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Cardiac megakaryocytes in SARS-CoV-2-positive autopsies

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Thromboembolic phenomena are an important complication of infection by severe acute respiratory coronavirus 2 (SARS-CoV-2). Increasing focus on the management of the thrombotic complications of Coronavirus Disease 2019 (COVID-19) has led to further investigation into the role of platelets, and their precursor cell, the megakaryocyte, during the disease course. Previously published postmortem evaluations of patients who succumbed to COVID-19 have reported the presence of megakaryocytes in the cardiac microvasculature. Our series evaluated a cohort of autopsies performed on SARS-CoV-2-positive patients in 2020 (n = 36) and prepandemic autopsies performed in early 2020 (n = 12) and selected to represent comorbidities common in cases of severe COVID-19, in addition to infectious and noninfectious pulmonary disease and thromboembolic phenomena. Cases were assessed for the presence of cardiac megakaryocytes and correlated with the presence of pulmonary emboli and laboratory platelet parameters and inflammatory markers. Cardiac megakaryocytes were detected in 64% (23/36) of COVID-19 autopsies, and 40% (5/12) prepandemic autopsies, with averages of 1.77 and 0.84 megakaryocytes per cm^2, respectively. Within the COVID-19 cohort, autopsies with detected megakaryocytes had significantly higher platelet counts compared with cases throughout; other platelet parameters were not statistically significant between groups. Although studies have supported a role of platelets and megakaryocytes in the response to viral infections, including SARS-CoV-2, our findings suggest cardiac megakaryocytes may be representative of a nonspecific inflammatory response and are frequent in, but not exclusive to, COVID-19 autopsies.

Keywords: COVID-19, megakaryocytes, platelets, SARS-CoV-2

Introduction

As of this writing, 530 million cases of Coronavirus Disease 2019 (COVID-19), caused by the novel severe acute respiratory coronavirus 2 (SARS-CoV-2), have been documented worldwide since its initial detection.[1] The symptomatology of COVID-19 is heterogeneous and complex, ranging from mild, asymptomatic infection to respiratory failure leading to multiorgan failure and death. Currently, over 970,000 deaths due to COVID-19 have been reported in the United States alone. COVID-19 has an estimated case fatality rate of 1–10%, and increased risk of mortality has been associated with older age, as well as cardiovascular and pulmonary comorbidities.[2–4]

Similar to other members of the coronavirus family known to spread in humans, SARS-CoV (SARS) and Middle East respiratory syndrome (MERS-CoV), SARS-CoV-2 primarily effects the respiratory system. Patients may present with mild symptoms such as fever and cough, with progression to acute respiratory distress syndrome (ARDS), coagulopathy,

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multiorgan failure and death in severe cases.[2–5] Severe COVID-19 is associated with a robust inflammatory response with immune dysfunction, including cytokine release syndrome (CRS), which contributes to pulmonary pathology and development of ARDS.[6,7] The immune response to SARS-CoV-2 is dynamic, and differs between patients with mild and severe infections.[7] Laboratory findings in severe COVID-19 include elevations in inflammatory cytokines, including tumor necrosis factor-α (TNF-α), and interleukins (IL), including IL-1, IL-6, IL-8, IL-18, and other inflammatory markers including C-reactive protein (CRP), D-dimer, and fibrinogen.[5] Elevations in inflammatory cytokines and D-dimer have been associated with critical illness and mortality.[7,8] Other laboratory findings in severe COVID-19 include prolonged prothrombin and activated partial thromboplastin time, lymphopenia, and variably, thrombocytopenia.[5,9]

Although initial reports emphasized the respiratory dysfunction associated with severe COVID-19 infection, thromboembolic phenomena were quickly recognized as a common and significant complication of COVID-19. Thrombosis has been reported in 30–40% of patients hospitalized with COVID-19, and pulmonary embolism is the most common reported complication.[10,11] Macroscopic thrombosis and microthrombi are reported in ~20% and 50% of autopsies, respectively.[12–14] Histologic examination of lungs from patients who died from COVID-19 frequently demonstrate thrombotic phenomena, including venous thromboemboli as well as capillary microthrombi, at an increased prevalence compared to other respiratory viruses such as influenza and SARS-CoV.[13,15] Notably, procoagulation abnormalities have also been described in SARS and MERS.[16]

Increasing focus on the management of the thrombotic complications of COVID-19 has led to investigations into of the role of platelets, and their precursor cell, the megakaryocyte, during the disease course. In addition to their well-described contribution to thrombosis, platelets also play diverse roles in innate and adaptive immunity and act as inflammatory effectors in processes such as atherosclerosis.[17–19] Platelet count, size, and immaturity are associated with critical illness and all-cause mortality in COVID-19.[20] Platelet activation occurs in inflammatory states, including viral infection, and contributes to the development of ARDS.[21–24] Enhanced platelet activation has been previously studied in other respiratory viral infections. Platelets have been demonstrated to internalize other viral particles, including human immunodeficiency virus (HIV), hepatitis C virus, and dengue with resultant platelet activation and demonstrable degranulation with release of complement (C3).[23,25,26] COVID-19 is associated with alteration of platelet number, size and function, include hyperactivity and increased aggregation, and increased thromboxane generation.[16,20,27] Alterations in platelet activation have been described in patients with severe infection, and increased activation has been associated with poorer patient outcomes, including thrombosis or death.[16,20,28]

Megakaryocytes, the precursor cell of platelets, are normally present in human bone marrow and lungs and shed platelets into circulation. Pulmonary megakaryocytes are hypothesized to play a role in platelet homeostasis, although their contribution to overall platelet production is unclear.[22,29] Increased pulmonary megakaryocytes have been previously reported in conditions such as diffuse alveolar damage, sepsis, and shock.[30–33] Platelets produced by pulmonary megakaryocytes are active contributors to alveolar damage and repair responses in Diffuse alveolar damage (DAD).[21,22] There are numerous reports of increased pulmonary megakaryocytes in COVID-19.[34–41]

Curiously, in addition to the lung, megakaryocytes have also been identified postmortem in cardiac tissue of COVID-19 patients.[12] Typical acute cardiac findings in COVID-19 autopsies attributable myocardial infarction and early ischemic injury, mural fibrin thrombi, and mild epicardial inflammation.[12] Commonly, manifestations of chronic cardiac disease are identified, including myocardial hypertrophy, coronary atherosclerosis, and focal myocardial fibrosis.[42] Despite interest in cardiac findings in COVID-19 based on early reports of myocarditis, which have not been supported by subsequent autopsy series, review of the autopsy literature does not reveal widespread reporting of this subtle finding in COVID-19 cases.[43] Rapkiewicz *et al.* reported the presence of megakaryocytes in the cardiac microvasculature in seven of seven cases, and Tombolini *et al.* reported in two of two cases.[12,44] However, there have been no large series investigating whether this is exclusive to covid infection or seen in other inflammatory processes.

In our series, we examined cardiac tissue obtained from 36 consecutive autopsies of COVID-19 patients performed at our institution to identify the presence of megakaryocytes in microvasculature. Additionally, we examined cardiac tissue from 12 control, non-COVID cases performed prior to the start of the COVID-19 pandemic. These cases were selected following retrospective review of finalized autopsy reports to represent nonspecific histopathologic findings overlapping with features of COVID-19, including diffuse alveolar damage or thromboemboli and comorbidities frequently associated with severe

COVID-19, including atherosclerotic and ischemic heart disease or immunosuppression (see summary tables for clinical information).

Materials and Methods

PATIENT SAMPLES

This study was performed with the approval of the Institutional Review Board at Brigham and Women's Hospital. Cases were retrieved from the Anatomic Pathology files of Brigham and Women's Hospital and included 36 patients with laboratory-confirmed COVID-19 who underwent autopsy between April–June 2020, and 12 patients who underwent autopsy pre-COVID-19 pandemic between January–April 2020. Prepandemic control cases were selected to include causes of death overlapping with features of severe COVID-19 (including thromboemboli or pulmonary embolus [$n = 4$], diffuse alveolar damage [$n = 2$], bronchopneumonia [$n = 2$]), or based on preexisting conditions (atherosclerotic coronary artery disease and/or ischemic heart disease [$n = 3$], interstitial lung disease [$n = 2$], metastatic carcinoma [$n = 1$]). Of the COVID-19 patients, 32 (88.89%) patients had tested positive for SARS-CoV-2 by RT-PCR of nasopharyngeal swabs in a CLIA-certified laboratory during hospital admission, and the remainder were positive by serology (IgG).

