Diagnostic synergy of non-invasive cardiovascular magnetic resonance and invasive endomyocardial biopsy in troponin-positive patients without coronary artery disease

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Aims
Only few data are available regarding a direct comparison of both non-invasive CMR and invasive EMB with respect to conformity of procedure-derived diagnoses in the same patients. The aim of this study was to elucidate the diagnostic performance of non-invasive cardiovascular magnetic resonance imaging (CMR) and endomyocardial biopsy (EMB) in patients with troponin-I (TnI) positive acute chest pain in the absence of significant coronary artery disease (CAD).

Methods and results
One thousand one hundred and seventy-four consecutive patients who were admitted with TnI-positive acute chest pain between March 2004 and July 2007 underwent coronary angiography. In 1012 patients (86%), significant CAD (stenosis >50%) was detected as underlying reason for the acute chest pain. In 82 out of the remaining 162 patients without significant CAD, further workup was performed including both CMR and EMB. Cardiovascular magnetic resonance imaging alone enabled a diagnosis in 66/82 (80%) and EMB alone in 72/82 (88%) patients (P=0.31). Myocarditis was the most frequent diagnosis by both CMR and EMB in this cohort and was detected with a higher frequency by EMB (58 vs. 81%; P<0.001). With the combined approach comprising CMR and EMB, a final diagnosis could be established applying the ‘Believe-The-Positive-Rule’ in 78/82 patients (95%). This combined approach turned out to yield more diagnoses than either CMR (P<0.001) or EMB (P=0.03) as single techniques, respectively. Comparison of diagnostic CMR procedures with the corresponding diagnostic EMBs demonstrated a substantial match of diagnoses (kappa =0.70).

Conclusion
Cardiovascular magnetic resonance imaging and EMB have good diagnostic performances as single techniques in patients with TnI-positive acute chest pain in the absence of CAD. The combined application of CMR and EMB yields a considerable diagnostic synergy by overcoming some limitations of CMR and EMB as individually applied techniques.

Keywords
Acute coronary syndrome • Troponin • CMR • EMB • Myocarditis

Introduction
Measurement of troponin release has enabled further detailed classification and risk stratification in acute chest pain syndromes.1 Coronary artery disease (CAD) constitutes the number one cause for troponin elevations in the setting of acute chest pain. Hence, a diagnosis of an acute coronary syndrome (ACS) is usually made in such clinical situations. Non-obstructed coronary arteries are,
However, found on immediate cardiac catheterization in a sizeable subgroup of 10% and more patients with troponin elevation.\(^2\)–\(^4\) The absence of coronary lesions in this setting confronts the clinician with a broad variety of possible underlying aetiologies including myocarditis, different cardiomyopathies, aortic disease, embolic and vasospastic infarction, pulmonary embolism, arrhythmias, valvular heart disease, sepsis, and further rare conditions.\(^5\)\(^–\)\(^6\) Due to this heterogeneous spectrum of differential diagnoses, a commonly accepted diagnostic pathway to identify the underlying disease in those individuals is still missing. However, these patients represent a high-risk group even in the absence of CAD\(^7\) and a diagnosis is the basis for targeting therapy.

Non-invasive cardiovascular magnetic resonance imaging (CMR) and invasive endomyocardial biopsy (EMB) represent powerful tools for specific diagnoses in such patients.\(^7\)\(^–\)\(^9\) However, both approaches have particular weaknesses limiting their diagnostic performance: EMB constitutes an invasive procedure with a low but definite risk of complications and is prone to sampling error. Conversely, CMR is limited by its resolution capacity and can only be performed in the absence of certain contraindications.\(^10\) Only few data are available regarding a direct comparison of both procedures with respect to conformity of procedure-derived diagnoses in the same patients or the additional value of a combined application for diagnostic workup of patients with troponin-I (TnI) positive chest pain in the absence of CAD.\(^8\)\(^,\)\(^12\)

Therefore, we sought to evaluate the diagnostic performance of CMR and EMB for the first time in a large TnI-positive patient cohort without significant CAD, comparing both techniques with respect to their diagnostic yield. Furthermore, we compared the diagnostic performance of each technique with a combined sequential approach. We hypothesized that a combined approach of CMR and EMB would yield incremental diagnostic synergy by overcoming some of the inherent limitations of CMR and EMB as individually applied techniques.

