CLINICAL RESEARCH

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

Natasha L. Altman, MD,[a] Amber A. Berning, MD,[b] Cara E. Saxon, MD,[c] Kylie E. Adamek, MD,[d] Jessica A. Wagner, BS,[a] Dobromir Slavov, PhD,[a] Robert A. Quaife, MD,[a] Edward A. Gill, MD,[a] Wayne A. Minobe, BS,[a] Eric R. Jonas, BS,[a] Ian A. Carroll, PhD,[e] Sophia P. Huebler, MS,[e] Joshua Raines, MD,[c] John C. Messenger, MD,[a] Amrut V. Ambardekar, MD,[a] Luisa Mestroni, MD,[a] Rachel M. Rosenberg, MS,[a] Jessica Rove, MD,[f] Thomas B. Campbell, MD,[g] Michael R. Bristow, MD, PhD[a,e]

VISUAL ABSTRACT

Altman NL, et al. J Am Coll Cardiol Basic Trans Science. 2022;■(■):■–■.
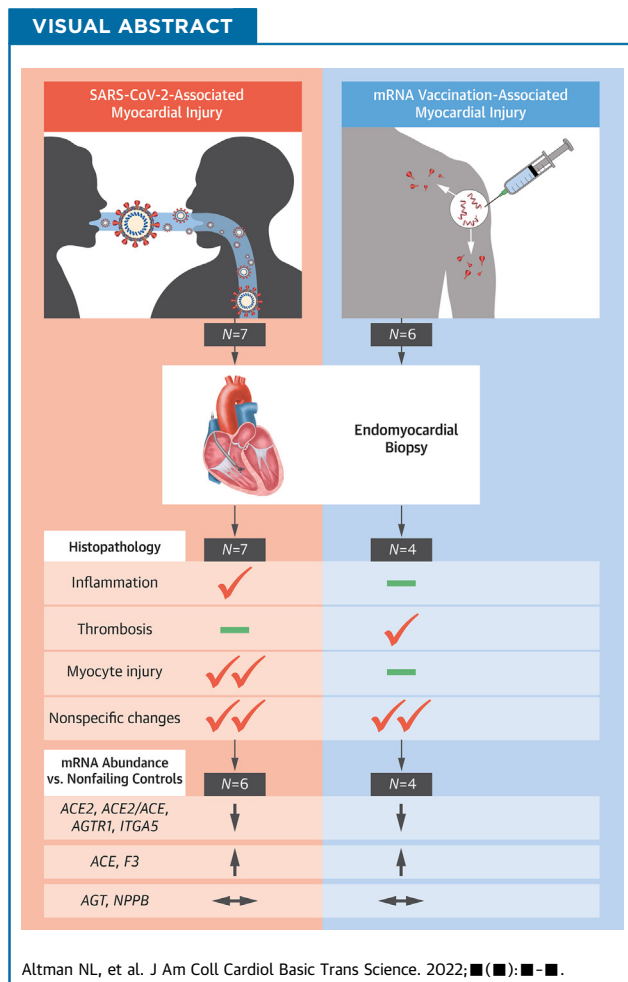
HIGHLIGHTS

- Patients with biomarker, imaging, or electrocardiographic evidence of myocardial injury associated with recent COVID-19 infection exhibited myocardial histopathologic findings ranging from myocarditis (1 patient) and nonspecific abnormalities (4 patients) to no abnormalities (2 patients).

- Patients with myocardial injury associated with recent mRNA vaccination exhibited histopathologic findings of microvascular thrombosis (1 patient) and nonspecific abnormalities (3 patients).

- 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 *ITGA5* and up-regulation in *ACE* and *F3* (tissue factor).

- COVID-19 and post–mRNA vaccine myocardial injury may have a common molecular pathology.

From the [a]Division of Cardiology, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA; [b]Department of Pathology, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA; [c]Department of Internal Medicine, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA; [d]Division of Cardiovascular Medicine, Vanderbilt University Medical Center, Nashville, Tennessee, USA; [e]ARCA Biopharma, Westminster, Colorado, USA; [f]Division of Cardiothoracic