The electronic medical record was searched in all cases for medical comorbidities, admission status (ICU or non-ICU), SARS-CoV-2 detection methodology, antiplatelet medications, and use of extracorporeal membrane oxygenation. Available premortem laboratory values, including the latest documented result within 1 week of the patient's death, were evaluated, including platelet counts, mean platelet volume (MPV), prothrombin time (PT), partial thromboplastin time (PTT), C-reactive protein (CRP), D-dimer, fibrinogen, and IL-6 were assessed.

All patients underwent unrestricted autopsy with complete anatomic dissection, except for patients 14, 44, and 45 in whom the brain was excluded from the autopsy permission. The presence of gross pulmonary emboli and microthrombi at autopsy were reported. Hematoxylin and eosin-stained histologic sections were reviewed by multiple surgical pathologists (K.L.G., R.F.P., O.P.).

HISTOLOGY AND IMMUNOHISTOCHEMISTRY (IHC)

Hematoxylin and eosin-stained histologic sections for COVID-19 autopsies were reviewed by surgical pathologists (R.F.P., K.LG.). Histologic descriptions for control autopsies were recorded from finalized autopsy reports. IHC was performed on a single representative block from each case, consisting of left and/or right ventricle, using a modified protocol described by Klairmont *et al.*[45] Staining for CD42b was performed on a BOND III Immunostainer (Leica Biosystems, Buffalo Grove, IL, USA). Pretreatment (heat-induced epitope retrieval) at low pH (ER1; Leica Biosystems) was performed for 30 min, followed by incubation with antihuman CD42b rabbit monoclonal antibody (clone SP219; ABCAM, Waltham, MA, USA), diluted 1:1000 for 1 h. Detection was performed using DAB Refine Polymer Detection Kit (Leica Biosystems). Slides were then briefly immersed in dilute copper sulfate solution, counterstained, dehydrated through solvents, and coverslipped. IHC stains were reviewed for the presence of megakaryocytes by three surgical pathologists (K.L.G., R.F.P., O.P.). Reviewing pathologists were not blinded to the COVID-19 status of the decedent. Megakaryocytes were enumerated in each slide. To account for differences in sampling, tissue present on each slide was individually measured to express the number of megakaryocytes present per cm^2.

STATISTICAL ANALYSES

Laboratory results were compared between COVID-19-positive and COVID-19-negative cohorts, cases with and without detectable cardiac megakaryocytes between cohorts and within the same autopsy cohorts. The raw number of megakaryocytes detected and number of megakaryocytes per cm^2 was compared between COVID-19-positive and egative autopsies. Statistical analysis performed with GraphPad Prism software (San Diego, CA, USA) included unpaired two-tailed *T*-test and Fischer's test.

Results

CLINICOPATHOLOGIC DATA

Clinicopathologic data in 36 patients with COVID-19 and 12 patients without COVID-19 are summarized in Table 1. In the COVID-19-positive cohort, 14 female and 21 male decedents with an average age of 68.1 years (range, 43–96 years), were assessed. In the control prepandemic group, three females and nine males, with an average age of 68.2 years (range, 54–77 years), were assessed. Documented antiplatelet therapies included aspirin (6/12 control [50.00%], 4/36 COVID-19 [11.11%]) and clopidogrel

Table 1. Clinical summary and preexisting conditions

Control (Non-COVID) Cases Clinical Summary and Preexisting Conditions

Case #	Age	Sex	Status	ECMO	Cause of Death	Autoimmune/ Inflammatory	Neoplastic	Cardiopulmonary / Vascular	Antiplatelet Medication	Anticoagulation
1	70	F	non-ICU	No	Pulmonary embolus	Remote h/o thyroid cancer	Thyroid carcinoma (remote)	–	–	–
2	54	M	ICU	No	Pulmonary embolus	Lung transplant status	Non-Hodgkin lymphoma	Cystic fibrosis	–	Apixaban, Heparin
3	64	F	ICU	No	Usual interstitial pneumonia	Lung transplant status	–	Usual interstitial pneumonia, CAD	–	Heparin
4	76	F	non-ICU	No	Pulmonary embolus	–	–	COPD	Aspirin	–
5	76	M	ICU	Yes	Atherosclerotic coronary artery disease	–	Colorectal carcinoma (remote)	–	Aspirin	Heparin
6	71	M	ICU	No	Usual interstitial pneumonia with diffuse alveolar damage	–	–	CAD, valvular heart disease	Aspirin	Enoxaparin
7	77	M	ICU	No	Bronchopneumonia in the setting of coronary artery disease	–	Metastatic GI small cell neuroendocrine carcinoma	CKD	–	–
8	64	M	ICU	No	Multisystem organ failure due to thromboembolus	–	–	Flu-like illness (COVID -)	–	–
9	71	M	non-ICU	No	Metastatic oral squamous cell carcinoma	–	–	CAD, IVC thrombus	–	Rivaroxaban
10	55	M	ICU	No	Atherosclerotic coronary artery disease and ischemic heart disease	–	–	Flu-like illness (COVID -), ERSD, DM	Aspirin	Bivalirudin
11	65	M	ICU	No	Diffuse alveolar damage	–	Metastatic adenocarcinoma of unknown primary	UIP, HCM	Aspirin	–

Table 1. (*Continued*)

Control (Non-COVID) Cases Clinical Summary and Preexisting Conditions

Case #	Age	Sex	Status	ECMO	Cause of Death	Autoimmune/ Inflammatory	Neoplastic	Cardiopulmonary / Vascular	Antiplatelet Medication	Anticoagulation
12	76	M	ICU	No	Bronchopneumonia	–	Incidental prostatic adenocarcinoma	HTN, CKD	Aspirin	Heparin

COVID-19+ Cases Clinical Summary and Preexisting Conditions

Case #	Age	Sex	Status	ECMO	SARS-CoV-2 detection method	Interval between SARS-CoV-2 test detection and death	Cause of Death	Autoimmune/ Inflammatory	Neoplastic	Cardiopulmonary/ Vascular	Antiplatelet medication	Anticoagulation
13	57	M	non-ICU	No	PCR	0	COVID-19 pneumonia with DAD	–	–	DM HTN Neurologic impairment	None	None
14	68	F	ICU	No	PCR	1	COVID-19 pneumonia	Febrile neutropenia	–	CAD COPD DM HTN	Clopidogrel	None
15	90	M	non-ICU	No	PCR	9	COVID-19 pneumonia with DAD	–	Prostatic adenocarcinoma-ma	CKD DM HTN Stroke Dementia	None	Heparin
16	77	M	ICU	No	PCR	3	COVID-19 infection with superimposed bacterial pneumonia	–	–	CKD DM	–	Heparin
17	58	M	ICU	No	PCR	2	COVID-19 pneumonia	–	–	CF HTN Stroke Neurologic impairment	Aspirin	Heparin
18	54	F	ICU	No	PCR	6	COVID-19 pneumonia	–	Anaplastic astrocytoma	Hemiplegia Stroke	None	Enoxaparin
19	53	M	ICU	No	PCR	18	COVID-19 pneumonia	–	–	CKD DM HTN NASH	Aspirin	Heparin
20	90	M	non-ICU	No	PCR	6	COVID-19 pneumonia	–	–	DM HTN	None	None
21	48	M	ICU	No	PCR	0	COVID-19 pneumonia	–	–	Atherosclerosis Hepatic steatosis	None	None

Table 1. (*Continued*)