### Methods

**Patient population**

This retrospective study was based on a routine clinical setting in 1174 patients who presented to our emergency department between March 2004 and July 2007 with TnI-positive chest pain and who underwent cardiac catheterization with coronary angiography as required for the management of ACS\(^13\)\(^,\)\(^14\) in the course of their hospital stay. Those patients presenting with symptoms of, e.g. fulminant pulmonary embolism (with right heart failure) or acute aortic dissection and in whom immediate diagnosis was made by other methods than cardiac catheterization (e.g. computed tomography or ventilation–perfusion scintigraphy) followed by appropriate therapy (e.g. anticoagulation, surgery) without the need for cardiac catheterization were not included to this analysis. Within this study cohort of 1174 patients, only those who did not demonstrate significant CAD were available for further diagnostic workup based on CMR and EMB. Figure 1 gives an overview of the whole study cohort including a flow-chart of patient management. In 1012 patients (86%), significant CAD was detected as the underlying reason for the acute chest pain, while the remaining 162 patients (14%) did not demonstrate significant CAD, constituting the core study group. Out of this group, 116 patients (72%) were investigated by means of CMR only (\(n = 27\)), EMB only (\(n = 7\)), or had both procedures (\(n = 82\)). In the remaining 46 patients (28%) of the non-CAD-group contraindications, lack of availability of the procedures or lack of patient consent for CMR and/or EMB prevented further intensive diagnostic workup, resulting in conservative management. All 82 individuals included in this study gave individual written informed consent for both diagnostic procedures, CMR and EMB, respectively.

### Cardiovascular magnetic resonance imaging—protocol and data analysis

Cardiovascular magnetic resonance imaging was performed in the great majority of all patients (73/82) prior to the second cardiac catheterization which was done to obtain EMBs. Time to CMR after hospital admission was 2 days for the median (range 1–4 days) and time to EMB after hospital admission was 4 days for the median (range 2–6 days). ECG-gated CMR imaging was performed in breath-hold with the use of a 1.5-T Magnetom Sonata (Siemens Medical Solutions, Erlangen, Germany). Both cine and late-gadolinium enhancement (LGE) short-axis CMR-images were prescribed every 10 mm (slice thickness 6 mm) from base to apex. In-plane resolution was typically 1.2 × 1.8 mm. Cine CMR was performed with the use of a steady state free precession sequence. LGE images were acquired on average 5–10 min after contrast administration with the use of a segmented inversion recovery gradient echo technique\(^15\) with constant adjustment of inversion time to null normal myocardium. The contrast dose [Omniscan (gadodiamide), Amersham Health Deutschland, Braunschweig, Germany] was 0.1 mmol/kg. The RV myocardium was completely covered by the appropriate short-axis stack.

The diagnosis of LGE required visual identification of elevated signal within the myocardium in two orthogonal views. In addition, a consensus regarding the presence or absence of LGE had to be reached between two experienced observers. If no consensus was reached, a third observer’s decision was binding. On the short-axis contrast images, patterns of LGE were classified as subendocardial, midwall, subepicardial, and transmural or any combination of those and the number of left-ventricular segments with LGE was quantified using a standard left ventricular 17-segment model.\(^16\)\(^–\)\(^18\) Classification of myocardial segments with respect to the presence of myocardial damage was made dichotomously based on visual identification of LGE. In addition, the extent of LGE was planimetered on the short-axis contrast images with the use of an image intensity level ≥2 SD above the mean of remote myocardium to define LGE. Regional parameters were assessed by dividing each short axis into 12 circumferential segments. For each segment, the extent of LGE was measured with the use of the NIH Image Analysis software package (National Institutes of Health, Bethesda, MD) as described previously.\(^9\)\(^,\)\(^19\) All CMR scans had sufficient quality, being therefore eligible for analysis. There was good interobserver agreement when analysing left-ventricular function (kappa 0.91; \(P < 0.001\)) and segmental myocardial damage based on LGE imaging (kappa 0.81; \(P < 0.001\)).

### Cardiac catheterization and endomyocardial biopsy

Significant CAD was defined as coronary artery stenosis >50% in diameter in at least one coronary artery segment or documented CAD in the past (stenosis >50% during prior coronary angiography >4 weeks ago). For EMB sampling a Cordis\(^\text{TM}\) or Meyer\(^\text{TM}\) bioptome (Meyer Medical Systems) was used. Endomyocardial biopsies were taken under fluoroscopic control from the right (septum) and/or left
ventricle (free wall) with five to six samples being collected from each ventricle in order to reduce sampling error.

Endomyocardial biopsies were preferentially taken from the ventricle demonstrating LGE: Those patients demonstrating LGE exclusively in the LV free lateral wall underwent selective LV biopsies, while those patients demonstrating LGE in the septal wall or having no LGE at all underwent either biventricular or selective RV biopsies, if possible. Thirty-three of eighty-two patients had biopsies from both ventricles, another thirty-three of eighty-two patients had selective LV biopsies, and sixteen of eighty-two patients underwent selective RV biopsies. No complications related to EMB occurred in the eighty-two patients.

