinarians and technicians at the CNIC.

CMR protocol

A baseline CMR scan was performed in each pig immediately before AMI induction to assess baseline LV mass and LVEF. A second CMR study was performed 7 days after AMI in order to assess IS and LV performance. Pigs were anesthetized by intramuscular injection of ketamine, xylazine, and midazolam as described above, and anesthesia was maintained by continuous intravenous infusion of midazolam (0.2 mg/kg/h). All studies were performed using a Philips 3-Tesla Achieva Tx whole-body scanner (Philips Medical Systems) equipped with a 32-element cardiac phased-array surface coil. To evaluate global LV motion, segmented cine SSFP sequences were performed to acquire 13 to 15 contiguous short-axis slices covering the heart from the base to the apex (FOV of 280×280 mm; slice thickness of 8 mm without gap; TR 2.8 ms; TE 1.4 ms, FA 45°; cardiac phases 25; voxel size 1.8×1.8 mm; and 3 NEX [number of excitations]). Delayed enhancement imaging of infarct size was performed 10 to 15 minutes after intravenous administration of 0.20 mmol of gadopentate dimeglumine contrast agent per kg of body weight¹⁶ using an inversion-recovery spoiled turbo field echo sequence (FOV of 280×280 mm; 13 to 15 short-axis slices with a thickness of 8 mm and no gap; TR 5.6 ms; TE 2.8 ms; voxel size 1.6×1.6 mm; time interval optimized to null normal myocardium; trigger delay longest; bandwidth, 304 Hz per pixel; and 2 NEX).

CMR data analysis

All CMR images were analyzed using dedicated software (QMass MR 7.5; Medis) by 2 observers experienced in CMR analysis and blinded to the LVH induction procedure. LV cardiac borders were traced in each cine image to obtain LV end-diastolic volume (LVEDV), end-systolic volume (LVESV), and LVEF. In the tracing convention used, the papillary muscles were included as part of the LV cavity volume. LVEDV and LVESV were determined using a summation of disks (“Simpson’s rule”) method. Ejection fraction (EF) was computed as $EF = (LVEDV - LVESV) / LVEDV$. LV epicardial borders were also traced on the end-diastolic images, with LV mass computed as the end-diastolic myocardial volume (ie, the difference between the epicardial and endocardial volumes)

multiplied by myocardial density (1.05 g/mL). Values of LV volume and LV mass normalized to body surface area were calculated with Brody's formula.²¹ Myocardial necrosis (IS), expressed as a percentage of LV mass (the gold standard for IS quantification), was defined according the extent of delayed gadolinium enhancement.¹⁸ Necrosis was identified as hyperintense regions, defined as >50% of maximum myocardial signal intensity (full width at half maximum), with manual adjustment when needed. If present, a central core of hypointense signal within the area of increased signal was included in the late gadolinium enhancement analysis.

Measurement of cTnI and total CK

Blood samples (3 mL) were taken from the femoral vein into clot activator tubes for serum total CK analysis and into lithium-heparin tubes for analysis of plasma cTnI. Samples were prepared from blood by centrifugation separation. Total CK was measured by an enzymatic method, according to the manufacturer's recommendations, and cardiac TnI was assayed using an automated colorimetric immunoassay with reagents provided by the manufacturer. Both biomarkers were determined on a Dimension RxL Max system (Siemens Healthcare Diagnostics). Blood samples for biomarker measurement were obtained from a femoral vein at baseline (before AMI procedure), minute 0 (just before coronary occlusion), minute 60 (immediately before reperfusion), and every hour over 6 hours following reperfusion.

Immunofluorescence Analysis of cTnI in Myocardial Tissue Samples

MI greatly influences myocardial protein tissue content,²² prompting us to measure myocardial tissue content of cTnI in an additional set of 8 pigs (n=4 banded-LVH; n=4 sham-operated controls). When they reached the target weight (60 to 70 kg), these pigs underwent the baseline CMR scan and were sacrificed (they did not undergo AMI induction). Myocardial samples were collected within minutes of euthanasia from the anterior and posterolateral mid-ventricular wall, fixed in formalin, embedded in paraffin, and analyzed by immunofluorescence to measure expression of cTnI and cardiomyocyte perimeter. A detailed description is given below.

Heart samples from banded LVH (n=4) and normal pigs (n=4) were fixed in 10% formalin at 4°C for 24 hours and embedded in paraffin. For epitope unmasking, heart sections (5 µm) were treated with 10 mmol/L of trisodium citrate 0.05% Tween 20 (pH 6.0) for 3 minutes at 125°C. Sections were then permeabilized with 0.25% Triton X-100 in PBS for 10 minutes and blocked with 2% BSA in PBS (blocking buffer) for 1 hour at room temperature. Blocked sections were incubated with a mouse monoclonal anti-cardiac troponin I

antibody (2 µg/mL, ab19615; Abcam, Cambridge, MA) or control mouse IgG (sc2025; Santa Cruz Biotechnology, Santa Cruz, CA) in blocking buffer for 2 hours at room temperature. After incubation with secondary anti-mouse Alexa-647 antibody (Invitrogen, Carlsbad, CA) for 45 minutes at room temperature, sections were stained with fluorescein isothiocyanate-conjugated lectin from wheat (FITC-WGA; 100 µg/mL in PBS, L-4895; Sigma-Aldrich, St. Louis, MO) overnight at 4°C to delineate the perimeter of cardiomyocytes. Finally, nuclei were stained by incubating slides with 4',6-diamidino-2-phenylindole, and slides were mounted with Mowiol-Dabco mounting medium. Images were captured with a fluorescence confocal microscope (Leica SPE, DM 2500; Leica Microsystems, Buffalo Grove, IL) fitted with an ×40 objective. ImageJ software (National Institutes of Health, Bethesda, MD) was used to quantify mean fluorescence intensities corresponding to cTnI and cardiomyocyte cross-sectional area. The captured images of 20 random fields in a section from each pig (4 pigs per group) were quantified, and an average value was calculated for each pig.

Statistical Analysis

For quantitative variables, data are expressed as mean±SD and compared by parametric methods. Non-normal data are reported as medians and interquartile range (IQR) and were compared by nonparametric methods. Owing to the small sample size in the experimental study, all data were compared using nonparametric methods irrespective of the normality test. For categorical data, percentages were compared using exact methods.

Multiple linear regression analysis was used to study the influence of indexed LV mass on total CK and cTnI values, adjusted for infarct size measured by CMR. Variance of data on peak and AUC of total CK and cTnI release tends to be proportional to the mean; therefore, we used a square-root transformation for these variables when included in the regression analysis. Indexed LV mass was analyzed either as a continuous or as a categorical variable. For the experimental study, pigs were categorized according the presence or absence of LVH (yes/no) at baseline (before MI), which perfectly coincided with banding or sham operation, respectively. In the clinical study, patients were classified into 3 categories (LVH tertiles) according to indexed LV mass and sex.²³ Nonparametric ROC (receiver operating characteristic) curve analyses were performed in the clinical study to assess the discriminatory capacity of AUC of cTnI and total CK to predict LV dysfunction at 1 week and 6 months after STEMI. In addition, several predictive multiple regression models were estimated based on our human data to correct infarct sizing for troponin when LVH is present (upper tertile of indexed LV mass), being the final model selected according to

parsimonious criteria and predictive capacity. All statistical analyses were performed using commercially available software (Stata 12.0; StataCorp LP, College Station, TX).

