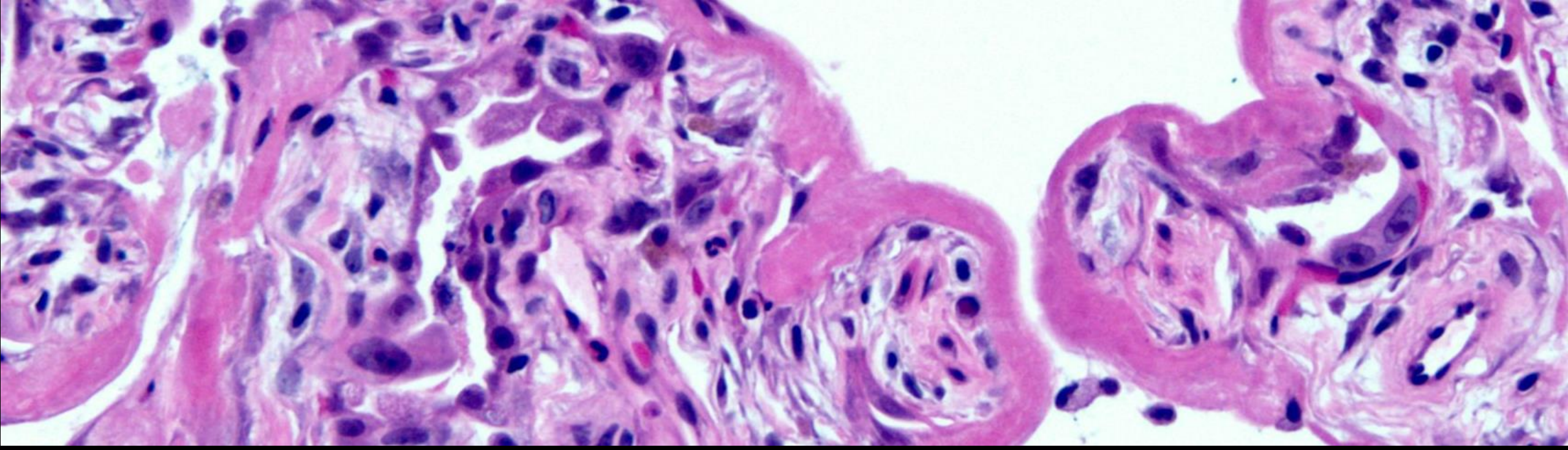


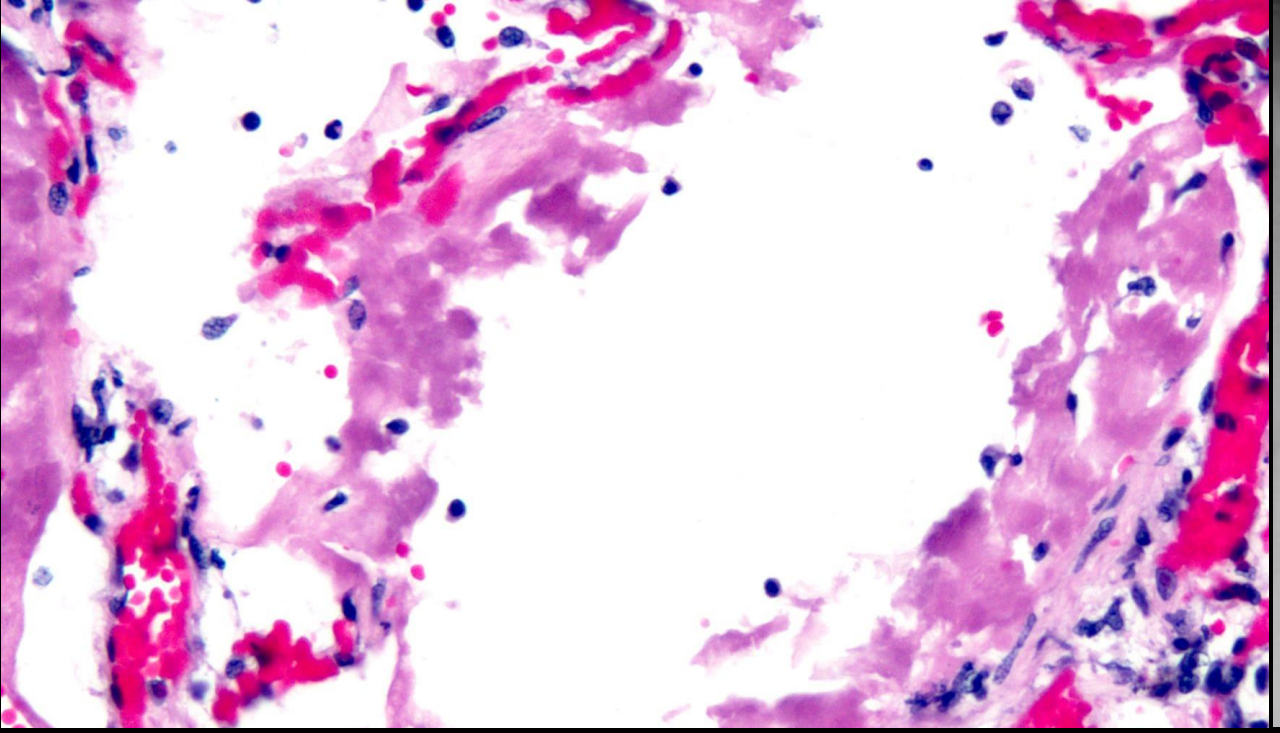
COVID-19 LUNG

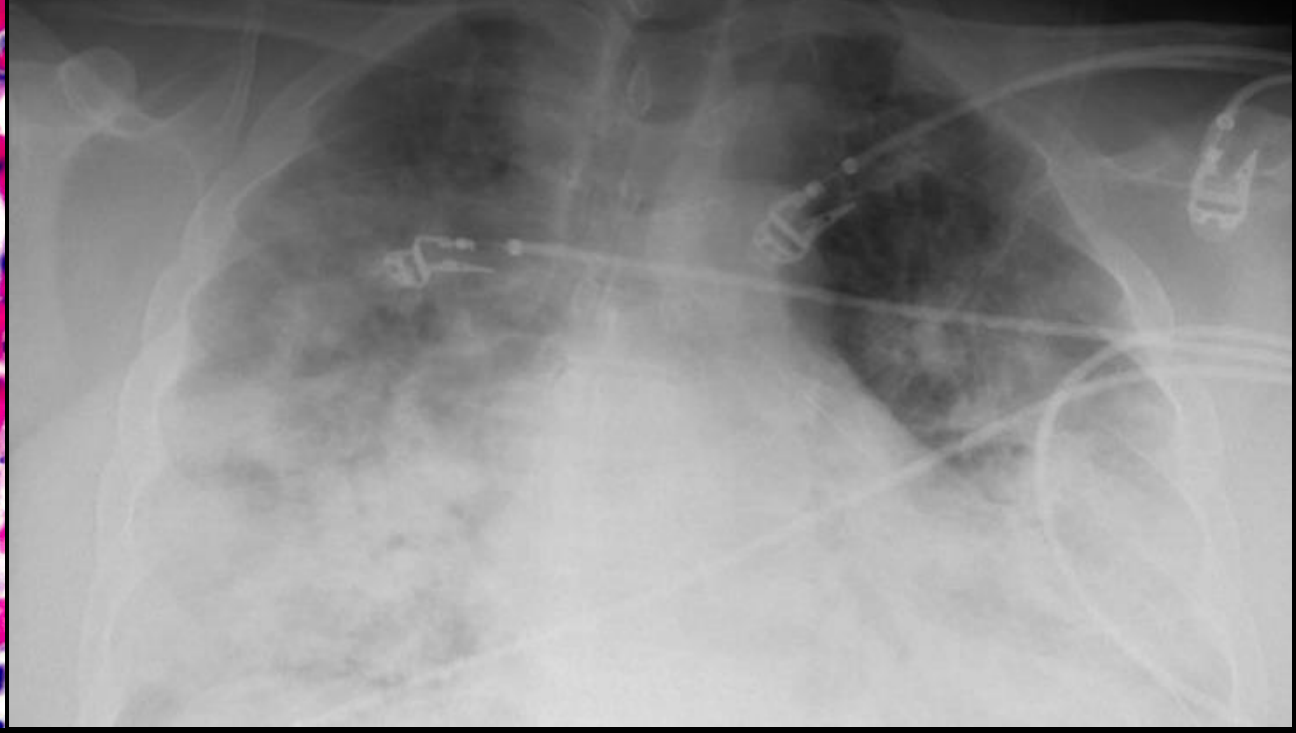
PATHOLOGIC FINDINGS FROM AUTOPSIES AND BEYOND