Histopathological analysis
Endomyocardial biopsies were stained with Masson’s trichrome as well as Giemsa and examined by light microscopy. For immunohistology, tissue sections were treated with an avidin–biotin immuno-peroxidase method (Vectastain-Elite ABC Kit, Vector, Burlingame, CA), with application of the following monoclonal antibodies: CD3 (T cells; Novocastra Laboratories, Newcastle, UK), CD68 (macrophages, natural killer cells; DAKO, Hamburg, Germany), and HLA-DR-α (DAKO, Hamburg, Germany). Cardiac amyloidosis was investigated histologically including congo-red staining.

Detection of viral genomes
DNA and RNA were extracted with the use of proteinase-K digestion followed by extraction with phenol/chloroform. Nested polymerase chain reaction/reverse transcriptase–polymerase chain reaction was performed for the detection of enteroviruses (including coxsackieviruses of group B, various coxsackieviruses of group A and echoviruses), parvovirus B19 (PVB19), adenoviruses, human cytomegalovirus, Epstein-Barr virus, and human herpes virus type 6 (HHV6). As a control for successful extraction of DNA and RNA, oligonucleotide sequences were chosen from the glyceraldehyde-3-phosphate-dehydrogenase gene. Specificity of all viral amplification products was confirmed by automatic DNA sequencing.

Diagnostic categories by cardiovascular magnetic resonance imaging and endomyocardial biopsy
All CMR- and EMB-based diagnostic categories were based on well-established and widely accepted definitions.

Cardiovascular magnetic resonance imaging
Diagnoses were based on assessment of left-ventricular size, regional and global wall motion, thickness of the interventricular septum, and the presence and pattern of LGE. Additionally, the presence or absence of pericardial effusion was assessed visually in cine- and contrast images analysing short- and long-axis views. Therefore, the potential spectrum of CMR-based diagnoses included myocarditis (comprising also perimyocarditis), myocardial infarction, HCM, DCM, TTCM, cardiac sarcoidosis, and amyloidosis.

Endomyocardial biopsy
Biopsy results were used for diagnosis and subsequent patient classification according to the WHO/ISFC Task Force Report. Myocardial inflammation indicative of myocarditis was defined as ≥14 infiltrating leukocytes/mm² (CD3⁺ T-lymphocytes and/or CD68⁺ macrophages) with further differentiation between active myocarditis requiring additional myocyte damage and borderline myocarditis (without myocyte damage), respectively. In addition, expression of HLA class II molecules in professional antigen-presenting immune cells and endothelium was visually assessed after immuno-peroxidase staining. Detection of virus genome in the absence of immunohistological myocardial inflammation was classified as presence of virus genomes which for the purpose of this analysis was counted as diagnosis, since previous studies showed a relationship to patient symptoms and outcomes. Trichrome staining furthermore allowed diagnoses of HCM, myocardial infarction, and amyloidosis, respectively, and was supplemented by congo-red-staining and electron microscopy where appropriate. TTCM was a ‘CMR-only-diagnosis’, as to date there is no consensus on pathognomonic histologic findings.

Scoring considerations
All patients were analysed scoring CMR and EMB as individual techniques, as well as using a combined approach comprising both techniques. This combined approach was defined as being diagnostic, if a diagnosis could be established with at least one procedure, following the ‘Believe-The-Positive-Rule’. If neither EMB nor CMR could establish a diagnosis, or if the two procedures did not yield the same diagnosis, the combined approach was classified as ‘non-diagnostic’.

Statistical analysis
Data for continuous variables are expressed as mean and standard deviation for normally distributed values and median and interquartile range
(IQR) for non-normally distributed values. Categorical variables are expressed as the number and percentage, respectively. Comparisons between groups were done by use of Mann–Whitney U-test for non-normally distributed continuous variables and Student’s t-test for normally distributed variables. χ2 test and Fisher’s exact test were used for comparison of categorical variables. To assess interprocedural conformity of diagnoses kappa statistics was calculated. Comparison of diagnostic methods was performed using McNemar’s Test for correlated proportions. Propensity score analysis was carried out to determine the probability of a patient’s being assigned to the combined approach by using a multivariable logistic regression model. To build the propensity score, the following four clinically based criteria were used as independent variables: (i) age; (ii) left-ventricular ejection fraction (LVEF); (iii) presence of renal insufficiency; and (iv) the severity of comorbidities. The combined approach was preferentially performed in patients < 80 years, in those with an LVEF < 60%, in those without the presence of renal insufficiency (defined as glomerular filtration rate > 60 mL/min), and in those without severe comorbidities. The severity of comorbidities was calculated using the Charlson Comorbidity Score (categorised dichotomously into <3 and 3+). A two-tailed P-value < 0.05 was considered statistically significant.