COVID-19+ Cases Clinical Summary and Preexisting Conditions												
Case #	Age	Sex	Status	ECMO	SARS-CoV-2 detection method	Interval between SARS-CoV-2 test detection and death	Cause of Death	Autoimmune/ Inflammatory	Neoplastic	Cardiopulmonary/ Vascular	Antiplatelet medication	Anticoagulation
22	53	M	ICU	No	PCR	5	COVID-19 pneumonia with DAD	–	–	Acute bacterial pneumonia; OSA	None	Heparin
23	69	F	ICU	No	PCR	24	COVID-19 pneumonia	–	–	CAD COPD DM HTN Stroke	Aspirin	Heparin
24	76	M	ICU	No	PCR	7	COVID-19 pneumonia	–	Non-Hodgkin lymphoma	CAD CHF CKD DM OSA	None	Apixaban, heparin
25	49	F	ICU	No	PCR	9	COVID-19 pneumonia	Hepatitis C (polysubstance abuse)	–	–	None	Enoxaparin, heparin
26	77	M	ICU	No	PCR	25	COVID-19 pneumonia	Rheumatoid arthritis	–	HTN	None	Enoxaparin
27	57	M	ICU	No	PCR	31	COVID-19 pneumonia	–	–	HTN	None	Enoxaparin
28	96	F	non-ICU	No	PCR	11	Aortic atherosclerosis-is with intestinal necrosis in setting of Acute MI	–	–	DVT HTN Stroke with neurologic impairment	None	Enoxaparin
29	63	M	ICU	No	PCR	18	COVID-19 pneumonia	–	–	DM HTN	None	Heparin
30	66	F	ICU	No	Serology	1	Gastrointestinal stromal tumor	SLE	GIST	Acute aspiration pneumonia; Cardiomegaly DM HTN	None	Heparin
31	66	F	ICU	No	PCR	34	COVID-19 pneumonia	–	–	Asthma CAD COPD HTN	None	Enoxaparin
32	82	F	non-ICU	No	PCR	8	COVID-19 pneumonia	–	–	CAD CHF CKD DM HTN	None	None

Table 1. (*Continued*)

COVID-19+ Cases Clinical Summary and Preexisting Conditions												
Case #	Age	Sex	Status	ECMO	SARS-CoV-2 detection method	Interval between SARS-CoV-2 test detection and death	Cause of Death	Autoimmune/ Inflammatory	Neoplastic	Cardiopulmonary/ Vascular	Antiplatelet medication	Anticoagulation
33	80	F	ICU	No	PCR	44	Multisystem organ failure	–	–	DVT HTN Neurologic impairment	None	Enoxaparin, heparin
34	75	M	non-ICU	No	PCR	2	Lung squamous cell carcinoma	–	Lung squamous cell carcinoma	HTN	None	Enoxaparin
35	43	M	ICU	No	PCR	33	COVID-19 pneumonia	Common variable immunodeficiency	–	Asthma Bronchiectasis	None	None
36	66	F	ICU	No	PCR	7	COVID-19 pneumonia	SLE Rheumatoid arthritis Pulmonary fibrosis CKD Interstitial lung disease MGUS	–	CAD HTN	None	Heparin
37	50	M	ICU	No	PCR	8	COVID-19 pneumonia	Urinary tract infection Aspergillus pneumonia	B-ALL	–	None	None
38	70	M	non-ICU	No	PCR	0	Pulmonary Embolus in setting of COVID-19	–	–	Atherosclerosis DM	None	None
39	81	M	non-ICU	No	PCR	Unknown	COVID-19 pneumonia	–	–	HTN OSA Dementia	None	None
40	66	M	ICU	No	PCR	16	COVID-19 pneumonia	–	–	Atherosclerosis CAD COPD DM HTN	None	Heparin
41	48	M	ICU	No	PCR	6	Gallbladder perforation	–	–	Liver cirrhosis DM	None	Heparin

Table 1. (*Continued*)

COVID-19+ Cases Clinical Summary and Preexisting Conditions												
Case #	Age	Sex	Status	ECMO	SARS-CoV-2 detection method	Interval between SARS-CoV-2 test detection and death	Cause of Death	Autoimmune/ Inflammatory	Neoplastic	Cardiopulmonary/ Vascular	Antiplatelet medication	Anticoagulation
42	91	F	non-ICU	No	PCR	Unknown	Coronary atherosclerotic disease and ischemic heart disease	–	–	DM HTN Dementia	None	None
43	45	M	ICU	Yes	PCR	24	COVID-19 pneumonia	–	–	HTN Neurologic impairment	None	Heparin
44	76	F	non-ICU	No	PCR	22	Acute Myeloid Leukemia	EBV viremia	AML	DVT HTN	None	Heparin
45	80	F	ICU	No	PCR	44	COVID19 leading to multisystem organ failure	–	–	DVT HTN Neurologic impairment	None	Enoxaparin
46	86	M	ICU	No	PCR	21	Systemic amyloidosis	–	Prostatic adenocarcinoma Rectal adenocarcinoma	Cardiac amyloidosis with CHF CKD DM HTN	Aspirin	Heparin
47	80	M	non-ICU	No	PCR	Unknown		Neurodegenerative disease	–	–	Dementia HTN	Neurologic impairment
None			None	48	66	F	ICU	No	Serology	4	COVID-19	pneumonia
			Aplastic		Anemia	–	–	None	None			

use in one COVID-19 patient (1/36, 2.78%). Anticoagulation, including active outpatient and inpatient medications, was documented in seven control (7/12, 58.33%) and 24 COVID-19 (24/36, 66.67%) cases (Table 1). Twenty-five (25/36, 69%) of COVID-19 patients were admitted to the ICU and one underwent Extracorporeal membrane oxygenation (ECMO), compared with nine (9/12, 75%) of the prepandemic patients. No prepandemic patients underwent ECMO. The average interval between SARS-CoV-2 detection and death was 13.6 days (range, 0–44 days).

LABORATORY DATA

Premortem laboratory results for control and COVID-19 patients are summarized in Table 2. Average values are provided when adequate data are available, with exclusion of laboratory results outside of the assay's quantifiable range (reported as multiple of upper limit of normal). In the COVID-19-positive cohort, average laboratory values were as follows: platelet count = 237.1 K/μl (range, 37–642), MPV = 10.99 fl (range, 9.2–13.2), CRP = 109.0 mg/l (range, 6.1 – >300), fibrinogen = 514.1 mg/dl (range, < 60–758), IL-6 = 152.8 pg/ml (range, 8.1 – >400), PT = 20.8 sec (range, 12.6–64.4), PTT = 44.7 sec (range, 13.5 – >150).

In the prepandemic cohort, average laboratory values were as follows: platelet count = 250.5 K/μL (range, 77–520), MPV = 11.29 fl (range, 9.9–11.8), CRP = 60.2 mg/l (range, 8.3–144.1), fibrinogen = 464.2 mg/dl (range, 203–602), PT = 23.5 sec (range, 13.0–37.5), PTT = 67.8 sec (range, 29.8–138.3). D-dimer levels, expressed as multiples of the upper limit of normal, averaged 6.19 in the COVID-19 cohort (range, 1.5–10) and 7.00 in the prepandemic cohort (range, 4–8). An IL-6 measurement was only documented in one prepandemic case, with a value of 69.6 pg/ml.

Platelet parameters, including platelet count, MPV, CRP, fibrinogen, IL-6, d-dimer, PT, PTT values were reflective of inflammatory states and comparable between SARS-CoV-2 and prepandemic cohorts, with no statistically significant differences as described above. Troponin values approached statistical significance, and were higher in the prepandemic cohort, with an average of 829.5 ng/l in the prepandemic cohort compared to 44.7 ng/l in the COVID-19-positive cases ($P = 0.0054$; difference between means and SEM = 784.8 ± 258.2). This result is likely reflective of intentional selection of cases with cardiac comorbidities for the non-COVID cohort.

