Decreased Serine\textsuperscript{207} phosphorylation of troponin T as a biomarker for left ventricular remodelling after myocardial infarction

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Aims
Chronic heart failure following myocardial infarction (MI) is characterized by progressive left ventricular remodelling (LVR). Despite significant improvements in MI management, LVR remains a frequent complication. Although several risk factors have been identified, such as infarct size, LVR is difficult to predict in clinical practice.

Methods and results
Using a rat model of MI and phosphoproteomic technology, we discovered that remodelling is associated with decreased levels of myocardial and plasma serine\textsuperscript{208}-phosphorylated troponin T (TnT). To confirm the association in human plasma, we developed new specific polyclonal antibodies against human/rat serine\textsuperscript{207}/208-phosphorylated TnT and tested plasma obtained in the first week after MI from patients with low, intermediate, and high remodelling a year later. We observed a significant decrease of serine\textsuperscript{207}-phosphorylated TnT and of the serine\textsuperscript{207}-phosphorylated TnT/total TnT ratio in those with intermediate or high LVR. These differences remained statistically significant when adjusted for other determinants of LVR. In contrast, baseline B-type natriuretic peptide levels were not associated with LVR.

Conclusion
The level of circulating phosphorylated TnT could be a new biomarker of LVR.

Keywords
Myocardial infarction • Echocardiography • Remodelling • Troponin T • Biomarker

Introduction
Despite significant improvements in management of myocardial infarction (MI), left ventricular remodelling (LVR) remains a major complication and a strong predictor of both heart failure (HF) and death after MI.\textsuperscript{1–3} Although several variables, such as MI size, have been identified as risk factors, LVR remains difficult to predict in clinical practice.\textsuperscript{1,3} Better prediction could allow an individualized approach with more intense therapy and follow-up for such high-risk patients.

Proteomic technology makes it possible to examine global alterations in protein expression and provides new insights into cellular mechanisms involved in myocardial dysfunction.\textsuperscript{4} It may also lead to the identification of new diagnostic and therapeutic markers.\textsuperscript{5} Moreover, recently described proteomic methods may give access, not only to global proteome patterns, but also to post-translational protein modifications.\textsuperscript{6} These are highly relevant in biomarker research in view of the importance of these modifications in protein regulation and their potential variance in different pathological states.\textsuperscript{7} For example, various diseases, including cardiovascular diseases, are caused or accompanied by the deregulation of phosphorylation.\textsuperscript{8} The phosphorylation status of contractile proteins is altered in HF, potentially contributing to decreased pump function.\textsuperscript{9} More recently, inhibition of the protein tyrosine phosphatase PTP1B was shown to reverse endothelial dysfunction in HF.\textsuperscript{10}

The aim of the present study was to use a relevant animal model,\textsuperscript{11–12} closely related to the human disease to detect cardiac phosphoproteins with phosphorylation modulated according to the extent of...
LVR. After the identification of a candidate biomarker detectable in plasma, we developed specific tools for accurate quantification and validated it in a cohort of patients screened for LVR 1 year after MI.

Methods

Animals

Myocardial infarction was induced in 10-week-old male Wistar rats (n = 19) (Charles River, France) by left coronary ligation according to the method described by Pfeffer et al.11 and modified by Mulder et al.12 Another 16 rats underwent the same protocol except that the snare was not tied, this was the sham-operated or control group. All rats were allowed standard rat chow and drinking water ad libitum and maintained on a 12-h/12-h light/dark cycle. Detailed methods are provided as Supplementary material online.

Patients

Left ventricular remodelling was analysed in a population of patients enrolled during 2002–2004. The patients were included in the multicentre REVE (REmodelage VEntriculaire) study,3 which was designed to prospectively analyse the impact of gene polymorphisms on LVR after MI.13 For the purpose of the present study, we limited the study population to patients included in the Lille centre because those patients also had plasma samples stored at −80°C that were available for western blot analysis. The Ethics Committee of the Lille University Hospital (France) approved patient recruitment and all patients gave informed consent before inclusion. Inclusion criteria were hospitalization for a first anterior Q-wave MI with at least three LV segments of the infarct zone akinetic at predischarge echocardiography. Exclusion criteria were: inadequate echographic image quality, life-limiting non-cardiac disease, significant valvular disease, or prior Q-wave MI. Echographic studies were performed immediately before hospital discharge and at 1 year after MI, as previously described,3 and images were recorded on optical disks. All echocardiograms were analysed at the Lille Core Echo Laboratory (Lille, France), as previously described.3 Left ventricular volumes and ejection fraction were calculated according to a modified Simpson’s rule. The LV was divided into a 16-segment model to evaluate regional systolic function. Left ventricular remodelling was expressed as the percentage change in LV end-diastolic volume (LVEDV) from baseline (predischarge) to 1 year afterwards: \( \frac{\text{LVEDV}_{1\text{yr}} - \text{LVEDV}_{\text{baseline}}}{\text{LVEDV}_{\text{baseline}}} \times 100 \). Blood samples were obtained in EDTA tubes for all patients at inclusion during the initial hospitalization. Plasma was processed and stored at −80°C and each sample was thawed only once.