SANJAY MUKHOPADHYAY, MD
@smlungpathguy

I have no disclosures relevant to this talk

Sanjay Mukhopadhyay, MD
Director, Pulmonary Pathology
Cleveland Clinic





COVID-19 FACTS

DIFFUSE ALVEOLAR DAMAGE AND ACUTE RESPIRATORY DISTRESS SYNDROME

ARDS
is associated with increased risk of death
In COVID-19

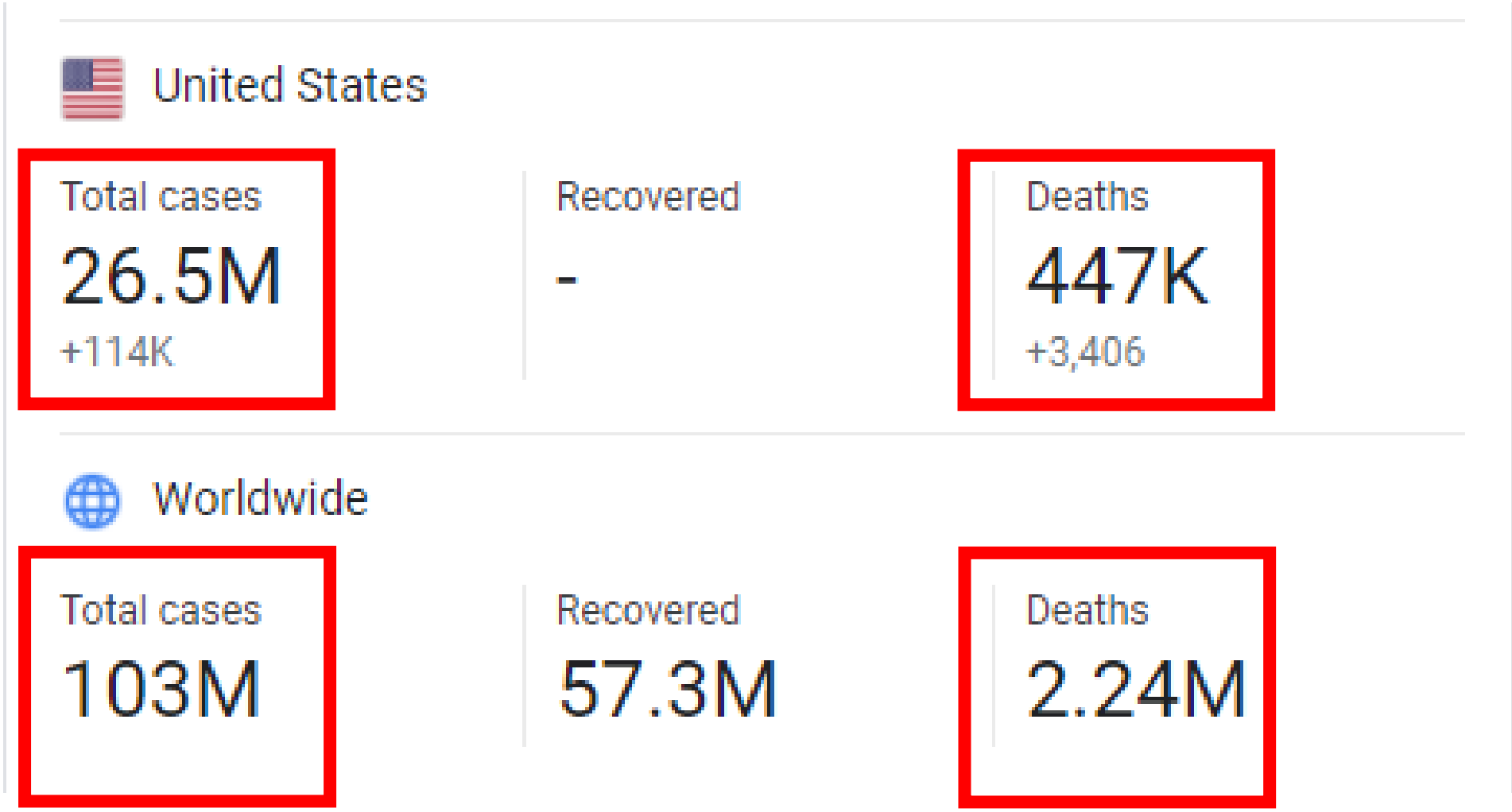
	Total (n=191)	Non-survivor (n=54)	Survivor (n=137)	p value
Treatments*				
Antibiotics	181 (95%)	53 (98%)	128 (93%)	0·15
Antiviral treatment	41 (21%)	12 (22%)	29 (21%)	0·87
Corticosteroids	57 (30%)	26 (48%)	31 (23%)	0·0005
Intravenous immunoglobin	46 (24%)	36 (67%)	10 (7%)	<0·0001
High-flow nasal cannula oxygen therapy	41 (21%)	33 (61%)	8 (6%)	<0·0001
Non-invasive mechanical ventilation	26 (14%)	24 (44%)	2 (1%)	<0·0001
Invasive mechanical ventilation	32 (17%)	31 (57%)	1 (1%)	<0·0001
ECMO	3 (2%)	3 (6%)	0	0·0054
Renal replacement therapy	10 (5%)	10 (19%)	0	<0·0001
Outcomes				
Sepsis	112 (59%)	54 (100%)	58 (42%)	<0·0001
Respiratory failure	103 (54%)	53 (98%)	50 (36%)	<0·0001
ARDS	59 (31%)	50 (93%)	9 (7%)	<0·0001
Heart failure	44 (23%)	28 (52%)	16 (12%)	<0·0001

Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study

Fei Zhou, Ting Yu*, Ronghui Du*, Guohui Fan*, Ying Liu*, Zhibo Liu*, Jie Xiang*, Yeming Wang, Bin Song, Xiaoying Gu, Lulu Guan, Yuan Wei, Hui Li, Xudong Wu, Jiuyang Xu, Shengjin Tu, Yi Zhang, Hua Chen, Bin Cao*

Zhou F et al. *Lancet* 2020;395(10229):1054-62.

COVID-19: February 3, 2020



Deaths in 1 year: COVID-19 vs. Cancer (US)

COVID-19: **281,000**

Lung cancer: 142,081

Breast cancer: 42,466

Prostate cancer: 33,330

Melanoma: 6,850

Thyroid cancer: 2,180

Pathology of COVID-19

Xu: postmortem biopsy from 1 patient

Tian: lobectomies from 2 patients

YouTube video: ARDS

New York Times video

COVID-19 GLOBAL DEATHS

>3000

>102,700

320,609

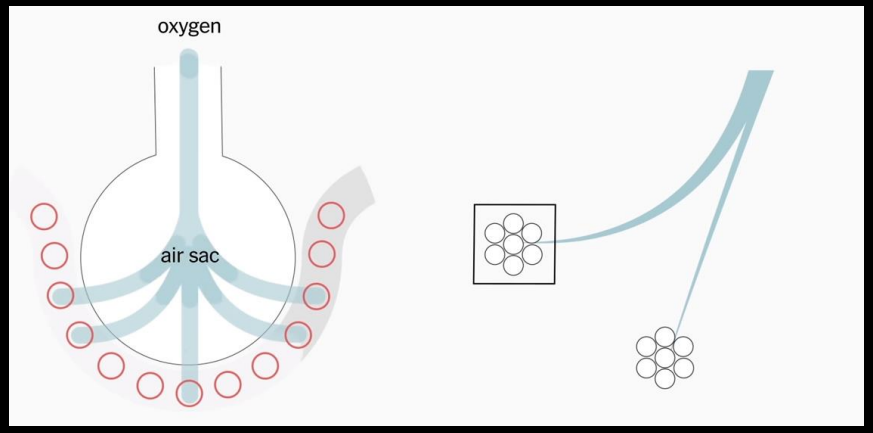
Xu Z et al. *Lancet Respir Med* 2020;8(4):420-422.
Tian S, et al. *J Thorac Oncol* 2020;15(5):700-704.

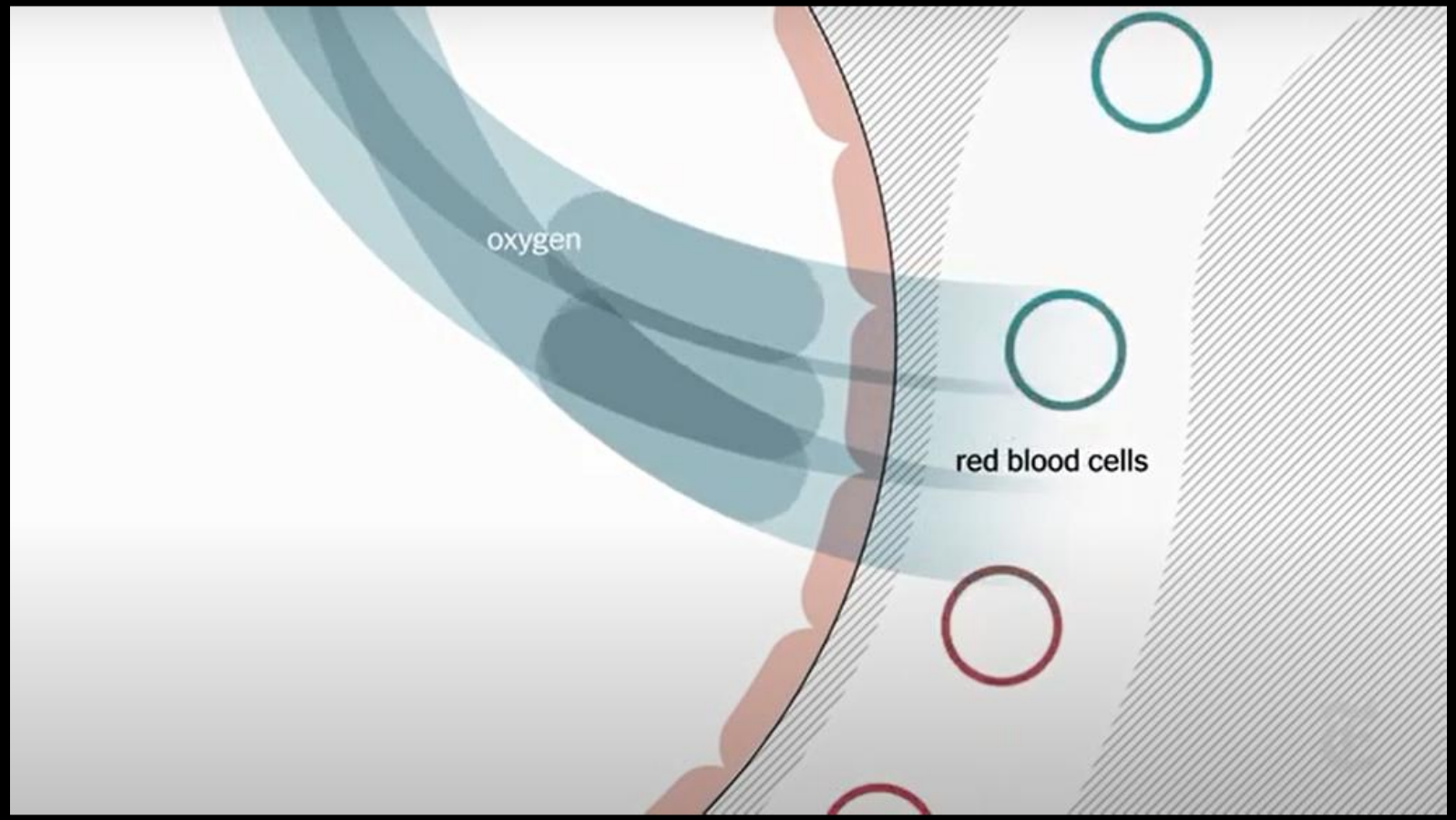
New York Times, April 6, 2020

How Coronavirus Attacks the Body | NYT News

1,423,427 views • Apr 6, 2020

The New York Times
3.22M subscribers





How Coronavirus Attacks the Body. Produced by Joshua Kessel, New York Times, April 6, 2020. https://www.youtube.com/watch?v=BuzP-uLctYE

ARDS vs. DAD

Acute Respiratory Distress Syndrome (ARDS)