AUTOPSY FINDINGS

The postmortem interval was 1–2 days in all cases. Causes of death are summarized in Table 1. Pulmonary thromboemboli were documented in 67% of COVID-19 cases (24/36 cases) compared with 50% of preCOVID control cases (6/12 cases), $P = 0.3250$. There was no correlation between antiplatelet therapy and the presence of thromboemboli (correlation coefficient = −0.04957). There was no relationship between platelet count and antiplatelet therapy in either cohort (in the COVID+ cohort, $P = 0.8916$; in prepandemic autopsies, $P = 0.8875$).

Gross and histologic cardiac findings frequently included manifestations of chronic disease (Table 3). Most frequent gross findings included cardiomegaly and/or ventricular hypertrophy and dilation in 55% of COVID-19 cases (20/36) and 50% of prepandemic cases (6/12), CAD in 55% of COVID-19 cases (20/36), and 42% of prepandemic cases (5/12). Histologic findings included at least moderate myocyte hypertrophy documented in 5% of COVID-19 cases (2/36) and 42% of prepandemic cases (5/12). Acute ischemic changes, including myocardial infarction and microinfarction, were identified in 22% of COVID-19 cases (8/36) and 42% of prepandemic cases (5/12). Myocarditis was reported in one COVID-19 patient.

CARDIAC MEGAKARYOCYTES

Megakaryocytes were detected by CD42b IHC in the cardiac microvasculature of 23 (64%) of cases in our COVID-19 autopsy series (see Figure 1A, Table 3). An average of 5.8 megakaryocytes were detected per section, or 1.77 megakaryocytes per cm^2. The average age of COVID-19 cases with megakaryocytes was 67.2 years, compared with 69.6 years in the remaining cases. The interval between SARS-CoV-2 detection and death was unknown in three cases. The interval was not statistically different between groups, with an average interval of 12.9 days in the patients with megakaryocytes and 15 days in the remaining cases ($P = 0.644$). Of the COVID-19-positive patients who had megakaryocytes identified in the cardiac microvasculature, 17 patients (17/23, 74%) were admitted to the ICU and none underwent ECMO. Nineteen patients (19/ 23, 82.61%) had COVID-19 pneumonia listed as the primary cause of death, with diffuse alveolar damage in three cases and superimposed bacterial pneumonia in one case. The causes of death in the remaining cases included malignancy ($n = 2$), multisystem organ failure ($n = 2$), and

Table 2. Premortem laboratory values

Control Cases (Non-COVID) Premortem Laboratory Values

Case #	Platelet count F: 150–400 K/μl, M: 150–450 K/μl	MPV 8.4–12.0 fl	CRP 0.0–3.0 mg/l	D-dimer <500 ng/ml	xULNL	IL-6 < 1.8 pg/ml	Fibrinogen 200–450 mg/dl	PT 11.5–14.5 sec	PTT 23.8–36.6 sec	Troponin F: 0–9 ng/l, M: 0–14 ng/l
1	–	–	–	–	–	–	–	–	–	–
2	190	9.9	8.3	–	–	–	283	17.2	–	13
3	77	13.7	–	–	–	–	587	14.3	–	19
4	–	–	–	–	–	–	–	–	–	–
5	103	11.8	–	–	–	–	449	37.5	138.3	–
6	390	11.5	53.6	–	–	–	–	20	–	–
7	–	–	–	–	–	–	–	–	–	–
8	–	–	–	–	–	–	–	–	–	–
9	520	11.2	144.1	>4000	8	–	400	32.5	57.2	27
10	348	11	23.5	1998	4	–	–	13	29.8	1911
11	86	10.3		>4000	8	–	602	36.2	45.9	2939
12	290	10.9	71.3	>4000	8	69.6	–	17.3	–	68
Overall average	251	11.3	60.2	–	8	–	464	23.5	67.8	830
Median	251	11	57	–	8	–	457	20	57	68
Average (megakaryocytes present)	255	10.9	62.5	–	–	–	–	24.5	–	1504
Median	290	11	62	–	8	–	602	20	46	1504
Average (no megakaryocytes)	248	11.52	58.6	–	6	–	429.8	22.9	75.1	493
Median	190	11	24	–	6	–	425	17	57	23

COVID-19+ Cases Premortem Laboratory Values

Case #	Platelet count F: 150–400 K/μl, M: 150–450 K/μl	MPV 8.4–12.0 fl	CRP 0.0–3.0 mg/l	D-dimer <500 ng/ml	xULNL	IL-6 < 1.8 pg/ml	Fibrinogen 200–450 mg/dl	PT 11.5–14.5 sec	PTT 23.8–36.6 sec	Troponin F: 0–9 ng/l, M: 0–14 ng/l
13	116	11.9	78.2	>4000	8	–	<60	33.3	>150	265

Table 2. (*Continued*)

COVID-19+ Cases Premortem Laboratory Values										
Case #	Platelet count F: 150–400 K/μl, M: 150–450 K/μl	MPV 8.4–12.0 fl	CRP 0.0–3.0 mg/l	D-dimer <500 ng/ml	xULNL	IL-6 < 1.8 pg/ml	Fibrinogen 200–450 mg/dl	PT 11.5–14.5 sec	PTT 23.8–36.6 sec	Troponin F: 0–9 ng/l, M: 0–14 ng/l
14	120	12.2	253	>4000	8	–	501	64.4	44.1	243
15	268	11	155	1935	2.8	–	–	–	–	57
16	170	12.2	>300	>4000	8	–	639	14.3	84.4	88
17	114	13.2	6.3	>5000	10	–	–	–	–	174
18	161	11.4	264	1374	2.7	–	–	15.8	25.5	11
19	362	11	70	>4000	8	234	513	14.5	72.7	7
20	–	–	–	–	–	–	–	–	–	–
21	–	–	–	–	–	–	–	–	–	–
22	153	12.6	104	>4000	8	395	331	38.4	–	40
23	569	9.2	144	1683	3.4	8.1	640	15.8	–	122
24	160	10.6	45	2131	4.3	–	593	19.8	32.9	66
25	264	12.5	48	>4000	8	80	493	23.3	37.2	52
26	642	10.3	–	725	1.5	400	480	13.8	29.2	–
27	348	9.8	140	2176	4.4	112	672	15.5	35.1	295
28	146	10.1	7.7	879	1.8	–	–	12.6	–	–
29	288	9.7	6.1	>5000	10	–	664	13.5	29.8	20
30	331	11.7	69	>4000	8	–	371	28.4	51.2	26
31	468	9.6	–	1084	2.2	23.4	727	13.7	39.2	38
32	–	–	–	–	–	–	–	–	–	–
33	388	11.7	–	3362	6.7		332	15.9	51.1	50
34	309	10.3	–	–	–	–	–	14.2	–	47
35	309	10.3	166	>4000	8	91.9	758	13.4	33.1	91
36	137	10.8	64	–	–	57.3	–	14.7	57.8	93

Table 2. (*Continued*)

COVID-19+ Cases Premortem Laboratory Values										
Case #	Platelet count F: 150–400 K/μl, M: 150–450 K/μl	MPV 8.4–12.0 fl	CRP 0.0–3.0 mg/l	D-dimer <500 ng/ml	xULNL	IL-6 < 1.8 pg/ml	Fibrinogen 200–450 mg/dl	PT 11.5–14.5 sec	PTT 23.8–36.6 sec	Troponin F: 0–9 ng/l, M: 0–14 ng/l
37	10	9.9	279	2848	5.7	369	473	18	27.7	797
38	–	–	–	–	–	–	–	–	–	–
39	–	–	–	–	–	–	–	–	–	–
40	157	11.2	80	>4000	8	>400	–	–	66.4	29
41	47	11.6	44	>4000	8	114	123	34.2	47.4	8
42	284	9.6	–	–	–	–	–	–	–	–
43	217	11.1	226.2	>4000	8	32.5	483	13.7	67.3	73
44	86	10.3	>300	>4000	8	68.6	626	17.5	42.4	56
45	388	11.7	–	3362	6.7		332	15.9	51.1	50
46	65	12.6	96.1	–	–	–	–	18	44.3	296
47	–	–	–	–	–	–	–	–	–	–
48	37	9.7	53.3	1405	2.8	–	531	29	13.5	9
Overall average	237	11	109	1914	6	153	514	21	45	115
Median	194	11	79	1809	8	92	507	16	43	56
Average (megakaryocytes present)	284	11	104	1705	6	168	551	21	44	94
Median	278	11	78	1683	8	102	553	16	37	55
Average (no megakaryocytes)	143	11	136	3105	7	122	466	18	49	166
Median	112	11	80	2848	8	69	478	18	47	56