ABBREVIATIONS AND ACRONYMS

ACE = angiotensin I-converting enzyme gene

ACE2 = angiotensin-converting enzyme 2 gene

AGT = angiotensinogen gene

AGTR1 = angiotensin II receptor type 1 gene

ANG II = angiotensin II

BNP = B-type natriuretic peptide

CMR = cardiac magnetic resonance

COVID-19 = Infectious disease caused by the SARS-CoV-2 virus

EM = electron microscopy

ITGA5 = integrin subunit alpha 5 gene

F3 = coagulation factor III (tissue factor) gene

IVS = interventricular septum

LGE = late gadolinium enhancement

LM = light microscopy

LV = left ventricular

LVEF = left ventricular ejection fraction

NDC = nonischemic dilated cardiomyopathy

NPPB = natriuretic peptide B gene

RV = right ventricular

S = SARS-CoV-2 Spike

TnI = troponin I

SUMMARY

SARS CoV-2 enters host cells via its Spike protein moiety binding to the essential cardiac enzyme angiotensin-converting enzyme (ACE) 2, followed by internalization. COVID-19 mRNA vaccines are RNA sequences that are translated into Spike protein, which follows the same ACE2-binding route as the intact virion. In model systems, isolated Spike protein can produce cell damage and altered gene expression, and myocardial injury or myocarditis can occur during COVID-19 or after mRNA vaccination. We investigated 7 COVID-19 and 6 post–mRNA vaccination patients with myocardial injury and found nearly identical alterations in gene expression that would predispose to inflammation, coagulopathy, and myocardial dysfunction. (J Am Coll Cardiol Basic Trans Science 2022;■:■–■) © 2022 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

C OVID-19[1-3] infection and SARS-COV-2 mRNA-based vaccines[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,[7,8] where it has a prevalence approaching COVID-19 myocarditis[7], 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.[9] For both COVID-19 infections and post-vaccine injury, the number of proven myocarditis cases has been small,[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,[11] resulting in lysosomal degradation of the complex[12] and ACE2 down-regulation that may be associated with cell and tissue damage.[13] The host cell response to ACE2 down-regulation includes changes in gene expression,[13,14] some of which may be pathologic.[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

Surgery, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA; and the ᵍDivision of Infectious Diseases, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA.

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 micro-array. 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 precluding 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 × 10³/μL. Using RNA extracted from right ventricular (RV) septal endomyocardial biopsy (EmBx) tissue[15,16] 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 study[15,16,18] (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)	Age,[a,b,c] y	Sex, F/M	Race/Ethnicity	LVEF,[d] %	ECG Findings	3-Vessel CAD[aa]	Bx (CMR if no Bx) Days After Covid Dx or mRNA Vax
A. COV (7)	53 ± 15	3/4	1W, 4WH, 2B	42 ± 20	6/7 abnormal	3/7	18 (8-31)
B. mRNA Vax (6)	36 ± 13	2/4	6W	52 ± 15	5/6 abnormal	0/6	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	$57^{e} \pm 5$	–	0/17	–
F. EmBx NDC Failing (33)	48 ± 11	7/26	21W, 6WH, 5B, 1I	$34^{f} \pm 10$	–	0/33	–

TABLE 1 Continued

Group (n)	Major Symp-toms	Peak TnI, 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^{g} (0-7)
C. NF Expl IVS (20)	–	–	–	–	–	–
D. Failing Expl IVS (25)	–	–	–	–	–	–
E. EmBx NF (17)	–	–	–	–	–	–
F. EmBx NDC Failing (33)	–	–	–	–	–	–

Values are mean $\pm$ SD or median (Q1, Q3). Comparisons are within groups A, B, C, and D and A, B, E, and F. [a]$pt < 0.05$ for groups A, B, C, and D [b]$P < 0.05$ for groups A, B, E, and F [c]$P < 0.05$ for groups A, B, C, and D vs all other groups [d]$P < 0.05$ for groups A, B, C, and D vs all other groups, except for A vs B [e]$P < 0.05$ vs groups A and F [f]$P < 0.05$ vs groups A, B, and E [aa]$P < 0.05$ for groups A, B, C, and D

↑, elevated above upper limit of normal; A = Asian, B = Black; Bx = biopsy; CAD = coronary artery disease; Cov = COVID-19 patients; EmBx = endomyocardial biopsy; Expl = explanted heart form unused transplant donors or end-stage heart failure patients undergoing transplantation; Hosp = hospitalization, I = Pacific Islander or Native American; IVS = interventricular septum; NDC = nonischemic dilated cardiomyopathy; NF = nonfailing; Vax = post–mRNA vaccine patients; W = White, WH = White Hispanic.

extracted RNA for presence of SARs-CoV-2 as described in the Supplemental Material; all were negative. The EmBx study control samples were all from before 2020.

These studies were conducted according to Declaration of Helsinki guidelines, and the protocols were approved by the University of Colorado Multiple Institutional Review Board. All patients signed informed written consent.