Results

Patients and clinical presentation

There were slightly more males than females in the study group (61 vs. 39%). Study patients were significantly younger than the CAD patients (median 44 years (range 35–66 years) vs. 68 years (range 59–75 years); P < 0.001). Further patient characteristics including clinical and serological parameters are displayed in Table 1. The study population was divided into quintiles according to the propensity score. Those 82 patients who underwent the combined approach demonstrated a significantly higher number of patients in higher quintiles (median propensity score 0.50) compared with the remaining 80 patients who did not undergo the combined approach (median propensity score 0.19; P < 0.001).

General findings by cardiovascular magnetic resonance imaging, endomyocardial biopsy, and the ‘combined approach’

The findings for the use of CMR and EMB as ‘stand-alone’ techniques are displayed in Figure 2. Myocarditis was the overall most frequent diagnosis by both CMR and EMB. However, the total spectrum of diagnoses comprised myocardial infarction, HCM, DCM, cardiac amyloidosis, and TTCM. Figure 3 displays some examples of ‘non-myocarditis-CMR-diagnoses’. The number of correct diagnoses with EMB (72/82; 88%) was similar to the number with CMR (66/82; 80%; P = 0.31). The combined approach of CMR and EMB yielded a diagnosis in 78/82 patients (95%), which was superior to both CMR (P < 0.001) and EMB (P = 0.03) as individually applied techniques (Figure 4).

Conformity of method-based diagnoses in the same patients

Forty-eight of the 82 patients had conclusive diagnoses both by EMB and CMR. The other 34 patients having a diagnosis only in a single procedure (CMR or EMB; n = 33) including the diagnosis of TTCM, which is a CMR-only diagnosis or having no conclusive diagnosis in either procedure (n = 1), were excluded from kappa-statistics. Forty-five of the 48 patients (94%) showed a match of diagnoses between CMR and EMB and only 3/48 (6%) demonstrated a mismatch. Comparison of diagnostic CMR- and EMB-procedures yielded a substantial interprocedural agreement (kappa = 0.70).

Of the 42 patients with a CMR-diagnosis of myocarditis, 41 patients (98%) had also a diagnosis out of the spectrum of myocarditis by EMB. Hypertrophic cardiomyopathy and myocardial infarction were diagnosed in agreement in 1/1 and 3/4 patients, respectively. Mismatch of CMR- and EMB-diagnoses occurred in three patients (Table 2).

Diagnosing myocarditis by endomyocardial biopsy

The diagnosis of myocarditis including virus genome presence was more often made by EMB than by CMR (81 vs. 58%; P = 0.004). On EMB, active myocarditis was diagnosed in 26% (n = 17),

Table 1 Combined approach patients’ characteristics

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Value (n = 82)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years; median; IQR)</td>
<td>44; 35–66</td>
</tr>
<tr>
<td>Gender,* n (%)</td>
<td>Male/female 50/32 (61/39)</td>
</tr>
<tr>
<td>Risk factors,* n (%)</td>
<td>Family history of MI 9 (11)</td>
</tr>
<tr>
<td></td>
<td>Diabetes 8 (10)</td>
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<tr>
<td></td>
<td>Hypertension 19 (23)</td>
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<tr>
<td></td>
<td>Current smoking 17 (21)</td>
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<tr>
<td></td>
<td>Hyperlipidaemia 12 (15)</td>
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<tr>
<td>Resting ECG findings,* n (%)</td>
<td>Pathological 77 (94)</td>
</tr>
<tr>
<td></td>
<td>Normal 5 (6)</td>
</tr>
<tr>
<td></td>
<td>ST-segment changes 72 (88)</td>
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<tr>
<td></td>
<td>Bundle branch block 5 (6)</td>
</tr>
<tr>
<td></td>
<td>Sinus rhythm 76 (93)</td>
</tr>
<tr>
<td></td>
<td>Atrial fibrillation 6 (7)</td>
</tr>
<tr>
<td>Maximum creatine kinase (in U/L; median; IQR)</td>
<td>190; 97–486</td>
</tr>
<tr>
<td>Maximum creatine kinase MB (in U/L; median; IQR)</td>
<td>25; 15–49</td>
</tr>
<tr>
<td>Maximum troponin-I (in µg/dL; median; IQR)</td>
<td>5.3; 1.5–20</td>
</tr>
<tr>
<td>Maximum C-reactive protein (in mg/dL; median; IQR)</td>
<td>3.4; 0.9–6.9</td>
</tr>
<tr>
<td>Creatinine on admission (in mg/dL; median; IQR)</td>
<td>0.9; 0.8–1</td>
</tr>
<tr>
<td>LV-parameters (median; IQR)</td>
<td>EDV (mL) 139; 120–181</td>
</tr>
<tr>
<td></td>
<td>EF (%) 53; 42–58</td>
</tr>
<tr>
<td>LGE, n (%)</td>
<td>Positive 53 (65)</td>
</tr>
<tr>
<td>Pericardial effusion,* n (%)</td>
<td>Positive 54 (66)</td>
</tr>
</tbody>
</table>

