JACC REVIEW TOPIC OF THE WEEK

Pathological Evidence for SARS-CoV-2 as a Cause of Myocarditis

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Rika Kawakami, MD,[a],* Atsushi Sakamoto, MD,[a],* Kenji Kawai, MD,[a] Andrea Gianatti, MD,[b] Dario Pellegrini, MD,[b] Ahmed Nasr, MD,[b] Bob Kutys, PA,[a] Liang Guo, PHD,[a] Anne Cornelissen, MD,[a] Masayuki Mori, MD,[a] Yu Sato, MD,[a] Irene Pescetelli, MD,[b] Matteo Brivio, MD,[b] Maria Romero, MD,[a] Giulio Guagliumi, MD,[b] Renu Virmani, MD,[a] Aloke V. Finn, MD[a,c]

ABSTRACT

To investigate whether severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2)–induced myocarditis constitutes an important mechanism of cardiac injury, a review was conducted of the published data and the authors' experience was added from autopsy examination of 16 patients dying of SARS-CoV-2 infection. Myocarditis is an uncommon pathologic diagnosis occurring in 4.5% of highly selected cases undergoing autopsy or endomyocardial biopsy. Although polymerase chain reaction–detectable virus could be found in the lungs of most coronavirus disease-2019 (COVID-19)–infected subjects in our own autopsy registry, in only 2 cases was the virus detected in the heart. It should be appreciated that myocardial inflammation alone by macrophages and T cells can be seen in noninfectious deaths and COVID-19 cases, but the extent of each is different, and in neither case do such findings represent clinically relevant myocarditis. Given its extremely low frequency and unclear therapeutic implications, the authors do not advocate use of endomyocardial biopsy to diagnose myocarditis in the setting of COVID-19. (J Am Coll Cardiol 2021;77:314–25) © 2021 by the American College of Cardiology Foundation.

Infection with the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) confers significant risk of morbidity and mortality. Previous epidemiological data suggest that approximately 20% of hospitalized patients have evidence of cardiac injury as indicated by elevated levels of high-sensitivity troponins (hs-cTnI) (1). In 1 study in which troponin I was measured within 24 h of admission to assess cardiac damage, 36% of patients had elevated troponin I concentrations. After adjusting for disease severity and relevant risk factor differences, even small amounts of myocardial injury were associated with increased mortality (2). However, much remains unknown about the nature of myocardial injury in patients with coronavirus disease-2019 (COVID-19). In the absence of obstructive epicardial coronary disease but evidence of myocardial injury (defined as positive troponin with or without wall motion abnormalities), physicians often default to the diagnosis of myocarditis as the underlying cause using data such as clinical and imaging markers of myocyte injury. Indeed, most published cases of presumed myocarditis induced by COVID-19 infection were based on blood troponin levels or cardiac magnetic resonance imaging (CMR) without tissue diagnosis (3–5). However, direct evidence for myocarditis in the setting

HIGHLIGHTS

- Whether myocarditis is a cause of myocardial injury in patients with COVID-19 is uncertain.

- Myocarditis is uncommon in autopsy or EMB in cases of COVID-19.

- Further work is needed to fully understand the cardiac effects of COVID-19 infection.

of SARS-CoV-2 remains very limited (6,7). A complete understanding of the pathogenesis of cardiac injury in the setting of COVID-19 infection is critical for the development of appropriate treatments.

PATHOPHYSIOLOGY OF COVID-19–INDUCED MYOCYTE INFECTION/INJURY

Acute myocarditis is a disease with variable clinical progression and presentation, making it one of the most challenging diagnoses in cardiology. Several mechanisms are hypothesized to be involved in the pathogenesis of COVID-19-induced myocarditis. To date, there is very little evidence supporting direct destruction of cardiomyocytes through the occurrence of virus-mediated lysis with damage to cardiac structures, resulting in myocyte injury and cardiac dysfunction. The quantification of viral load in 39 consecutive autopsy cases from Germany demonstrated that SARS-CoV-2 could be documented in 24 of 39 (61.5%) hearts with 16 of 39 (41%) having copy numbers higher than 1,000 copies per ug RNA (6). Virus replication of SARS-CoV-2 defined by detection of the (−) strand replicate of the RNA genome was documented in 5 of the patients with the highest viral load, but in situ hybridization confirmed the virus presence in interstitial cells within cardiac tissue but not in myocytes. Virus presence was not associated with increased infiltration of mononuclear cells into the myocardium, and no myocarditis was present in any of these cases according to the Dallas criteria.

Another potential mechanism of cardiac injury that has been proposed is direct entry of the virus into endothelial cells in the heart without necessarily entering myocytes. Direct endothelial infection has been documented in autopsy hearts as well as in glomerular endothelial cells using electronic microscopy with identification of virus particles, although in some cases their appearance and location within cells was not typical of coronavirus infected cells (7-9). We believe other techniques such as in situ hybridization should be used to confirm such findings. In our own experience using both techniques, we have not been able to document a single case of endothelial infection by SARS-CoV-2 in the heart. From these data, it seems difficult to conclude that endothelial tropism is a major mechanism of COVID-19-induced cardiac injury without more confirmation.

Another, and perhaps better supported, idea is that cardiac injury can be induced via hyperactivation of the immune system characterized by the release of multiple inflammatory mediators, including interleukins, tumor necrosis factors, and so on. Circulating levels of these factors that exceed normal thresholds can result in collateral damage. The term *cytokine storm* has been used to describe this condition, which has been reported in severely ill COVID-19 patients (10). Microvascular and macrovascular thrombi that result from the activation of platelets, neutrophils, and other proteins may contribute to vascular occlusion and cell death (11). We recently reported a case of myocardial infarction and cardiogenic shock caused by cardiac microvascular thrombosis in a young woman with COVID-19 (12). In this case, the virus was not detectable by polymerase chain reaction (PCR) in the heart. However, the exact pathogenesis of cardiac injury induced by COVID-19 infection remains to be elucidated, but we believe the most compelling evidence points toward cytokine-storm-related effects.