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, $2^{-\Delta Ct}$ transformations were used. Two group comparisons were by Wilcoxon rank-sum tests. Analysis of variance with the Holm-Sadako 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 $\pm$ SD.
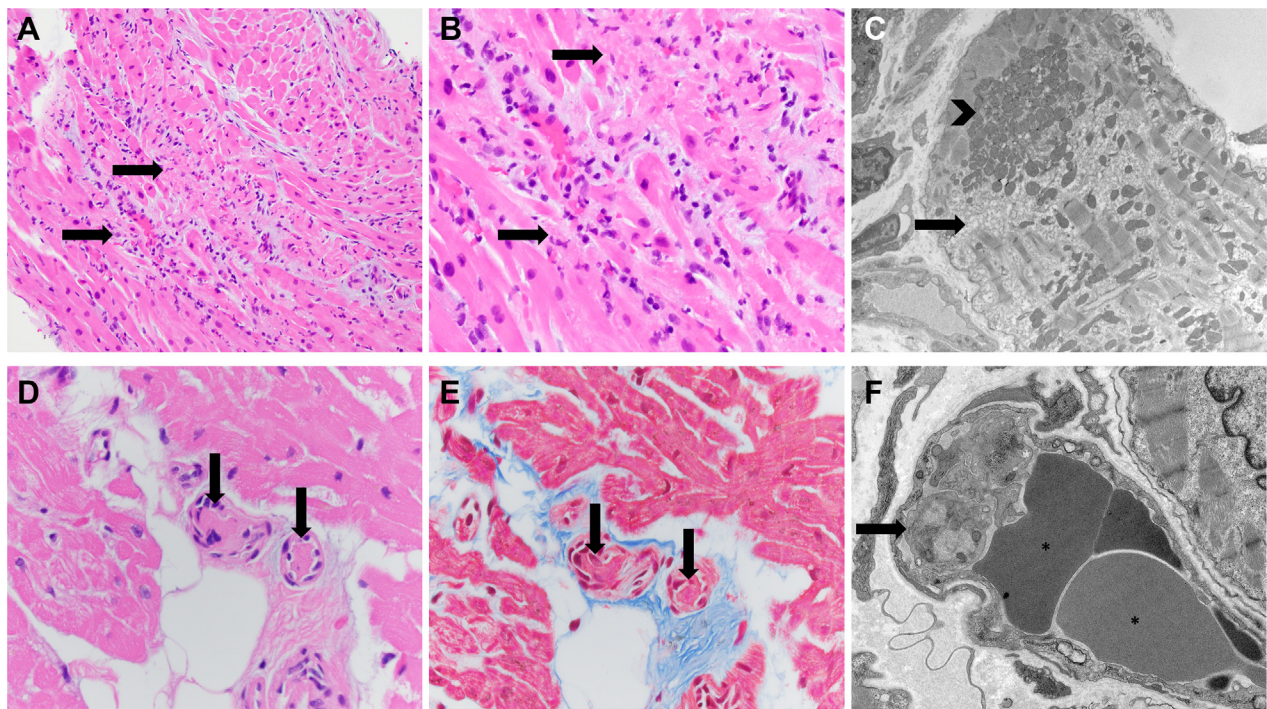
Linear discriminant analysis was used to create weighted linear combinations of mRNA abundance analyzed from ΔCt values from the 7 measured genes as the independent variable to differentiate among 4 groups within the dependent variable of nonfailing explanted heart IVS, failing IVS, and COVID-19 and 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.

EmBx AND SURGICAL BIOPSY, RNA EXTRACTION, AND RT-qPCR. See Supplemental Material, Section 1.0 and Table S1.

HISTOPATHOLOGY AND CMR METHODS. See Supplemental Material, Sections 2.0 and 3.0.

ARTICLE IN PRESS

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. ■, NO. ■, 2022   Altman *et al*   5
■ 2022:■–■   COVID-19– or mRNA Vaccination–Related Myocardial Injury

FIGURE 1 Endomyocardial Biopsies of a COVID-19 Patient and a Post–mRNA Vaccine Case

COVID-19 patient 7: **(A)** Endomyocardial biopsy (EmBx) demonstrating infiltrating inflammatory cells with associated myocyte injury **(arrows)** (×200); **(B)** higher-power view of myocyte injury, characterized by fragmentation of myocytes and early necrosis **(arrows)** (×400); **(C)** electron microscopy (EM) showing myocyte damage characterized by myocytolysis and decreased contractile elements **(arrow)** with adjacent unremarkable mitochondria **(arrowhead)**. Post–mRNA vaccine case 3: **(D)** EmBx with small-caliber vessels exhibiting microvascular thrombosis **(arrows)** (×600); **(E)** trichrome stain highlighting microvascular thrombosis **(arrows)** (×600); **(F)** EM with nonocclusive platelet-rich thrombus **(arrow)** interacting with endothelial cells of an interstitial vessel with adjacent red blood cells **(asterisks)**.

