Cardiac MRI in Suspected Acute Myocarditis After COVID-19 mRNA Vaccination

Befunde der kardialem MRT bei Patienten mit Verdacht auf eine akute Myokarditis nach COVID-19-mRNA-Impfung

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Key words
vaccine, myocarditis, cardiac MRI, COVID-19

ABSTRACT

Purpose To evaluate cardiac MRI characteristics in patients with suspected hypersensitivity myocarditis following mRNA COVID-19 vaccination.

Materials and Methods Patients clinically suspected of acute myocarditis after COVID-19 vaccination were retrospectively analyzed and compared against a healthy control group. Cardiac MRI protocol included parameters such as T1 and T2 relaxation times, extracellular volume (ECV), T2 signal intensity ratio, and late gadolinium enhancement (LGE). Lymph node size was assessed in the patient group on the injection side. Student t-test, analyses of variance (ANOVA) with Tukey post-hoc test, and χ² test were used for statistical analysis.

Results 20 patients with clinically suspected post-vaccine myocarditis (28 ± 12 years; 12 men) and 40 controls (31 ± 11 years; 25 men) were evaluated. According to the 2018 Lake Louise criteria (LLC), patients with clinically suspected myocarditis were further subdivided into an LLC-positive group (n = 9) and an LLC-negative group (n = 11). The mean time of symptom onset after vaccination was 1.1 ± 1.2 days (LLC-positive) and 6.5 ± 9.2 days (LLC-negative). Group differences in inflammatory variables between myocarditis patients and control subjects were more pronounced in the LLC-positive group (e.g., T1 relaxation time: 1041 ± 61 ms [LLC positive] vs. 1008 ± 79 ms [LLC-negative] vs. 970 ± 25 ms [control]; p < .001; or T2 signal intensity ratio 2.0 ± 0.3 vs. 1.6 ± 0.3 [LLC-negative] and vs. 1.6 ± 0.3 [control], p = .012). LLC-positive patients were significantly faster in receiving an MRI after initial symptom onset (8.8 ± 6.1 days vs. 52.7 ± 33.4 days; p = .001) and had higher troponin T levels (3938 ± 5850 ng/l vs. 9 ± 11 ng/l; p < .001). LGE lesions were predominantly located at the subepicardium of the lateral wall. Axillary lymphadenopathy was more frequent in the LLC-positive group compared to the LLC-negative group (8/9 [89 %] vs. 0/11 [0 %], p < 0.001).

Conclusion Vaccine-induced myocarditis should be considered in patients with acute symptom onset after mRNA vaccination, especially if elevated serum troponin T is observed. Imaging findings of vaccine-induced myocarditis are similar to virus-induced myocarditis, allowing for the use of the Lake Louise Criteria for diagnostic purposes.

Key Points:
- Vaccine-induced hypersensitivity myocarditis can be confirmed with cardiac MRI
- Especially patients with sudden onset of symptoms and elevated serum troponin T had positive cardiac MRI findings
- Cardiac MRI characteristics of vaccine-induced myocarditis are similar to those in virus-induced myocarditis

Citation Format
Introduction

Acute myocarditis after vaccination is a rare complication which has been previously described for the influenza and small-pox vaccines [1]. The incidence of myocarditis for these vaccines is estimated at around 0.24 per 100,000 compared to the diagnosis of myocarditis in a non-corona virus 19 disease (COVID-19) specifically starting in early 2021, there have been a few case reports and case series regarding acute myocarditis after mRNA-COVID-19-impfung and in principle refers to inflammation of the myocardium (HSM), a rare and possibly fatal type of myocarditis which is poorly understood and is associated with a recent start of pharmaceutical therapy [9, 10].

Materials and Methods

This retrospective study was approved by the institutional ethics committee and the requirement for written informed consent was waived. The study was performed in concordance with the Declaration of Helsinki and International Conference on Harmonization of Good Clinical Practice.