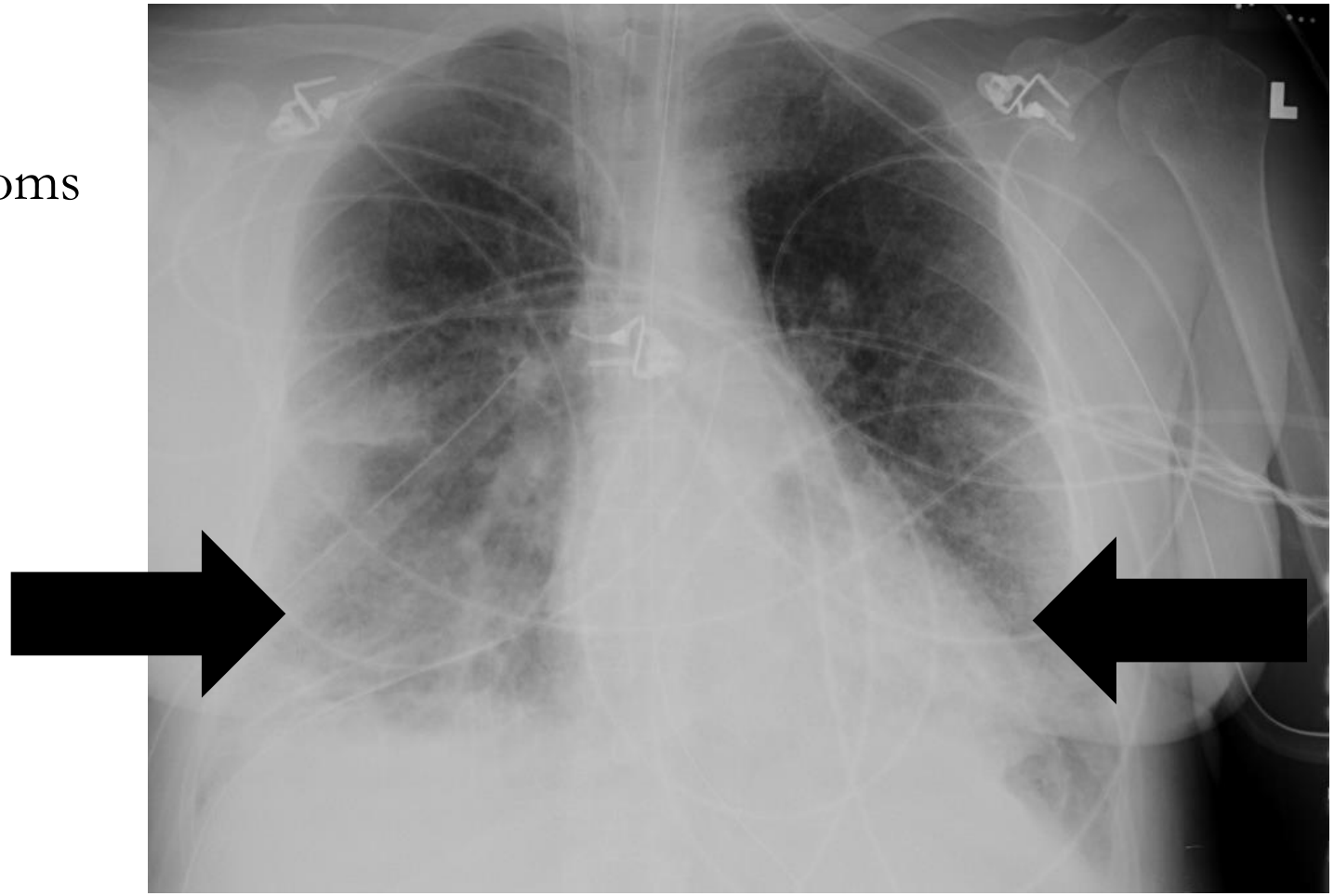
… is defined by clinical criteria

Diffuse Alveolar Damage (DAD)

… is defined by histologic criteria

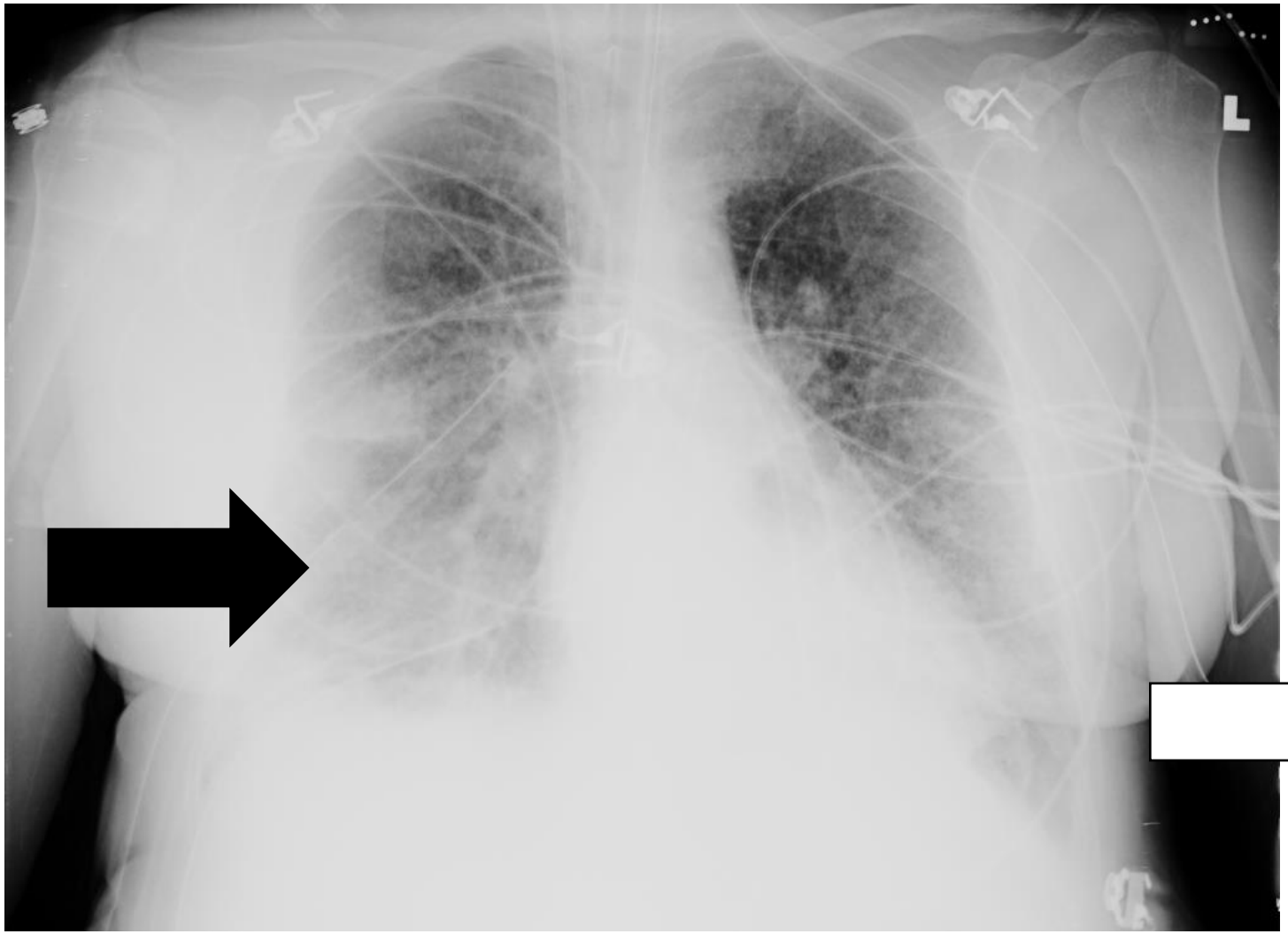
ARDS

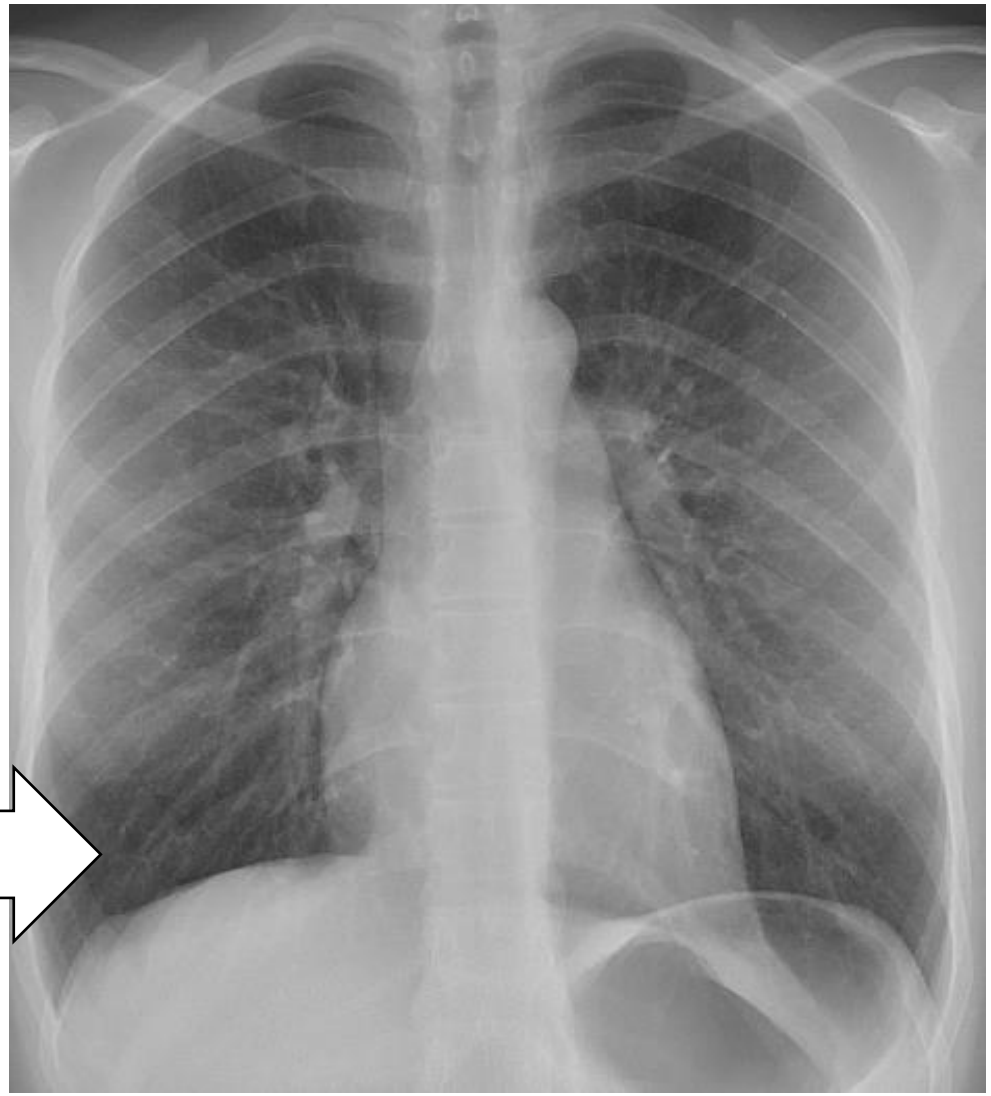
- Acute = onset within 1 week of a known clinical insult or new or worsening symptoms
- Respiratory failure not explained by heart failure or fluid overload
- Hypoxia (low oxygen levels)
- Bilateral lung opacities on chest imaging