Table 3. Significant autopsy findings

Control (Non-COVID) Cases Significant Autopsy Findings

Case #	Megakaryocytes present	# Megakaryocytes /cm 2	Gross cardiac findings	Histologic cardiac findings	Pulmonary emboli
1	No	–	–	Myocyte hypertrophy	Yes
2	No	–	–		Yes
3	No	–	Cardiomegaly, CAD	Focal replacement fibrosis, myocyte hypertrophy	No
4	No	–	Cardiomegaly, left ventricular hypertrophy, left atrial dilation	Myocyte hypertrophy	Yes
5	No	–	Cardiomegaly, biventricular hypertrophy and dilation, left atrial dilation, CAD s/p CABG, saphenous vein graft thrombosis	Acute myocardial infarction, multifocal remote myocardial infarcts	No
6	Yes	1.96	Cardiomegaly, four chamber hypertrophy, valvular heart disease	Myocyte hypertrophy	Yes
7	Yes	0.33	Cardiomegaly, CAD	Acute myocardial infarction, multifocal remote myocardial infarcts	No
8	Yes	1.28	–	Multifocal acute to subacute thromboembolic microinfarctions	Yes
9	No	–	CAD	Remote myocardial infarction, myocyte hypertrophy	No
10	No	–	Cardiomegaly, CAD s/p CABG	Acute and remote myocardial infarction	No
11	Yes	0.30	–	Focal acute myocardial infarction	No
12	Yes	0.36	–	–	Yes

COVID+ Cases Significant Autopsy findings

Case #	Megakaryocytes present	# Megakaryocytes /cm 2	Gross cardiac findings	Histologic cardiac findings	Pulmonary emboli
13	Yes	3.25	Cardiomegaly	–	Yes
14	Yes	0.91	Cardiomegaly, CAD s/p CABG	Healing transmural myocardial infarction and microinfarcts, subendocardial myocyte vacuolization	No
15	Yes	0.48	Cardiomegaly, biventricular dilation, CAD	Healed subendocardial microinfarcts (focal)	No
16	Yes	2.86	Cardiomegaly, biventricular dilation, CAD	–	Yes (clinical)
17	Yes	0.77	Cardiomegaly, left ventricular hypertrophy, CAD	Remote myocardial infarction	Yes (clinical)

Table 3. (*Continued*)

COVID+ Cases Significant Autopsy findings					
Case #	Megakaryocytes present	# Megakaryocytes /cm 2	Gross cardiac findings	Histologic cardiac findings	Pulmonary emboli
18	Yes	4.58	CAD	Remote myocardial infarction, Acute subendocardial microinfarcts	Yes
19	Yes	3.75	–	–	Yes
20	No	0.64	Cardiomegaly, CAD	Subendocardial myocyte vacuolization	No
21	No	2.72	Cardiomegaly, CAD s/p CABG	Healing transmural myocardial infarction and microinfarcts, subendocardial myocyte vacuolization	Yes
22	No	5.56	–	Myocarditis, myocyte hypertrophy	Yes
23	No	0.42	Cardiomegaly, left ventricular hypertrophy	Remote microinfarctions	No
24	No	0.63	Cardiomegaly		Yes
25	No	0.42	Cardiomegaly, left ventricular hypertrophy, CAD	Remote myocardial infarction	No
26	No	0.40	Cardiomegaly		No
27	No	1.81	CAD	Vascular congestion	Yes
28	No	0.79	Cardiomegaly, biventricular hypertrophy and dilation, CAD	Acute and healing subendocardial infarction	Yes
29	No	0.69	Left ventricular hypertrophy, CAD	–	Yes
30	No	3.85	CAD	Focal acute microinfarcts, myocyte hypertrophy	No
31	No	0.79	Cardiomegaly, biventricular dilation, left ventricular hypertrophy	Focal acute ischemic changes	No
32	No	1.23	–	–	Yes
33	No	2.78	Cardiomegaly, CAD s/p CABG	–	No
34	No	1.00	–	–	No
35	No	0.40	Cardiomegaly, biventricular dilation and hypertrophy, biatrial enlargement	Focal acute ischemic changes	Yes
36	No	–	Cardiomegaly, biventricular hypertrophy and left atrial enlargement	Acute myocardial infarction	Yes
37	No	–	CAD with acute plaque change	Replacement fibrosis	No

Table 3. (*Continued*)

COVID+ Cases Significant Autopsy findings					
Case #	Megakaryocytes present	# Megakaryocytes /cm 2	Gross cardiac findings	Histologic cardiac findings	Pulmonary emboli
38	No	–	Biventricular hypertrophy	–	No
39	No	–	Right ventricular dilation	–	Yes
40	No	–		–	No
41	No	–	Atrial enlargement, patent foramen ovale, CAD	–	No
42	No	–	Cardiomegaly	Remote myocardial infarction and microinfarcts	Yes
43	No	–	CAD	Acute subendocardial microinfarcts, remote myocardial infarction	Yes
44	No	–	CAD s/p CABG	Amyloidosis, remote myocardial infarction	Yes
45	No	–	Dilated cardiomyopathy, CAD	–	No
46	No	–	Benign myxoma, CAD	–	No
47	No	–	Cardiomegaly, biventricular dilation	–	No
48	No	–	–	N/A	No

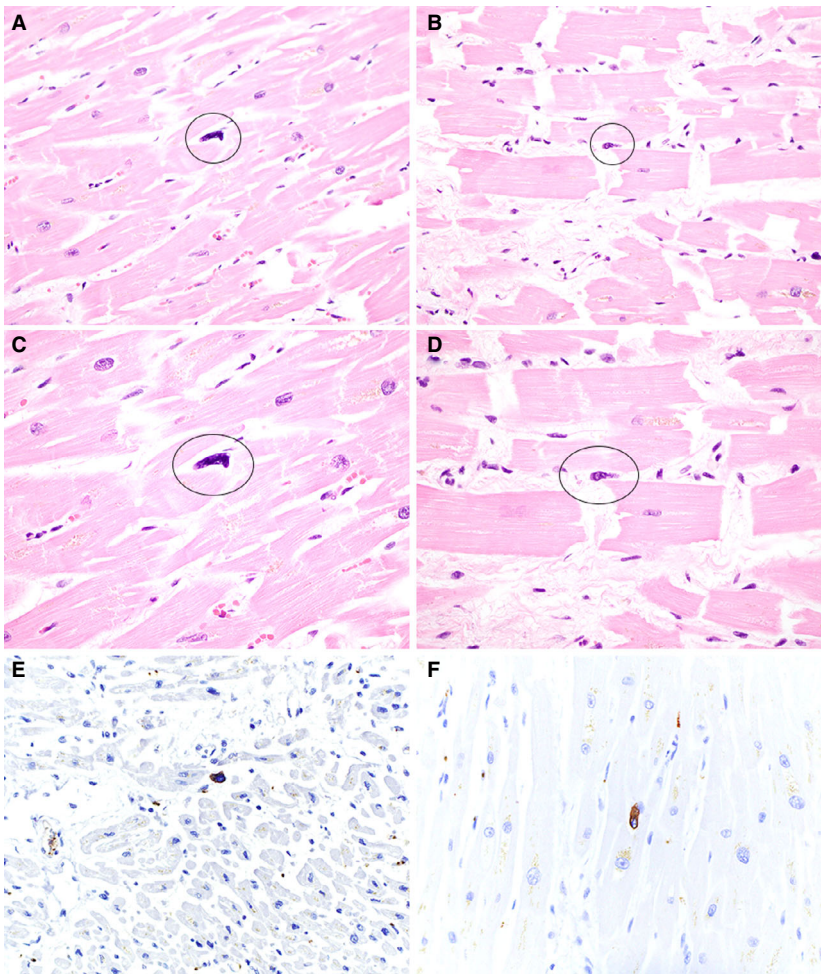

Figure 1. Cardiac megakaryocytes were identified in both COVID-19-positive and prepandemic autopsies. Megakaryocytes are challenging to identify on H&E alone, in part due to their unusual morphology that differs significantly from bone marrow megakaryocytes. The morphologic features, such as lobulated nuclei, condensed chromatin and scant cytoplasm, and presence between cardiomyocytes can help megakaryocyte identification. Morphology was similar in COVID-19-positive (**A,C**) and prepandemic cases (**B, D**). Immunohistochemistry for CD42b allows for enumeration of megakaryocytes in COVID-19-positive (**E**) and prepandemic decedents (**F**).

atherosclerosis. Pulmonary emboli were reported in 13 (13/23, 56%), compared with five (5/19, 38%) of cases without megakaryocytes.