Study Patients

All patients referred for cardiac MRI to the Department of Diagnostic and Interventional Radiology at the University Hospital Bonn last summer with suspected acute myocarditis after receiving one of the approved mRNA COVID-19 vaccines in Germany (Pfizer/BioNTech or Moderna) were included for analysis. Reasons for referral for cardiac MRI were exertional dyspnea, chest pain, or
palpitations. All participants must have had received at least one dose of a COVID-19 vaccine approved for use in Germany.

Patients were referred by local medical offices and university centers. Healthy volunteers, and outpatients who were referred for chest pain to rule out structural heart disease were retrospectively identified and age matched as the control group (n = 40). All control patients had an unremarkable history of cardiovascular disease, did not have any known cardiovascular risk factors, and demonstrated unremarkable cardiac MRI results. All patients with suspected myocarditis tested negative for COVID-19 before cardiac MRI using a PCR-test. Clinical patient information was retrieved through the internal hospital information system.

Cardiac MRI protocol

Each multiparametric cardiac MRI examination was performed with the same clinical whole-body MRI system (Ingenia 1.5T; Philips Healthcare, Best, The Netherlands). Signal reception was achieved using a 32-channel torso coil with a digital interface. A signal intensity correction algorithm (CLEAR: Constant Level Appearance; Philips Medical Systems) was used to correct for torso-coil related signal inhomogeneities. For functional analysis, electrocardiogram-gated steady state free-precession cine images were obtained in the short-axis, two-chamber, three-chamber, and four-chamber views. A transversal respiratory-gated fat-suppressed T2-weighted fast spin echo sequence (Philips MultiVane XD, Philips Healthcare, Best, The Netherlands) was utilized for the assessment of lymphadenopathy and lung pathologies. T2-weighted short-tau inversion-recovery sequences in the short axis and transversal views were acquired for visualization of myocardial edema and calculation of T2 signal intensity ratio. Myocardial T1 and T2 mapping in end-diastolic short axis views with acquisition of apical, midventricular, and basal sections was performed for analysis. A six-echo gradient spin-echo sequence (GraSE) was applied for myocardial T2 mapping before intravenous contrast injection [12]. A standard 3(3)3(3)5 modified Look-Locker inversion recovery (MOLLI) acquisition scheme was used for myocardial T1 mapping [13]. Post-contrast T1 maps using the same acquisition scheme were obtained 10 minutes after contrast administration. Contrast enhancement was achieved using a 0.2 mmol/kg of body weight bolus of gadoterate meglumine (Clariscan; GE Healthcare, Chicago, IL). Segmented inversion-recovery gradient-echo sequences for LGE imaging were obtained in short axis, two-chamber, four-chamber, and transversal views. The Look-Locker method was utilized to determine the optimal inversion time for LGE image acquisition as previously described [14]. Prior to the cardiac MRI scan, blood samples were drawn for blood count and hematocrit assessment. A summary of the sequence parameters is provided in the supplement (Table A1).