Two-dimensional gel electrophoresis, mass spectrometry, western blot analysis, enzymatic assays, and quantification

Two-dimensional (2D) gel electrophoresis was performed as previously described.14 Left ventricular proteins (500 μg) from control

![Figure 1](https://academic.oup.com/eurheartj/article-abstract/32/1/115/443446/1)

Figure 1 Representative 2D gels of rat LV phosphoproteins and proteins. An average image of 2D gels was established for LV phosphoproteome and LV proteome of control (n = 4) and 2 months MI rats (n = 4). Phosphorylated polypeptidic spots modulated in rats with MI are indicated by a number. The insert is detailed in Figure 2. The positions of Mw are indicated on the left and the pl on the bottom of the gels.
(n = 4) and MI (n = 4) rats at 2 months after surgery were analysed, mixed on a dry 24-cm strip with a pH linear gradient of 3–10 (Immobilin DryStrip, GE Healthcare). Detailed methods are provided as Supplementary material online.

**Troponin T and phospho-troponin T-specific antibodies**

Anti-peptide polyclonal antibodies against the 202–215 conserved sequence of rat troponin T (TnT), specific for either TnT (AQTE RKSGKRQTER) or phosphorylated Ser208-TnT (AQTERK(pS)GKRQTER), were developed according to a standard protocol (immunization of 3 months, P.A.R.I.S Company, France). Detailed methods are provided as Supplementary material online.

**Statistical analysis**

Continuous variables are expressed as mean ± SD or as median with 25th and 75th percentiles unless otherwise indicated. Variables that did not follow normal distribution were log transformed prior to statistical testing. Differences in end-diastolic volume (EDV) (1 year vs. baseline) were assessed by the paired Student’s t-test. Differences between groups were compared by an unpaired bilateral Student’s t-test or by ANOVA followed by Scheffe’s F procedure for post hoc comparisons. Categorical data were tested by the χ² test or Fisher’s exact test as appropriate. The relationship between the percentage change in EDV and the ratio of Ser208-phosphorylated TnT to total TnT was tested by linear regression. Independent correlates of change in EDV were identified by multiple linear regression. Variables with a P-value < 0.05 on univariate analysis were entered into the model. Colinearity was excluded by means of a correlation matrix between candidate predictors. A value of P < 0.05 was considered statistically significant. Analyses were performed with SAS software (release 9.1, SAS Institute, Inc., Cary, NC, USA).

**Results**

**Differential phosphoproteomic analysis of the left ventricle in control and myocardial infarction model rats at 2 months after surgery**

This study investigated cardiac phosphoproteomic changes associated with LVR and dysfunction in an experimental rat model of MI. Detailed echocardiographic, haemodynamic, and histomorphometric parameters are presented in Supplementary material online, Table S1.
At 2 months after surgery, proteomic analysis revealed different LV phosphoproteomic patterns between the groups, with 32 ± 5 phosphorylated spots detected in control rats and 52 ± 8 in MI rats ($P = 0.013$), although their LV proteome patterns were similar (309 ± 49 and 284 ± 29 spots) (Figure 1). Using the 2D gel containing the most spots, we found 69 spots differentially phosphorylated between the groups (Figure 1 indicated by numbers).

We focus on three spots labelled 34, 35, and 36 presented in enlarged 2D gel (Figure 2A) that showed a decrease (by a mean factor of 4.3) in phosphorylation in the LV of MI rats compared with controls (Figure 2B). These three spots were identified by mass spectrometry (MS) to cardiac TnT (Figure 2C). We were able to rule out the possible degradation of TnT as previously described for HF. Supplementary material online, Table S2 presents a detailed MS analysis for these three spots. Troponin T itself is a well-established biomarker in cardiovascular diseases, and elevated levels of serum TnT are associated with altered clinical outcomes in conditions including MI and HF. We therefore explored the process of decreased TnT phosphorylation after MI.