ARDS

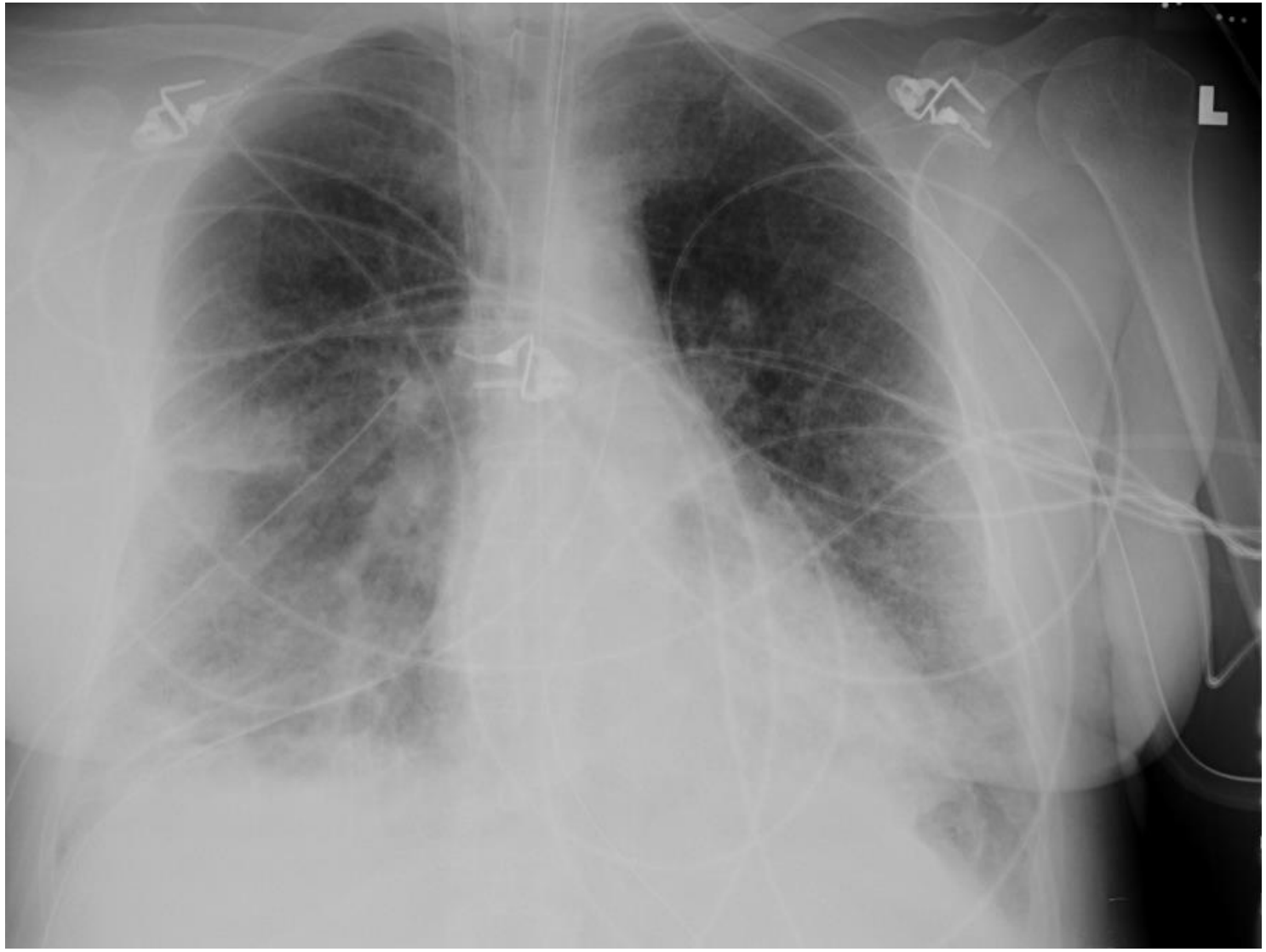
Normal



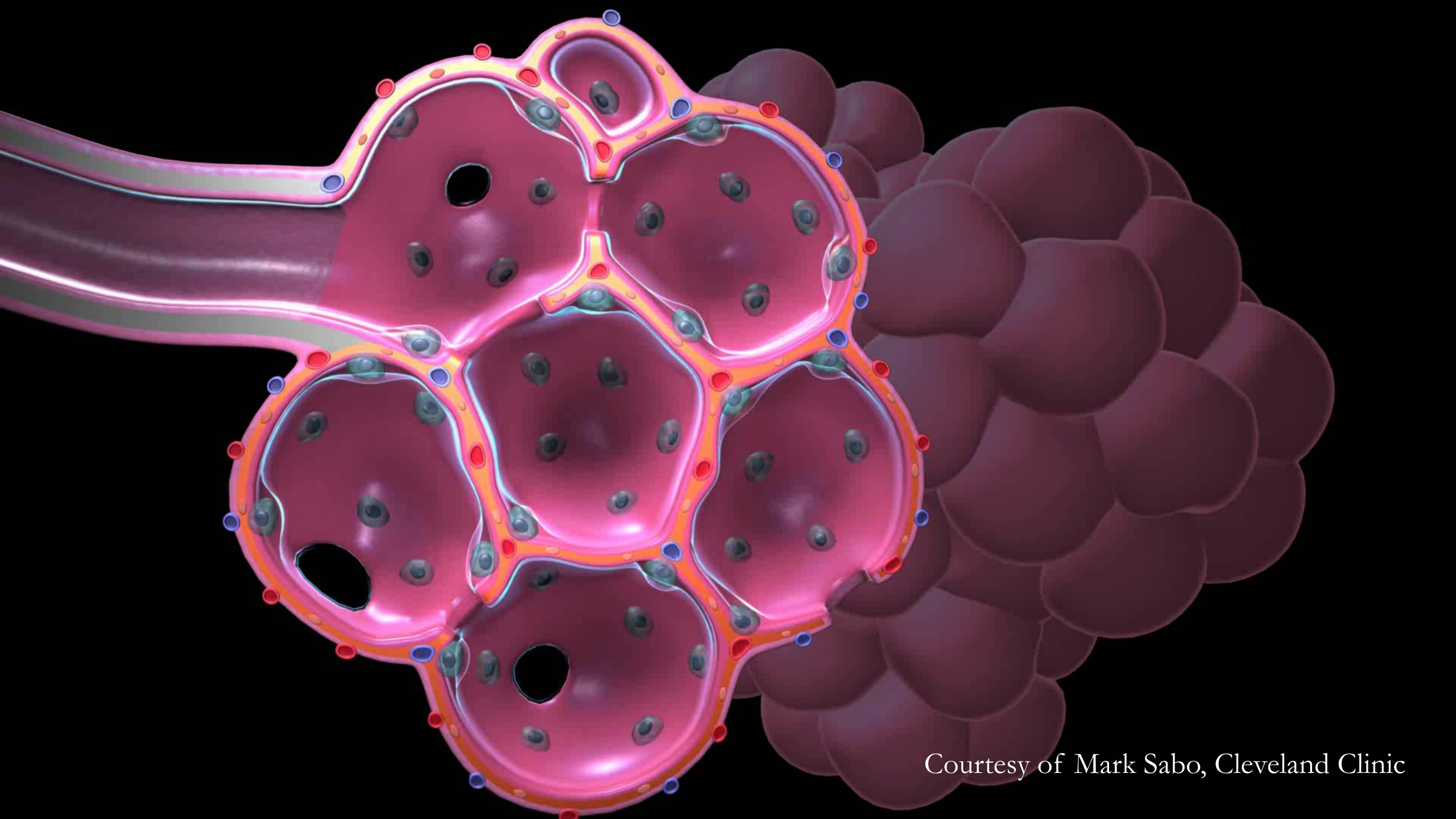


What causes ARDS?

- **Sepsis/Infection**
- Shock
- Toxic inhalants
- Drug overdose
- Chemotherapy
- Toxic ingestants
- Aspiration
- Irradiation
- Pancreatitis
- Massive blood transfusion
- Major trauma




Courtesy of Mark Sabo, Cleveland Clinic



Courtesy of Mark Sabo, Cleveland Clinic

SARS-CoV-2 detected by IHC in upper airways and lungs but **NOT** in heart, liver, kidney