Image Analysis

Image analysis was performed by a board-certified radiologist (J.A.L. with 9 years of experience in cardiac MRI) and a radiology resident (D.K. with 2 years of experience in cardiac MRI) using dedicated software (IntelliSpace Portal, version 10.6.32.82.; Philips Medical Systems). Readers were blinded to the clinical information. Papillary muscles were included in the blood pool for volumetric quantification of the left and right ventricles. The presence of focal areas of regional high signal intensities in a non-ischemic distribution pattern on T2 short-tau inversion-recovery and on LGE images was visually assessed by consensus agreement of the two readers. Quantitative markers of myocardial edema (T2 signal intensity ratio) and myocardial injury and fibrosis (enhanced volume percentage on short-axis LGE images using full width half maximum technique) were calculated as previously reported [15]. Motion correction was achieved using a software-implemented algorithm (fast elastic image registration, IntelliSpace Portal, version 10.1) for myocardial T1 and T2 relaxation maps, and global T1 and T2 relaxation times as well as hematocrit-corrected global ECV values were calculated, as previously described [15, 16]. For the assessment of the 2018 Lake Louise criteria, institution specific cutoffs (≥1000 ms for myocardial T1 relaxation times and ≥55.9 ms for myocardial T2 relaxation times) were used as previously described [17]. LGE distribution was classified according to the American Heart Association 17 segment heart model as previously described [18]. Additionally, LGE localization was classified according to the involvement of the subepicardial, midventricular, subendocardial, or transmural wall and if a patchy distribution was noted or not. Imaging protocol in this study was the same as used in our previous studies of patients with suspected acute myocarditis [16, 19]. Axial T2 weighted images were assessed for axillary lymph node enlargement of the vaccination injection side. For the control group, the largest lymph node of either side was used. The largest short axis diameter measured in millimeter was recorded, with any measurement over 10 mm considered enlarged.

Statistical Analysis

Prism (version 8.4.1; GraphPad Software, San Diego, Calif., USA) was used for statistical analysis. Data are given as means ± standard deviation or as percent to absolute frequency. Normal distribution was checked using the Shapiro-Wilk test. For comparison of continuous variables and inter-individual variables the Student t test was used. Mann-Whitney-U test was used for non-normal distributed data. Dichotomous variables were compared by using the χ2 test. One-way analysis of variance (ANOVA) followed by Tukey post hoc multiple comparison tests was performed to compare variables in the three participant groups. The level of statistical significance was set to P <.05.

Results

Patient characteristics

A total of 60 subjects were included in this retrospective study: 20 patients with clinically suspected myocarditis (mean age 28 ± 12 years, 12 men) and 40 healthy control individuals (mean age 31 ± 11 years, 25 men). Patients with suspected vaccine-induced myocarditis had a mean time of symptom onset of 4.1 ± 7.3 days after the last
Table 1 Clinical and cardiac MRI characteristics of patients with suspected myocarditis after COVID-19 vaccination and control subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>LLC-positive patients (n = 9)</th>
<th>LLC-negative patients (n = 11)</th>
<th>Control group (n = 40)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BioNTech*</td>
<td></td>
<td></td>
<td></td>
<td>.26</td>
</tr>
<tr>
<td>1st dose</td>
<td>2 (22%)</td>
<td>3 (27%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>2nd dose</td>
<td>6 (67%)</td>
<td>8 (73%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Moderna*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st dose</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>2nd dose</td>
<td>1 (11%)</td>
<td>0 (0%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>First vs. second dose*</td>
<td></td>
<td></td>
<td></td>
<td>.80</td>
</tr>
</tbody>
</table>