Results

Clinical Study

Baseline characteristics and CMR data are presented in Table 1. Patients in the upper LVH tertile showed a \approx 50% higher CMR-measured absolute and indexed LV mass, compared with those in the lower LVH tertile (Figure 1). Infarct size (% of LV mass) and LVEF on 1-week CMR did not differ between the lower and upper LVH tertiles (Figure 2).

Peak cTnI (median [IQR]) was 154.0 ng/mL (90.4 and 266.3 ng/mL) in the upper LVH tertile versus 98.1 ng/mL (36.2 and 191.3 ng/mL) in the lower LVH tertile ($P=0.02$; Figure 3A). AUC of cTnI (median [IQR]) was 3640 ng/mL (2335 and 5143 ng/mL) in the upper LVH tertile versus 2539 ng/mL (995 and 4616 ng/mL) in the lower LVH tertile ($P=0.03$; Figure 3B). In contrast, no significant differences were found in peak and AUC of total CK between the upper

and lower LVH tertiles (Figure 4). Biomarker release data in the 3 population tertiles are summarized in Table 2.

In the regression analysis, higher LV mass was associated with significantly higher peak and AUC cTnI, after adjustment for IS evaluated by the gold standard (% of LV mass on 1-week CMR). This difference was observed both when indexed LV mass was categorized (upper versus lower LVH tertiles; $P=0.009$ and $P=0.01$ for peak and AUC cTnI, respectively; Figure 5A and 5B) and when it was evaluated as a continuous variable ($P<0.001$ and $P=0.003$ for peak and AUC cTnI, respectively; see Table 3). In contrast, indexed LV mass showed no significant association with peak or AUC of total CK, either when it was categorized or when it was evaluated as a continuous variable.

The discriminatory capacity of AUC of cTnI to predict post-STEMI LV dysfunction (LVEF \leq 40%) on 1-week CMR was significantly worse for patients with LVH (upper LVH tertile, 0.71; 95% confidence interval [CI], 0.55 to 0.87; lower LVH tertile, 0.92; 95% CI, 0.84 to 0.99; $P=0.02$; Figure 6A). A sensitivity analysis using other clinically relevant LVEF cutoffs showed similar results (Figure 6B). AUC of cTnI was similarly poor at predicting long-term (6 months post-STEMI) severe

Table 1. Characteristics and Cardiac Magnetic Resonance Data in the Clinical Study

	Lower Tertile of Indexed LV Mass (n=47)	Middle Tertile of Indexed LV Mass (n=46)	Upper Tertile of Indexed LV Mass (n=47)	P Value*	P Value†
Age, y	58.7 (11.9)	59.7 (12.0)	56.7 (10.6)	0.45	0.23
Male, %	89.4	89.1	89.4	1.00	1.00
Hypertension, %	40.4	45.7	31.9	0.41	0.40
Dyslipidemia, %	53.2	44.4	36.2	0.26	0.15
Diabetes, %	12.8	30.4	14.9	0.08	1.00
Smoking (active), %	51.1	50.0	55.3	0.91	0.84
Body mass index, kg/m ²	27.9 (3.1)	28.3 (3.7)	28.0 (4.4)	0.86	0.94
Body surface area, m ²	1.93 (0.15)	1.90 (0.14)	1.89 (0.17)	0.40	0.20
Symptom-onset-to-balloon time, minute	190.8 (61.1)	186.0 (57.7)	212.7 (77.3)	0.12	0.13
TIMI grade 3 post-PTCA, %	68.1	69.6	76.6	0.67	0.49
Prereperfusion i.v. metoprolol, %	46.8	54.3	44.7	0.49	1.00
LV mass, g	93.1 (12.9)	110.1 (13.6)	136.6 (22.2)	<0.0001	<0.0001
Indexed LV mass, g/m ²	48.1 (5.1)	57.7 (4.5)	72.3 (10.9)	<0.0001	<0.0001
Infarct size (% of LV) at day 5 to 7	21.4 (12.6)	19.7 (11.1)	23.0 (10.7)	0.37	0.52
LVEF (%) at day 5 to 7	44.4 (9.7)	47.4 (9.4)	44.2 (7.3)	0.17	0.92
LVEF (%) at 6 months	47.18 (10.85)	50.8 (10.0)	46.48 (9.90)	0.11	0.75

Continuous variables are reported as means (SD) and compared by parametric methods (1-way ANOVA for comparison between all tertiles, and 2-tailed Student *t* test for comparison between upper and lower tertile of indexed LV mass). Categorical variables are reported as percentages and compared by Fisher's exact test. LV indicates left ventricle; LVEF, left ventricular ejection fraction; PTCA, percutaneous transluminal coronary angioplasty; TIMI, thrombolysis in myocardial infarction.

*P value for comparison between all tertiles.

†P value for comparison between upper and lower tertile of indexed LV mass.

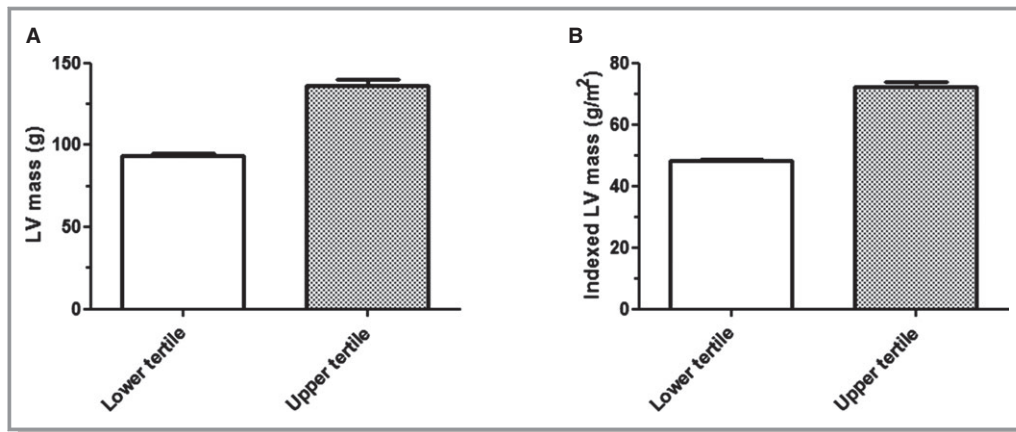


Figure 1. Left ventricular mass differences in patients presenting with STEMI. Patients in the upper tertile showed $\approx 50\%$ higher absolute LV mass (A) and indexed LV mass (B): 136.6 ± 22.2 g and 72.3 ± 10.9 g/m² in the upper tertile versus 93.1 ± 12.9 g and 48.1 ± 5.1 g/m² in the lower tertile, respectively ($P < 0.0001$ for both comparisons). Bars represent mean and SEM. LV indicates left ventricular; STEMI, ST-segment elevation myocardial infarction.