Volume 26, Number 9—September 2020

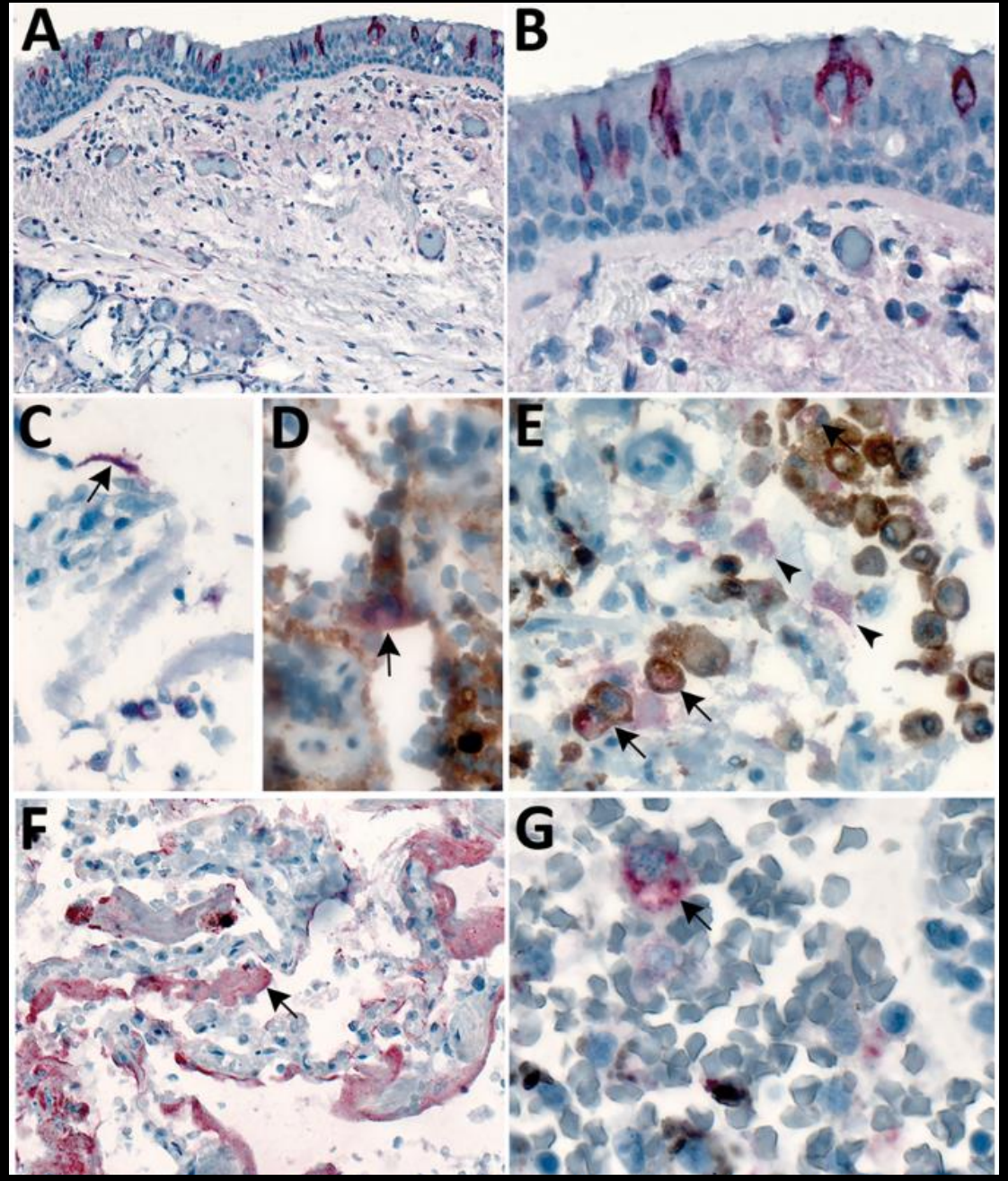
Synopsis

Pathology and Pathogenesis of SARS-CoV-2 Associated with Fatal Coronavirus Disease, United States

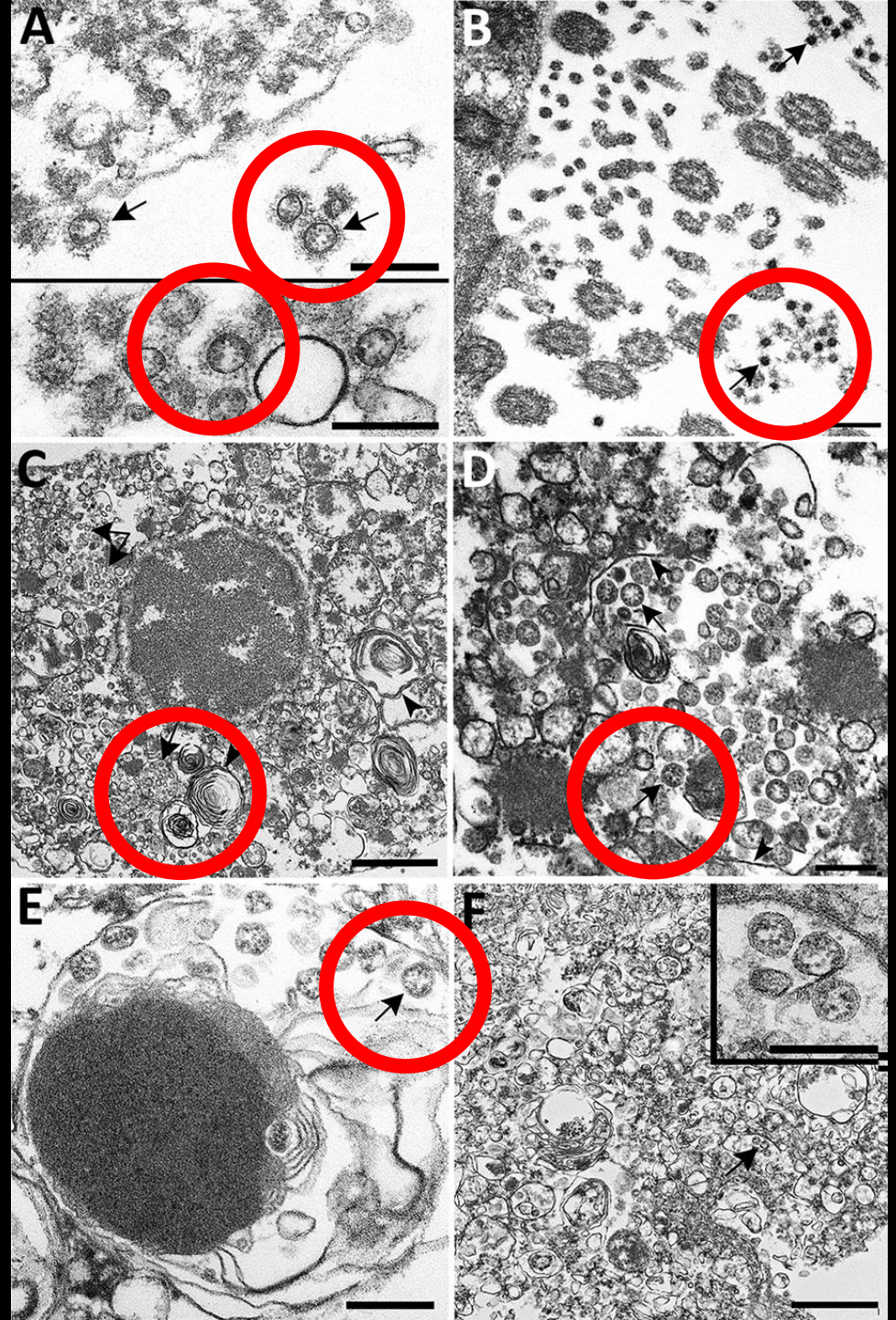
Roosecelis B. Martines[1]✉, Jana M. Ritter[1], Eduard Matkovic, Joy Gary, Brigid C. Bollweg, Hannah Bullock, Cynthia S. Goldsmith, Luciana Silva-Flannery, Josilene N. Seixas, Sarah Reagan-Steiner, Timothy Uyeki, Amy Denison, Julu Bhatnagar, Wun-Ju Shieh, Sherif R. Zaki, and COVID-19 Pathology Working Group

Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (R.B. Martines, J.M. Ritter, E. Matkovic, J. Gary, B.C. Bollweg, C.S. Goldsmith, L. Silva-Flannery, J.N. Seixas, S. Reagan-Steiner, T. Uyeki, A. Denison, J. Bhatnagar, W.-J. Shieh, S.R. Zaki); Synergy America Inc., Atlanta (H. Bullock).

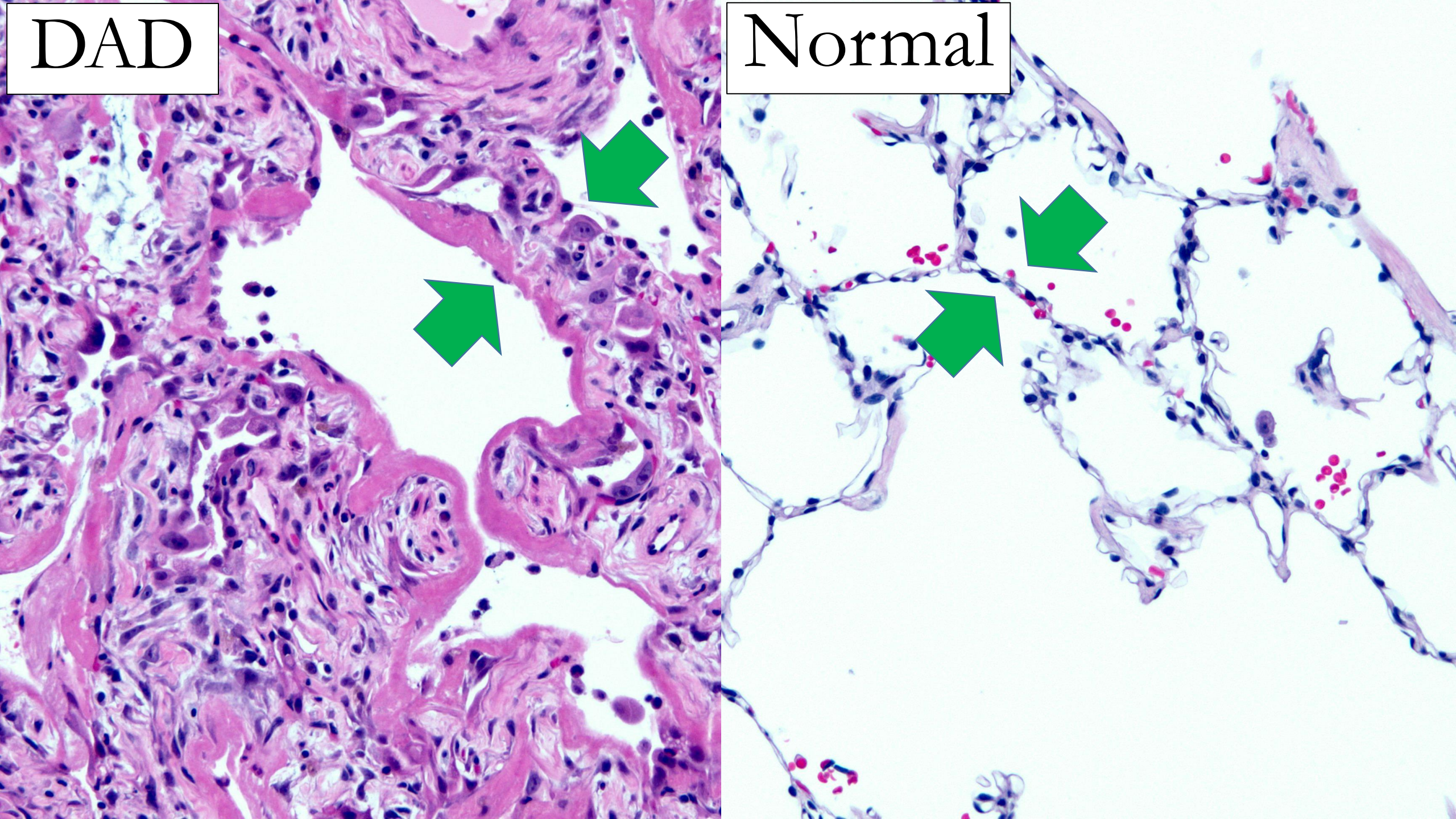
Martines RB, et al. *Emerg Infect Dis* 2020. PMID 32437316