CLINICAL AND PATHOLOGICAL DIAGNOSIS OF COVID-19-INDUCED MYOCARDIAL INJURY

Despite having endured the COVID-19 pandemic for over 6 months, many questions still remain about the best approaches for determining causes of cardiac injury in both hospitalized and nonhospitalized patients. In most cases, cardiac injury appears to result within the context of the overall respiratory infection rather than the first manifestation of disease. Coagulation abnormalities that may result as a part of the immune response to the disease have been shown to predispose these patients toward thrombotic processes, both in the venous and arterial circulation (11). A single-center observational study on COVID-19 subjects with ST-segment elevation myocardial infarction (STEMI) documented higher troponin levels, multivessel thrombosis, and stent thrombosis when compared with COVID-19 patients without STEMI (13). However, a substantial number of cases in subjects with STEMI had no evidence of coronary occlusion. In 1 case series from New York involving 18 patients with a confirmed diagnosis of COVID-19 and

TABLE 1   Published Cases of COVID-19 With Examination of the Heart at Autopsy or Biopsy for the Presence of Myocarditis

PMID	First Author (Ref.), Journal	Total Number of Cases	Type of Sample	Age, yrs, Mean (Range)	Sex
32267502	Sala et al. (24), Eur Heart J	1	EMB	43	F
32275347	Tavazzi et al. (25), Eur J Heart Fail	1	EMB	69	M
32529795	Escher et al. (26), ESC Heart Fail	5	EMB	49 (36–62)	4 M 1 F
32562489	Wenzel et al. (27), Cardiovasc Res	2	EMB	39, 36	2 M
32432787	Pesaresi et al. (19), Eur Rev Med Pharmacol Sci	1	Aut	84	F
32085846	Zhe et al. (35), Lancet Resp Med	1	Aut	50	M
32275742	Barton et al. (36), Am J Clin Pathol	2	Aut	77, 42	2 M
32682491	Beadley et al. (37), Lancet	14	Aut	71 (42–84)	6 M 8 F
32434133	Buja et al. (38), Cardiovasc Path	23	Aut	NA (34–76)	12 M 7 F 4 NA
32374815	Wichmann et al. (39), Ann Intern Med	12	Aut	73 (52–87)	9 M 3 F
32422076	Lax et.al. (40), Ann Internal Med	11	Aut	80 (66–91)	8 M 3 F
32291399	Tian et al. (41), Modern Pathol	4	Aut	73 (59–81)	3 M 1 F
32325026	Varga et al. (8), Lancet	3	Aut	66 (58–71)	2 M 1 F
NA	Bryce et al. (28), medRxiv	25	Aut	69 (34–94)	NA
32552178	Beigmohammadi et al. (42), Int J Surg Pathol	7	Aut	68 (46–84)	5 M 2 F
32766543	Rapkiewicz et al. (43), EClinicalmedicine	7	Aut	57 (44–65)	3 M 4 F
32968776	Basso et al. (29), Eur Heart J	21	Aut	69 (44–86)	15 M 6 F
32689809; 32473124	Fox et al. (7), Circulation; Fox et al. (44), Lancet Respir Med‖	22	Aut	69 (44–79)	NA
32730555	Lindner et al. (6), JAMA Cardiol	39	Aut	85 (78–89)	16 M 23 F
Total		201 cases	EMB; 9 Aut; 192	(34–94)	89 M 61 F 51 NA

*"Borderline myocarditis" cases counted as not diagnostic of myocarditis. †Very low level of virus was detected by PCR (likely contamination by circulating virus rather than direct infection). ‡5 patients had virus detected in other tissues although not clearly stated which tissues virus was detected in. §12 patients showed new ECG abnormality including atrial fibrillation, premature ventricular beats, bundle branch block, and ST-segment abnormality. Only 5 cases were evaluated by cardiac ultrasound without any evidence of impaired cardiac function. ‖The same case series was reported in other papers (PMID 32689809, 32473124). ¶Virus load was lower than 1,000 copies in 8 patients and was above 1,000 copies in 16 patients.

Aut = autopsy; CAD = coronary artery disease; CHF = chronic heart failure; CKD = chronic kidney disease; COVID-19 = coronavirus disease-2019; DCM = dilated cardiomyopathy; DM = diabetes mellitus; EF = ejection fraction; EM = electron microscopy; EMB = endomyocardial biopsy; HCM = hypertrophic cardiomyopathy; HD = heart disease; HF = heart failure; H/O = history of; HTN = hypertension; IC = immunocompromised; Malig = malignancy; NA = no available information; PAD = peripheral artery disease; TEM = transmission electron microscopy; VD = valve disease.

ST-segment elevation, 44% received a diagnosis of acute coronary thrombosis causing myocardial infarction, and 56% had evidence of noncoronary myocardial injury (defined as nonobstructive disease on coronary angiography) (14). It is precisely in cases such as these, or in situations where troponin levels are elevated but clinical suspicion of acute coronary syndrome is low, where diagnostic dilemmas may occur. Because the clinical presentation of myocarditis may vary and may include vague or nonspecific symptoms such as fatigue, dyspnea, palpitations, and chest discomfort, the diagnosis of myocarditis in this setting is not straightforward.