IQR, interquartile range; LV, left ventricular; EF, ejection fraction; LGE, late-gadolinium enhancement.

*Indicates that variable is categorical.
borderline myocarditis in 61% \((n=40)\), and virus genome presence in 13% \((n=9)\). Nested-PCR identified virus genome in 62% \((n=41)\) of the 66 patients with an EMB-based diagnosis of myocarditis. The majority of patients having either active myocarditis \((59%; n=10)\) or borderline myocarditis \((55%; n=22)\) demonstrated also virus genome presence by PCR-analysis. PVB19 was the most commonly found virus with 36% \((n=24)\) in those patients with EMB-based myocarditis. The second most common finding was a co-infection of PVB19 and HHV6 \((14%, n=9)\). Further viruses detected \((12%; n=8)\) included HHV7, Epstein-Barr, and coxsackie B3 with the latter found in a single patient only.

### Diagnosing myocarditis by cardiovascular magnetic resonance imaging

In 48 patients \((58\%)\) with CMR-based myocarditis, LGE was localized subepicardially \((73%; n=35)\) and intramurally \((27%; n=13)\), respectively. Pre-dilection site of LGE was the basal and mid-ventricular posterolateral free wall and the interventricular septum as previously described.8,9 Concomitant pericardial effusion was found in 77% \((n=37)\) of patients with myocarditis on CMR.

Detailed quantification of LGE revealed a significantly increased number of segments with detection of LGE in those patients with EMB-based active myocarditis \((\text{median } \text{LGE-positive segments } 3.0; \text{IQR } 2–4)\) compared with those with EMB-based borderline myocarditis or virus genome presence \((\text{median } \text{LGE-positive segments } 2.0; \text{IQR } 0–3; P = 0.022)\). When comparing LGE-analysis in patients with EMB-based active vs. borderline myocarditis alone, the difference in the extent of LGE still remained statistically significant \((P = 0.046)\).

### Detailed analysis of diagnostic vs. non-diagnostic procedures

To investigate parameters potentially accounting for the ability to make a diagnosis of CMR and EMB, respective patient data were opposed for detailed analysis. Characteristics of diagnostic vs. non-diagnostic CMR-examinations are displayed in Table 3.

Endomyocardial biopsy-based active myocarditis had a higher incidence in the diagnostic CMR-group \((28\% \text{ vs. } 6\%)\), whereas borderline myocarditis was more frequently detected in EMBs in the non-diagnostic CMR-group \((63\% \text{ vs. } 43\%)\) without reaching statistical significance. Compared with the non-diagnostic CMR-group, patients with a diagnostic CMR showed significantly higher maximum release of serum creatine kinase (CK) and creatine kinase-MB (CK-MB). Individuals with diagnostic CMR examinations had significantly larger ventricles than those with non-diagnostic examinations. Time interval from onset of symptoms to the date of the CMR-scan, LVEF, and maximum of C-reactive protein in the serum did not differ significantly between the groups.

Comparison of diagnostic vs. non-diagnostic EMB-groups did not show relevant differences of time intervals from symptom-onset to the date of the EMB, time intervals between CMR- and EMB-procedures. However, the limited number of patients with non-diagnostic EMB-results precluded a detailed meaningful statistical analysis. Selective RV-EMBs were diagnostic in 13/16, selective LV-EMBs in 27/33, and biventricular EMBs in 32/33. Thus, there was no significant difference in the diagnostic yield between these groups \((P > 0.05)\).
The present study was performed on a large TnI-positive patient group who had CAD ruled out by early coronary angiography and demonstrates that both CMR and EMB are highly valuable diagnostic tools to establish underlying diagnoses. While myocarditis was diagnosed more often by EMB, including more subtle patterns, LGE-CMR had an excellent performance in the setting of histologically more distinct forms of myocarditis. When CMR and EMB were applied individually, diagnostic failure occurred in a non-negligible patient-fraction, mainly due to method-inherent

**Discussion**

**General observations**

The present study was performed on a large TnI-positive patient group who had CAD ruled out by early coronary angiography and demonstrates that both CMR and EMB are highly valuable
of limitations. Application of a combined approach, comprising both CMR and EMB yielded a substantial diagnostic synergy by overcoming some of the limitations of the individual techniques.