Megakaryocytes were identified in five (5/12, 40%) of prepandemic autopsies (Figure 1B). An average of 2.6 megakaryocytes were detected per section, or 0.84 megakaryocytes per cm^2. The average age of prepandemic cases with megakaryocytes was 70.6 years, compared with 66.6 in the remaining cases. All five patients were admitted to the ICU, and causes of death included bronchopneumonia (n = 2, with atherosclerotic coronary artery disease contributing in one case), diffuse alveolar damage (n = 2, with usual interstitial pneumonia contributing in one case), and multisystem organ failure due to thromboembolism in one case. Pulmonary emboli were reported in three cases (3/5, 60%), compared with three (3/7, 43%) cases without megakaryocytes.

COVID-19 autopsies with detected megakaryocytes, average laboratory values were as follows: platelet count = 284.3 K/μl (range, 120–642), MPV = 11.07 fl (range, 9.2–13.2), CRP = 103.8 mg/l (range, 6.3 – >300), fibrinogen = 551.0 mg/dl (range, < 60–748), IL-6 = 168.1 pg/ml (range, 8.1–400), PT = 21.14 sec (range, 12.6–64.4), PTT = 43.50 sec (range, 25.5 – >150). D-dimer levels, expressed as multiples of upper limit of normal, averaged 5.99 (range, 1.5–10). In prepandemic autopsies with detected megakaryocytes, average laboratory values were as follows: platelet count = 255.3 K/μl (range, 86–390), MPV = 10.90 fl (range, 10.3–11.5), CRP = 62.45 mg/l (range, 53.6–71.3), PT = 24.50 sec (range, 17.3–36.2). PTT was only documented in one case, resulting at 45.9 sec. D-dimer levels, expressed as multiples of upper limit of normal, averaged 8.00 (range, 4–8). Fibrinogen and IL-6 were only documented once, resulting at 602 mg/dl and 69.6 pg/ml, respectively.

We compared laboratory values in cases with and without cardiac megakaryocytes, and between COVID-19 and prepandemic autopsies with detectable megakaryocytes (Table 4). Platelet counts were significantly higher in COVID-19-positive cases, with

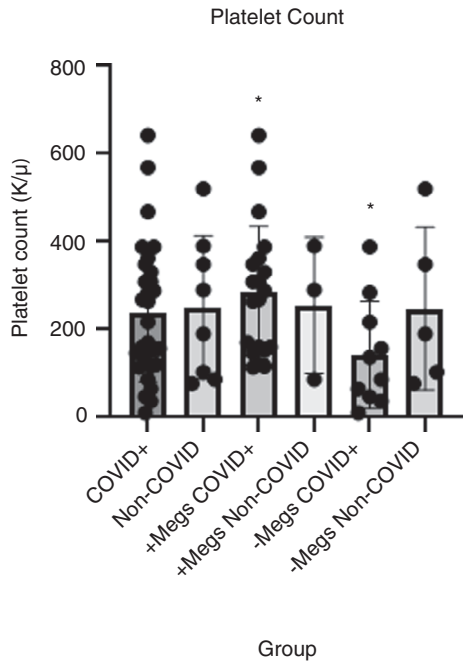

Figure 2. Comparison of platelet counts between all COVID-19 and prepandemic autopsies.

megakaryocytes present compared to those without megakaryocytes, with an average of 284 K/μl in cases with megakaryocytes present, and 143 K/μl in cases without ($P = 0.0157$; difference between means and SEM = -141.5 ± 55.03). All other platelet parameters showed no statistically significant differences between groups (Figure 2). In COVID-19 autopsies, the average number of megakaryocytes per section and per cm^2 was 5.78 (range, = 1–22) or 1.77 per cm^2 (range, = 0.40–3.85). In prepandemic autopsies, an average of 2.6 megakaryocytes were identified per section (range, = 1–5) or 0.84 per cm^2 (range, = 0.30–1.96). However, there was no statistically significant difference in megakaryocyte quantification between COVID-19-positive and prepandemic autopsies with detectable megakaryocytes. There was no relationship between antiplatelet therapy and number of cardiac megakaryocytes ($P = 0.4234$),

Discussion

Postmortem examinations remain a powerful tool to understand pathologic manifestations of disease. Undoubtably, they have been invaluable in our understanding of COVID-19. Although there has been much interest in the effects of COVID-19 on cardiac tissue, there are limited reports of cardiac megakaryocytes in the literature. We report the presence of megakaryocytes within the microvasculature of 23 (23/36, 64%) of COVID-19 autopsies in our institution, and in five prepandemic cases without COVID-19 (5/12, 40% of selected cases). This finding is frequent, but not ubiquitous in COVID-19 autopsies. The presence of megakaryocytes in the heart of non-COVID patients suggests this may be a nonspecific inflammatory response, rather than a response to viral infection. Given the focus on platelets and thrombosis in COVID-19 manifestations, it is important to consider that the presence of megakaryocytes in the heart is not exclusive to COVID-19 pathology and that this finding should not be overinterpreted as a finding characteristic of SARS-CoV-2 infection.

The finding of megakaryocytes within the cardiac microvasculature is not a novel finding, although there are few contemporary studies describing this phenomenon. As part of investigative efforts to determine the origin of megakaryocytes, Brill and Halpern examined multiple organs in a series of autopsies which represented neoplastic, infectious, inflammatory, and cardiovascular etiologies.[46] In 45 cases with available cardiac tissue, megakaryocytes were detected in 13% of cases. The largest study to date, published by Smith and Butcher in 1952, reviewed 180 total autopsy cases and compared the incidence of megakaryocytes in the heart and other tissues between in-hospital deaths and "sudden deaths" such as accidental injuries.[47] By assessment of routine histologic sections, the authors reported the presence of megakaryocytes in the heart, lung, liver, spleen, kidney, adrenal and pituitary glands, brain, lymph nodes, pancreas as well as the marrow. Cardiac megakaryocytes were described in 16% of "sudden death" cases, compared with 45% of hospital deaths.

The morphology of cardiac megakaryocytes differs from that of their marrow-based counterparts, rendering them difficult to detect on routine histologic sections (Figure 1). Megakaryocytes in peripheral capillaries are smaller in size, with less cytoplasm, and frequently have hypolobated, round nuclei that may be distorted by the shape of vascular channels. Therefore, it can be challenging to distinguish true megakaryocytes from degenerating cardiac myocyte nuclei. The unique morphology of cardiac megakaryocytes, and sparse distribution among the tissue, suggests that this finding may be underreported in routine assessment. The megakaryocyte-specific immunohistochemical detection method provides a useful means for accurate identification.