LVEF depression ($\leq 35\%$) for patients with LVH (upper LVH tertile, 0.74; 95% CI, 0.59 to 0.90; lower LVH tertile, 0.92; 95% CI, 0.84 to 1.00; $P = 0.04$). In contrast, the discriminatory capacity of AUC of total CK to predict LV dysfunction did not differ significantly between LVH tertiles at any cutoff or time point.

Animal Study

Six pigs (4 of 10 banded, and 2 of 8 sham-operated) died during AMI induction as a result of refractory ventricular fibrillation. The final population for analytical purposes was

thus 12 pigs (6/group). Baseline characteristics and CMR data are presented in Table 4. Banded pigs had $\approx 30\%$ higher absolute and indexed LV mass (Figure 7). Infarct size (% of LV mass) and LVEF on day 7 CMR did not differ between banded and sham-operated groups (Figure 8).

Peak cTnI (median [IQR]) was 183.4 ng/mL (171.4 and 194.3 ng/mL) in the banded group versus 153.1 ng/mL (132.6 and 169.7 ng/mL) in sham-operated controls ($P = 0.04$; Figure 9A). AUC of cTnI (median [IQR]) was 55 230 ng/mL (52 537 and 57 598 ng/mL) in the banded group versus 45 817 ng/mL (41 998 and 50 365 ng/mL) in sham-operated controls ($P = 0.04$; Figure 9B). In contrast, peak and

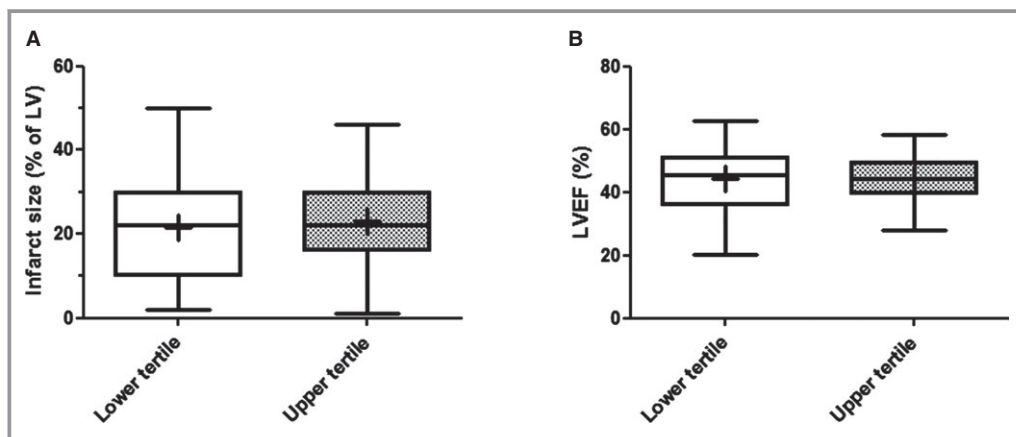


Figure 2. Distributional plots of infarct size (A) and left ventricular ejection fraction (B) measured by magnetic resonance in STEMI patients 1 week after infarction and classified in upper and lower tertiles of indexed LV mass. No between-tertile differences were detected: infarct size $23.0 \pm 1.6\%$ in the upper LVH tertile versus $21.4 \pm 1.8\%$ in the lower tertile; LVEF $44.2 \pm 1.1\%$ in the upper tertile versus $44.4 \pm 1.4\%$ in the lower tertile ($P > 0.50$ for both comparisons). Box plots show median values and interquartile range (Tukey's method); crosses represent mean values. LV indicates left ventricular; LVEF, LV ejection fraction; LVH, LV hypertrophy; STEMI, ST-segment elevation myocardial infarction.

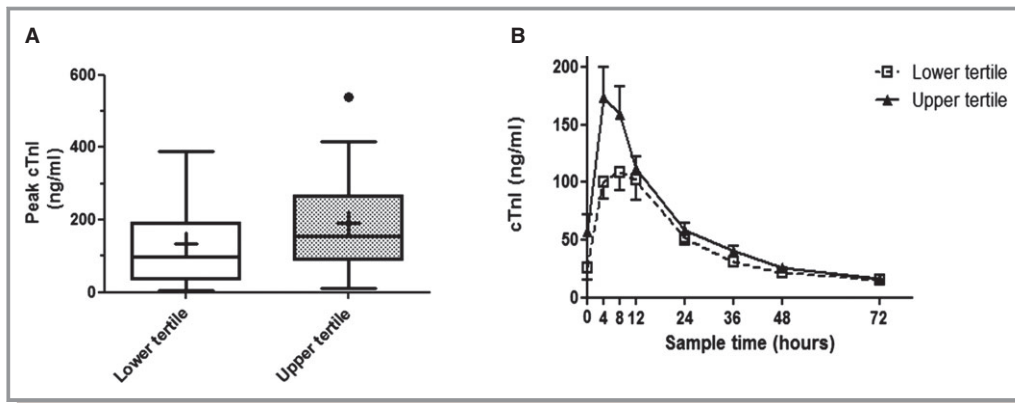


Figure 3. Differences in peripheral blood concentrations of cTnI between lower and upper tertiles of indexed LV mass in patients presenting with STEMI. A, Box plots of peak cTnI, showing median values and interquartile range (Tukey’s method); crosses represent mean values. B, Time profile of cTnI readings after STEMI (mean±SEM). cTnI indicates cardiac troponin I; LV, left ventricular; STEMI, ST-segment elevation myocardial infarction.

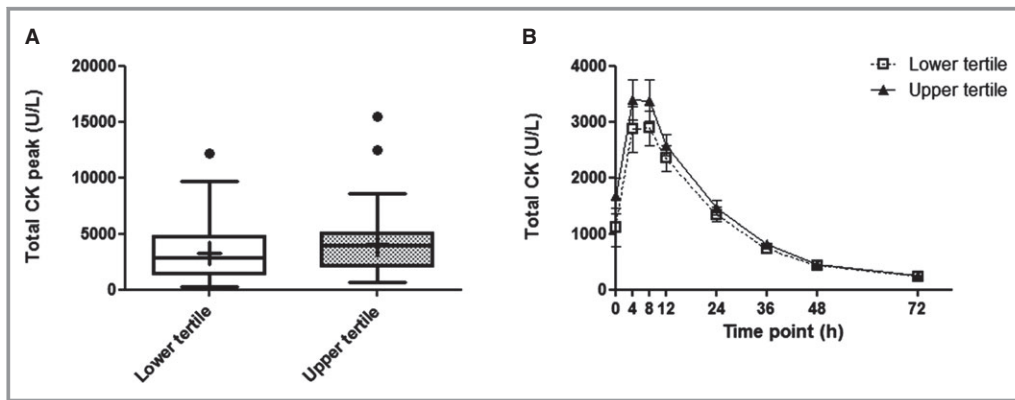


Figure 4. A, Peak total CK in STEMI patients in the lower and upper LVH tertiles in the clinical study. Box plots show median values and interquartile range (IQR; Tukey’s method) of total CK; crosses represent mean values. The median (IQR) of the peak in the upper tertile was 4003 (2064 to 5065) versus 2829 UI/L (1348 to 4720) in the lower tertile ($P>0.10$). B, AUC of total CK in the lower and upper LVH tertiles. Data are presented as mean±SEM. The median (IQR) AUC of total CK in the upper tertile was 67 841 (40 759 to 99 692) versus 54 365 UI/L (28 573 to 83 894) in the lower tertile ($P>0.10$). AUC indicates area under the curve; CK, creatine kinase; LVH, left ventricular hypertrophy; STEMI, ST-segment elevation myocardial infarction.