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 and 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 and 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 and Supplemental Material). Six patients had elevated peak D-dimer, and all 7 were anti-coagulated and/or received antiplatelet agents (Table 1 and 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 (patent 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
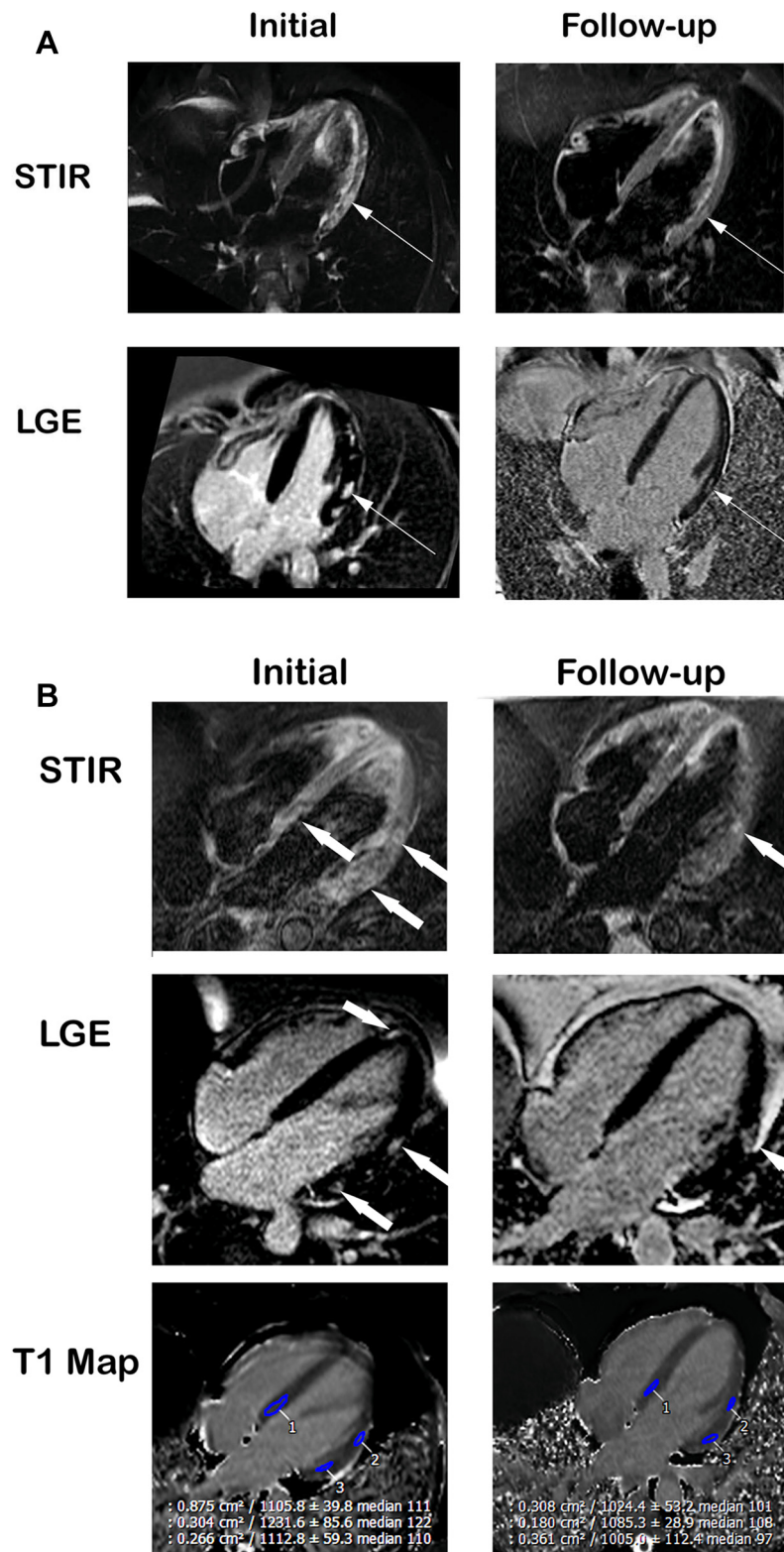
of 71%, 55%, 48%, and 20% (Supplemental Table S2). On light microscopy (LM), patient 7 had a moderate amount of lymphocytic and macrophage infiltrate (Figure 1A and Supplemental Figures S1Ato 1D) consistent with myocarditis and evidence of myocyte injury (Figure 1B). Electron microscopy (EM) revealed 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 >12,000 ng/L, ST-segment elevation, and LVEF of 50%. Angiography revealed left main and right coronary artery high-grade stenoses requiring 3-vessel coronary artery bypass grafting. Histopathology in these CAD patients revealed myocyte hypertrophy in patients 4 and 5 and interstitial fibrosis in patient 4. EM revealed mild nonspecific myocyte injury and some loss of contractile elements in patient 5, with no abnormalities detected in patients 3 and 4.

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 and 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. TnI levels were elevated in all 5 patients who had them determined (Table 1 and Supplemental Table S4). LVEFs ranged from 30% to 65% (mean 51.7 ± 14.6%) (Table 1 and 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

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.

