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Myocardial Injury and Altered Gene Expression Associated With SARS-CoV-2 Infection or mRNA Vaccination

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HIGHLIGHTS

- Patients with biomarker, imaging, or electrocardiographic evidence of myocardial injury associated with recent COVID-19 infection exhibited myocardial histopathologic findings ranging from myocarditis (n = 1) and nonspecific abnormalities (n = 4) to no abnormalities (n = 2).
- Patients with myocardial injury associated with recent mRNA vaccination exhibited histopathologic findings of microvascular thrombosis (n = 1) and nonspecific abnormalities (n = 3).
- Despite the variability in histopathologic findings, mRNA expression of candidate genes, selected for protein gene product likelihood of producing myocardial dysfunction, inflammation, or a prothrombotic state in response to Spike protein, exhibited similar changes, consisting of down-regulation in ACE2, ACE2/ACE ratio, AGTR1, and ITGaS and up-regulation in ACE and F3 (tissue factor).
- COVID-19 and post-mRNA vaccine myocardial injury may have a common molecular pathology.

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COVID-19\textsuperscript{1-3} infection and SARS-CoV-2 mRNA-based vaccines\textsuperscript{4-8} are both infrequently associated with myocardial injury that is typically diagnosed clinically as myocarditis. The observation that mRNA platform-based vaccines are associated with an incidence of myocardial injury/myocarditis that exceeds background rates in certain younger age groups,\textsuperscript{7,8} where it has a prevalence approaching COVID-19 myocarditis\textsuperscript{2}, is a surprising and important observation that needs further investigation. Specifically, the question of whether these 2 disorders are similar or different from histopathologic and molecular perspectives has not yet been addressed. The common exposure of both to SARS-CoV-2 Spike (S) protein provides the basis for testing the hypothesis that the pathophysiologies are similar.

Although both types of myocardial injury are commonly referred to as myocarditis, histopathologic evidence of myocardial inflammation is necessary to confirm that diagnosis.\textsuperscript{9} For both COVID-19 infections and post-vaccine injury, the number of proven myocarditis cases has been small\textsuperscript{3,10} and alternative pathologic processes that could account for tissue injury and cardiac magnetic resonance (CMR) imaging characteristics suggestive of myocarditis have not been extensively investigated. These include microvascular thrombosis and direct cardiac myocyte injury unrelated to an inflammatory process, and this constellation of tissue pathology could be mediated by changes in gene expression in response to exposure to SARS-CoV-2 S protein. During COVID-19 infection as well as after mRNA vaccination, S protein is bound to and internalized with its host cell receptor, angiotensin-converting enzyme (ACE) 2,\textsuperscript{11} resulting in lysosomal degradation of the complex\textsuperscript{12} and ACE2 down-regulation that may be associated with cell and tissue damage.\textsuperscript{13} The host cell response to ACE2 down-regulation includes changes in gene expression,\textsuperscript{13,14} some of which may be pathologic.\textsuperscript{13} Therefore, it is possible that patients exhibiting myocardial injury after mRNA vaccination or with COVID-19 infection share a similar molecular phenotype related to ACE2-S protein binding and internalization, the elucidation of which could lead to mechanism-based preventative strategies.

**METHODS**

**CLINICAL RESEARCH PROTOCOLS.** This single-center study was carried out at the University of Colorado Anschutz Medical Campus and University of Colorado Hospital. The study was initially designed to investigate 4 aims in an American Heart Association COVID 19 Rapid Response grant, awarded in April 2020, in patients with COVID-19-associated myocardial injury. The aims were to: 1) determine if the SARS-CoV-2 virion could be detected in cardiac myocytes; 2) assess the degree of inflammatory
reaction vs direct myocardial injury; 3) measure by means of rapid turnaround reverse-transcription (RT) quantitative polymerase chain reaction (qPCR) the mRNA expression of candidate genes including ACE2, ACE, and other genes whose protein products could be involved in myocardial dysfunction, inflammation, and coagulopathy; and 4) measure global gene expression by means of RNA sequencing and microarray. When mRNA vaccine myocardial injury began to appear in May 2021, those patients were added to the protocol and eventually investigated in another funded study with only minor amendments to the COVID-19 protocol and retention of aims 2, 3, and 4. The present report presents data relevant to aims 1-3 and 2-3 of the COVID-19 and mRNA vaccine protocols, respectively.