AUC of total CK did not differ significantly between banded and sham-operated groups.

In the regression analysis, higher LV mass was associated with a significantly higher peak and AUC cTnI, after

adjustment for IS evaluated by the gold standard (% of LV mass on day 7 CMR). This difference was observed both when indexed LV mass was categorized (yes/no; $P<0.001$ and $P=0.001$ for peak and AUC cTnI, respectively; Figure 10A

Table 2. Biomarker Release Data in the Clinical Study

	Lower Tertile of Indexed LV Mass (n=47)	Middle Tertile of Indexed LV Mass (n=46)	Upper Tertile of Indexed LV Mass (n=47)
Peak of cTnI, ng/mL	98.1 (36.2 to 191.3)	98.3 (29.4 to 200.3)	154.0 (90.4 to 266.3)
AUC of cTnI, ng/mL	2539 (995 to 4616)	2021 (741 to 4913)	3640 (2335 to 5143)
Peak of total CK, UI/L	2829 (1348 to 4720)	2176 (897 to 4471)	4003 (2064 to 5065)
AUC of total CK, UI/L	54 365 (28 573 to 83 894)	45 573 (21 230 to 74 260)	67 841 (40 759 to 99 692)

Data are presented as median (interquartile range). Data are not adjusted by actual infarct size as measured by cardiac magnetic resonance. AUC indicates area under the curve; CK, creatine kinase; cTnI, cardiac troponin I; LV, left ventricle.

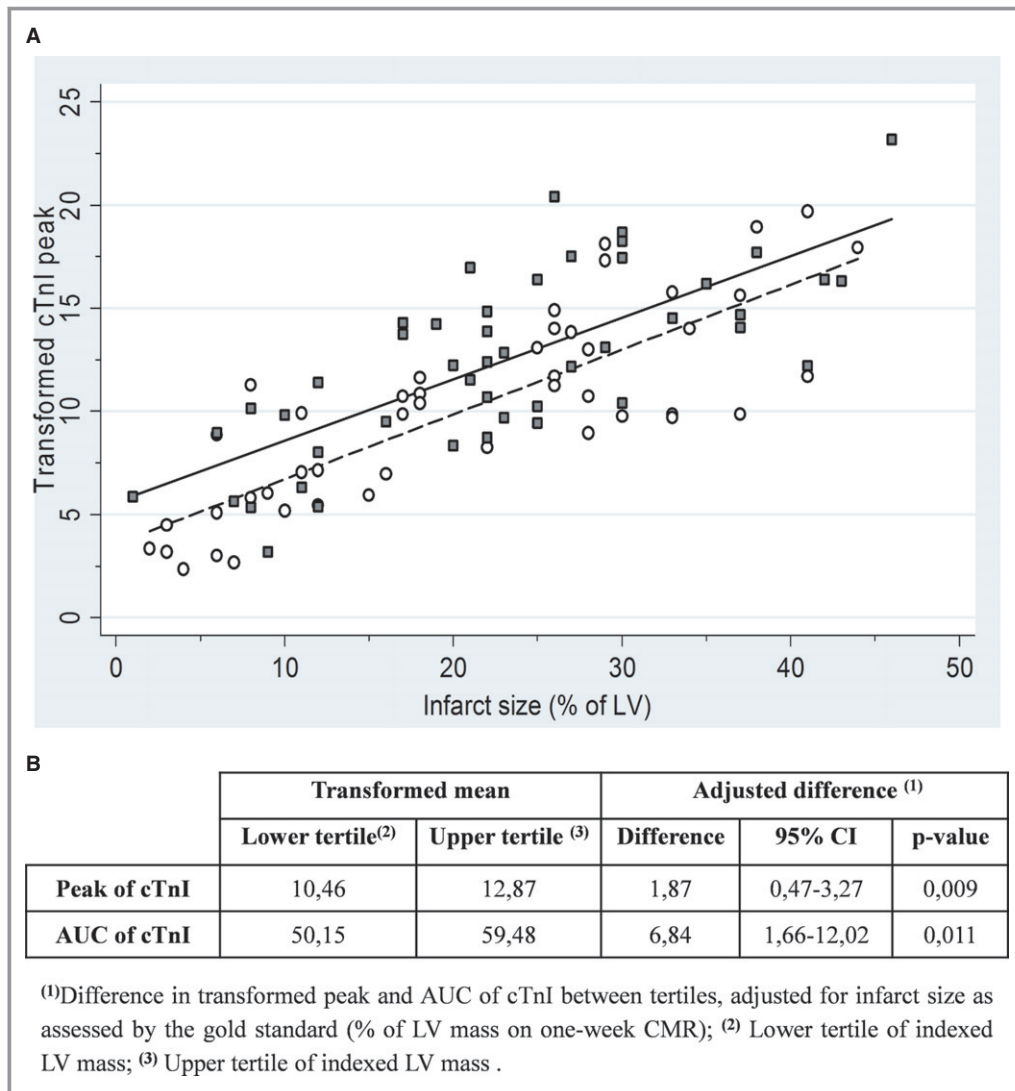


Figure 5. A, Scatter plots and fitted values of transformed (square-root) peak cTnI in STEMI patients versus infarct size evaluated by the gold standard (% of LV mass on 1-week CMR). Plots are shown for patients in the lower tertile of indexed LV mass (white circles and dash line) and in the upper tertile (gray squares and solid line). B, Differences in transformed peak and AUC of cTnI between the upper and lower indexed LV mass tertiles, adjusted for infarct size as assessed by the CMR gold standard. AUC indicates area under the curve; CI, confidence interval; CMR, cardiac magnetic resonance; cTnI, cardiac troponin I; LV, left ventricle; STEMI, ST-segment elevation myocardial infarction.

and 10B) and when it was evaluated as a continuous variable ($P=0.023$ and $P=0.035$ for peak and AUC cTnI, respectively; see Table 5). In contrast, indexed LV mass showed no

significant association with peak or AUC of total CK, either when it was categorized or when it was evaluated as a continuous variable.

Table 3. Elevated LV Mass in STEMI Patients in the Clinical Study Is Significantly Associated With High Peak and AUC of cTnI, After Adjustment for Infarct Size

	Adjusted Difference	95% CI	P Value
Transformed peak of cTnI	0.87	0.41 to 1.33	<0.001
Transformed AUC of cTnI	2.59	0.87 to 4.30	0.003

The table shows adjusted differences in transformed peak and transformed AUC of cTnI for every 10-g/m² increase in indexed LV mass, adjusted for infarct size as assessed by the gold standard (% of LV mass on 1-week CMR). AUC indicates area under the curve; CI, confidence interval; CMR, cardiac magnetic resonance; cTnI, cardiac troponin I; LV, left ventricle; STEMI, ST-segment elevation myocardial infarction.