### Diagnostic performance of endomyocardial biopsy and cardiovascular magnetic resonance imaging as individually applied techniques

This study is the first to provide data on both non-invasive and invasive tissue characterization in a large population of TnI-positive patients with acute chest pain but non-obstructed coronaries. Using either a non-invasive (CMR) or an invasive approach (EMB) yielded a good diagnostic performance (80 vs. 88%), comprehensive for various non-CAD aetiologies known to be associated with the release of troponin.\(^7\)\(^8\)

The advantage of EMB compared with CMR observed in this study can be attributed to the powerful diagnostic capabilities of EMB based on immunohistochemistry and nested-PCR enabling further differential classification within the diagnostic groups. The leading diagnosis ‘myocarditis’ was established in a significantly higher percentage of patients by use of EMB compared with CMR alone. This is mainly explained by the presence of different expressions of myocarditis in our collective, including active myocarditis, but also more subtle forms such as borderline myocarditis and finally, intramyocardial virus genome presence without myocardial inflammation. Since several previous studies have shown a clinical and prognostic value of a mere virus genome presence in the absence of myocardial inflammation,\(^29\)\(^30\)\(^35\)\(^36\) we included virus genome presence to the EMB-based diagnoses of myocarditis.

The more subtle forms of EMB abnormalities such as borderline myocarditis and virus genome presence were often missed by...
LGE-CMR-imaging alone, possibly due to the limited spatial resolution of CMR. Other research groups are propagating contrast-enhanced T1-weighted and/or T2-weighted imaging modalities for detection of tissue hyperaemia and oedema in the early phases of inflammatory or ischaemic myocardial damages.\textsuperscript{37–39} However, these imaging modalities have been prone to inconsistencies and inaccuracies regarding image quality and reproducibility. Therefore, we did not routinely apply these techniques in this patient cohort. Newly developed CMR-sequences seem to overcome some of these limitations\textsuperscript{40,41} and application of these imaging modalities in patients with TnI-positive chest pain in the absence of CAD in relation to EMB and LGE-imaging results is highly desired.

The diagnosis of myocarditis was more frequently made by CMR in patients with active myocarditis compared with those with borderline myocarditis, since patients with active myocarditis showed more segments with the finding of LGE in accordance with higher serum values of cardiac enzymes as markers of the severity of myocardial damage compared with subjects with borderline myocarditis or virus genome presence. Significantly higher maximum peaks of CK and CK-MB, respectively, were observed in the diagnostic CMR-group (LGE-positive) compared with the non-diagnostic CMR-group (LGE-negative) (Table 3). This indicates that a higher extent of myocardial damage was related with a greater diagnostic capability of LGE-CMR in our study.

Therefore, we hypothesize that (i) EMB is superior to LGE-CMR in diagnosing myocarditis because of its ability to capture minor forms of myocarditis and (ii) that the value of LGE in the CMR-based diagnosis of myocarditis is related to the histological degree and extent of inflammation as detected on EMB. This is exemplarily demonstrated in Figure 5.

**Figure 5** Exemplary comparison of ‘active’ vs. ‘borderline myocarditis’. The upper and lower rows display histologic and cardiovascular magnetic resonance findings in active myocarditis and borderline myocarditis, respectively (lymphocytes, necrosis, and late-gadolinium enhancement are indicated by arrowheads). In the patient with borderline myocarditis cardiovascular magnetic resonance was not able to diagnose myocarditis due to low extent of inflammation.

**Diagnostic performance of cardiovascular magnetic resonance imaging and endomyocardial biopsy as a sequential or combined approach**

A sizeable minority of patients could, however, not be diagnosed using either CMR or EMB alone (20 vs. 12%). The combined approach as serial investigation comprising both CMR and EMB improved the diagnostic performance reducing the number of patients without a final diagnosis to just 5%. In the 48 patients in whom both techniques were able to establish a diagnosis, this diagnosis was identical in 45 (94%) between CMR and EMB (interprocedural match). Our data indicate that it is reasonable to initially perform CMR in all patients. If CMR is able to establish a diagnosis, EMB is unlikely to change this diagnosis. However, if a diagnosis cannot be established by CMR, but is clinically needed for instance in a patient with persistent symptoms, EMB should be employed as a second step. There is, however, one important caveat to this approach: The use of CMR only to establish the diagnosis of myocarditis will result in less detailed information about the degree of inflammation, the presence of special forms of myocarditis (such as giant cell or eosinophilic myocarditis which require specific therapies), or the presence and type of virus.