The World Health Organization defines myocarditis as an inflammatory disease of the myocardium diagnosed by established histological, immunological, immunohistochemical, and molecular criteria with endomyocardial biopsy (EMB) used to gain certainty on the diagnosis and to potentially identify etiology (15). The current indication for EMB is "a strong reason to believe that the results will have a meaningful effect on subsequent therapeutic decisions" according to the American College of Cardiology guidelines (16). These guidelines state that EMB should be performed in the setting of new-onset heart failure with hemodynamic compromise or new

TABLE 1 Continued

H/O Underlying HD, Type	Impaired Cardiac Function	Virus Detected	Type and Cells	Case With Evidence of Myocarditis* According to Authors
0	EF 43%	No	(T-cell)+ necrosis (limited)	1
0	EF 34%	Yes	Low-grade inflammation, no necrosis	0
NA	4/5 Impaired	5	Active lymphocytic myocarditis	1
1 HTN, 1 HF, 1 CAD	EF 60% EF 30%	2	Lymphocyte infiltration no necrosis	0
NA	NA	Yes (TEM)	Virus in myocytes by TEM, no inflammatory cells	0
0	NA	No	Interstitial inflammatory cells	0
1 HTN	NA	NA	0	0
9 HTN, 3 CAD, 4 HF, 8 CKD, 5 DM, 5 obesity	NA	+†	Lymphocyte infiltration with necrosis	1
10 HTN, 5 DM 9 obesity	NA	NA	Lymphocytic myocarditis	1
2 HTN, 6 CAD 2 CKD, 1 PAD 3 DM, 3 obesity	NA	NA‡	Lymphocytic myocarditis	1
9 HTN, 5 DM, 3 CAD, 2 Malig	NA	NA	Focal lymphocytic infiltrate	0
1 DM, HTN	NA	NA	0	0
2 HTN, 1 CAD 1 DM, 1 obesity	1/3 EF low	NA	0, Endothelialitis	0
HTN 63%, CAD 31%, DM 40%	NA	NA	2 cases of interstitial chronic inflammation	0
4 HTN, 1 IC 1 DM, 1 VD	NA	NA	Inflammation and necrosis but no myocarditis	0
6 HTN, 5DM 5 obesity	NA	Non in 4 cases (EM)	1 case of focal lymphocytic infiltration with myocardial necrosis	1
16 HTN, 7 DM, 3 CAD, 2 CKD	2/21 died due to cardiogenic shock or cardiac arrest§	NA	Multifocal lymphocyte infiltration with myocardial necrosis	3
18 HTN, 1 CAD, 11 DM, 4 CKD, 9 obesity	2/22 HF	NA	Scattered single myocyte necrosis without significant lymphocyte infiltration	0
17 HTN, 32 CAD, 7 DM	NA	24¶	NA	0
Myocarditis Author Reported				
Myocarditis 9 (4.5%)				

ventricular arrhythmias (Class I recommendation), but the timing and exact criteria for EMB in cases of myocarditis, especially in the setting of COVID-19 infection, remain uncertain.

Histological evidence from cardiac biopsies and autopsy hearts for myocarditis was initially diagnosed by the Dallas criteria (1985) and defined as histological evidence of inflammatory infiltrates within the myocardium associated with myocyte degeneration or necrosis (myocytolysis) of non-ischemic origin (17). In the absence of necrosis and the presence of lymphocytic infiltrate, myocarditis was defined as borderline. In addition to a sampling error in EMBs containing PCR-detectable viral pathogens, the Dallas criteria were absent in 50% of virus positive cases (18). For these reasons, the use of Dallas criteria alone to diagnose myocarditis has been discouraged. More recently, immunohistochemical

criteria have been added to address these shortcomings. By immunohistochemistry, myocarditis is defined according to the presence of an abnormal inflammatory infiltrate, defined as follows: ≥ 14 leukocytes/mm^2 including up to 4 monocytes/mm^2 with the presence of CD3-positive T-lymphocytes ≥ 7 cells/ mm (19). Cell counts must be accompanied by evidence of myocyte degeneration and necrosis of non-ischemic origin.

The appearance of inflammatory infiltrates alone in the absence of myocyte necrosis can be seen in normal myocardium as has been reported by us and others in individuals dying a noninfectious death (20,21). From our experience, it is the pattern of inflammatory infiltrate (concentrated collection of inflammatory cells [predominance of lymphocytes versus macrophages] around myocytes), extent, and presence of myocyte necrosis that defines

myocarditis (16). Molecular biology diagnosis is defined as histological evidence for myocarditis associated with positive viral PCR to confirm the diagnosis of infectious myocarditis. Virus-negative myocarditis is defined as histological myocarditis with negative viral PCR.

Practically speaking, EMB is not routinely used to diagnose myocarditis and patients may be given the diagnosis of COVID-19 myocarditis based upon circumstantial evidence such as abnormal echocardiography or magnetic resonance imaging examinations. In 1 series where cardiac involvement was evaluated (using CMR) in 15 patients who reported cardiac symptoms but were considered recovered from COVID-19, 58% had abnormal CMR findings consisting mostly of myocardial edema, fibrosis, and impaired right ventricular function (3). In another series of 100 recovered patients, 78% had detectable cardiac involvement via CMR with 75% having detectable troponin levels (4). These studies raise the possibility that months after infection, ongoing myocardial inflammation with resultant left ventricular dysfunction may occur. In the following text, we will discuss data regarding the findings of EMB in cases of suspected COVID-19-induced myocarditis or unexplained heart failure and the low overall diagnostic yield of such investigations. Our opinion on the role of EMB in cases of suspected COVID-19 myocarditis is given later in this review.