The initial protocol investigated recently diagnosed COVID-19 patients who were hospitalized and had evidence of myocardial injury up to 6 months after qPCR-confirmed COVID-19 infection or mRNA vaccination. Evidence for myocardial injury was defined as any of the following: an otherwise unexplained elevation in troponin I (TnI) or B-type natriuretic peptide (BNP) biomarkers, a left ventricular ejection fraction (LVEF) <50% or a new decline, a global longitudinal strain of <16%, or electrocardiographic ST–T changes or sustained ventricular tachycardia or fibrillation. TnI or BNP levels were used for primary screening of COVID-19 study candidates. Exclusion criteria included clinical instability preceding cardiac catheterization, evidence that ventricular dysfunction was secondary to respiratory failure and hypoxemia, moderate pulmonary hypertension (mean ≥35 mm Hg), international normalized ratio >1.8 on no anticoagulation, or a platelet count <100 × 10^9/µL. Using RNA extracted from right ventricular (RV) septal endomyocardial biopsy (EmBx) tissue or from intraoperative myocardial biopsies, we sought, by means of rapid-turnaround (results within 48 hours) qPCR measurements, to assess the expression of ACE2, ACE, and other candidate genes whose altered expression might predispose to cardiac pathology that would be amenable to therapy, and to relate changes in gene expression to myocardial histopathology and imaging characteristics.

When the first case of mRNA vaccine–associated apparent myocarditis was encountered in May 2021, an amendment to the original institutional review board (IRB)-approved protocol was submitted and approved to allow investigation of patients who met criteria for myocardial injury after vaccination, and subsequently a dedicated grant application and protocol was awarded and approved to investigate these patients. This protocol was identical to the COVID-19 version, except that CMR was required and added to injury biomarkers for screening of possible myocardial injury cases.

For both COVID-19 and post-vaccination myocardial injury patients, 2 versions of informed consent forms were used, one for all patients who underwent endomyocardial or operative ventricular biopsy with gene expression, and another for patients who underwent clinical evaluation without invasive or gene expression testing in order to allow use of laboratory and imaging data. Three patients were investigated by the noninvasive method, 1 COVID-19 patient (patient 7, who had an EmBx for clinical purposes but declined consent for the additional biopsies used for gene expression) and 2 mRNA vaccine cases (cases 1 and 2, who were investigated while the invasive protocol was pending IRB approval). Thus all 7 COVID-19 patients underwent ventricular tissue biopsy for histopathology, with 6 having myocardial gene expression measurements, and 4 of 6 mRNA vaccine patients underwent EmBx for assessment of histopathology and gene expression.

Because the COVID-19 subjects were anticipated to have a range of left ventricular (LV) function, the study design included control samples from both nonfailing (LVEF ≥0.50) and failing (LVEF <0.35) LVs, using biobanked mid-distal interventricular septum (IVS) samples from explanted organ donor or cardiac transplant recipient hearts, respectively, which were age and sex matched to the COVID-19 group. Such samples have been shown to be suitable gene expression proxies for EmBx-obtained starting material,17 and data reported in the Supplemental Material confirm the appropriateness of these control samples. For ACE2/ACE ratio measurements we also used control samples from a previously conducted EmBx study15,16,18 (Effect of Beta-blockers on Structural Remodeling and Gene Expression in the Failing Human Heart [BORG]; NCT01798992), which measured ACE2 and ACE mRNA abundance with the use of microarray; an expression ratio is internally controlled and allows for cross-platform comparisons between studies. Nonfailing control samples from BORG included 4 patients with LVEF ≥50% at baseline, as well as 13 reverse-remodeled nonischemic dilated cardiomyopathy (NDC) patients biopsied at the 3- or 12-month end point who satisfied the criteria of LVEF ≥50%.15 Failing NDC (F/NDC) control subjects (n = 33) with LVEF <50% at the 3- or 12-month end point were also included from the BORG study.15 For the biobanked explanted hearts, 14 (7 each of nonfailing and failing) were procured after January 1, 2020, and had PCR performed on
TABLE 1 Patient Characteristics of COVID-19, mRNA Vaccine, Explanted Heart, and EmBx Control Samples

| Group (n)                        | Major Symptoms | Peak Tnl, ng/mL | Peak BNP, pg/mL | Peak D-Dimer, ng/mL | Peak CRP, mg/L | Hosp, days |
|----------------------------------|----------------|----------------|----------------|---------------------|----------------|------------|
| A. COV (7)                       | SOB, fatigue   | 6/7 †           | 5/7 †          | 6/7 †               | 6/7 †          | 10 (7-27) |
| B. mRNA Vax (6)                  | Chest pain     | 6/6 †           | -              | 4/6 †               | 4/6 †          | 2.0 † (0-7) |
| C. NF Expl IVS (20)              | -              | -              | -              | -                   | -              | -          |
| D. Failing Expl IVS (25)         | -              | -              | -              | -                   | -              | -          |
| E. EmBx NF (17)                  | -              | -              | -              | -                   | -              | -          |
| F. EmBx NDC Failing (33)         | -              | -              | -              | -                   | -              | -          |