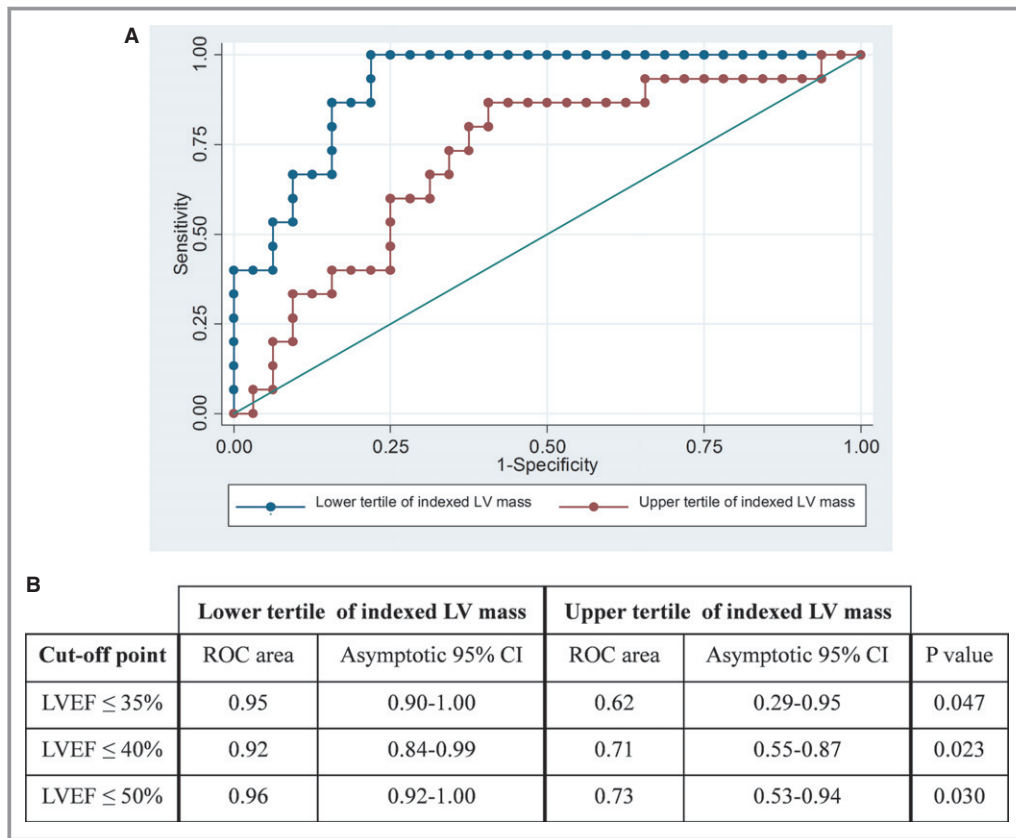


Figure 6. A, Discriminatory capacity of AUC of cTnI to predict LV dysfunction (LVEF ≤40%) on 1-week CMR in STEMI patients in the lower and upper indexed LV mass tertiles. B, Sensitivity analysis performed according to clinically relevant LVEF cut-offs. AUC indicates area under the curve; CI, confidence interval; CMR, cardiac magnetic resonance; cTnI, cardiac troponin I; LV, left ventricle; LVEF, LV ejection fraction; ROC, receiver operating characteristic; STEMI, ST-segment elevation myocardial infarction.

Histological evaluation showed that ventricular cardiomyocytes from banded pigs were significantly larger than those of sham-operated pigs ($P=0.021$; Figures 11 and 12A). Quantification of total cTnI per cardiomyocyte on immunostained sections revealed significantly higher cTnI content per cell in banded pigs ($P=0.043$; Figures 11 and 12B).

Discussion

The present study shows that, for a given infarct size determined by CMR, peak and AUC of cTnI are unusually high in patients with LVH and in pigs with induced LVH. In contrast, LVH had no effect on peak or AUC of total CK.

Table 4. Characteristics and Cardiac Magnetic Resonance Data of the Animal Study Population

	Sham-Operated Group (n=6)	Banded Group (n=6)	P Value
Weight, kg	63.8 (6.1)	66.9 (2.8)	0.23
Body surface area, m ²	1.12 (0.07)	1.16 (0.03)	0.23
LV mass, g	81.7 (8.3)	107.4 (16.7)	0.004
Indexed LV mass, g/m ²	72.9 (3.4)	92.8 (13.8)	0.004
Baseline LVEF, %	54.9 (5.2)	57.6 (3.9)	0.42
Infarct size (% of LV)	27.8 (5.0)	25.0 (3.3)	0.42
LVEF (%) at day 7	38.2 (8.0)	39.7 (6.3)	0.87

Continuous variables are reported as means (SD) and compared by a nonparametric test (Wilcoxon's rank-sum test). LV indicates left ventricle; LVEF, left ventricular ejection fraction.

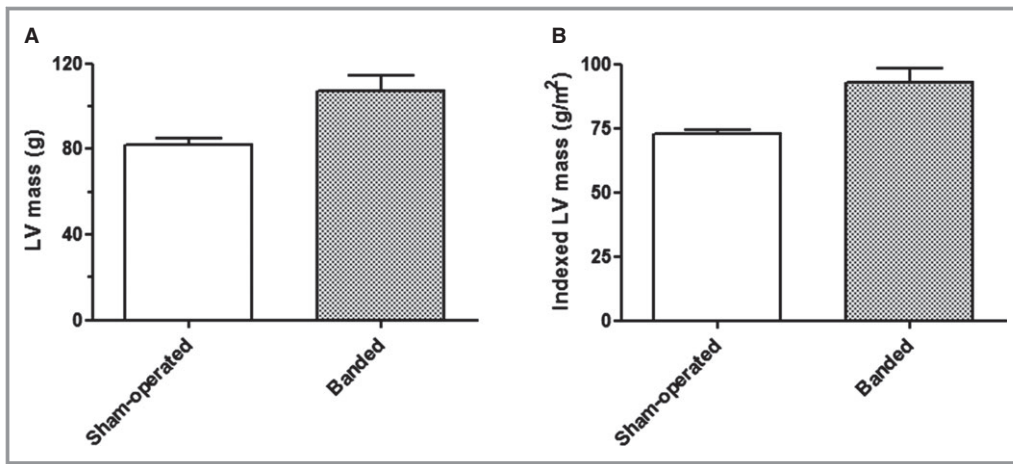


Figure 7. LV mass differences between sham-operated pigs and pigs with banding of the aorta to induce LVH. Absolute (A) and indexed (B) LV mass were $\approx 30\%$ higher in the banded pigs: 107.4 ± 16.7 g and 92.8 ± 13.8 g/m² in the banded group versus 81.7 ± 8.3 g and 72.9 ± 3.4 g/m² in the sham-operated group ($P < 0.01$ for both comparisons). Bars represent mean and SEM. LV indicates left ventricle; LVH, left ventricular hypertrophy.