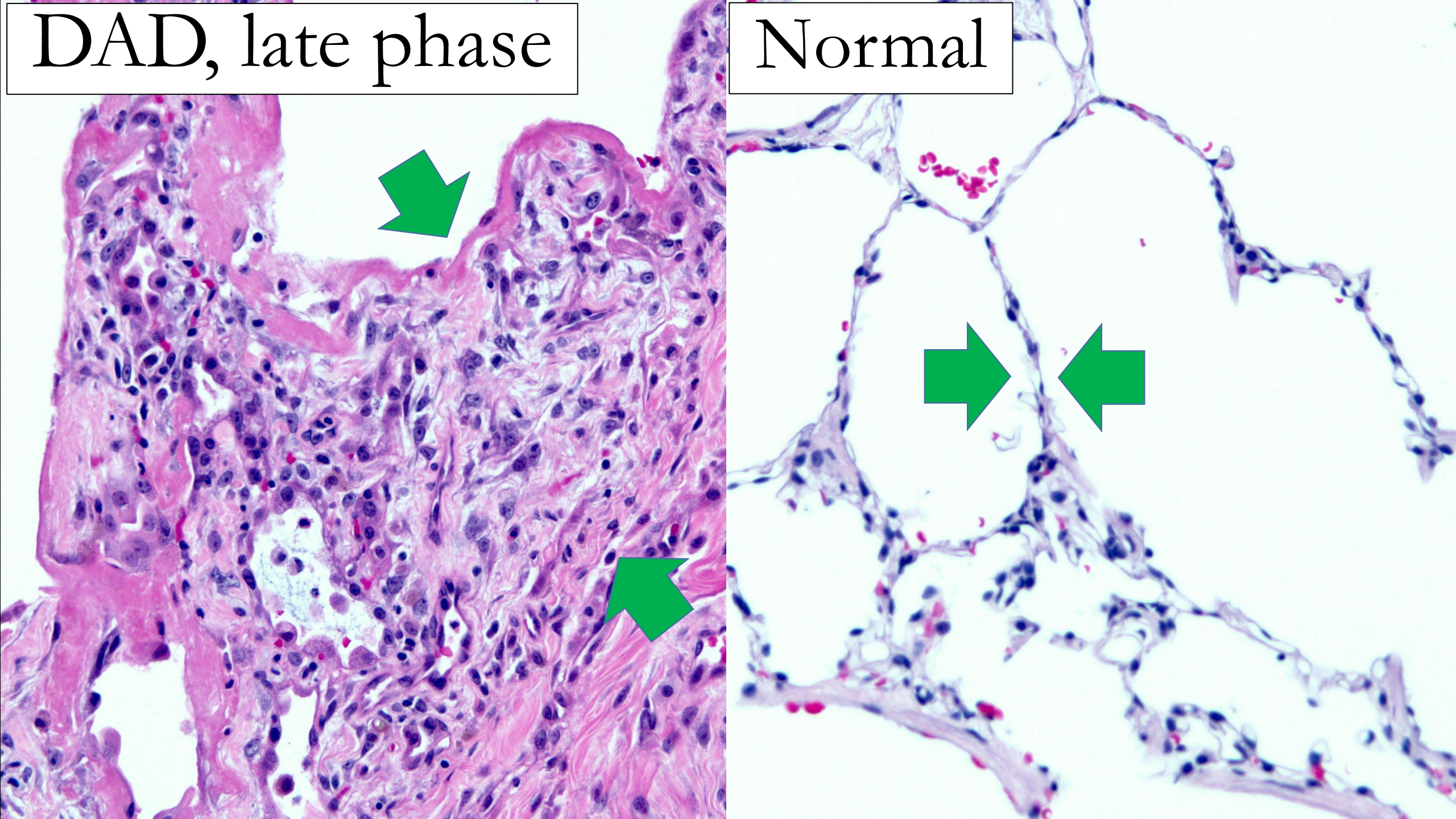


Martines RB, et al. *Emerg Infect Dis* 2020. PMID 32437316



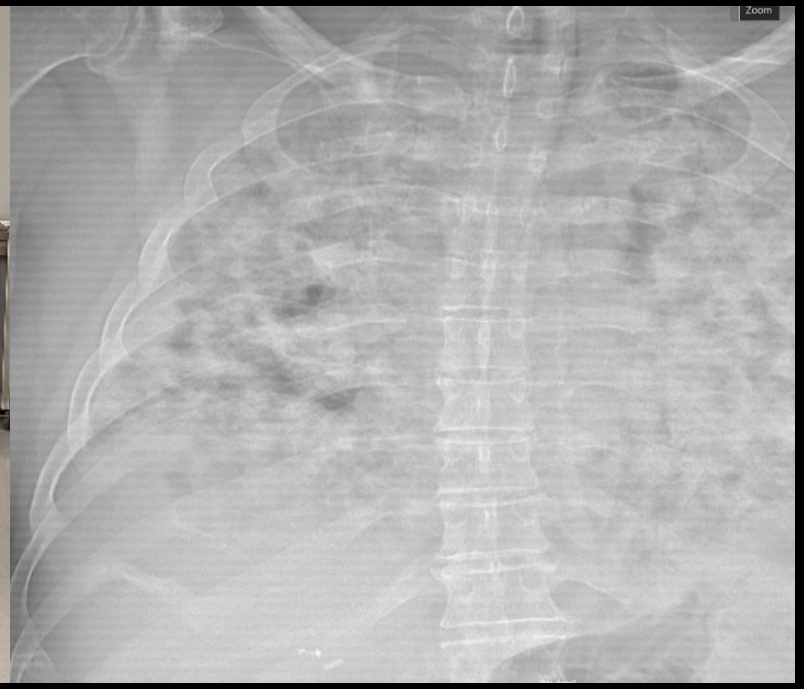
A. Alveolar space
B. Ciliated cells upper airway
C. Type 2 pneumocyte (virus and surfactant)
D. Membrane bound vacuole
E. Alveolar macrophage
F. Hyaline membrane

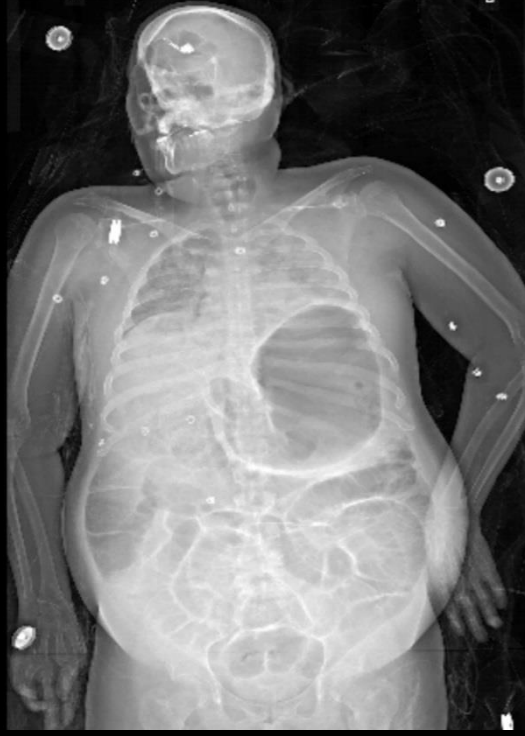

DAD
Normal


DAD, late phase
Normal

ARDS is the result of sudden, **severe injury to the lung** from any cause

When ARDS develops, **oxygen levels drop** and mechanical ventilation may be required to keep patients alive





COVID-19 AUTOPSIES OK, USA

FIRST PUBLISHED REPORT OF COMPLETE COVID-19 AUTOPSIES IN THE LITERATURE

COVID-19 Autopsies, Oklahoma, USA

Lisa M. Barton, MD, PhD,[1] Eric J. Duval, DO,[1] Edana Stroberg, DO,[1] Subha Ghosh, MD,[2] and Sanjay Mukhopadhyay, MD[3]

From the [1]Office of the Chief Medical Examiner, Oklahoma City, OK; [2]Section of Thoracic Imaging, Imaging Institute, Cleveland Clinic, Cleveland, OH; and [3]Department of Pathology, Cleveland Clinic, Cleveland, OH.

Key Words: Coronavirus; COVID-19; SARS-CoV-2; Autopsy; Diffuse alveolar damage; Acute lung injury; Pulmonary pathology.

Am J Clin Pathol 2020;XX:1-9

DOI: 10.1093/AJCP/AQAA062

April 10: Barton et al
First published report of complete autopsies

GLOBAL DEATHS

>102,700

320,609

Jan 2020	Feb 2020	March 2020	April 2020	May 2020

Barton LM et al. *Am J Clin Pathol* 2020;153(6):725-733. PMID 32275742

Impact

Covid-19 autopsies, oklahoma, usa

..., E Stroberg, S Ghosh, S **Mukhopadhyay** - American journal of ..., 2020 - academic.oup.com

Objectives To report the methods and findings of two complete autopsies of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) positive individuals who died in Oklahoma (United States) in March 2020. Methods Complete postmortem examinations ...

☆ 99 Cited by 458 Related articles All 24 versions »

COVID-19 Autopsies, Oklahoma, USA

Overview of attention for article published in American Journal of Clinical Pathology, April 2020

SUMMARY | News | Blogs | Twitter | Facebook | Wikipedia | Reddit

Title	COVID-19 Autopsies, Oklahoma, USA
Published in	American Journal of Clinical Pathology, April 2020
DOI	10.1093/ajcp/aqaa062
Pubmed ID	32275742
Authors	Lisa M Barton, Eric J Duval, Edana Stroberg, Subha Ghosh, Sanjay Mukhopadhyay

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COVID-19: What the Autopsies Reveal

Full Transcript

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Study: Autopsies Can Help Determine If Patient Died From Or With COVID-19

Cleveland Clinic doctors were involved in the first study published in the U.S. that examined complete autopsies of individuals with COVID-19. [Cleveland Clinic]