Values are mean ± SD or median (Q1, Q3). Comparisons are within groups A, B, C, and D and A, B, E, and F. *P < 0.05 for groups A, B, C, and D; †P < 0.05 for groups A, B, E, and F; ‡P < 0.05 for groups A, B, C, and D vs all other groups; †‡P < 0.05 for groups A, B, C, and D vs all other groups, except for A vs B †P < 0.05 vs groups A and F †P < 0.05 vs groups A, B, and E; †‡P < 0.05 for groups A, B, C, and D

| Group (n)                        | LVEF, %         | Age, y          | Sex, F/M | Race/Ethnicity | ECG Findings | 3-Vessel CAD* | Bx (CMR if no Bx) Days After Covid Dx or mRNA Vax |
|----------------------------------|-----------------|----------------|----------|---------------|--------------|--------------|-----------------------------------------------|
| A. COV (7)                       | 53 ± 15         | 3/4            | 1W, 4WH, 2B | 6/7 abnormal   | 3/7          | 18 (8-31)   |
| B. mRNA Vax (6)                  | 36 ± 13         | 2/4            | 6W       | 52 ± 15       | 5/6 abnormal | 26 (11-76)  |
| C. NF Expl IVS (20)              | 50 ± 11         | 9/11           | 11W, 4WH, 2B | 67 ± 8        | –             | 0/20        |
| D. Failing Expl IVS (25)         | 51 ± 11         | 10/15          | 18W, 6WH, 1A | 20 ± 8        | –             | 10/25       |
| E. EmBx NF (17)                  | 44 ± 16         | 7/10           | 13W, 1WH, 1B, 1A | 57 ± 5       | –             | 0/17        |
| F. EmBx NDC Failing (33)         | 48 ± 11         | 7/26           | 21W, 6WH, 5B, 11 | 34 ± 10      | –             | 0/33        |

**Statistical Analyses and Data Presentation.** All statistical analyses of mRNA abundance and other nonnormally distributed data used nonparametric methods, with estimates of central tendency expressed as median (Q1, Q3). To derive a quantitative comparison between control and COVID-19 or post-mRNA vaccine groups, the mRNA abundance values were calculated for every pair of observations between COVID-19 or post-vaccination and control groups and summarized as fold difference (FD) by median (Q1, Q3). Statistical comparisons of gene mRNA abundance were calculated with the use of ΔCt values, by means of the Kruskal-Wallis test followed by Dunn’s test for the 4 group differences, and a Benjamini-Hochberg correction for false discovery in the 7 transcripts being measured. For display in figures, 2 ΔCt transformations were used. Two group comparisons were by Wilcoxon rank-sum tests. Analysis of variance with the Holm-Sidak post hoc test for multiple pairwise comparisons or unpaired t-tests were used to compare age and LVEF baseline characteristics, with results presented as mean ± SD.

Linear discriminant analysis was used to create weighted linear combinations of mRNA abundance analyzed from ΔCt values from the 7 measured genes as the independent variable to differentiate among 4 groups within the dependent variable of nonfailing explanted heart IVS, failing IVS, and COVID-19 and post-mRNA myocardial biopsies. The 4 post-vaccine subjects’ mRNA abundance values were subsequently entered to obtain a model-implied probability of group membership, quantifying the comparative similarity in gene-wise mRNA abundance to values in the respective dependent variable groups.

Box and whisker plots were constructed for mRNA abundance values for the nonfailing IVS, failing IVS, and Covid-19 groups.

A P value or false discovery rate of 0.05 was considered to be statistically significant, and all analyses were conducted in R version 4.1.1 or GraphPad Prism 9.

**Histopathology and CMR Methods.** See Supplemental Material, Sections 2.0 and 3.0.
RESULTS

STUDY POPULATIONS. The COVID-19 and post-mRNA vaccination patients did not differ by age or sex from nonfailing and failing control subjects (Table 1). For groups A, B, C, and D (COVID-19 or mRNA vaccine patients vs explanted heart control subjects) and groups A, B, E, and F (COVID-19 or mRNA vaccine patients vs EmBx control subjects) there was a higher proportion of White Hispanics and Blacks in the COVID-19 group (Table 1). In groups A, B, C, and D, LVEF differed between all groups except between the COVID-19 and mRNA vaccine groups, with those groups having LVEF values intermediate between nonfailing and failing control subjects.