**Decreased troponin T phosphorylation in the left ventricle of myocardial infarction rats**

By immunoprecipitation with a specific antibody against TnT followed by western blot analysis with phospho-serine (Ser), -threonine (Thr), and -tyrosine (Tyr) antibodies, we determined that TnT was phosphorylated on Ser- and Thr residues. The extent of phosphorylation on TnT Ser residues in the LV of rats 2 months after MI decreased significantly (Figure 3A), but we observed no significant modulation of phosphorylation on TnT Thr residues (Figure 3B). In addition, total TnT expression did not differ between control and MI rats (Figure 3C).

In view of the suggestion that reduced troponin I (TnI) phosphorylation of Ser23/24 might account for the functional difference in troponin according to the presence of HF, we used specific antibodies against either TnI or Ser23/24 phosphorylated TnI to perform western blot analysis of the samples above, seeking to confirm that the modulation of phosphorylation was specific to TnT. In our model, we observed no variation in the expression of either TnI or Ser23/24 phosphorylated TnI 2 months after MI (Supplementary material online, Figure S1A and B). This is consistent with previous reports that overall phosphorylation status of TnI does not differ significantly between sham-operated and MI pigs.

**Decrease in Ser208-phosphorylated troponin T in the left ventricle and plasma of rats with myocardial infarction, assessed with specific antibodies**

Bioinformatic analysis (PhosphoSitePlus™), consistently with a previous study with mouse TnT, suggested that only serine at position 208 (in the rat TnT sequence) could be phosphorylated and that the amino acids surrounding Ser208 were consensus sites for PKC and for protein phosphatase 2A (PP2A) (Supplementary material online, Figure S2A).

We synthesized the corresponding phosphopeptide AQTERK(pS)GKRQTER, including amino acid residues 202–215 of the rat TnT sequence (1–298 aa), with phosphorylated Ser208. Polyclonal antibodies were raised against the peptide sequence and purified against both the phosphorylated and non-phosphorylated TnT peptides. Both antibodies detected TnT from rat LV at 34 kDa. We characterized the specificity of the antibody for the Ser208-phosphorylated form of TnT with both the Ser208-phosphorylated and non-phosphorylated peptides. Antibody binding was abolished only with the Ser208-phosphorylated peptide. For further confirmation, we treated LV proteins with
alkaline phosphatase to remove all phosphate moieties. Again, antibody binding was abolished. In contrast, binding of the antibody purified against the non-phosphorylated peptide was abolished by incubation with both peptides; this indicates that this antibody recognized total TnT and not only its non-phosphorylated form (Supplementary material online, Figure S2A).

Next, we examined the antibody’s cross-reactivity with TnI, the sequence of which had 5 of 14 amino acids in common with rat TnT and 0 of 14 with human TnT; serine residues were absent in both species. We also used specific antibodies for immunoprecipitation of total or Ser208-phosphorylated TnI from LV proteins. Western blot analysis with Ser208-phosphorylated and total TnT antibodies produced no signal and thus demonstrated a lack of cross-reactivity with TnI (Supplementary material online, Figure S2B).

We used these specific antibodies to semi-quantify precisely the degree of Ser208-phosphorylation of TnT in MI rats. Using the antibody against total TnT, we found no modification of TnT expression in the LV 2 months after MI and confirmed the decreased level of the Ser208-phosphorylated form of TnT in this LV (Figure 4). The ratio of Ser208-phosphorylated TnT to total TnT was thus significantly lower in these rats (Figure 4).