Clinical parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>LLC-positive patients (n = 9)</th>
<th>LLC-negative patients (n = 11)</th>
<th>Control group (n = 40)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24 ± 6</td>
<td>32 ± 15</td>
<td>30 ± 11</td>
<td>.06</td>
</tr>
<tr>
<td>Men*</td>
<td>7 (88)</td>
<td>5 (56)</td>
<td>25 (63)</td>
<td>.36</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70 ± 6</td>
<td>74 ± 14</td>
<td>75 ± 14</td>
<td>.30</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173 ± 7</td>
<td>171 ± 7</td>
<td>175 ± 10</td>
<td>.40</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.6 ± 2.8</td>
<td>25.1 ± 3.8</td>
<td>24.5 ± 3.6</td>
<td>.60</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.8 ± 0.1</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>.53</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>63 ± 8</td>
<td>74 ± 15</td>
<td>69 ± 14</td>
<td>.08</td>
</tr>
<tr>
<td>Time to symptom begin (days)</td>
<td>1.1 ± 1.2</td>
<td>6.5 ± 9.2</td>
<td>NA</td>
<td>.10</td>
</tr>
<tr>
<td>Time to MRI (days) ‡</td>
<td>8.8 ± 6.1</td>
<td>52.7 ± 33.4</td>
<td>NA</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Troponin T (highest) (&lt; 14 ng/l) †</td>
<td>3938 ± 5850</td>
<td>9 ± 11</td>
<td>NA</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>White blood cell count, (n/cells)</td>
<td>7.7 ± 1.2</td>
<td>7.0 ± 3.2</td>
<td>7.2 ± 2.0</td>
<td>.58</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>41 ± 3</td>
<td>41 ± 2</td>
<td>41 ± 4</td>
<td>.86</td>
</tr>
<tr>
<td>Lymph node swelling* ††</td>
<td>8 (89%)</td>
<td>0 (0%)</td>
<td>1 (3%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Largest lymph nodes (cm) ††</td>
<td>1.15 ± 0.25</td>
<td>0.61 ± 0.14</td>
<td>0.59 ± 0.24</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Cardiac MRI parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>LLC-positive patients (n = 9)</th>
<th>LLC-negative patients (n = 11)</th>
<th>Control group (n = 40)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDV (ml)</td>
<td>155 ± 20</td>
<td>143 ± 28</td>
<td>143 ± 34</td>
<td>.34</td>
</tr>
<tr>
<td>LVEDVi (ml/m²) †</td>
<td>84 ± 10</td>
<td>77 ± 12</td>
<td>75 ± 12</td>
<td>.05</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>57 ± 7</td>
<td>54 ± 14</td>
<td>61 ± 4</td>
<td>.34</td>
</tr>
<tr>
<td>Interventricular septal thickness (mm)</td>
<td>8.4 ± 1.9</td>
<td>8.1 ± 2.0</td>
<td>9.0 ± 1.9</td>
<td>.37</td>
</tr>
<tr>
<td>T2 signal intensity ratio††</td>
<td>2.0 ± 0.3</td>
<td>1.6 ± 0.3</td>
<td>1.6 ± 0.3</td>
<td>.012</td>
</tr>
<tr>
<td>Visible myocardial edema* ††</td>
<td>8 (89%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Visible late gadolinium enhancement* ††</td>
<td>9 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>T1 relaxation time, native (ms) †</td>
<td>1041 ± 61</td>
<td>1008 ± 79</td>
<td>970 ± 25</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>T2 relaxation time (ms) †</td>
<td>58 ± 5</td>
<td>54 ± 5</td>
<td>52 ± 2</td>
<td>.007</td>
</tr>
<tr>
<td>Extracellular volume fraction (%)</td>
<td>27 ± 4</td>
<td>24 ± 2</td>
<td>25 ± 3</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Numbers are given as mean ± standard deviation. Analysis of Variance (ANOVA) with Tukey post hoc tests were applied unless otherwise noted. NA, not applicable; LLC, Lake Louise Criteria; LVEDV, left ventricular end diastolic volume; LVEDVi, left ventricular end-diastolic volume index.

*Data are absolute frequencies with percentages in parentheses. † Mann-Whitney U test used as normality could not be assumed. ‡ χ² test.

‡ P = < .05 LLC positive vs control group
§ P = < .05 LLC positive vs LLC negative
† P = < .05 LLC positive vs control group

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COVID-19 vaccine. Fifteen participants (n = 15 out of 20, 75 %) received a second dose while five (n = 5 out of 20, 25 %) received their first dose. Altogether, 19 (n = 19 out of 20, 95 %) patients received the Pfizer/BioNTech vaccine while 1 person (n = 1 out of 20, 5 %) received the Moderna vaccine. Seventeen out of 20 patients (85 %) reported chest pain, 11 out of 20 (55 %) reported experiencing exertional dyspnea, 2 out of 20 (10 %) described hot flashes, and 1 out of 20 (5 %) complained of new onset of fatigue.