However, our data also show that performing both procedures yields the highest number of final diagnoses. Establishing the maximum number of diagnoses in this patient group may be beneficial, if a prognostic impact of the underlying diagnosis can be shown. Recent data by Kindermann et al.\textsuperscript{42} indicate that the presence of inflammation in EMBs has prognostic implications. Data from our group (Mahrholdt et al.\textsuperscript{9}) indicate that the presence and distribution of LGE may also have prognostic implications.
Limitations and failure of cardiovascular magnetic resonance imaging

To date CMR is the most accurate method for non-invasive tissue characterization. However, image quality can be limited by trigger problems (e.g. rhythm disturbances) and other artefacts (e.g. breath-holding- and motion-artefacts). This may result in inaccuracies when judging global and regional function or the presence of LGE. The late-enhancement technique (segmented IR-GRE) provides a resolution of only 1.2 \times 1.8 \text{ mm} in plane while histopathology is able to characterize cellular and intracellular structures at a resolution of a few micrometres.

Ricciardi et al.\textsuperscript{13} investigated the role of LGE-CMR in detecting ‘microinfarction’. They found that myonecrosis of only 2 g [corresponding CK levels: median 229; range 146–709 (U/L)] could be detected by LGE-CMR. In our non-diagnostic CMR group, however, CK levels were even lower [median 108; IQR range 75–203 (U/L)]. We therefore conclude that subtle tissue pathology, as seen in many clinical cases of myocarditis, can be missed by LGE-CMR.

Our results expand the sparse database regarding the correlation of CMR and EMB findings. A sensitivity of 90\% with an even higher specificity of LGE was reported in patients with active myocarditis by EMB.\textsuperscript{8} DeCobelli et al.\textsuperscript{44} found LGE in 84\% of cases with biopsy-proven active myocarditis, but only in 44\% of cases with borderline myocarditis. In a group of patients with less pronounced inflammation, Gutterlet et al.\textsuperscript{12} reported a low sensitivity (27\%) at a preserved high specificity of 80\% for LGE. Our recent findings are nicely in line with the aforementioned data underlining the strengths of CMR in active inflammation.

Limitations and failure of endomyocardial biopsy

The major limitation of EMB remains the so-called ‘sampling error’. However, we believe that our EMB technique minimized the frequency of sampling errors. The degree of sampling error depends (i) on the number of biopsies taken per patient and (ii) on the methods applied for ex vivo analysis.

Critics of EMB often refer to Hauck et al.\textsuperscript{10} who demonstrated in post-mortem tissue of patients with histologically proven lymphocytic myocarditis that with the examination of only five biopsies, the histological diagnosis of myocarditis/ borderline myocarditis was false negative in 55\% of the cases, whereas more biopsies reduced this sampling error significantly. As we took five to six samples from each ventricle, false negative results can consequently be expected to be lower. Furthermore, previous studies including the study by Hauck et al.\textsuperscript{10} evaluating the sensitivity of EMB in diagnosing myocarditis were only based on histological analyses according to the Dallas criteria. However, as recently discussed by Baughman,\textsuperscript{28} the Dallas criteria may no longer be adequate for workup of biopsies in patients with suspected myocarditis. Immunohistological and molecular-pathological methods—as applied in our study—were shown to be more sensitive for the diagnosis of myocardial inflammation even in the absence of focal lymphocytic infiltration and have been successfully used to identify patients responding to immunomodulatory therapy independent of Dallas criteria.\textsuperscript{29,30}

Endomyocardial biopsy could not establish a diagnosis in 10 patients. Their CMR-results are displayed in Table 2. Failure of EMB to diagnose myocarditis in five subjects who showed myocarditis on CMR may still represent EMB-sampling errors, as the applied EMB methods were the same and consistent in the EMB-non-diagnostic and the EMB-diagnostic patients. Three patients were found to have TTCM on CMR. However, TTCM is mainly a clinical diagnosis\textsuperscript{45} with various histological findings that do not serve as diagnostic criteria for the disease.