We next investigated whether the modulation of Ser208 phosphorylation of TnT could also be detected in the plasma. Total

Figure 4 Decrease of Ser208-phosphorylated TnT in LV and plasma of rats 2 months after MI, assessed with specific antibodies. Total TnT and Ser208-phosphorylated TnT were quantified in LV (50 μg) and plasma (1 μL) control (n = 16) (white box) and MI (n = 19) (black box) rats at 2 months. Pre-operative plasma was obtained from the same animals before surgery for control (dotted grey box) and MI (dotted black box) rats. The positions of Mw are indicated on the right. Data are expressed as means of an arbitrary unit (AU) ± SEM, except for the ratio of the Ser208-phosphorylated form of TnT to total TnT. ‡P < 0.001.
Ser207-phosphorylated TnT sequence (Supplementary material online). Because our antibodies also specifically recognized the human Ser207-phosphorylated TnT might be a biomarker of LVR in MI patients. Circulating phosphorylated TnT was modestly (by a factor of 1.7) but significantly increased in their plasma of rats with MI (Figure S2A). The ratio of Ser207-phosphorylated TnT to total TnT in the plasma was significantly decreased in patients with left ventricular remodelling did not differ in the LVR tertiles. Ser207-phosphorylated TnT and its ratio to total TnT decreased very significantly in patients with larger MIs, as shown by a higher wall motion score index. The remodelling process occurred despite nearly systematic administration of medications known to reduce remodelling including angiotensin-converting enzyme inhibitors and beta-blockers.

Blood samples for biological analysis were obtained at 7 ± 3 days after MI. As Figure 5 shows total TnT expression in the population did not differ in the LVR tertiles. Ser207-phosphorylated TnT and its ratio to total TnT decreased very significantly in patients with intermediate or high remodelling. The relationship between this ratio and the percentage change in EDV between baseline and 1 year (expressed as a continuous variable) was analysed by linear regression and found to be statistically significant (P = 0.0003). Multivariable analyses to determine independent correlation of LVR found three variables independently associated with the change in EDV: the ratio of Ser207-phosphorylated TnT to total TnT (P = 0.002), the wall motion systolic index (P = 0.002), and hypertension (P = 0.045) (Table 2).

Table 1  Analysis of left ventricular remodelling in patients with anterior myocardial infarction: baseline characteristics are presented according to tertiles of left ventricular remodelling defined as percent change in end-diastolic volume from baseline to 1-year follow-up

<table>
<thead>
<tr>
<th></th>
<th>1st tertile (n = 30)</th>
<th>2nd tertile (n = 31)</th>
<th>3rd tertile (n = 31)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent change in EDV from baseline to 1 year (mean ± SD [range])</td>
<td>−11.3 ± 10.6 [−43.3−0]</td>
<td>7.4 ± 4.5 [0.6−14.7]</td>
<td>39.7 ± 22.5 [16.5−101.3]</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.9 ± 11.8</td>
<td>58.1 ± 13.7</td>
<td>59.4 ± 11.6</td>
<td>0.89</td>
</tr>
<tr>
<td>Women</td>
<td>7 (23%)</td>
<td>7 (23%)</td>
<td>10 (32%)</td>
<td>0.63</td>
</tr>
<tr>
<td>Hypertension</td>
<td>12 (40%)</td>
<td>11 (35%)</td>
<td>20 (65%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3 (10%)</td>
<td>10 (32%)</td>
<td>11 (35%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Reperfusion therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Thrombolysis</td>
<td>15 (50%)</td>
<td>19 (61%)</td>
<td>16 (52%)</td>
<td>0.63</td>
</tr>
<tr>
<td>Primary PCI</td>
<td>10 (33%)</td>
<td>8 (27%)</td>
<td>12 (40%)</td>
<td>0.55</td>
</tr>
<tr>
<td>Peak creatine kinase (IU/L) (n = 90)</td>
<td>2073 [1153–3030]</td>
<td>2452 [1765–3794]</td>
<td>2635 [1157–3945]</td>
<td>0.60</td>
</tr>
<tr>
<td>Peak troponin I (ng/mL) (n = 90)</td>
<td>103 [40–190]</td>
<td>92 [59–217]</td>
<td>134 [46–200]</td>
<td>0.44</td>
</tr>
<tr>
<td>NT-proBNP (pg/mL) (n = 57)</td>
<td>1396 [823–2214]</td>
<td>1095 [595–2696]</td>
<td>1632 [1057–3760]</td>
<td>0.69</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>51.2 ± 8.1</td>
<td>50.0 ± 11.3</td>
<td>46.6 ± 10.0</td>
<td>0.16</td>
</tr>
<tr>
<td>Wall motion systolic index (n = 91)</td>
<td>1.82 ± 0.18</td>
<td>1.83 ± 0.13</td>
<td>1.94 ± 0.14</td>
<td>0.005</td>
</tr>
<tr>
<td>Multivessel coronary artery disease (n = 91)</td>
<td>9 (31%)</td>
<td>9 (29%)</td>
<td>14 (45%)</td>
<td>0.35</td>
</tr>
<tr>
<td>Final TIMI grade 3 flow in infarct-related vessel (n = 91)</td>
<td>28 (96%)</td>
<td>25 (81%)</td>
<td>25 (81%)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

EDV, end-diastolic volume; PCI, primary coronary intervention; ACE, angiotensin-converting enzyme. The variables were available for all 92 patients unless otherwise indicated.