COVID-19 PATIENTS WITH MYOCARDIAL INJURY. The 7 COVID-19 patients (Table 1, Supplemental Table S2) were investigated at median [Q1, Q3] of 18 [8, 31] days after diagnosis. At the time of investigation, all but one had remained hospitalized after initial admission and another (subject 2) (Supplemental Table S2) had been readmitted. None of the patients had severe respiratory distress requiring mechanical ventilation. Six of 7 patients had elevated C-reactive protein (CRP) on admission (Table 1, Supplemental Table S2), which had declined into the normal range by the time of biopsy in all but one (Supplemental Table S2). Multiple other protocol-designated proinflammatory cytokines were within normal limits at the time of biopsy (Supplemental Table S3, Supplemental Material). Six patients had elevated peak D-dimer, and all 7 were anticoagulated and/or received antiplatelet agents (Table 1, Supplemental Table S2). All 7 patients had been treated with remdesivir and dexamethasone (Supplemental Table S2). Six of the 7 patients signed consent for the full research protocol that included RNA extraction for gene expression measurements in ventricular myocardial biopsy (4 EmBx, 2 surgical biopsy) samples that were additional to those used for histopathology, and 1 patient (patient 7) consented to histopathology only.

Four of the 7 COVID-19 patients (1, 2, 6, and 7) had no history of heart disease and had respective LVEFs
FIGURE 2  Cardiac Magnetic Resonance Imaging of Post-mRNA Vaccine Cases

A

Initial  Follow-up

STIR

LGE

B

Initial  Follow-up

STIR

LGE

T1 Map
of 71%, 55%, 48%, and 20% (Supplemental Table S2). On light microscopy (LM), patient 7 had a moderate amount of lymphocytic and macrophage infiltrate (Figure 1A, Supplemental Figures S1A to 1D) consistent with myocarditis and evidence of myocyte injury (Figure 1B). Electron microscopy (EM) revealed myocytolysis and loss of contractile elements (Figure 1C). In patient 1, coronary angiography detected a small distal posterior descending artery dissection without other abnormalities. LM revealed no significant abnormality in patients 1 and 2, and evidence of scattered myocyte hypertrophy in patient 6. Patient 1 had evidence of mild nonspecific myocyte injury on EM. Patients 2 and 6 had normal EMs.

Patients 3, 4, and 5 had 3-vessel coronary artery disease (CAD), known previously in patient 4, who had an ischemic cardiomyopathy (Supplemental Table S2). This patient developed worsening heart failure following COVID-19 infection, requiring LV assist device implantation. Patient 5 presented with a COVID-19 diagnosis and an LVEF of 30% with 3-vessel disease on angiography that presumably antedated his infection. A third CAD patient (patient 3) had no cardiac history but presented with high-sensitivity Tn elevation of 1,050 ms) except region 2 (1,085 ms), which was abnormal on the LGE images (Figure 2).

RT-PCR using 4 different sets of primers (Supplemental Material, Section 1.0) was not positive for SARS-CoV-2 in any of the 6 patients who had RNA extraction, and EMs from all 7 biopsied COVID-19 subjects revealed no evidence of viral particles (Supplemental Table S2).

**Patients with myocardial injury presenting after receiving a COVID-19 mRNA-based vaccine.**

We investigated 6 post-mRNA vaccination patients who presented with a clinical diagnosis of myocarditis, with symptom onset from 3 to 17 days (median [Q1, Q3] 4.5 [3, 12] days) after their first, second, or third dose of mRNA-1273 or BNT162b2 vaccine (Table 1, Supplemental Table S4). Ages ranged from 26 to 55 years (mean 36.3 ± 12.7 years), and 5 of the 6 presented with chest pain. Tnl levels were elevated in all 5 patients who had them determined (Table 1, Supplemental Table S4). LVEFs ranged from 30% to 65% (mean 51.7 ± 14.6%) (Table 1, Supplemental Table S4). One patient (case 2) had a questionable history of RV dysfunction, and the other 5 had no cardiac history. Case 2 developed severe LV dysfunction and incipient cardiogenic shock 5 days after her first dose of mRNA vaccine and was considered too unstable for EmBx. She was treated with high-dose methylprednisolone, dobutamine, and venoarterial extracorporeal membrane oxygenation (ECMO) for 1 week and clinically stabilized (Supplemental Table S4). After ECMO discontinuation, CMR without gadolinium infusion demonstrated increased T1 and T2 native parametric values suggesting myocardial edema consistent with an inflammatory process and myocarditis, with an LVEF of 48%. She continued to improve on guideline-directed heart failure medical therapy and was discharged.

Case 6 was a 47-year-old woman with no cardiac history who developed symptoms of heart failure 10 days after her first dose of BNT162b2, with a subsequent LVEF measured at 37% by means of transthoracic echocardiogram (Supplemental Table S4). Eighty-nine days after vaccination, she developed PCR-proven COVID-19, without further decline in cardiac function. She was enrolled as an outpatient in the post-vaccination myocardial injury protocol 46 days after her COVID-19 diagnosis, and CMR imaging returned an LVEF of 58% with mild RV dysfunction.