FIGURE 3   Box and Whisker Plots for mRNA Abundance
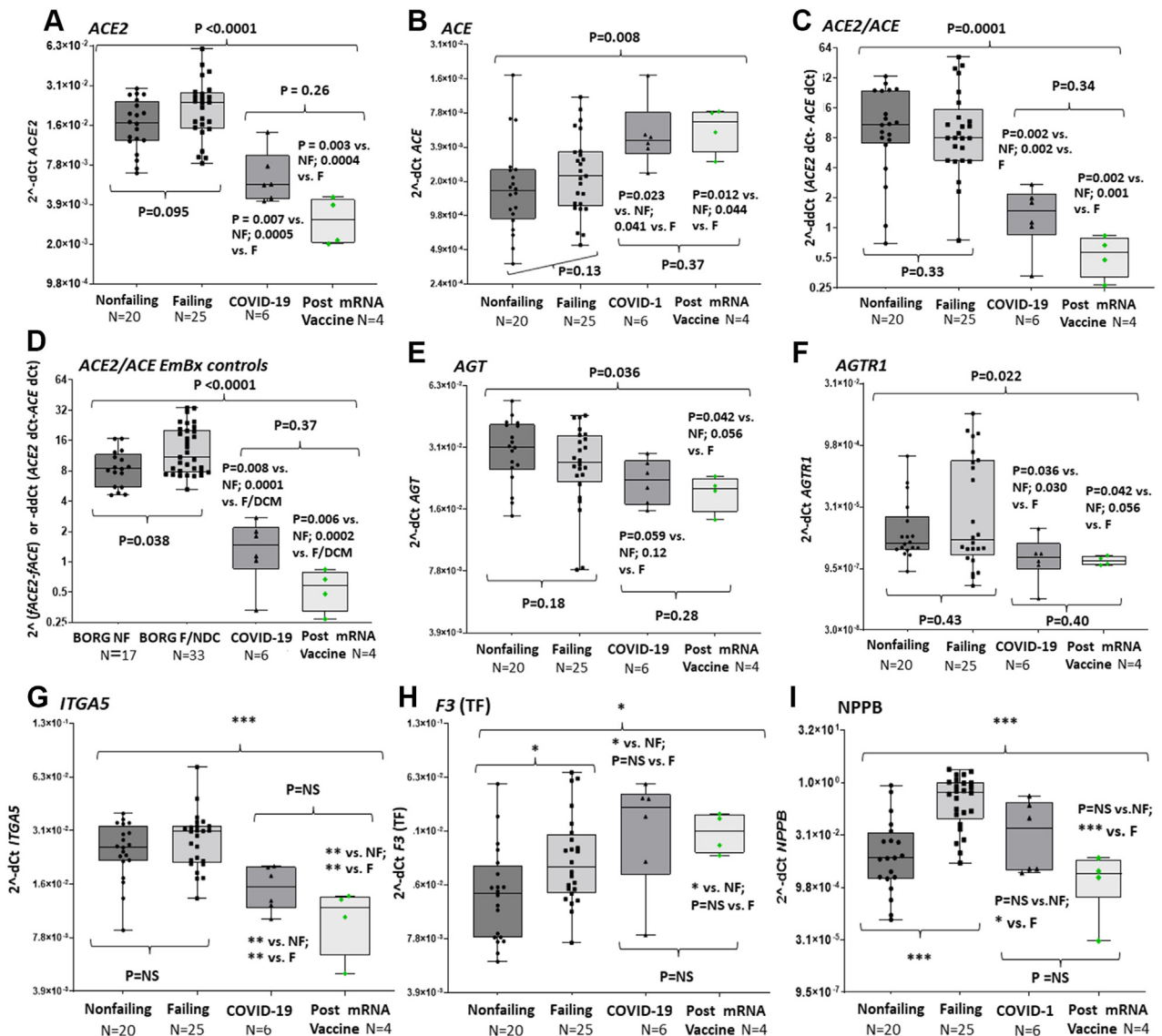
Box (median and [Q1, Q3]) and whisker plots for mRNA abundance as $2^{-\Delta Ct}$ (2^{Δ}-dCt) referenced to GAPDH[22,48] for explanted heart interventricular septum (nonfailing [NF]: n = 20; failing [F]: n = 25 [15 nonischemic dilated cardiomyopathy, 10 ischemic cardiomyopathy]), 17 endomyocardial biopsy (EmBx) NF control samples from the BORG study,[18,22] 33 F nonischemic cardiomyopathy (NDC) patients from BORG, 6 COVID-19 subjects, and 4 post-vaccine myocardial injury subjects. *ACE2/ACE* from the BORG study is calculated from microarray fluorescence units that are log2 transformed. Significance levels are indicated above or below box plot: *P < 0.05; **P < 0.01; ***P < 0.001; NS (nonsignificant): P ≥ 0.05. *ACE*, angiotensin I–converting enzyme gene; *ACE2*, angiotensin-converting enzyme 2 gene; *AGT*, angiotensinogen gene; *AGTR1*, angiotensin II receptor type 1 gene; *ITGA5*, integrin subunit alpha 5 gene; *F3*, coagulation factor III (tissue factor) gene; NPPB = natriuretic peptide B.

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[17] 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 and 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 and 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 and 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 and 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 1 receptor and a biosensor of angiotensin II generation,[19] 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 and Table 2**).

Expression of *ITGA5* mRNA, a possible CoV-2 co-receptor[15,20] whose encoded protein binds to and is co-regulated with *ACE2* in eccentric remodeling,[15] 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 and Table 2**). The full-length transcript (flTF) of the *F3* or tissue factor (TF) gene[21,22] 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 flTF having higher expression in failing vs nonfailing control samples (by 1.6-fold; *P* = 0.027) (**Figure 3H and 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 and 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.