WHAT IS THE EVIDENCE FOR SARS-CoV-2 AS A CAUSE OF MYOCARDITIS?

Coronaviruses are a family of enveloped, positive-sense, single-stranded, and highly diverse RNA viruses. To date, there have been 3 documented, highly pathogenic, and lethal human coronaviruses: SARS-CoV, Middle East Respiratory Syndrome-CoV, and SARS-CoV-2. SARS-CoV-2 shares approximately 79.5% genomic homology with SARS-CoV, but only about 50% with Middle East Respiratory Syndrome-CoV, indicating that SARS-CoV-2 is closer to SARS-CoV (22). SARS-CoV was highly lethal, but its presence was mitigated effectively by intense public health measures. Although many similarities exist between SARS-CoV and SARS-CoV-2, important differences also exist in transmissibility and disease pathogenesis. Nonetheless, lessons learned from the effects of SARS-CoV may have some degree of relevance to our current understanding of how SARS-CoV-2 might affect the heart. Autopsy data from 20 patients who died during the SARS outbreak in 2003 identified viral RNA in the hearts of 7 of these patients (23). Immunohistochemical staining of postmortem myocardial tissue using macrophage-specific marker (CD68) revealed a significant amount of macrophage infiltration, which was increased in patients who had evidence of SARS-CoV (by PCR) in their hearts with only minor elevation in those without SARS-CoV. By contrast, immunohistochemical staining for T-cell specific (CD3) cell surface markers showed that myocardial lymphocytic infiltration was minimal with no differences in lymphocyte counts between those with and without SARS-CoV in their hearts. Although virus entry occurs predominantly via the angiotensin-converting enzyme receptor 2 for both SARS-CoV and SARS-CoV-2, organ tropism may be different given the differing clinical presentations and infectiousness of the 2 pathogens.

To date, 9 cases have been reported where EMB was performed in COVID-19-positive subjects (24-27). The diagnostic criteria for myocarditis were met in only 2 of these cases. In all studies, there was no direct evidence of SARS-CoV-2 within cardiomyocytes. For the most part, direct proof that SARS-CoV-2 infects myocytes leading to virus-induced necrosis and cell death with troponin release is lacking. In 1 of the cases that met the authors' diagnostic criteria for myocarditis, a 43-year-old woman had an inverted Takotsubo pattern in the setting of COVID-19 infection (24). The final diagnosis was virus-negative immune-mediated myocarditis, because the PCRs of EMB samples were negative for the SARS-CoV-2 genome. In another case, a 48-year-old man with newly diagnosed heart failure in the setting of COVID-19 infection underwent EMB (26). Histological assessment met the Dallas criteria with necrosis of myocytes and pronounced myocardial inflammation with macrophages and lymphocytes, and PCR of the EMB sample was positive for the viral genome (albeit at a very low level). A summary of the results of prior studies published to date on EMB samples in the setting of COVID-19 infection is shown in **Table 1**.

Another method to demonstrate the effect of COVID-19 on the heart is through autopsy studies. Although many studies have focused on the pulmonary findings of COVID-19, few studies have been conducted specifically examining the effects of COVID-19 on the heart. In the largest series of autopsies conducted in a New York hospital in subjects dying of COVID-19 infection, hearts from 25 cases were evaluated (28). Most hearts showed evidence of pre-existing atherosclerotic or hypertensive heart disease, with 60% of cases showing nonspecific patchy, mild interstitial chronic inflammation within the myocardium without associated myocyte necrosis. More recently, and as mentioned in the previous text,

TABLE 2 Relevant Clinical and Laboratory Information of the 16 COVID-19 Autopsy Cases Examined by CVPath

Case	Age (yrs)	Sex	Relevant Past Medical History	History of CV Disease	Duration of Hospitalization (Days)	Max Ventilatory Support	ICU Stay	ECG Suggestive of Ischemia*	Troponin I Level (ng/l)	D-Dimer (ng/ml)
1	80	M	None	None	7	CPAP	No	No	N/A	N/A
2†	28	M	None	None	0	None	No	N/A	N/A	N/A
3	80	F	GIM	None	3	CPAP	No	No	N/A	1,790
4	65	F	HM	None	11	Mechanical	Yes	No	79	26,421
5	80	F	None	None	3	None	No	No	N/A	N/A
6	43	F	None	None	3	Mechanical	Yes	Yes	45,844	662
7	86	M	None	CAD (post-stent implantation), PM implantation	14	CPAP	No	No	N/A	N/A
8	57	M	None	None	9	CPAP	No	N/A	N/A	4,918
9	81	M	None	OMI (post-stent implantation), A Fib, Valve surgery	1	Mechanical	Yes	Yes	125,000	N/A
10	66	M	None	OMI (post-stent implantation)	7	Mechanical	Yes	No	381	35,000
11	73	M	HM	None	3	CPAP	No	No	N/A	2,672
12	63	M	None	CAD (post-stent implantation)	17	Mechanical	Yes	No	1,000	5,000
13	86	M	None	None	29	CPAP	No	No	N/A	N/A
14	57	M	None	None	8	Mechanical	Yes	No	N/A	N/A
15	56	M	None	None	2	Mechanical	Yes	No	25	19,118
16	82	F	Liver cirrhosis, breast cancer	None	5	CPAP	No	No	N/A	N/A
Total	**70 (57–80)**	**Male 11 (69%)**	**—**	**Coronary stent implantation 4 (25%)**	**6 (3.0–9.5)**	**Mechanical 7 (44%)**	**7 (44%)**	**2 (13%)**		

*Ischemic ECG changes defined as ST-segment elevation/depression >0.1 mV, new left bundle branch block, inverted T-wave. †Died suddenly at home (not hospitalized).