DECREASE IN HUMAN SER207-PHOSPHORYLATED TROPSIN T IN PLASMA OF PATIENTS WITH LEFT VENTRICULAR REMODELLING

Our finding of a specific decrease of Ser208-phosphorylated TnT in the plasma of rats with MI suggested that circulating phosphorylated TnT might be a biomarker of LVR in MI patients. Because our antibodies also specifically recognized the human Ser207-phosphorylated TnT sequence (Supplementary material online, Figure S2A), we measured circulating phosphorylated TnT in a population of 92 patients with anterior MI who subsequently underwent systematic echocardiographic follow-up throughout the first year after MI to quantify LVR. Left ventricular EDV increased from 57.7 ± 13.3 mL/m² at baseline to 64.4 ± 19.4 mL/m² at 1 year (P < 0.0001). Table 1 summarizes the baseline characteristics of population according to tertiles of LVR. The first tertile comprised the patients with the least remodelling, the second those with intermediate remodelling, and the third, those with the most remodelling. Patients with the greatest LVR were those with larger MIs, as shown by a higher wall motion score index. The remodelling process occurred despite nearly systematic administration of medications known to reduce remodelling including angiotensin-converting enzyme inhibitors and beta-blockers.
Discussion

Proteomic techniques are widely used to study disease-related biomarkers in humans. Analysis of diseased human tissue samples may be compromised, however, by factors including disease stage, tissue heterogeneity, genetic variability, and the patient’s medical or treatment history. Alternatively, proteomics can be applied to appropriate animal models for which heterogeneity and sample preparation variability can be minimized and the findings can subsequently be validated in humans. Here, we used a well-characterized rat model in which the induction of anterior MI leads to LVR and dysfunction and to HF. This model is an accurate representation of the LVR that occurs frequently in humans despite treatment as illustrated by the patients described above (Table 1). Major advances in the prevention of LVR in humans, such as angiotensin-converting enzyme inhibitors, were initially discovered with this experimental model. Moreover, while obtaining human cardiac biopsies adequate for proteomic analysis is difficult, an experimental model of MI offers easy access to abundant LV tissue.

Recent studies show that the balance between the kinases and phosphatases that coordinate phosphorylation of the regulatory proteins involved in cardiomyocyte contractility is disrupted in HF, and this imbalance initiates cardiac remodelling. We thus postulated that proteins with post-translational alterations in response to MI might be used as markers of events linked to this disease. We focused on the decreased phosphorylation of TnT, mainly because total TnT is itself an established circulating biomarker of myocardial damage and its plasma levels have prognostic implications in patients with MI or HF. This suggested to us that the phosphorylated form of TnT might be an interesting biomarker of post-MI LVR. The finding that the level of Ser208-phosphorylated TnT decreased in the plasma of rats with MI, proportionally to its decrease in their LV, corroborates the hypothesis that a small blood sample may reveal the physiological and pathological state of any tissue.

Since our aim was to search for candidate circulating biomarkers for clinical practice, we did not attempt to analyse the causes or consequences of decreased Ser208 phosphorylation of TnT, mainly because total TnT is itself an established circulating biomarker of myocardial damage and its plasma levels have prognostic implications in patients with MI or HF. This suggested to us that the phosphorylated form of TnT might be an interesting biomarker of post-MI LVR. The finding that the level of Ser208-phosphorylated TnT decreased in the plasma of rats with MI, proportionally to its decrease in their LV, corroborates the hypothesis that a small blood sample may reveal the physiological and pathological state of any tissue.