ROC analysis shows that cTnI release during a STEMI is a less-accurate predictor of short- and long-term LV impairment in the presence of LVH. Moreover, cardiomyocytes from pigs with LVH have a higher cTnI content compared with cardiomyocytes from nonhypertrophied hearts. The same findings were consistently found in patients enrolled in a contemporary clinical trial and in a controlled large-animal study.

Quantification of the extent of myocardial necrosis after STEMI is of clinical and research interest. CMR has become the gold standard for accurate *in vivo* quantification of IS.¹

However, CMR is not widely available and is logistically challenging. Biomarkers of cardiac damage released to the systemic circulation after STEMI have, for many years, been used as surrogate markers of IS. For several decades, peak and AUC of total CK was the preferred biomarker. The more specific cardiac troponins were introduced later and are increasingly used. Peak and AUC of total CK and cTnI have been shown to correlate well with IS measured by gold-standard techniques. Owing to their cardiac specificity, troponins have become the reference biomarker for estimating IS and thus for predicting long-term clinical outcomes

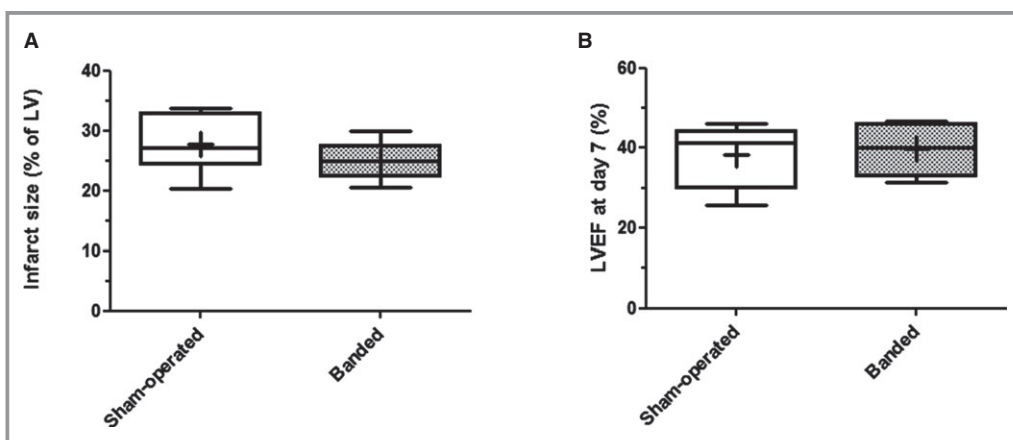


Figure 8. Distributional plots of infarct size (A) and LVEF (B) measured by magnetic resonance in sham-operated and banded pigs 1 week after infarction. No differences were detected between groups for either parameter: infarct size, $25.0 \pm 3.3\%$ banded versus $27.8 \pm 5.0\%$ sham-operated; LVEF, $39.7 \pm 6.3\%$ banded versus $38.2 \pm 8.0\%$ sham-operated ($P > 0.40$ for both comparisons). Box plots show median values and interquartile range (Tukey's method); crosses represent mean values. LV indicates left ventricle; LVEF, LV ejection fraction.

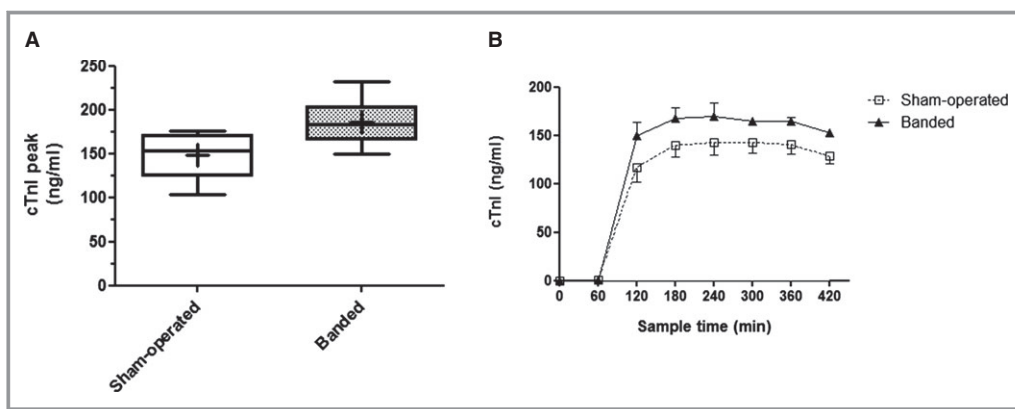


Figure 9. Postinfarction differences in peripheral blood concentrations of cTnI between sham-operated and pigs with banding of the aorta to induce LVH. A, Box plots of peak cTnI, showing median values and interquartile range (Tukey’s method); crosses represent mean values. B, Time profile of cTnI readings after induced MI (mean±SEM). cTnI indicates cardiac-troponin I; LVH, left ventricular hypertrophy; MI, myocardial infarction.

after STEMI,^{4,24} and, consequently, many experimental studies and clinical trials use cardiac troponins as the primary outcome measure.^{8,25,26} It is therefore important to identify confounders that affect the release and quantification of cardiac troponins. Given the high prevalence of LVH in the general population (20% to 25%),^{11,12} and especially in the MI population (50%),²⁷ it is of great importance to know whether LV mass affects the systemic release of biomarkers after an AMI. Notably, stratification according to hypertension—the most frequent cause of LVH—may be insufficient to control the effect of LV mass on biomarker release, given that approximately one third of patients with hypertension are unaware that they are hypertensive.²⁸

Our analysis of total CK and cTnI showed that whereas hypertrophied hearts release more cTnI than control hearts upon AMI, hypertrophy does not affect total CK release. The CK system is important for intracellular energy production and utilization and is abundantly present in tissues with high metabolic demand. There are 4 electrophoretically distinct CK isoenzymes—BB, MB, MM, and the mitochondrial forms

(mi-CK)—with CK-MB predominating in the adult heart.²⁹ Animal models of LVH consistently show that hypertrophic cardiomyocytes switch to a fetal CK isoenzyme pattern, increasing expression of CK-MB and CK-BB at the expense of reduced expression of CK-MM and mi-CK in order to increase energy yield; these changes balance out so that total CK levels in the hypertrophied myocardium are unaltered.^{30–35} In contrast, it is intuitive to argue that cardiomyocyte content of troponin would increase with hypertrophy as part of the general increase in cardiomyocyte content of contractile units and associated contractile proteins. Thus, in the setting of LVH, the release of troponins will be disproportionately high after STEMI, whereas total CK release will not be affected. Regarding this specific issue, we estimated a final predictive model to correct infarct sizing for troponin when LVH is present. Thus, it was estimated that, in patients with LVH (upper tertile of indexed LV mass), peak of cTnI overestimates infarct size by 30%, as measured on CMR. Most research on the CK system took place in the 1980s and 1990s, when the troponins were still

Table 5. Experimentally Increased LV Mass in Pigs Is Significantly Associated With High Peak and AUC of cTnI, After Adjustment for Infarct Size

	Adjusted Difference	95% CI	P Value
Transformed peak of cTnI	0.67	0.12 to 1.22	0.023
Transformed AUC of cTnI	9.62	0.82 to 18.42	0.035
Transformed peak of total CK	2.72	–27.06 to 32.51	0.841
Transformed AUC of total CK	72.18	–446.31 to 590.68	0.760

On the other hand, increased LV mass in pigs is not associated with higher peak and AUC of total CK. The table shows adjusted differences in transformed peak and transformed AUC of cTnI and total CK for every 10-g/m² increase in indexed LV mass, adjusted for infarct size as assessed by the gold standard (% of LV mass on 1-week CMR). AUC indicates area under the curve; CK, creatine kinase; CMR, cardiac magnetic resonance; cTnI, cardiac troponin I; LV, left ventricle.