TABLE 2 Median Fold (Q1, Q3) Difference vs Explanted Heart IVS of EmBx Nonfailing of Failing Control Samples

Control Group	ACE2		ACE		ACE2/ACE		AGT	
	COV	Vax	COV	Vax	COV	Vax	COV	Vax
NF Expl IVS (n = 20)	−2.7ᵃ (1.6-4.0)	−5.7ᵃ (3.6-8.0)	2.8ᵃ (1.7-6.3)	3.6ᵃ (1.9-6.9)	−7.9ᵃ (3.9-16.2)	−20.0ᵃ (10.7-38.4)	−1.5 (1.1-1.9)	−1.6ᵃ (1.2-2.1)
Failing Expl IVS (n = 25)	−3.4ᵃ (2.0-5.2)	−7.0ᵃ (4.4-11.7)	2.1ᵃ (1.2-4.3)	2.6ᵃ (1.3-4.7)	−6.6ᵃ (3.6-17.6)	−16.7ᵃ (9.6-36.2)	−1.3 (0.9-1.7)	−1.4 (1.1-1.9)
NF EmBx (n = 17)	–	–	–	–	−6.1ᵃ (4.0-10.6)	−16.2ᵃ (10.2-24.8)	–	–
Failing EmBx (n = 33)	–	–	–	–	−9.0ᵃ (5.1,-18.8)	−25.7ᵃ (14.5-38.9)	–	–

TABLE 2 Continued

Control Group	AGTR1		ITGA5		F3 (TF)		NPPB	
	COV	Vax	COV	Vax	COV	Vax	COV	Vax
NF Expl IVS (n = 20)	−2.7ᵃ (1.4-17.3)	−2.8ᵃ (1.8-9.7)	−1.7ᵃ (1.2-2.2)	−2.4ᵃ (1.8-3.4)	2.6ᵃ (1.3-4.4)	2.4ᵃ (1.5-3.5)	3.2 (1.4-32.8)	−20.0 (10.9-38.4)
Failing Expl IVS (n = 25)	−4.4ᵃ (1.2-22.4)	−3.2 (1.4-27.5)	−1.8ᵃ (1.4-2.6)	−2.6ᵃ (2.0-3.9)	1.7 (1.3-2.9)	1.5 (0.9-2.2)	−7.9ᵃ (1.8-173)	−16.7ᵃ (9.6-36.2)
NF EmBx (n = 17)	–	–	–	–	–	–	–	–
Failing EmBx (n = 33)	–	–	–	–	–	–	–	–

ᵃ$P < 0.05$ from data in Figure 3.
  Abbreviations as in Table 1.

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 time[23] the initial protocol was developed, TnI and BNP were used as biomarkers of myocardial injury. All 7 COVID-19 patients had elevations 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,[5,25] there have also been reported cases with no histopathologic evidence of inflammation.[4,6]

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

FIGURE 4 Scatterplot of Linear Discriminant Analysis by Group, Defined by mRNA Abundance of 7 Candidate Gene Transcripts
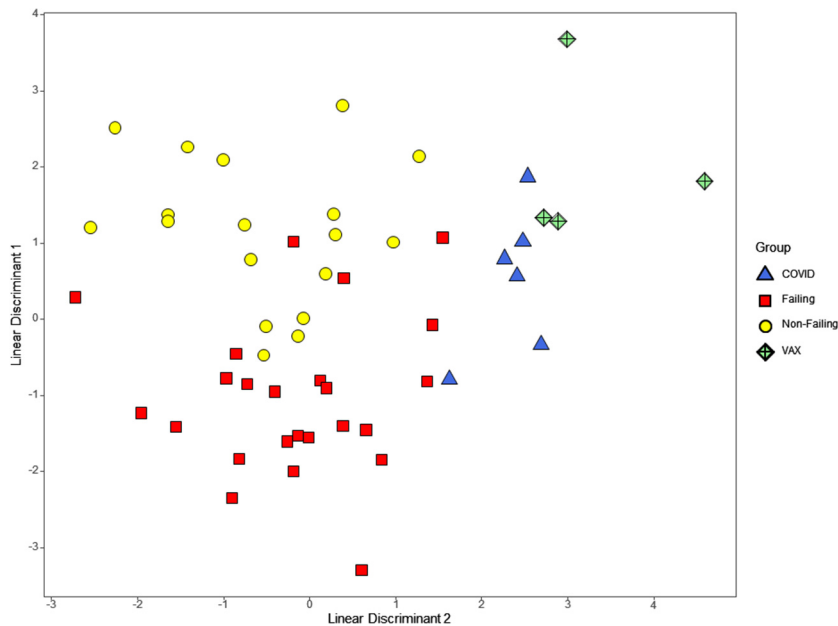
The 4 mRNA vaccine (VAX) patients classified with the 6 patient COVID-19 group with 97.7 ± 2.2% posterior probability. The probabilities for being classified with the nonfailing (n = 18) or failing (n = 25) explanted heart group are, respectively, 0.2 ± 0.3% and 2.1 ± 2.1% (Table 3). In the nonfailing group, 2 of the 20 hearts are not represented, because mRNA abundance of *AGTR1* was too low to be measured and expression of all 7 genes was required for the linear discriminant analysis.