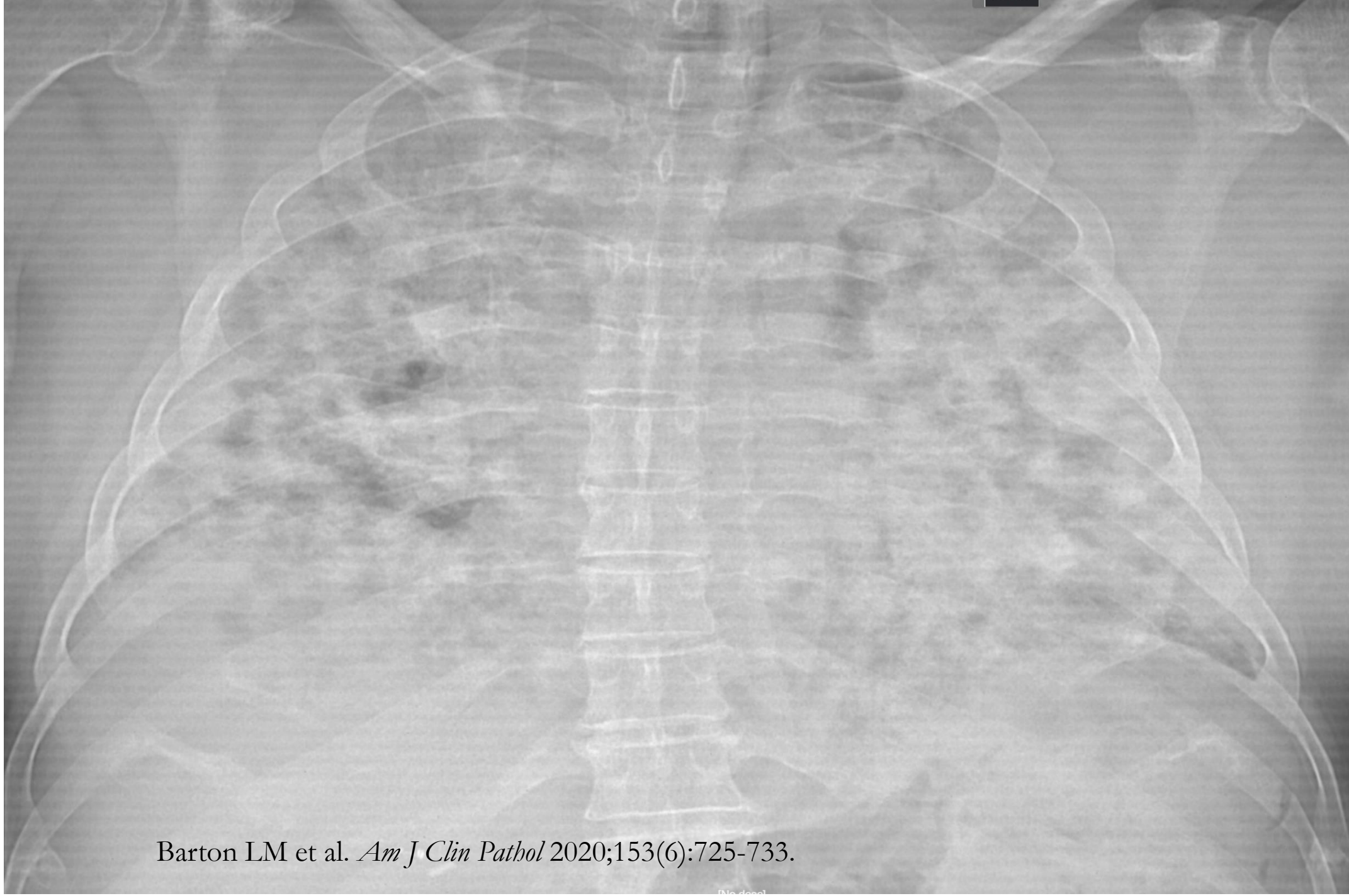
Case 1: 77/M

H/o fever, chills x 6 days

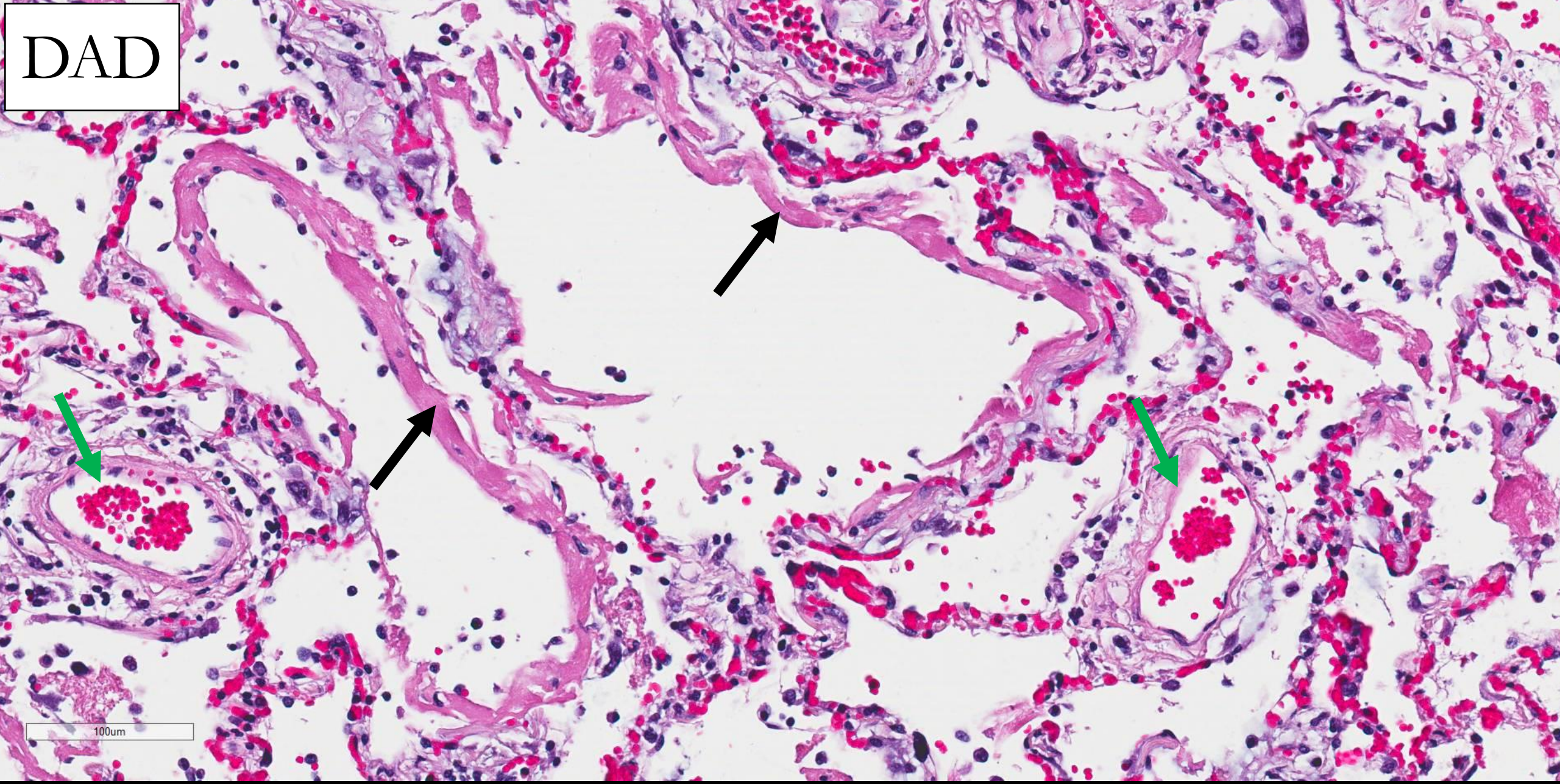
H/o hypertension, remote splenectomy

Never tested for COVID-19 pre-mortem

Arrested en route to hospital

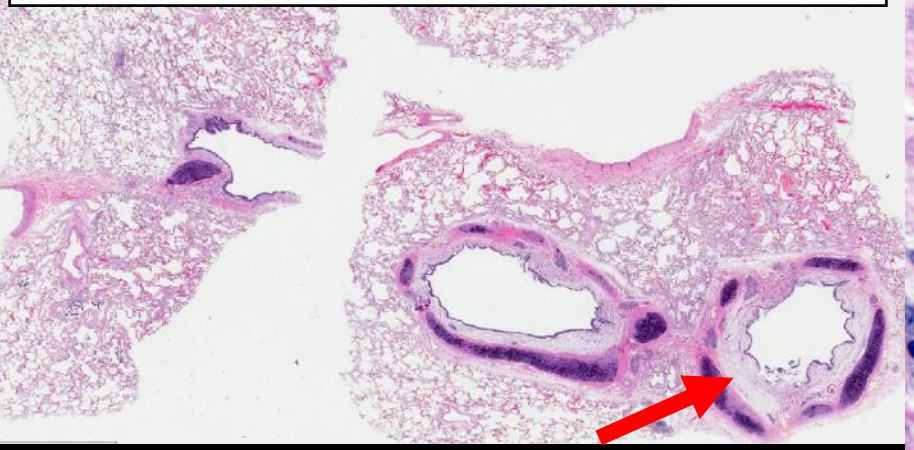


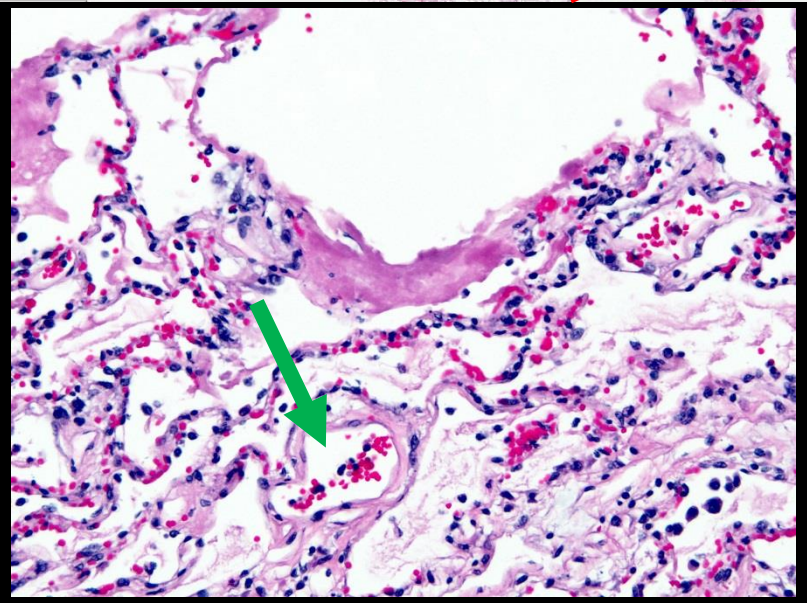
Barton LM et al. *Am J Clin Pathol* 2020;153(6):725-733.