AFib = atrial fibrillation; CAD = coronary artery disease; CPAP = continuous positive airway pressure therapy; CV = cardiovascular; ECG = electrocardiogram; GIM = gastrointestinal malignancy; HM = hematology malignancy; Mechanical = mechanical ventilation; N/A = not available; OMI = old myocardial infarction; PCI = percutaneous coronary intervention; PM = pacemaker.

Lindner et al. (6) quantified viral load in 39 consecutive autopsy cases from Germany and demonstrated that SARS-CoV-2 could be documented in 24 of 39 (61.5%); yet, none of these cases were found to meet the criteria for myocarditis. Recently Basso et al. (29) reported a multicenter autopsy case series examining 21 hearts from COVID-19 cases. They found myocarditis in 3 cases (3 of 21; 14.2%), defined as the presence of an inflammatory infiltrate associated with myocyte injury not due to other causes and observed in multiple foci (29). All 3 cases had CD3-positive lymphocytes (2 had CD4, 1 had CD8 predominance) as well as macrophages. The high rate of myocarditis reported in this paper may in part be due to selection bias, as cases were referred from 4 separate institutions. A recently published literature review of 277 autopsied hearts across 22 publications found that myocarditis was present in 20 hearts (7.2%), with closer examination by the authors revealing that most cases were likely not functionally significant, and in their opinion, the true prevalence was much lower

(<2%) (30). A list of the findings of published papers that included pathological analysis of hearts from COVID-19-positive subjects using specific and well-adopted criteria for the diagnosis of myocarditis are listed in Table 1. Of the collective 201 hearts (or EMB samples) examined in published series, in 9 cases (4.5%), investigators found some evidence of myocarditis of unclear extent and nature. In most of these cases, analysis for the virus in the heart was not performed and evidence for direct infection of cardiac myocytes has not been shown.

An import caveat to the analysis of these studies (as well as to the clinical diagnosis of SARS-CoV-2) is the possibility of false-negative results, which in the case of tissue samples, might be caused by either sampling errors or by degradation of viral RNA during the process of fixation prior to analysis (31). Indeed, a recent case report documented the occurrence of 2 subjects with clinically suspected myocarditis whose nasopharyngeal swab tests for COVID-19 were negative but the virus was recovered by PCR from EMB

TABLE 3   Pathological Findings of 16 COVID-19 Autopsy Cases Examined by CVPath

Case	Age (yrs)	Sex	Autopsy Determined Cause of Death	Heart Weight (g)	Pericardial Effusion at Autopsy	Quantification of Effusion (ml)
1	80	M	DAD	385	Yes	50-100
2	28	M	AH	444	No	<20
3	80	F	DAD	415	Yes	50-100
4	65	F	DAD	350	Yes	50-100
5	80	F	DAD, AH, PT	304	Yes	50-100
6	43	F	AMI, DAD, PT	278	Yes	50-100
7	86	M	DAD, AH	477	Yes	50-100
8	57	M	DAD, BP	393	Yes	50-100
9	81	M	AMI	900	Yes	50-100
10	66	M	DAD, AH	450	Yes	50-100
11	73	M	DAD, AH, PT	468	Yes	50-100
12	63	M	DAD, AH, PT	641	Yes	50-100
13	86	M	DAD, AH, PT	384	Yes	50-100
14	57	M	DAD, PT	373	Yes	50-100
15	56	M	DAD, PT	353	Yes	50-100
16	82	F	DAD, AMI	475	Yes	50-100
Total	**70 (57–80)**	**Male 11 (69%)**	**AMI 3 (19%), DAD 13 (81%), PT 8 (50%)**	**404 (368–470)**	**15 (94%)**	

Continuous values are expressed by a median (interquartile range). *Degree of inflammation in epicardium was classified into 3: mild ($\geq$50 and <100 per mm^2), moderate ($\geq$100 and <500 per mm^2), and severe ($\geq$500 per mm^2). †Additional examination by immunohistochemistry was done (see Figure 1). ‡Neutrophil infiltration in the area of acute myocardial infarction.

AH = alveolar hemorrhage; AMI = acute myocardial infarction; BP = bronchial pneumonia; DAD = diffuse alveolar damage due to coronavirus; LA = left atrium; Lym = lymphocytes; ND = not detected; Neut = neutrophils; Plasma = plasma cells; PT = pulmonary thrombus; RA = right atrium; y/n = yes or no; other abbreviations as in Table 2.

specimens (27). It is important to recognize the reliability of SARS-CoV-2 RNA tests when examining diagnostic pathology reports.

In addition to the possibility of false-negative PCR results during tissue sampling, it is also likely that given the low overall expression of ACE2 receptor in myocardial cells, tropism of SARS CoV-2 for the heart is not likely (32). Takotsubo syndrome is another potential cause of myocardial injury in the setting of electrocardiographic abnormalities and troponin-positive results with normal coronaries and should also be considered. In such cases, patients are known to have regional wall motion abnormalities, injury to myocytes, and infiltration of lymphocytes and macrophages in autopsy specimens. However, to be sure of the diagnosis, this requires exclusion of myocarditis. Troponin levels and CMR signs of edema can be found in both entities and each can mimic the other.

CVPath EXPERIENCE

In a series of 384 autopsy cases (239 cardiac, 51 noncardiac, and 94 non-natural deaths), inflammatory infiltrates were found in the heart in 18% of all cases, with multifocal infiltrates in 9%. Incidental infiltrates were most frequent in natural noncardiac deaths (31%), but were also seen in drug-related deaths (20%) and cardiac deaths (16%), and were least frequent in traumatic deaths (16%) (21). We previously reported autopsy findings in the hearts of patients dying during viral infection (in this case during the AIDS epidemic) and showed that focal mild inflammatory infiltrates not meeting the definition of myocarditis in the heart were not uncommon (31%) (20). We also found focal mild mononuclear inflammatory infiltrates in 10 of 24 (42%) hearts in sudden traumatic deaths. Because these foci were not associated with myocyte necrosis, they were not diagnosed as myocarditis.