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 control samples 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-19[27,28] 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 cells[13] 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

TABLE 3 Linear Discriminant Analysis Posterior Probabilities and Standardized Coefficients

Group	Probability of Vaccine Patient Membership (95% CI)	Gene Predictor (by mRNA ΔCt[a])	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
–	–	*NPPB*	1.04	−0.66
–	–	TF (*F3*)	0.35	−0.02

[a]ΔCts normalized for between-gene differences in absolute expression; values are inversely related to mRNA abundance.

angiotensin II production[19] and whose mRNA 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 (flTF) 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,[13,14,29] 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,[13,37] 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.

ADDRESS FOR CORRESPONDENCE: Dr Natasha Altman, Division of Cardiology, University of Colorado Anschutz Medical Campus, 12631 East 17th Avenue, B-130, Aurora, Colorado 80045, USA. E-mail: natasha.altman@cuanschutz.edu. OR Dr Michael Bristow, Division of Cardiology, University of Colorado Anschutz Medical Campus, 12631 East 17th Avenue, B-130, Aurora, Colorado 80045, USA. E-mail: michael.bristow@cuanschutz.edu.

PERSPECTIVES

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."

REFERENCES

1. Puntmann VO, Carerj ML, Wieters I, et al. Outcomes of cardiovascular magnetic resonance imaging in patients recently recovered from coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* 2020;5:1265-1273.

2. Italia L, Tomasoni D, Bisegna S, et al. COVID-19 and heart failure: from epidemiology during the pandemic to myocardial injury, myocarditis, and heart failure sequelae. *Front Cardiovasc Med.* 2021;8:713560.

3. Escher F, Pietsch H, Aleshcheva G, et al. Detection of viral SARS-CoV-2 genomes and histopathological changes in endomyocardial biopsies. *ESC Heart Fail.* 2020;7:2440-2447.

4. Rosner CM, Genovese L, Tehrani BN, et al. Myocarditis temporally associated with COVID-19 vaccination. *Circulation.* 2021;144:502-505.

5. Verma AK, Lavine KJ, Lin C-Y. Myocarditis after COVID-19 mRNA vaccination. *N Engl J Med.* 2021;385:1332-1334.

6. Larson KF, Ammirati E, Adler ED, et al. Myocarditis after BNT162b2 and mRNA-1273 vaccination. *Circulation.* 2021;144:506-508.

7. Patone M, Mei XW, Handunnetthi L, et al. Risks of myocarditis, pericarditis, and cardiac arrhythmias associated with COVID-19 vaccination or SARS-CoV-2 infection. *Nat Med.* 2022;28:410-422.

8. Oster ME, Shay DK, Su JR, et al. Myocarditis cases reported after mRNA-based COVID-19 vaccination in the US from December 2020 to August 2021. *JAMA.* 2022;327:331-340.

9. Kociol RD, Cooper LT, Fang JC, et al. Recognition and initial management of fulminant myocarditis: a scientific statement from the American Heart Association. *Circulation.* 2020;141:e69-e92.

10. Ozieranski K, Tyminska A, Jonik S, et al. Clinically suspected myocarditis in the course of severe acute respiratory syndrome novel coronavirus-2 infection: fact or fiction? *J Card Fail.* 2021;27:92-96.

11. Bayati A, Kumar R, Francis V, McPherson PS. SARS-CoV-2 infects cells after viral entry via clathrin-mediated endocytosis. *J Biol Chem.* 2021;296:100306.

12. Ogunlade BO, Lazartigues E, Filipeanu CM. Angiotensin type 1 receptor-dependent internalization of SARS-CoV-2 by angiotensin-converting enzyme 2. *Hypertension.* 2021;77:e42-e43.

13. Lei Y, Zhang J, Schiavon CR, et al. SARS-CoV-2 Spike protein impairs endothelial function via downregulation of ACE 2. *Circ Res.* 2021;128:1323-1326.

14. Sui Y, Jianping L, Venzon DJ, Berzofsky JA. SARS-CoV-2 Spike protein suppresses ACE2 and type I interferon expression in primary cells from macaque lung bronchoalveolar lavage. *Front Immunol.* 2021;12:658428.

15. Bristow MR, Zisman LS, Altman NL, et al. Dynamic regulation of SARS-Cov-2 binding and cell entry mechanisms in remodeled human ventricular myocardium. *J Am Coll Cardiol Basic Transl Science.* 2020;5:871-883.

16. Kao DP, Lowes BD, Gilbert EM, et al. Therapeutic molecular phenotype of β-blocker associated reverse remodeling in nonischemic dilated cardiomyopathy. *Circ Cardiovasc Genet.* 2015;8:270-283.