One strength of our study is that its combination of experimental and clinical data allows the confirmation of our results in a clinical setting. The patients included in our clinical study all had a first anterior Q-wave MI, very similar to the event mimicked by the experimental model. The patients with significant LVR 1 year after MI had lower levels of circulating Ser207-phosphorylated TnT at baseline. Moreover, the differences remained statistically significant when the analyses were adjusted for known determinants of LVR such as markers of infarct size. In addition, blood samples were taken before hospital discharge, i.e. before LVR is apparent. The determination of Ser207-phosphorylated TnT levels may therefore help to identify high-risk patients after MI. The observation that this level decreases significantly in patients with
intermediate remodelling suggests that it might be a sensitive indicator of LV dilation after MI. At the present time, it remains difficult in clinical practice to predict among the post-MI patients those who will undergo severe LVR and HF. Yet, an early identification of the patients prone to LVR could help to apply a more aggressive therapy for the high-risk group. Those patients would be candidate for rapid upitation of anti-remodelling drugs such as beta-blockers, ACE-inhibitors, and aldosterone antagonists. Repeat imaging of cardiac function in the high-risk group will help to select the patients needing prophylactic implantation of a defibrillator, or patients with extensive LVR who could ultimately be candidate to heart transplantation. Finally, improving the risk stratification process after MI would also allow a better selection of study populations to test new therapeutic strategies aiming at reducing LVR and HF after MI such as anti-apoptotic drugs, anti-proteolytic treatments, or cell therapy approaches. Finally, patients with extensive LVR could ultimately be candidate to heart transplantation.

Further studies are nonetheless needed to assess in more detail the potential of Ser207-phosphorylated TnT as a cardiovascular biomarker. In particular, its predictive value, either alone or in relation to total TnT, must be determined in large populations of patients with MI or HF and compared with currently established prognostic indicators. Although our data suggest that the association of Ser207-phosphorylated TnT with LVR may be greater than that of BNP, a quantitative ELISA test will be needed to confirm these findings; a comparison with other biomarkers such as C-reactive protein would also be of interest. For that purpose, we will need monoclonal antibodies specific for the Ser207-phosphorylated form of TnT. Future studies should not only analyse LVR but also major adverse cardiac events such as death or rehospitalization for HF. Finally, serial studies would also help to determine whether the decrease of Ser207-phosphorylated TnT is still observable during follow-up after MI.

In conclusion, Ser207/208 phosphorylation of TnT decreases during LVR after MI. We propose that the level of circulating phosphorylated TnT may serve as a new biomarker of outcome in post-MI or HF patients.

**Supplementary material**

Supplementary material is available at *European Heart Journal* online.

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**References**


Heart with a trunk: form fruste of Cantrell’s Syndrome

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A newborn presented with a prominent pulsatile supraumbilical tumour which was initially diagnosed as umbilical hernia by the caring paediatrician. On referral, clinical examination showed a subcutaneous pulse-synchronous pulsatile mass with diastasis of m. rectus abdominis (Panel A). Echocardiography demonstrated situs solitus with laevocardia and a secundum atrial septal defect. The apex of the left ventricle (LV) continued into a funnel-like diverticulum running caudally and ending supraumbilically in a pulsatile bulbous vesicle (Panel B). These findings were confirmed by cardiac magnetic resonance (CMR), where the anatomical course of the LV diverticulum was clearly delineated (Panel C), and a defect in the anterior midline abdominal wall, just covered by the skin, visualized. Echocardiography and CMR demonstrated that the diverticulum was entirely covered by myocardium, and therefore pulsatile. Surgical resection of the LV diverticulum (Panel D) was performed without the use of cardiopulmonary bypass through a partial inferior sternotomy, at the age of 3 weeks. Overlapping reconstruction of the abdominal wall without the use of any prosthetic material was performed. Postoperative course was uneventful. The patient was discharged in good clinical condition and with a normal cardiac function.

Cantrell’s pentalogy is a midline defect, including midline, suprapubic abdominal wall defects, a defect of the lower sternum, a deficiency of the anterior diaphragm, a defect of the diaphragmatic pericardium, and congenital heart disease. Clinical presentation can range from mild forms (fruste) similar to the case presented, to severe defects with omphalocele, ectopia cordis, and more severe congenital heart defects, such as tetralogy of Fallot.

Panel A: Clinical picture showing a diastasis of the m. rectus abdominis and a subcutaneous supraumbilical mass.
Panel B: Echocardiographic subcostal view showing the apex of the left ventricle continuing in a funnel-like diverticulum (**). Colour Doppler demonstrates blood flowing into the diverticulum.
Panel C: 3D Three-dimensional reconstruction of cardiac magnetic resonance CMR angiography delineates the entire course of the LV left ventricular diverticulum.
Panel D: Intra-operative view of the LV left ventricular diverticulum before surgical resection.