**Figure 2 Continued**

(A) Case 1, 4-chamber (4C) images, initial study shown top left: short T2 inversion recovery (STIR) imaging specifically identifies increased interstitial space from either myocyte loss or edema, showing increased signal in the lateral left ventricular (LV) wall suggesting acute myocardial edema. This is less obvious on follow-up imaging (top right) conducted 57 days later. Shown in the bottom panels are initial (left) and follow-up (right) post-gadolinium contrast phase-sensitive inversion recovery (PISR) 4C images. Note that the initial focal punctate late gadolinium enhancement (LGE) areas in the lateral wall (arrows) are less prominent on follow-up. (B) Case 3, initial STIR imaging shown in the top left panel, with follow-up study conducted 35 days later on the right. Note the septal, apical, and lateral foci of increased signal for edema (arrows) with a decreased signal on the follow-up study. Shown in the center panels are (left) initial LGE punctate foci in the lateral wall in 2 places and LV apex (arrows). Follow-up study (center right) shows a single less prominent LGE focus. The bottom panel images are T1 mapping native relaxation times showing 1 septal, 2 lateral anterior, and 3 lateral posterior values from regions of interest. Note that (left) initial values are ~1,105-1,230 ms for all 3 regions suggesting increased interstitial space, whereas in (right) the follow-up study the T1 values are normal (~1,050 ms) except region 2 (1,085 ms), which was abnormal on the LGE images (center right) of the lateral wall.
dysfunction and “minimal” late gadolinium enhancement (LGE) in the basal lateral visceral pericardium without clear myocardial uptake (Supplemental Table S4). EmbX performed 182 days after vaccination and 93 days after her COVID-19 diagnosis revealed rare nuclear enlargement of cardiac myocytes, and EM showed only nonspecific changes (Supplemental Table S4).

The other 4 post-vaccine cases had uneventful clinical courses, and all underwent CMR with LGE assessment. The initial and follow-up CMRs for cases 1 and 3 are described in Figures 2A and 2B, respectively, and in Supplemental Table S4. Both patients had areas of focal punctate LGE on the initial study that were diminished but still present >1 month later. Cases 4, 5, and 6 also had focal areas of LGE, as
described in Supplemental Table S4. No post-vaccine patient had a positive nasal swab for SARS-CoV-2 (Supplemental Table S4). One subject had a myocardial RT-PCR with an S RNA Ct <40 (case 5: Ct = 37) (Supplemental Table S4), with Cts >40 for all other viral genome regions).

Per study protocol, cases 3, 4, 5, and 6 underwent EmBx for histopathology and gene expression performed at, respectively, 10, 29, 23, and 172 days after onset of chest pain or other cardiac symptoms and 13, 32, 40, and 182 days after vaccination (Supplemental Table S4). Histopathology for case 3 is shown in Figures 1D to 1F, where rare fibrin thrombi were identified within capillaries on both hematoxylin and eosin (Figure 1D) and trichrome (Figure 1E) stained sections. EM (Figure 1F) demonstrated aggregated platelets interacting with endothelial cells within an interstitial capillary that contained a nonocclusive thrombus. Although D-dimer levels were elevated on hospital admission in 3 of 5 post-mRNA vaccine patients, case 3’s D-dimer was within normal limits while peak CRP was elevated (47 mg/L) (Supplemental Table S4). The platelet count was normal, and the PF4 IgG antibody screen was negative. Proinflammatory biomarkers assessed at the time of biopsy/catheterization were normal (Supplemental Table S4). Cases 4, 5, and 6 demonstrated no evidence of an inflammatory infiltrate or microthrombi on LM, and only nonspecific abnormalities on EM with no evidence of loss of contractile elements (Supplemental Table S4).

MYOCARDIAL GENE EXPRESSION. For the 7 measured transcripts, IVS samples yielded very similar mRNA abundance values compared with simulated EmBx (Supplemental Table S5), confirming previous work showing that explanted heart mid-distal septum is suitable for EmBx control material.

COVID-19 patients. Myocardial mRNA abundance values calculated as $2^{-\Delta Ct}$ relative to GAPDH (groups A, B, C, and D) or as microarray log2 fluorescence intensity (f; groups E and F) are shown in Figure 3, and median (Q1, Q3) fold changes (vs nonfailing and failing IVS control samples) are presented in Table 2. Expression of ACE2 mRNA was markedly decreased in the 6 COVID-19 patients, compared with either nonfailing (by −2.7-fold; $P = 0.007$) or failing (by −3.4-fold; $P < 0.001$) explanted heart IVS control samples (Figure 3A, Table 2). In contrast, ACE mRNA expression was increased compared with nonfailing (by 2.8-fold; $P = 0.023$) and failing (by 2.1-fold; $P = 0.041$) IVS control samples (Figure 3B, Table 2). The $ACE2/ACE$ expression ratio was markedly reduced in COVID-19 subjects compared with nonfailing (by −7.9-fold; $P = 0.002$) or failing (by −6.6-fold; $P = 0.002$) IVS control samples, respectively (Figure 3C, Table 2). The $ACE2/ACE$ ratio was similarly reduced compared with EmBx control samples, in nonfailing (by −6.1-fold; $P = 0.008$) or failing (by −9.0-fold; $P < 0.001$) NDC (Figure 3D, Table 2).