We have examined the hearts of 15 subjects dying with COVID-19 infection from the Lombardy region outbreak (Papa Giovanni Hospital, Bergamo, Italy) and 1 case from the Medical Examiner in Baltimore, Maryland. The hearts of 15 unselected patients dying from COVID-19 at Ospedale Papa Giovanni XXIII (Bergamo, Italy) and undergoing autopsy were collected and sent to CVPath Institute (Gaithersburg, Maryland) for detailed pathological analysis. The study protocol was reviewed and approved by the ethical committee at Ospedale Papa Giovanni XXIII Bergamo (2020-0056) and by CVPath Institute IRB (RP0112), and was registered at ClinicalTrials.gov (NCT04367792). The specimens were anonymized before shipping, and the examining pathologist (R.V., M.R., R.K.) was blinded

TABLE 3 Continued

| Epicardial Inflammation | | | Myocardial Inflammation | | Nonischemic | Virus RT-PCR | |
Yes/No (Degree)*	Cell Type	AMI	Yes/No	Cell Type	Myocardial Necrosis	Heart	Lung
No		No	No†		No	+(LA)	+
Yes (Mild)	Lym	No	No		No	ND	ND
No		No	No†		No	ND	+
No		No	No		No	ND	+
Yes (Mild)	Lym	No	No†		No	ND	+
Yes (Mild)	Neut, Lym	Yes	Yes	Neut‡	No	ND	+
Yes (Mild)	Lym	No	No†		No	ND	+
Yes (Mild)	Lym	No	Yes†	Lym	No	ND	+
No		Yes	Yes	Neut‡	No	ND	ND
Yes (Moderate)	Lym	No	Yes	Lym	No	ND	+
Yes (Moderate)	Lym	No	No		No	+(RA)	+
No		No	No		No	ND	+
Yes (Mild)	Neut, Lym, Plasma	No	No		No	ND	+
No		No	No		No	ND	+
Yes (Mild)	Lym	No	No		No	ND	ND
Yes (Mild)	Lym	Yes	Yes	Neut‡	No	ND	ND
10 (63%)	**—**	**3 (19%)**	**5 (31%)**	**—**	**0 (0%)**	**2 (13%)**	**12 (75%)**

to the clinical details. All tissue specimens were fixed in 10% buffered formalin for at least 72 to 96 h prior to shipment. Whole hearts and lungs (either paraffin blocks or tissues), were sent to CVPath Institute for pathological examination.

As shown in Table 2, the average age of the subjects was 70 years, and 69% were men. In total, 4 subjects had a history of prior cardiovascular intervention and 4 had other relevant past medical histories. The average length of hospitalization until death was 6 days. A complete autopsy with detailed cardiac examination (histological sections examined from all 4 walls, at 2 levels of the heart) was conducted. Overall, none of the cases met the criteria for myocarditis, although 3 had evidence of either microvascular or epicardial thrombosis in the setting of acute myocardial infarction, and these cases all had neutrophilic infiltration. Inflammatory cell infiltration was found in the epicardium in 10 of 16 cases (63%), consisting mostly of lymphocytes, and myocardial mononuclear inflammatory cell infiltration was found in 5 of 16 (31%) cases (Table 3).

Total RNA was extracted from myocardial tissue (1 sample from each chamber of the heart) from each of the 16 cases. Concentration and quality of RNA samples were measured. Quantitative reverse transcriptase PCR was performed using specifically designed primers for SARS-CoV-2 (N1 and N2 from CDC EUA essay). Virus copy number was quantified based on the reverse transcriptase PCR results with standard control containing the complete nucleocapsid gene from SARS-CoV-2. RNase P was used as a control. The detection of SARS-CoV-2 RNA was determined by the amplification at Ct ≤40 (33). In only 2 cases was the virus detected in the heart by PCR and both were in the atria (1 left atrium and 1 right atrium) and not in the ventricles, and neither was accompanied by inflammation, although in most of the 16 cases the virus was detected in the lung (Table 3).

We also evaluated the nature of the inflammatory cells found in the myocardium in cases of (noninfectious) traumatic (control) versus COVID-19 deaths. We examined histological sections from the left ventricles from 5 randomly selected cases of control death from our CVPath registry and from 5 randomly selected cases of subjects dying of COVID-19 (see Table 2 for list of cases selected). We stained left ventricular myocardial sections using antibodies against CD3 (T-cell marker) and CD68 (macrophage). Overall, there was no difference in the total number of T cells and macrophages in the 2 groups (Figure 1). However, the cell counts in COVID-19 hearts were higher than what others have used to diagnose myocarditis. We believe because these cells are scattered and not accompanied by myocyte necrosis, these cases do not fulfill the criteria of myocarditis. Moreover, CD3 cells were significantly more frequent in control cases than in COVID-19 cases, whereas macrophages were more frequent in COVID-19 than in control cases, but we did not determine their exact location (i.e., interstitium vs. intravascular). Figure 1 contrasts these results with a typical case of mild myocarditis from the CVPath biobank, which showed greater CD3 abundance than