Group designation

The clinically suspected vaccine induced myocarditis group was further divided into a group which fulfilled the updated 2018 Lake Louise Criteria (LLC) for myocarditis (LLC positive group; n = 9; mean age 24 ± 6 years, men n = 7) and an LLC negative group which did not fulfill the criteria (LLC negative group; n = 11; mean age 32 ± 15 years, men n = 5). The average time to symptom onset was 1.1 ± 1.2 days for the LLC positive group and 6.5 ± 9.2 days for the LLC negative group. No significant difference was noticed between the groups regarding age (p = .06), sex (p = .36), weight (p = .30), height (p = .40), body mass index (p = .60), or body surface area (p = .53). LLC positive patients were significantly faster in receiving an MRI after initial symptom onset (mean 8.8 ± 6.1 days vs. 52.7 ± 33.4 days for the LLC negative group, p = .001) but also demonstrated much higher cardiac troponin T levels (cTnT; mean 3938 ± 5850 ng/l vs. 9 ± 11 ng/l, p < .001). A summary of patient characteristics is found in Table 1.

**Cardiac MRI findings**

A difference in T1 and T2 relaxation times was noted between the LLC positive group and the control group (T1: 1041 ± 61 vs. 970 ± 25 ms, p < .001; T2: 58 ± 5 vs. 52 ± 2 ms, p = .007) (see Fig. 1). LLC positive patients demonstrated an increased T2 signal intensity ratio (2.0 ± 0.3, p = .012) compared to the LLC negative group (1.6 ± 0.3) and the control group (1.6 ± 0.3), a higher rate of visual myocardial edema seen in 89 % of the patients (n = 8 out of 9) vs. 0 % (n = 0 out of 11) in the LLC negative and control group (n = 0 out of 40), as well as a higher rate of LGE (n = 9, 100 %) compared to 0 % in both the LLC negative (n = 0 out of 11) and control group (n = 0 out of 40). Focal non-ischemic LGE lesion distribution in the 9 LGE positive patients was as follows: basal inferior (n = 3, 33 %), basal inferolateral (n = 7, 78 %), basal anterolateral (n = 11, 11 %), mid anterior (n = 1, 11 %), mid anteroseptal (n = 1, 11 %), mid inferoseptal (n = 2, 22 %), mid inferolateral (n = 6, 67 %), mid anterolateral (n = 5, 56 %), apical anterior (n = 1, 11 %), apical septal (n = 1, 11 %), apical inferior (n = 2, 22 %), and apical lateral (n = 7, 78 %). LGE lesion distribution is summarized in Fig. 2. Subepicardial localization was the most commonly affected myocardial wall segment with involvement in 8 out of 9 patients (89 %), followed by the midwall (5 out of 9, 56 %), and subendocardial involvement (1 out of 9, 11 %). Only one patient demonstrated patchy LGE (11 %) and one (11 %) transmural involvement. A patient fulfilling the modified 2018 Lake Louise Criteria is demonstrated in Fig. 3, depicting positive LGE findings and prolonged T1 and T2 relaxation times. All controls had normal cardiac MRI results without structural abnormalities or signs of previous myocarditis. No group differences were noted for cardiac MRI parameters such as left ventricular ejection fraction (LVEF, p = .34), left ventricular end diastolic volume (LVEDV, p = .34), left ventricular end diastolic volume index (LVEDVI, p = .05), interventricular septal diameter (IVSD, p = .37), or extracellular volume (ECV, p = .23).

**Additional MRI findings**

Lymph node swelling was much more frequent in the cardiac LLC positive group (n = 8 out of 9, 89 %) compared to the cardiac LLC negative group (n = 0 out of 11, 0 %) with an average short axis diameter of the largest lymph node in the ipsilateral axilla where vaccination occurred of around 1.15 ± 0.25 cm in the LLC positive group.
group compared to 0.61 ± 0.14 cm for the LLC negative group and 0.59 ± 0.25 cm for the control group (p = <.001). Typical lymph node swelling is demonstrated in ▶ Fig. 4.