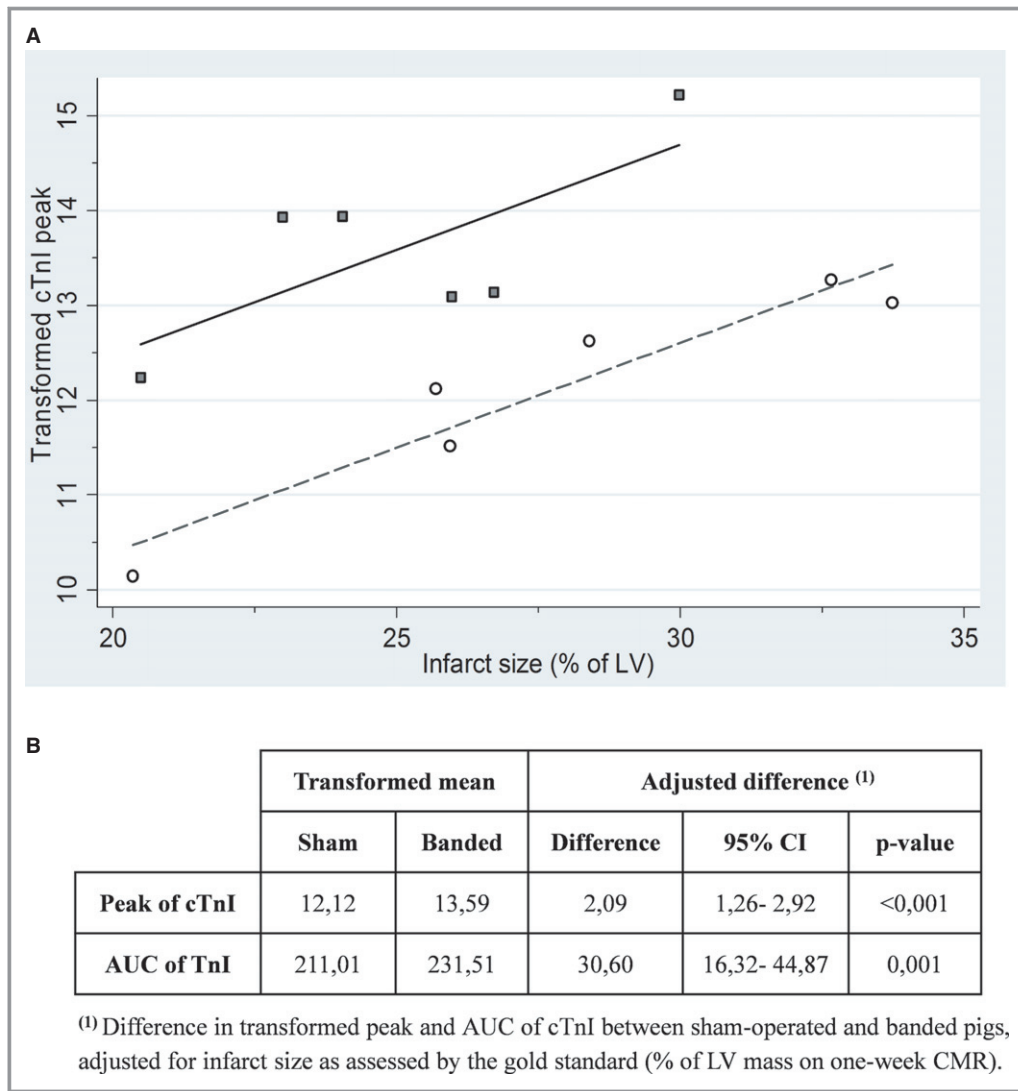


Figure 10. A, Scatter plots and fitted values of transformed (square-root) peak postinfarction cTnI versus infarct size evaluated by the gold standard (% of LV mass on 1-week CMR). Plots are shown for sham-operated (white circles and dash line) and banded pigs (gray squares and solid line). B, Differences in transformed peak and AUC of cTnI between sham-operated and banded pigs, adjusted for infarct size as assessed by the CMR gold standard. AUC indicates area under the curve; CI, confidence interval; CMR, cardiac magnetic resonance; cTnI, cardiac-troponin I; LV, left ventricle.

unknown. The subsequent development of specific methods to accurately quantify troponins has established the use of this biomarker, and the high sensitivity and specificity of troponins for detecting even mild cardiac damage has represented a big step forward in the early triage of patients with chest pain. The influence of LVH on cTnI release after myocardial damage reported here does not invalidate the use of troponins as surrogate markers, but shows the need to take LV mass into account as an important potential confounding factor in clinical and research evaluations. These considerations could be especially important in determining the significance of small increases in circulating troponin in patients presenting to the emergency depart-

ment, given that troponin readings in patients with LVH may give a false-positive diagnosis of MI.³⁶ In this regard, mild increases in troponin-I³⁷⁻⁴⁰ or troponin-T⁴¹ have been reported in patients with LVH in the absence of chest pain or any other acute condition. In addition, it has been recently reported that cardiac troponin concentrations correlate with LV mass index independent of coronary artery disease status in patients with aortic valve stenosis.^{42,43} Presence of a dynamic pattern (rise and/or fall) in cardiac troponin values along with clinical evidence of ischemia can be of help to confirm the diagnosis of MI in these contexts. Although the aims of our study were beyond these important issues, our data support that, for a given small stress to the myocar-