FIGURE 1 Myocardial Inflammatory Cell Infiltrates in Cases of Traumatic Death, COVID-19, and Myocarditis
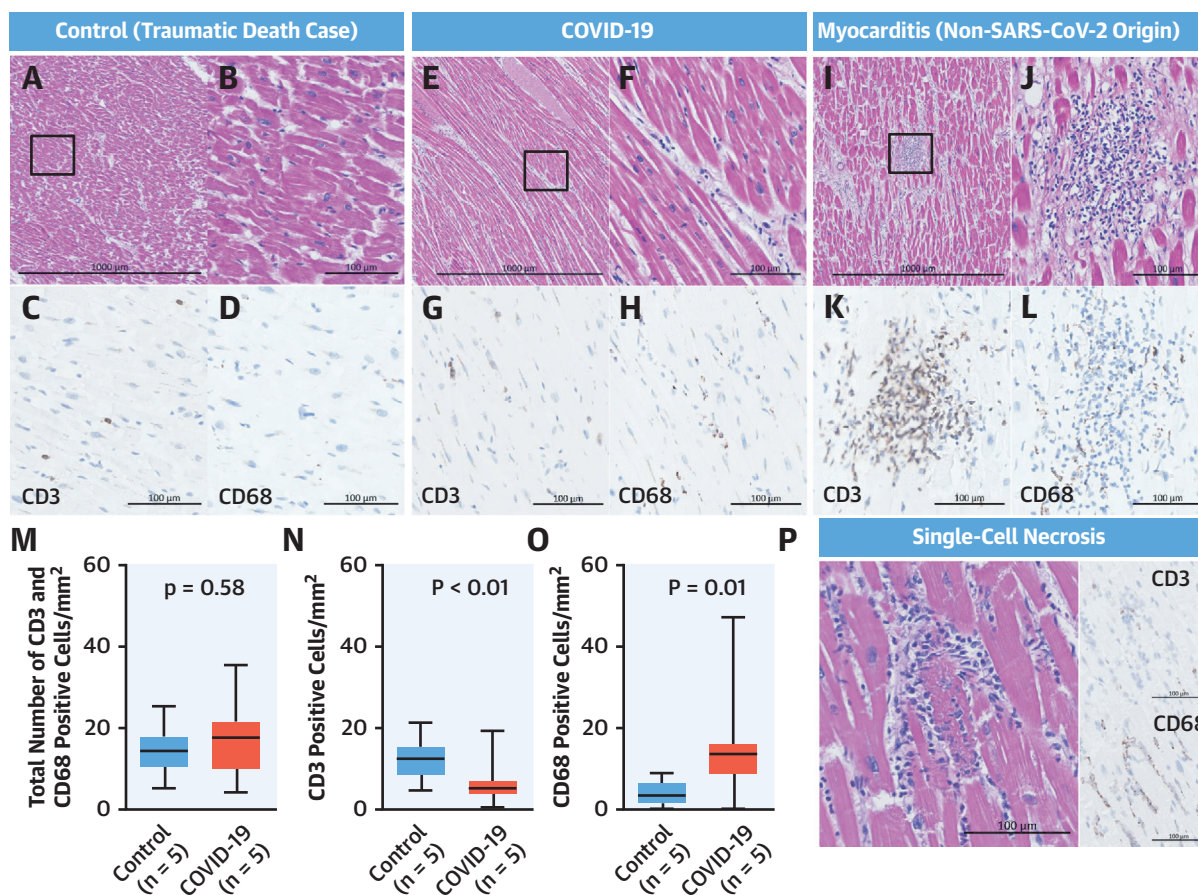
Representative low and high power of hematoxylin and eosin images, and immunostains for CD3 and CD68 from cases of traumatic death (control) **(A to D and P)**, COVID-19 **(E to H)**, and myocarditis **(I to L)**. **Bar graphs** represent total inflammatory (i.e., CD3 and CD68) cell counts in control subjects and COVID-19 cases (n = 5 each) **(M, N, and O)**. A photomicrograph of single-cell necrosis with CD3 and CD68 staining is shown in **P**. CD3 = cluster of differentiation 3; CD68 = cluster of differentiation 68; COVID-19 = coronavirus disease-2019; SARS-CoV-2 = severe acute respiratory syndrome-coronavirus-2.

macrophages, but both are typically more frequently observed in myocarditis than in control deaths and COVID-19 cases.

THE AUTHORS' POSITION ON USEFULNESS OF EMB IN CASES OF SUSPECTED COVID-19-INDUCED MYOCARDITIS

The EMB procedure itself comes with inherent risks, including cardiac perforation, pericardial tamponade, bleeding, and so on, although these have declined with the use of flexible bioptomes with smaller jaws. The incidence of complications was 1.2%, with perforation occurring in 0.42% and death in 0.03% (16). The reason for EMB should be based upon the anticipated yield of the disorder (presumed

probability and risk of sampling error) and the presence of an effective therapy. Certainly, the procedure should only be recommended in cases where benefits of the information obtained outweighs the potential risks of the procedure. The preponderance of autopsy and EMB evidence to date in cases of suspected myocarditis after COVID-19 infection suggests that myocarditis is an uncommon diagnosis. Moreover, the limited sampling of the heart (confined usually to the right ventricular septum) combined with the rarity of COVID-19 myocarditis makes diagnostic yield very unlikely, as has been shown in published cases where EMB has been conducted in cases of suspected myocarditis in the setting of COVID-19 infection. Further complicating the issue of histological diagnosis is that the typical criteria for myocarditis based