Due to our study size, it is difficult to correlate the presence of cardiac megakaryocytes and thrombotic events. Among the COVID-19 autopsy patients,

Table 4. Cardiac megakaryocytes: summary of significant autopsy findings and premortem labs in control (non-COVID) and COVID-19+ cases

Case #	Cause of Death	# Megakaryocytes /cm^2	Gross cardiac findings	Histologic cardiac findings	Pulmonary emboli	Platelet count F: 150–400 K/ul, M: 150–450 K/ul	MPV 8.4–12.0 fl	CRP 0.0–3.0 mg/l	D-dimer <500 ng/ml	xULNL	IL-6 ≤ 1.8 pg/ml	Fibrinogen 200–450 mg/dl	PT 11.5–14.5 sec	PTT 23.8–36.6 sec	Troponin F: 0–9 ng/l, M: 0–14 ng/l
control (non-COVID) autopsies															
6	Usual interstitial pneumonia with diffuse alveolar damage	1.96	Cardiomegaly-y, four chamber hypertrophy, valvular heart disease	Myocyte hypertrophy	Yes	390	11.5	53.6	–	–	–	–	20	–	–
7	Bronchopneumonia in the setting of coronary artery disease	0.33	Cardiomegaly, CAD	Acute myocardial-l infarction, multifocal remote myocardia-l infarcts	No	–	–	–	–	–	–	–	–	–	–
8	Multisystem organ failure due to thromboembolism	1.28	–	Multifocal acute to subacute thromboembolic microinfarctions	Yes	–	–	–	–	–	–	–	–	–	–
11	Diffuse alveolar damage	0.30	–	Focal acute myocardial-l infarction	No	86	10.3		>4000	8	–	602	36.2	45.9	2939
12	Bronchopneumonia	0.36	–	–	Yes	290	10.9	71.3	>4000	8	69.6	–	17.3		68
COVID-19 Positive Autopsies															
13	COVID-19 pneumonia with DAD	3.25	Cardiomegaly-y	–	Yes	116	11.9	78.2	>4000	8	–	<60	33.3	>150	265
14	COVID-19 pneumonia	0.91	Cardiomegaly-y, CAD s/p CABG	Healing transmural-l myocardia-l infarction and microinfarcts, subendocardial myocyte vacuolization	No	120	12.2	253	>4000	8	–	501	64.4	44.1	243

Table 4. (*Continued*)

control (non-COVID) autopsies

Case #	Cause of Death	# Megakaryocytes /cm^2	Gross cardiac findings	Histologic cardiac findings	Pulmonary emboli	Platelet count F: 150–400 K/ul, M: 150–450 K/ul	MPV 8.4–12.0 fl	CRP 0.0–3.0 mg/l	D-dimer <500 ng/ml	xULNL	IL-6 ≤ 1.8 pg/ml	Fibrinogen 200–450 mg/dl	PT 11.5–14.5 sec	PTT 23.8–36.6 sec	Troponin F: 0–9 ng/l, M: 0–14 ng/l
15	COVID-19 pneumonia with DAD	0.48	Cardiomegaly-y, biventricular dilation, CAD	Healed subendocardial microinfarcts (focal)	No	268	11	155	1935	2.8	–	–	–	–	57
16	COVID-19 infection with superimposed bacterial pneumonia	2.86	Cardiomegaly-y, biventricular dilation, CAD	–	Yes (clinical)	170	12.2	>300	>4000	8	–	639	14.3	84.4	88
17	COVID-19 pneumonia	0.77	Cardiomegaly-y, left ventricular hypertrophy, CAD	Remote myocardia-l infarction	Yes (clinical)	114	13.2	6.3	>5000	10	–	–	–	–	174
18	COVID-19 pneumonia	4.58	CAD	Remote myocardia-l infarction, Acute subendocardial microinfarcts	Yes	161	11.4	264	1374	2.7	–	–	15.8	25.5	11
19	COVID-19 pneumonia	3.75	–	–	Yes	362	11	70	>4000	8	234	513	14.5	72.7	7
20	COVID-19 pneumonia	0.64	Cardiomegaly-y, CAD	Subendocardial myocyte vacuolized-ion	No	–	–	–	–	–	–	–	–	–	–
21	COVID-19 pneumonia	2.72	Cardiomegaly-y, CAD s/p CABG	Healing transmural-l myocardial infarction and microinfarcts, subendocardial myocyte vacuolized-ion	Yes	–	–	–	–	–	–	–	–	–	–
22	COVID-19 pneumonia with DAD	5.56	–	Myocardia-is, myocyte hypertrophy	Yes	153	12.6	104	>4000	8	395	331	38.4	X	40

Table 4. (*Continued*)

control (non-COVID) autopsies															
Case #	Cause of Death	# Megakaryocytes /cm^2	Gross cardiac findings	Histologic cardiac findings	Pulmonary emboli	Platelet count F: 150–400 K/ul, M: 150–450 K/ul	MPV 8.4–12.0 fl	CRP 0.0–3.0 mg/l	D-dimer <500 ng/ml	xULNL	IL-6 ≤ 1.8 pg/ml	Fibrinogen 200–450 mg/dl	PT 11.5–14.5 sec	PTT 23.8–36.6 sec	Troponin F: 0–9 ng/l, M: 0–14 ng/l
23	COVID-19 pneumonia	0.42	Cardiomegaly, left ventricular hypertrophy	Remote microinfarctions	No	569	9.2	144	1683	3.4	8.1	640	15.8	X	122
24	COVID-19 pneumonia	0.63	Cardiomegaly		Yes	160	10.6	45	2131	4.3	–	593	19.8	32.9	66
25	COVID-19 pneumonia	0.42	Cardiomegaly-y, left ventricular hypertrophy, CAD	Remote myocardia-l infarction	No	264	12.5	48	>4000	8	80	493	23.3	37.2	52
26	COVID-19 pneumonia	0.40	Cardiomegaly-y	–	No	642	10.3	–	725	1.5	400	480	13.8	29.2	–
27	COVID-19 pneumonia	1.81	CAD	Vascular congestion-n	Yes	348	9.8	140	2176	4.4	112	672	15.5	35.1	295
28	Aortic atherosclerosis with intestinal necrosis in setting of Acute MI	0.79	Cardiomegaly-y, biventricular hypertrophy and dilation, CAD	Acute and healing subendocardial infarction	Yes	146	10.1	7.7	879	1.8	–	–	12.6	–	–
29	COVID-19 pneumonia	0.69	Left ventricular hypertrophy, CAD	–	Yes	288	9.7	6.1	>5000	10	–	664	13.5	29.8	20
30	Gastrointestinal stromal tumor	3.85	CAD	Focal acute microinfarcts, myocyte hypertrophy	No	331	11.7	69	>4000	8	–	371	28.4	51.2	26
31	COVID-19 pneumonia	0.79	Cardiomegaly-y, biventricular dilation, left ventricular hypertrophy	Focal acute ischemic changes	No	468	9.6	–	1084	2.2	23.4	727	13.7	39.2	38
32	COVID-19 pneumonia	1.23	–	–	Yes	–	–	–	–	–	–	–	–	–	–

Table 4. (*Continued*)