Expression of AGT mRNA was marginally significantly different across the 4 groups (Kruskal-Wallis $P = 0.036$) (Figure 3E), with no significant differences for COVID-19 compared with either nonfailing or failing control samples. Median values of AGTR1, a low-abundance mRNA that encodes the type I receptor and a biosensor of angiotensin II generation, was lower in COVID-19 patients compared with nonfailing (by −2.7-fold; $P = 0.036$) or failing (by −4.4-fold; $P = 0.030$) IVS control samples (Figure 3F, Table 2).

Expression of ITGA5 mRNA, a possible CoV-2 co-receptor whose encoded protein binds to and is co-regulated with ACE2 in eccentric remodeling, tracked with ACE2 mRNA expression by being reduced in COVID-19 compared with both nonfailing (by −1.7-fold; $P = 0.006$) and failing (by −1.8 fold; $P = 0.002$) IVS control samples (Figure 3G, Table 2). The full-length transcript (fITF) of the F3 or tissue factor (TF) gene was up-regulated in COVID-19 compared with nonfailing (by 2.6-fold; $P = 0.026$) but not compared with failing ( $P = 0.17$) IVS control samples, owing to fITF having higher expression in failing vs nonfailing control samples (by 1.6-fold; $P = 0.027$) (Figure 3H, Table 2). NPPB expression was, as expected, markedly increased in failing compared with nonfailing IVS control samples (by 46-fold; $P < 0.0001$; Figure 3I). NPPB values trended nonsignificantly higher in COVID-19 compared with nonfailing control samples (by 3.2-fold; $P = 0.23$) and were lower than in failing control samples (by −7.9-fold; $P = 0.034$) (Figure 3I, Table 2).

mRNA vaccination patients. Post-vaccination patients who had gene expression measured (cases 3, 4, 5, and 6) had mRNA abundance values that were similar to the COVID-19 data (Figure 3). The similarity is striking, and none of the individual gene mRNA abundances had $P$ values $<0.10$ (range 0.12-0.44) between the COVID-19 and post-vaccination groups (Figure 3). Moreover, compared with nonfailing or failing control samples from either explanted hearts or EmBx material from intact hearts, the directionality and statistically significant differences of the COVID-19 patients were nearly completely recapitulated in the post-vaccination myocardial injury patients. The only exception was AGTR1, where the decrease vs the failing group had a $P$ value of 0.030 in COVID-19 and 0.056 in post-vaccination subjects.
Linear discriminant analysis of the 7 genes’ mRNA expression in the 4 groups is presented in Figure 4 and Table 3, which for post-vaccination subjects yielded a posterior probability of 97.8% for model-implied COVID-19 group membership compared with probabilities of 1.9% and 0.2% for membership in the nonfailing and failing IVS groups, respectively. Based on the analysis coefficients (Table 3) NPPB was the most robust predictor of linear discriminant 1, and ACE2 the highest-order predictor of discriminant 2.

### DISCUSSION

**SUMMARY OF THE CLINICAL AND HISTOPATHOLOGIC FINDINGS.** Clinical and biomarker evidence of myocardial injury. Based on knowledge available at the time23 the initial protocol was developed, TnI and BNP were used as biomarkers of myocardial injury. All 7 COVID-19 patients had elevations of TnI of at least 1 of these; had elevated TnI and 5 had elevated BNP. In addition, 4 of the 7 COVID-19 patients had reduced LVEF, and 6 had electrocardiographic abnormalities. All 7 had either shortness of breath or chest pain symptoms, and none had pneumonia or required mechanical ventilation.

The 6 post-mRNA vaccination subjects exhibited clinical features in common with previously reported cases.4–8,25 All had LGE on CMR, and chest pain developing 3–17 days after vaccination with either BNT162b2 or mRNA-1273 vaccine. Five of the 5 subjects where TnI level was available had elevation, and 3 of 6 had reduced LVEF or abnormal global longitudinal strain on echocardiography.

**Histopathology in COVID-19 myocardial injury.** Three of the 7 patients had advanced underlying CAD, and 1 had a distal posterior descending artery dissection that was clinically incidental and possibly related to vascular effects of COVID-19.24 Despite clinical findings of myocardial injury, histopathology revealed myocarditis in only 1 patient, nonspecific changes in 4, and no abnormalities on LM or EM in 2. No COVID-19 patient had SARS-CoV-2 detectable in cardiac myocytes on EM, or in RNA detectable by RT-PCR.