Discussion

In this retrospective study, we report cardiac MRI findings of 20 suspected acute myocarditis cases after mRNA COVID-19 vaccination. Out of 20 clinically suspected cases, 9 showed cardiac MRI findings consistent with myocarditis according to the 2018 LLC with subepicardial LGE similar to findings in viral induced myocarditis. These 20 patients were further divided into an LLC positive and LLC negative group for further analysis while being compared to a control group. Significant differences regarding T1 and T2 relaxation times, T2 signal intensity ratios, LGE, and cTnT levels were observed between the groups.

There have been a few recent reports of adverse reactions to mRNA COVID-19 vaccines from Pfizer/BioNTech and Moderna including cases of acute myocarditis [1–4, 20, 21]. HSM is a rare entity, its true incidence remains unknown, although Lewin et al. estimated its prevalence at around 2.4 %, with 7 out of 288 transplanted hearts showing histopathological signs of HSM [22]. Most cases are self-limiting and resolve after the administration of the offending pharmaceutical is stopped [23]. Similar to viral induced myocarditis, we demonstrated prolonged T1 and T2 relaxation times, increased T2 signal intensity ratios, and markedly elevated serum cTnT levels in the LLC positive group compared to the LLC negative and control group. Recent case reports demonstrated comparable findings regarding focal edema, LGE, and prolonged T1 and/or T2 relaxation times in myocarditis after mRNA vaccination [24–26]. Chelal et al. found a similar LGE distribution with primarily subepicardial lateral wall involvement compared to our findings where 89 % of the LLC positive group showed subepicar-
A short-axis view of the left ventricle demonstrating positive late gadolinium enhancement findings (arrow heads) in a 25-year-old male presenting with chest pain, exertional dyspnea, and elevated troponin T. B Horizontal long-axis view depicting LGE along the left lateral wall stretching from the cardiac base all the way to the apical wall. Short-axis T1 C and T2 relaxation times D in the same patient demonstrating prolonged T1 and T2 times (arrows).

Our study includes a few limitations. While there is a correlation between increased cTnT and myocarditis-like findings on cardiac MRI, we cannot assume a causation as we do not know if patients with low cTnT received imaging at a later date due to normal lab values and thus had negative findings on cardiac MRI due to a larger temporal difference in time to symptom onset and time to MRI. Previous studies have indeed demonstrated a drop-off in positive cardiac MRI findings over the course of acute myocarditis [34], with consistently prolonged T1 and T2 relaxation times up to 8 weeks after initial cardiac MRI as the only consistently positive parameters of myocardial inflammation. With a significantly longer mean time to cardiac MRI for the LLC negative group compared to the LLC positive group, it is possible that these patients received imaging at too late of a date after symptom onset to visualize myocarditis-like findings on cardiac MRI. No myocardial biopsies were performed, in accordance with the standard of care for hemodynamically stable patients with suspected myocarditis at our institution. Additionally, our cohort populations are very small, with vaccine induced myocarditis being either rare or under-reported.

Conclusion

While it is difficult to prove a causal relationship between mRNA vaccination and acute myocarditis with such low incidences, we...
have observed a temporal relationship between mRNA vaccination and onset of myocarditis confirmed via cardiac MRI, especially when elevated cTnT levels were noted. Cardiac function was not found to be impaired in the LLC positive group. Vaccine-induced myocarditis demonstrates the same pathological findings on cardiac MRI as virus induced myocarditis, allowing for the application of the LLC for diagnostic purposes.

**CLINICAL RELEVANCE:**
- The diagnosis of vaccine-induced myocarditis correlates with elevated serum troponin T levels.
- Cardiac MRI shows changes similar to those seen in virus-induced myocarditis.
- The 2018 Lake Louise Criteria can be employed for diagnostic confirmation of suspected vaccine-induced myocarditis.

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**Conflict of Interest**
The authors declare that they have no conflict of interest.

**References**


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