Endomyocardial biopsy was confined to the RV in 16 and to the LV in 33 patients which is standard practice in most experienced centres.\textsuperscript{46} Although the restriction to one ventricle in these patients may have resulted in a lower number of EMB diagnoses when compared with biventricular biopsies, the high yield of 13/16 positive RV biopsies and 27/33 positive LV biopsies argues against this concern. Moreover, there was no statistical difference in the proportion of diagnostic EMBs between uni- vs. biventricular biopsies. Considering the multifocal and patchy pattern of myocarditis, one would expect the proportion of diagnostic EMBs to be higher in biventricular compared with univentricular biopsies. The reason for similar proportions of diagnostic EMBs between uni- vs. biventricular biopsies in this study may be due to our EMB approach preferentially taking EMBs in a standardized procedure from the ventricle demonstrating LGE. However, since the study size was limited, this observation has to be carefully interpreted and needs to be proven in future studies of larger size.

Failure of the combined approach

Table 2 gives an overview of the ‘combined-approach-failures’ comprising one missed diagnosis and three mismatches. The first patient of the three mismatches had EMB-proven amyloidosis but showed myocarditis on CMR. In this patient, amyloidosis was histologically not distributed in a typical generalized pattern but showed a multifocal deposition. The second mismatch patient had the CMR-diagnosis of DCM but showed borderline myocarditis on respective EMB. The minor expression of inflammation most likely caused CMR to miss the diagnosis as a consequence of limited spatial resolution. The third mismatch patient had myocardial infarction on CMR, but myocarditis on corresponding EMB.

This last finding is somewhat challenging because a subendocardial LGE-pattern, although reported in single cases of non-ischaemic inflammatory heart disease,\textsuperscript{47,48} is still widely believed to be exclusively caused by CAD. However, a subendocardial LGE in the absence of CAD, may be caused by coronary vasospasm.\textsuperscript{49} Coronary vasospasm may occur with or without associated intramyocardial inflammation.\textsuperscript{49} In all four patients with myocardial infarction on CMR in this study, coronary vasospasm was reproduced by acetylcholine testing (defined as 75\% lumen-reduction in the vessels supplying the corresponding infarct area as demonstrated by CMR with reproduction of the symptoms (leading to hospital admission) suggesting vasospastic infarction. Therefore, the EMB-diagnosis of myocardial inflammation in a patient with a vasospastic infarct may not represent a mismatch but an explanation for the sudden occurrence of coronary vasospasm.\textsuperscript{49}
Limitations
This study has the following limitations: first, this was not a prospective study and patients were not randomly assigned to receive either CMR, EMB, or both procedures but rather underwent these procedures based on clinical decision making and diagnostic availability. Second, CMR and EMB are of course not able to demonstrate all causes of TnI-positive chest pain syndromes. For instance, coronary vasospasm and pericardial inflammation did contribute to some extent to the clinical picture of our patients. Third, there was no gold-standard to which the results of CMR and EMB could be compared. Therefore, positive or negative predictive values or sensitivities and specificities could not be calculated.

Furthermore, our CMR approach to myocarditis was solely based on LGE-imaging. Unfortunately, hyperaemia and oedema sequences did not work on our scanner when the majority of this patient group was examined. Such sequences would of course be of interest in this setting. Moreover, the likelihood to detect the underlying pathogen (in particular in the case of viral myocarditis) is obviously higher, if EMB is performed immediately after onset of symptoms. Since the time to EMB after hospital admission was 4 days for the median in our study group, some non-diagnostic EMB results could be due to delayed biopsy.

Finally, the use of combined CMR- and EMB-procedures in this patient cohort helped us to guide further clinical management in some patients and to collect valuable data for future risk stratification: one patient with HCM could be correctly identified and risk-stratified with respect to prophylactic ICD implantation. Another patient with atypical cardiac amyloidosis was identified by our combined work-up. Work-up for coronary vasospasm (acetylcysteine testing) and anti-spastic treatment (long acting nitrates and calcium antagonists) could be initiated in those patients with unclear myocardial infarction in the absence of CAD. However, none of the patients who received a diagnosis of myocarditis had any specific therapy such as immunosuppression or antiviral therapy at this stage, but most of them are/were clinically monitored at regular intervals and data for prospective risk-stratification is/was collected. At our institution, immunosuppressive or antiviral therapy offered only to those patients who suffer from worsened chest pain in the absence of CAD and EMB could be compared. Therefore, positive or negative predictive values or sensitivities and specificities could not be calculated.

Conclusions
Late-gadolinium enhancement—cardiovascular magnetic resonance imaging showed an excellent diagnostic performance in histologically active forms of myocarditis, whereas EMB had its strengths in capturing histologically more subtle forms with lesser release of markers of cardiac injury. The combined application of CMR and EMB according to our protocol in patients with TnI-positive chest pain in the absence of CAD yielded a considerable diagnostic synergy. The combined approach was superior to each single technique and could overcome some of the well-known limitations of CMR and EMB as individually applied techniques.

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References
Diagnostic synergy of CMR and EMB