**Histopathology in post-mRNA vaccine myocardial injury.** Despite CMR findings consistent with myocarditis, on EmBx no post-vaccine patient had evidence of a myocardial inflammatory infiltrate. Case 3 had microvascular thrombosis on both LM and EM, a histopathologic finding that has not been previously reported in post-vaccination myocardial injury. The 3 other biopsied cases had nonspecific changes on EM, possibly related to a previous myocardial insult. CMR findings in cases 1, 3, 4, and 5 revealed LV punctate areas of LGE, initially interpreted as compatible with myocarditis but also potentially related to small infarcts.26 Although previous histopathologic reports based on EmBx or autopsy in mRNA vaccine-associated myocardial injury have demonstrated inflammatory infiltrates,5,27 there have also been reported cases with no histopathologic evidence of inflammation.4,26

In summary, it appears that histopathologic findings in myocardial injury associated with COVID-19 or mRNA vaccine can be due to causes other than myocarditis. Alternatively, recovery from inflammation or EmBx sampling error could explain the lack of biopsy-proven myocarditis.

**GENE EXPRESSION CHANGES.** Despite a lack of consistent histopathologic findings associated with
myocardial injury, the gene expression findings in COVID-19 and post-mRNA vaccination patients were quite uniform, revealing a pattern that was markedly different from control myocardium. In addition, ACE2/ACE ratio data from EmBx nonfailing and failing control samples were in the same range as IVS controls and were markedly higher than in either COVID-19 or post-vaccine myocardial injury patient samples.

Down-regulation of ACE2 was a striking gene expression finding in COVID-19 and post-vaccination patients. This change was the major contributor to a markedly reduced ACE2/ACE ratio, compared with either explanted heart IVS or EmBx nonfailing or failing control samples. ACE2 gene expression has previously been shown to be decreased at the mRNA level in human hearts of patients who died from COVID-1927,28 and in induced pluripotent stem cell human cardiac myocytes inoculated with SARS-CoV-2.29 Down-regulation in ACE2 gene expression in response to an S protein construct has also been shown in model systems for mRNA in bronchial alveolar cells13 and protein in human pulmonary artery endothelial cells.14 In addition, up-regulation of ACE gene expression compared with nonfailing IVS control samples contributed to the decreased ACE2/ACE ratio in COVID-19 and post-vaccination patients. If translated into protein changes these adjustments in ACE2 and ACE mRNA expression would lead to much higher myocardial concentrations of angiotensin II, the degree of which is determined by the relative activities of these enzymes.30 Although angiotensin II was not measured in myocardial samples, expression of a biosensor of angiotensin II, AGTR1, whose encoded type 1 receptor down-regulates with increased ACE activity and angiotensin II production19 and whose mRNA

### Table 3

| Group            | Probability of Vaccine Patient Membership (95% CI) | Gene Predictor (by mRNA ΔCt) | Coefficient, Linear Discriminant 1 | Coefficient, Linear Discriminant 2 |
|------------------|----------------------------------------------------|------------------------------|------------------------------------|------------------------------------|
| COVID-19         | 0.978 (0.840 to 0.999)                             | ACE2                         | 0.24                               | 0.97                               |
| Nonfailing       | 0.019 (1.2e−5 to 0.154)                            | ACE                          | 0.08                               | −0.37                              |
| Failing          | 0.002 (4.9e−7 to 0.013)                            | AGT                          | −0.23                              | 0.12                               |
|                  | −                                                  | AGTR1                        | 0.46                               | 0.13                               |
|                  | −                                                  | ITGA5                        | 0.03                               | 0.40                               |
|                  | −                                                  | NPPL                         | 1.04                               | −0.66                              |
|                  | −                                                  | TF (F3)                      | 0.35                               | −0.02                              |

ΔCts normalized for between-gene differences in absolute expression; values are inversely related to mRNA abundance.
up-regulates with reverse remodeling associated with neurohormonal inhibition,16 was decreased in COVID-19 and post-vaccination patients compared with nonfailing control samples. Expression of angiotensinogen (AGT), which encodes the protein that gives rise to all angiotensin peptides generated in the heart, was not reduced in COVID-19 compared with nonfailing or failing control samples, or in post-vaccination patients compared with failing control samples. If encoded, these mRNA changes indicate the myocardial renin-angiotensin system in COVID-19 patients was functionally imbalanced toward angiotensin II production, which has negative implications for cardiac myocyte and endothelial cell function as well as for promoting a procoagulant state and predisposing to inflammation.31,32

Compared with nonfailing IVS control samples, the full-length tissue factor (tTF) transcript of the F3 gene was up-regulated in COVID-19 and post-vaccination patients. On cell entry, certain viruses are known to up-regulate TF,33,34 the proximal initiator of the extrinsic coagulation pathway that also mediates inflammatory responses via protease-activated receptor signaling,22,35 which has been implicated in COVID-19 in the promotion of both thrombosis and inflammation.36