17. Hahn VS, Knutsdottir H, Luo X, et al. Myocardial gene expression signatures in human heart failure with preserved ejection fraction. *Circulation.* 2021;143:120-134.

18. Toni LS, Carroll IA, Jones KL, et al. Sequential analysis of myocardial gene expression with phenotypic change: use of cross-platform concordance to strengthen biologic relevance. *PLoS One.* 2019;14:e0221519.

19. Zisman LS, Asano K, Dutcher DL, et al. Differential regulation of cardiac angiotensin converting enzyme binding sites and AT₁ receptor density in the failing human heart. *Circulation.* 1998;98:1735-1741.

20. Beddingfield BJ, Iwanaga N, Chapagain PP, et al. The integrin binding peptide, ATN-161, as a novel therapy for SARS-CoV-2 infection. *J Am Coll Cardiol Basic Transl Science.* 2021;6:1-8.

21. Szotowski B, Goldin-Lang P, Antoniak S, et al. Alterations in myocardial tissue factor expression and cellular localization in dilated cardiomyopathy. *J Am Coll Cardiol Basic Transl Science.* 2005;245:1081-1089.

22. Eisenreich A, Rauch U. Regulation and differential role of the tissue factor isoforms in cardiovascular biology. *Trends Cardiovasc Med.* 2010;20:199-203.

23. Han H, Xie L, Liu R, et al. Analysis of heart injury laboratory parameters in 273 COVID-19 patients in one hospital in Wuhan, China. *J Med Virol*. 2020;92:819–823.

24. Courand PY, Harbaoui B, Bonnet M, Lantelme P. Spontaneous coronary artery dissection in a patient with COVID-19. *J Am Coll Cardiol Intv*. 2020;13:e107–e108.

25. Nguyen TD, Mall G, Westphal JG, Weingärtner O, Möbius-Winkler S, Schulze PC. Acute myocarditis after COVID-19 vaccination with mRNA-1273 in a patient with former SARS-CoV-2 infection. *ESC Heart Fail*. 2021;8:4710–4714.

26. Kwong RY, Chan AK, Brown KA, et al. Impact of unrecognized myocardial scar detected by cardiac magnetic resonance imaging on event-free survival in patients presenting with signs or symptoms of coronary artery disease. *Circulation*. 2006;113:2733–2743.

27. Sakamoto A, Kawakami R, Kawai K, et al. ACE2 (angiotensin-converting enzyme 2) and TMPRSS2 (transmembrane serine protease 2) expression and localization of SARS-CoV-2 infection in the human heart. *Arterioscler Thromb Vasc Biol*. 2021;41:542–544.

28. Bearse M, Hung YP, Krauson AJ, et al. Factors associated with myocardial SARS-CoV-2 infection, myocarditis, and cardiac inflammation in patients with COVID-19. *Mod Pathol*. 2021;34:1345–1357.

29. Bailey AL, Dmytrenko O, Greenberg L, et al. SARS-CoV-2 infects human engineered heart tissues and models COVID-19 myocarditis. *J Am Coll Cardiol Basic Transl Science*. 2021;6:331–345.

30. Crackower MA, Sarao R, Oudit GY, et al. Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature*. 2002;417: 822–828.

31. Sriram K, Insel PA. A hypothesis for pathobiology and treatment of COVID-19: the centrality of ACI/ACE2 imbalance. *Br J Pharmacol*. 2020;177: 4825–4844.

32. Mann DL. Angiotensin II as an inflammatory mediator: evolving concepts in the role of the renin angiotensin system in the failing heart. *Cardiovasc Drugs Ther*. 2002;16:7–9.

33. Antoniak S, Mackman N. Multiple roles of the coagulation protease cascade during virus infection. *Blood*. 2014;123:2605–2613.

34. Key NS, Vercellotti GM, Winkelmann JC, et al. Infection of vascular endothelial cells with herpes simplex virus enhances tissue factor activity and reduces thrombomodulin expression. *Proc Natl Acad Sci*. 1990;87:7095–7099.

35. Zelaya H, Rothmeier AS, Ruf W. Tissue factor at the crossroad of coagulation and cell signaling. *J Thromb Haemost*. 2018;16:1941–1952.

36. Rosell A, Havervall S, von Meijenfeldt F, et al. Patients with COVID-19 have elevated levels of circulating extracellular vesicle tissue factor activity that is associated with severity and mortality—brief report. *Arterioscler Thromb Vasc Biol*. 2021;41:878–882.

37. Nuovo GJ, Magro C, Shaffer T, et al. Endothelial cell damage is the central part of COVID-19 and a mouse model induced by injection of the S1 subunit of the spike protein. *Ann Diagn Pathol*. 2021;51:151682.

38. Röltgen K, Nielsen SCA, Silva O, et al. Immune imprinting, breadth of variant recognition, and germinal center response in human SARS-CoV-2 infection and vaccination. *Cell*. 2022;185:1025–1040. e14.