Case #	Cause of Death	# Megakaryocytes /cm^2	Gross cardiac findings	Histologic cardiac findings	Pulmonary emboli	Platelet count F: 150–400 K/ul, M: 150–450 K/ul	MPV 8.4–12.0 fl	CRP 0.0–3.0 mg/l	D-dimer <500 ng/ml	xULNL	IL-6 ≤ 1.8 pg/ml	Fibrinogen 200–450 mg/dl	PT 11.5–14.5 sec	PTT 23.8–36.6 sec	Troponin F: 0–9 ng/l, M: 0–14 ng/l
control (non-COVID) autopsies															
33	Multisystem organ failure	2.78	Cardiomegaly-y, CAD s/p CABG	–	No	388	11.7	–	3362	6.7	–	332	15.9	51.1	50
34	Lung squamous cell carcinoma	1.00	–	–	No	309	10.3	–	–	–	–	–	14.2	–	47
35	COVID-19 pneumonia	0.40	Cardiomegaly-y, biventricular dilation and hypertrophy, Bia trial enlargement	Focal acute ischemic changes	Yes	309	10.3	166	>4000	8	91.9	758	13.4	33.1	91

platelet counts were indeed significantly higher in cases with megakaryocytes present compared to those without megakaryocytes, with an average platelet count of 284.3 K/μl in cases with megakaryocytes present and 142.8 K/μl in cases without megakaryocytes ($P = 0.0157$; difference between means and SEM = -141.5 ± 55.03). However, it is unclear what contribution cardiac megakaryocytes make, if any, to overall platelet production. Cardiac megakaryocytes in this group may be reflective of increased megakaryopoiesis in the marrow and therefore thrombopoiesis, rather than a causal relation. Previously published postmortem evaluation of bone marrow in COVID-19 autopsies showed increased megakaryocytes in 15% of examined cases.[48] It has been postulated that elevations in IL-6 in severe COVID-19 may stimulate megakaryopoiesis and platelet production in marrow and pulmonary megakaryocytes.[49] There were no significant differences in clotting parameters (PT, PTT); a greater proportion of cases with megakaryocytes in the heart had pulmonary emboli either diagnosed clinically or detected at autopsy. In cases with megakaryocytes present, 13 COVID-19 cases (13/23, 56%) and three control cases (3/5, 60%) had pulmonary emboli present, compared with five (5/16, 38%) of the remaining COVID-19 cases and three (3/7, 47%) of the remaining control cases ($P = 0.3618$).

The role of platelets in disease has been more widely studied than that of their precursor cell. Platelets receive their mRNA repertoire from megakaryocytes via highly regulating pathways during thrombopoiesis.[50,51] Despite lacking a nucleus, platelets have active spliceosome machinery and can process mRNA, which may play a role in platelet activation.[52] Evidence of alterations in the platelet transcriptome and proteome have been described in diverse diseases, such as myocardial infarction, sepsis, malignancy, autoimmune disease such as lupus, and aging.[53] In the absence of disease, platelet gene expression has been demonstrated to be stable in individuals over time.[54] *In vitro* studies have demonstrated similar transcriptional and translational studies in response to sepsis in mice comparable to those in patient-derived platelets and demonstrate higher amounts of αIIβ directly correlating with survival.[55] High levels of procoagulant platelets have been implicated in noninfectious inflammatory conditions as well, such as arterial thrombotic disease.[56] Platelets are known to contribute to the development of atherosclerosis through induction of SOCS3 in plague macrophages, with resultant inflammatory cytokine production (Il6, Il1b, and TNF-α) and impaired phagocytic capacity, thus contributing to sustained plaque formation.[19]

There is some evidence that megakaryocytes may also play a role in the development or response to a noninfectious inflammatory condition, as increased numbers of circulating megakaryocytes have been detected in patients following acute myocardial infarction.[57] As megakaryocytes have a longer lifespan than the 9–11 day circulation period of their progeny, much is left to be discovered regarding their potential role in development of and response to disease states, as well as how these transcriptomic and proteomic changes are conveyed to platelets.

The role of platelets and megakaryocytes in SARS-CoV-2 infection is under active investigation and of interest, as characterization of the immune response to SARS-CoV-2 may aid in stratification of patients and improve management.[58] Whether the altered platelet function observed in COVID-19 is a result of viral interactions or systemic inflammation is unclear, both processes have potential to contribute to the observed platelet phenotype in severe COVID-19. Both platelets and megakaryocytes are thought to be active in innate immunity, and antiviral activity has been previously demonstrated during viral infections. Internalization of influenza by platelets has been previously described through a phagocytosis-like process with subsequent digestion.[18,59] SARS-CoV-2 virions has been identified in circulating platelets, and studies of platelet RNA expression demonstrate alterations in pathways associated with Mitogen-activated protein kinase activation, ubiquitination, antigen presentation, mitochondrial dysfunction, and upregulation of antiviral proteins such as Interferon-induced transmembrane protein 3 (IFITM3).[16,24.27,28] Interestingly, uptake of SARS-CoV-2 results in increased expression of pathways responsible for programmed cell death, suggestive of a probable role in viral clearance.[24] Taken together, these findings suggest platelets are active, not passive, factors in the acute response to infection by SARS-CoV-2.[60]

The potential role of megakaryocytes in the response to infection by SARS-CoV-2 is not well understood. In response to influenza and dengue virus, megakaryocytes overexpress IFITM3 and may play a regulatory role in response to infection.[61] There is conflicting evidence regarding the ability of SARS-CoV-2 to directly enter megakaryocytes. Retrospective analysis of published deep-sequencing and microarray data have not demonstrated Angiotensin-converting enzyme 2 (ACE2) expression on megakaryocytes.[62] Although megakaryocytes are not known to express the ACE2 receptor, recent studies have demonstrated expression of ACE2 in platelet *in vitro*.[63] Transcriptomic studies have shown expression of CD147 on primary megakaryocytes and proposed this receptor as a means of entry.[20] Barrett *et al.* recently reported visualization of SARS-COV-2 virions in megakaryocytes within the bone marrow and replicating SARS-CoV-2 detected in the pulmonary megakaryocyte from a deceased COVID-19 patient; however, this finding has not been identified by other authors.[20,49]

Much of our understanding of megakaryocyte function is based on study of the bone marrow; however, there is a growing body of evidence to suggest functional and phenotypic differences in other tissue locations, namely, a more robust immunoinflammatory function. The lung is the best described of these extramedullary sites. Flow cytometric and transcriptomic studies in murine models show a greater proportion of lung megakaryocytes express markers of terminal maturation, suggestive of priming for efficient platelet production.[64] Interestingly, lung megakaryocytes express greater levels of immune molecules compared to those residing in the marrow, with inducible immunophenotypic changes reminiscent of antigen-presenting cells. Lung megakaryocytes show elevated expression of markers including MHC II, CD80, CD40, ICAM-1, LFA-1, and CCR7 compared to their marrow counterparts.[64,65] Further studies are needed to elucidate a specific role for megakaryocytes and platelets in COVID-19, and more generally in immunoregulatory roles, and how this role differs in the setting of tissues outside of the bone marrow.

Limitations of our study include sample size and patient selection, as patients who undergo autopsy at our institution may not be representative of the overall population. Moreover, control cases were retrospectively selected from institutional records by the authors. Documentation is limited in some cases, and laboratory and prescription data were not universally available for reporting.

In summary, the presence of megakaryocytes in the heart is not unique to COVID-19, as these/our findings demonstrate the presence of megakaryocytes in the hearts of patients that died from noninfectious causes and other viral infections. Our findings suggest the presence of megakaryocytes in the cardiac microvasculature may be a nonspecific response to systemic inflammatory stimuli or pulmonary dysfunction, rather than a specific or coordinated response to infection. Notably, the presence of increased pulmonary and cardiac megakaryocytes has not been correlated with overall platelet production, nor has their abundance at autopsy been related to clinical thrombosis. The pathologic role of megakaryocytes found in cases of patients who died from SARS-CoV-2 infection is unclear. Randomized controlled trials of the use of aspirin or P2Y12 inhibitors have found no

benefit when used with heparin anticoagulation in both moderately ill and critically ill patients to date (RECOVERY, ACTIV-4A, REMAP-CAP),[66–68] suggesting that these megakaryocytes and the platelets they produce may play little role in the development of the thromboinflammation and microvascular thrombosis that characterizes severe COVID-19.

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Author contributions

EM Battinelli, O Pozdnyakova, R Padera, J Connors, and KL Gawelek designed the research study. G Pinkus implemented the immunohistochemical procedures. R Padera conducted autopsies of the COVID-19 cohort. KL Gawelek selected prepandemic cases, and gathered clinical and laboratory data for all cases. KL Gawelek, R Padera, and O Pozdnyakova reviewed the histology and immunostains. KL Gawelek and EM Battinelli performed statistical analysis. KL Gawelek wrote the article and constructed figures with discussion and feedback from all authors. EM Battinelli, O Pozdnyakova, R Padera, J Connors, and KL Gawelek edited the article.

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Conflict of interest

The authors declare that they have no affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this article.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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