A pattern of altered gene expression that was qualitatively identical to COVID-19 patients was found in the post-vaccination patients. Linear discriminant analysis yielded a high probability (97.8%) that the post-vaccine aggregate gene expression data were group-indistinguishable from COVID-19 patients. A difference between the post-vaccination subjects and the COVID-19 patients is that BNT162b2 and mRNA-1273 produce isolated S protein, while the COVID-19 patients were exposed to S protein as a component of the full SARS-CoV-2 virion. However, S protein-isolated constructs, as “pseudoviruses,” produce the same down-regulation of ACE2 as the full virion,15,14,29 indicating that it is S or cleaved S1 protein that is signaling ACE2 gene dysregulation. In addition, S protein constructs can produce vascular endothelial cell damage,33,37 and S-ACE2 binding results in down-regulation of ACE2 by sequestration into clathrin-coated pits and lysosomal degradation where breakdown products may inflict cell damage and modify gene expression.11,12

A somewhat surprising feature of the alterations in gene expression is that the changes were durable, noted up to 137 or 182 days post-infection diagnosis in COVID-19 or mRNA vaccination, respectively. This may reflect effects of the ongoing injury process, or sustained S protein levels that have been shown to remain in tissue for at least 60 days after mRNA vaccination.38 We did not measure S protein levels in the EmBx samples, but one of 4 biopsied patients had S RNA detectable in his sample taken 40 days after vaccination.

STUDY LIMITATIONS. An obvious major limitation of this study is the small number of patients investigated, 7 and 6 patients for COVID-19 and post-mRNA vaccination, respectively. In addition, 3 of the 13 patients had missing gene expression data owing to pending IRB approval or decline of consent for additional EmBx for gene expression. However, the uniformity of the gene expression changes in both groups of myocardial injury patients suggests a strong pattern that is unlikely to be affected by relatively unimportant missing data. Also, we reported the mRNA expression of only 7 candidate genes, which were selected for their potential diagnostic impact that might lead to development of therapeutic strategies based on measurements potentially available within hours of tissue sampling. The study design includes the use of both RNA sequencing and microarray platforms to measure global gene expression (aim 4), and to avoid batch effects these measurements are conducted in RNA stored at −80 °C until the study is completed or has reached the 2-year time point from earliest RNA extraction. Thus, these data are not yet available.

CONCLUSIONS

In this first study of direct and contemporaneous comparison of myocardial histopathology and gene expression between COVID-19 and post-mRNA vaccine myocardial injury living patients, myocardial gene expression was altered to predispose to inflammation, thrombosis, and contractile dysfunction. The described constellation of gene expression changes could potentially be amenable to preventative or, if diagnosed promptly, targeted therapy on presentation. Targeted therapies could include intensive angiotensin II inhibitory and/or anticoagulation strategies including tissue factor inhibition. However, further studies are required to define the full extent of the molecular pathology of COVID-19- and mRNA vaccine-associated myocardial injury, including the extent that and how these are triggered by exposure to S protein.

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COVID-19 vaccination and mRNA vaccines can be associated with myocardial injury that is usually diagnosed clinically as myocarditis. In model systems, SARS-CoV-2 Spike protein, the mechanism through which the virus enters hosts cells by binding to the essential cardiac enzyme ACE2 as well as being the immunogen produced by mRNA vaccines, can produce cell damage and unfavorable changes in gene expression. These observations suggest that myocardial injury during COVID-19 or after mRNA vaccination may be produced by the same Spike protein-based mechanism, which may be amenable to preventative or therapeutic strategies.

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**COMPETENCY IN MEDICAL KNOWLEDGE:** COVID-19 infection and mRNA vaccines can be associated with myocardial injury that is usually diagnosed clinically as myocarditis. In model systems, SARS-CoV-2 Spike protein, the mechanism through which the virus enters hosts cells by binding to the essential cardiac enzyme ACE2 as well as being the immunogen produced by mRNA vaccines, can produce cell damage and unfavorable changes in gene expression. These observations suggest that myocardial injury during COVID-19 or after mRNA vaccination may be produced by the same Spike protein-based mechanism, which may be amenable to preventative or therapeutic strategies.

**TRANSLATIONAL OUTLOOK:** Identification of similar patterns of altered gene expression in COVID-19- and mRNA vaccination–associated myocardial injury would imply a common mechanism, implicating Spike protein. This hypothesis could be further tested in model systems, in an example of “reverse translation.”
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KEY WORDS COVID-19, gene expression, mRNA vaccines, myocardial injury, myocarditis

APPENDIX For supplemental tables and figures, please see the online version of this paper.