CENTRAL ILLUSTRATION Histological Evidence of Myocarditis in COVID-19 Infection and Numbers of Infiltrated Inflammatory Cells in Myocardium in COVID-19 Autopsy Hearts Without Myocarditis and in Non–COVID-19 Myocarditis
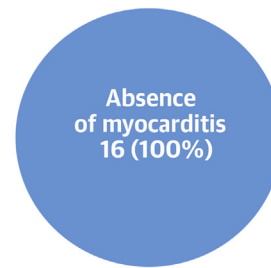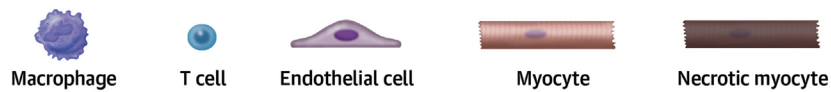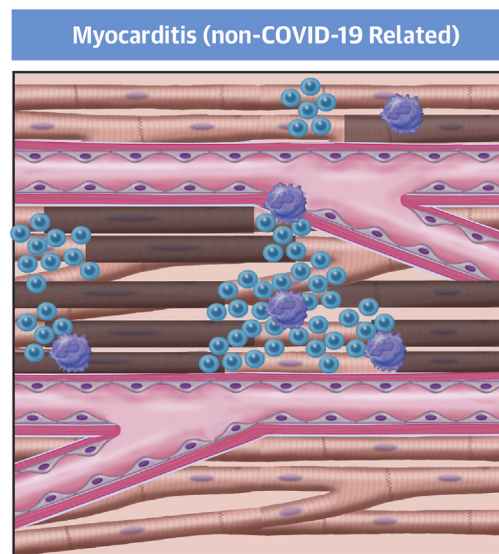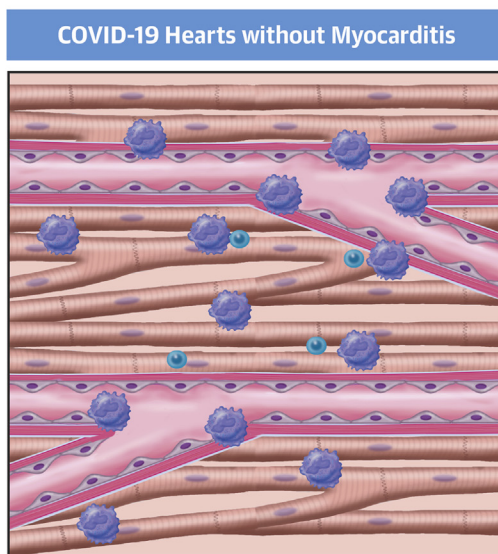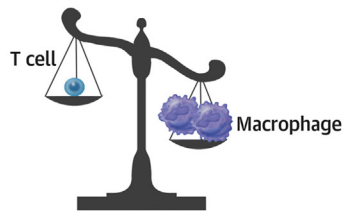

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According to published data on pathological evidence for myocarditis in subjects with coronavirus disease-2019 (COVID-19), the rate of myocarditis is 4.5%. In our experience with 16 COVID-19 autopsy cases, no case met diagnostic criteria for myocarditis. In a comparison of inflammatory cells in the myocardium of subjects dying from traumatic versus COVID-19 deaths (but without a diagnosis of myocarditis), there were less cluster of differentiation (CD) 3-positive cells in COVID-19 cases and more CD68-positive cells.

upon the presence of certain types of inflammation may be different in cases of COVID-19 where very ill patients tend to have lymphopenia (34). It remains unclear to what extent lymphocytic infiltration should be expected in these cases (as it is in typical myocarditis). Moreover, the therapeutic implications are even less clear given there is no data supporting the effectiveness of particular therapies for myocarditis in the setting of COVID-19. Thus, at present, the authors believe EMB should not be routinely performed in such cases and should perhaps be reserved for worst case scenarios, such as patients with new-onset fulminant heart failure with hemodynamic compromise in the setting of documented COVID-19 infection, and the absence of coronary artery disease.

CONCLUSIONS

SARS-CoV-2 infection carries significant morbidity and mortality, especially in the setting of concomitant cardiac injury. However, the mechanism by which the virus causes cardiac damage remains uncertain. Because of the known relationship between viral infection and myocarditis, COVID-19-induced myocarditis has been suggested to be a major mechanism. A thorough review of the literature describing both EMB and autopsy sample analysis suggests that myocarditis is an uncommon diagnosis occurring in 4.5% of highly selected cases undergoing autopsy or EMB (**Central Illustration**). Given the referral bias of autopsy studies, it is likely the number of COVID-19 cases complicated by myocarditis is even lower, because the majority of cases do not have evidence of myocardial injury and do not result in death. However, it should also be appreciated that myocardial inflammation involving infiltration by macrophages and T cells can also be seen in noninfectious deaths (control) and COVID-19 cases, but the extent of each is different, and in neither case do such findings represent clinically relevant myocarditis. Indeed, in the hearts of SARS-CoV-2 infected subjects, nonspecific inflammatory infiltrate characterized by macrophages was more abundant whereas T cells were lower compared with hearts from cases of control deaths (**Central Illustration**). This finding may be consistent with the known lymphope5nic effects of the virus, but deserves further investigation. Nonetheless, our data suggest evidence that COVID-19-induced myocarditis is an uncommon phenomenon. Given this fact and the unclear therapeutic implications of identifying viral myocarditis in subjects with COVID-19, the authors believe EMB should not be routinely used and perhaps should be reserved for worst case scenarios such as patients with new-onset fulminant heart failure with hemodynamic compromise in the setting of documented COVID-19 infection.

ADDRESS FOR CORRESPONDENCE: Dr. Aloke V. Finn, CVPath Institute Inc., 19 Firstfield Road, Gaithersburg, Maryland 20878, USA. E-mail: afinn@cvpath.org. Twitter: @alokefinn, @CVPath_MD.

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