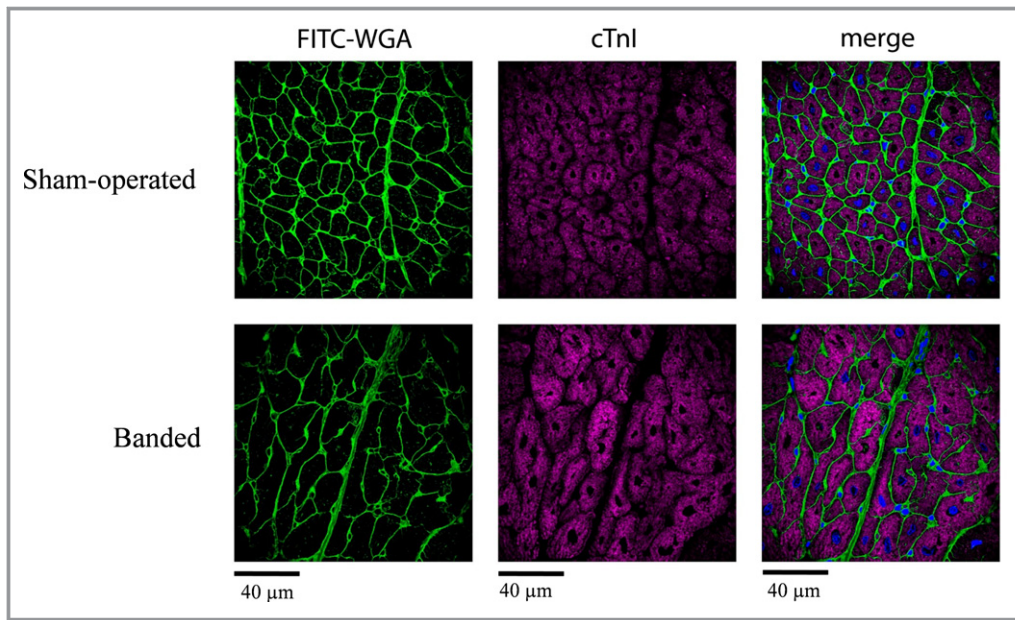


Figure 11. Representative immunofluorescence images of cTnI (pink, center) and cell membrane stained with FITC-WGA (green, left) in heart sections from sham-operated pigs (top) and hypertrophic (banded) pigs (bottom). Merged images are shown on the right. Nuclei are stained blue. cTnI indicates cardiac troponin I; FITC-WGA, fluorescein-isothiocyanate–conjugated lectin from wheat.

dium, troponin release in patients with LVH might be larger than in patients with normal LV mass.

In summary, our results show that LVH influences troponin release upon cardiac damage both in STEMI patients and in a controlled large-animal study, associated with higher expression of cTnI in cardiomyocytes from LVH hearts. These data have clinical implications given that many clinical trials use

troponin release as a surrogate endpoint of the extent of cardiac damage and IS.

Limitations

Total CK circulating levels are potentially influenced by skeletal muscle trauma (including animal handling, intramus-

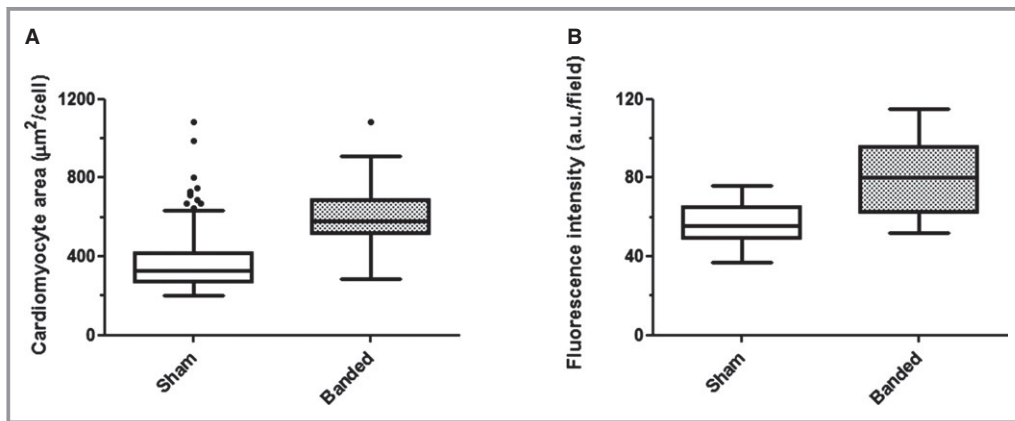


Figure 12. A, Cardiomyocyte area in banded and sham-operated pigs with no induced myocardial infarction. Box plots (Tukey’s method) represent individual data (all random analyzed fields) from all pigs (4 pigs/group). Median (IQR) cardiomyocyte size was 579.3 μm^2 (516.9 to 685.7) in banded pigs and 326.2 μm^2 (271.0 to 420.0) in sham-operated pigs ($P=0.021$). B, Immunofluorescence of cardiac troponin I in banded and sham-operated pigs with no induced myocardial infarction. Box plots (Tukey’s method) represent individual data (all random analyzed fields) from all pigs (4 pigs/group). Median (IQR) immunofluorescence intensity was 80 arbitrary units/field (62 to 96) in the banded pigs and 55.5 a.u./field (49.5 to 65) in sham-operated pigs ($P=0.043$). IQR indicates interquartile range.

cular injections, or surgical procedures).^{44,45} Although we cannot completely exclude a significant influence of animal manipulation in total CK release, baseline total CK values were not different among groups. Of note, blood sample collections for biomarker measurement were performed 4 to 5 months after thoracotomy, thus unlikely to interfere in total CK release.

CK-MB was not systematically measured in the clinical study because it is no longer routinely evaluated in our environment. Similarly, CK-MB was not assessed in the animal study owing to lack of specificity of available antibodies and species differences in detected CK-MB activity.⁴⁶ In addition, activity of CK-MB has been shown to be very low in pigs,⁴⁷ thus being much less cardiac specific of myocardial damage than expected in humans owing to a very low CK-MB/total CK ratio in the cardiac muscle.⁴⁸ Of note, because of previously mentioned increase expression of CK-MB during ventricular hypertrophy,^{30–35} we can speculate that CK-MB measurement overestimates actual infarct size similar to cTn. Although CK-MB has been used for years as the cardiac-specific biomarker of myocardial damage, cTn is currently the preferred biomarker because of its significantly higher cardiac tissue specificity.^{9,49}

None of the cTnI assays used in this study are considered ultrasensitive. However, the conclusions from the present work are not likely to be affected by this fact given that high sensitivity is not needed to measure the levels observed both in pigs and humans in the context of STEMI.

The Siemens Dimension RxL assay was designed for detection of human cTnI. However, several findings anticipate its acceptable use in blood samples derived from pigs: (1) There is an almost perfect match between pigs and humans regarding amino acid sequences of the residues (epitopes) recognized by the antibodies of this kit (see www.ifcc.org/ifcc-scientific-division/documents-of-the-sd/troponinassay-analyticalcharacteristics2013/) when performing a Basic Local Alignment Search Tool (BLAST) test; (2) this assay has been shown to have good cTnI immunoreactivity in serum collected from rats and dogs,⁵⁰ which have identical amino acid sequence in these epitopes to pigs; and (3) blood and cardiac tissue collected from pigs has been shown to have good cross-reactivity to the antibodies used in a previous first-generation kit from the same manufacturer, which recognized the same epitopes with the same method.^{51,52}

Conclusions

Peak and AUC of cTnI significantly overestimate infarct size in the presence of LVH, a phenomenon probably owing to the increased cTnI content in hypertrophied cardiomyocytes. Consistent with these findings, the discriminatory capacity of cTnI to predict LV dysfunction is significantly worse for

patients with LVH. LV mass should therefore be considered when infarct size and LV function are estimated by troponin release.

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Disclosures

None.

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