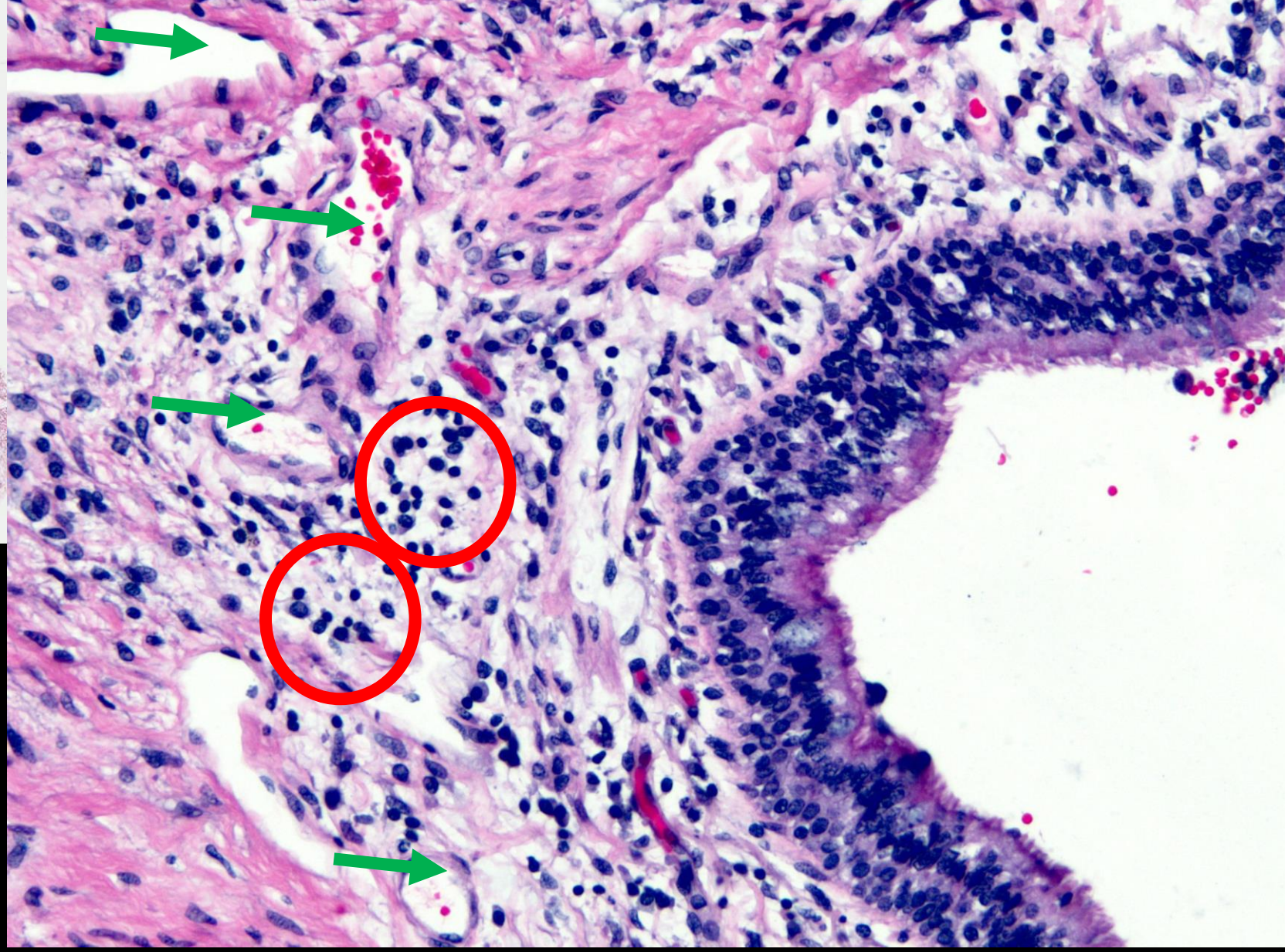


Barton LM et al. *Am J Clin Pathol* 2020;153(6):725-733.

Inflammation in COVID-19

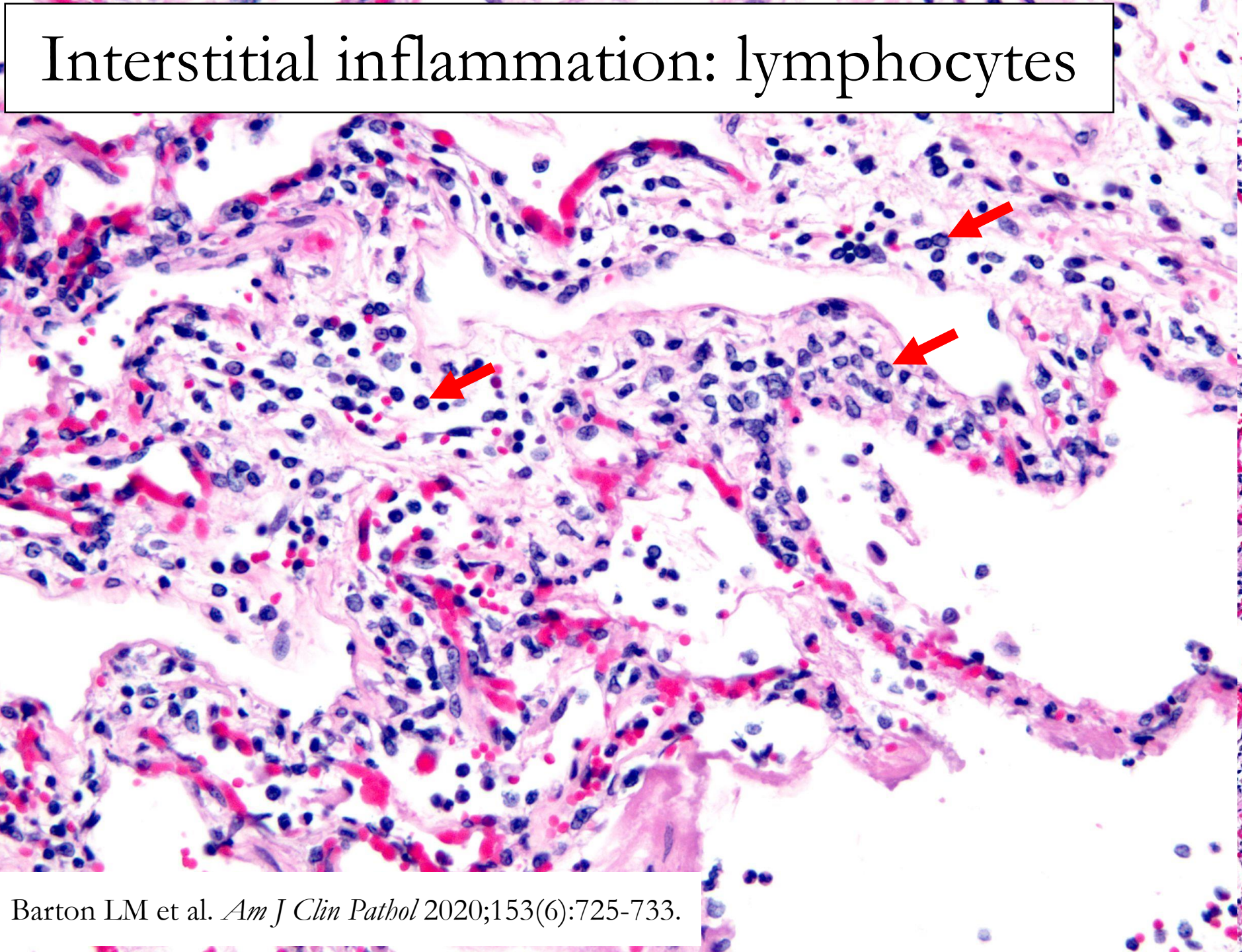


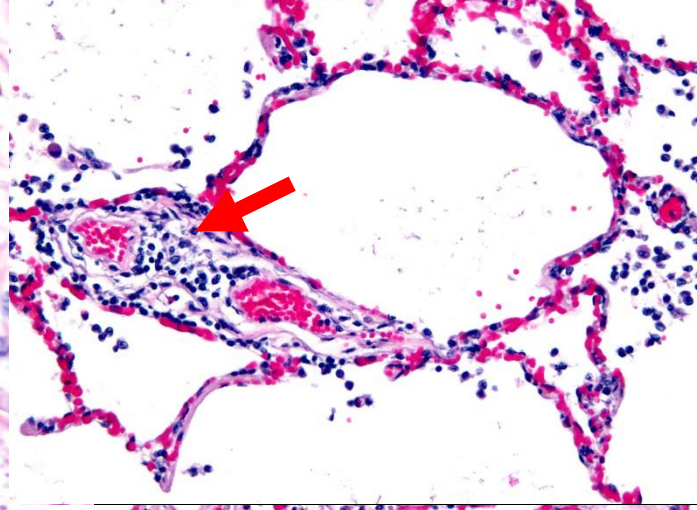


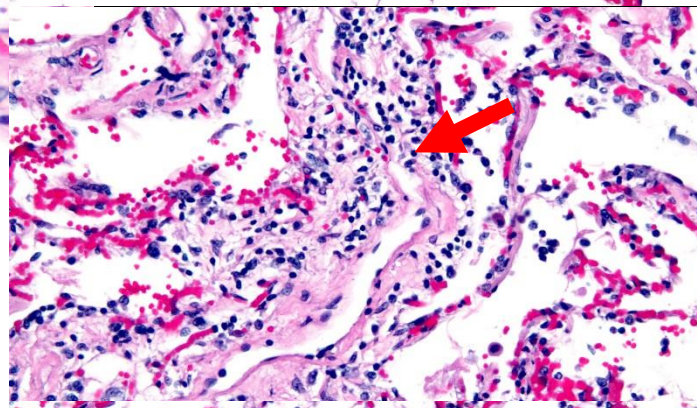


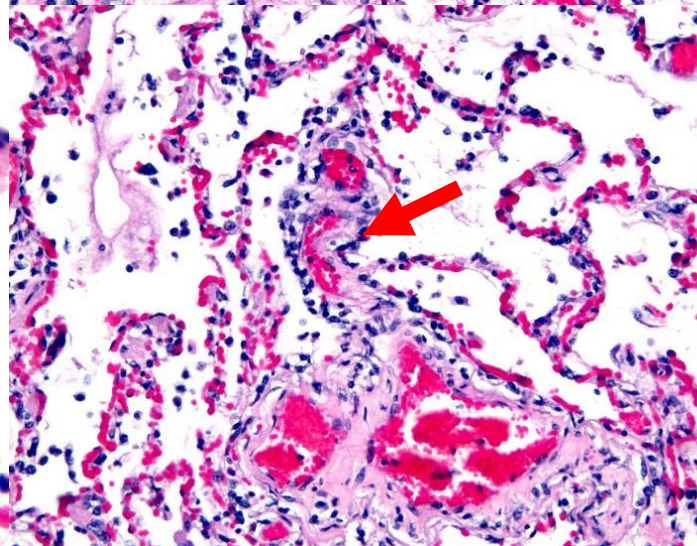
Barton LM et al. *Am J Clin Pathol* 2020;153(6):725-733.

Interstitial inflammation: lymphocytes





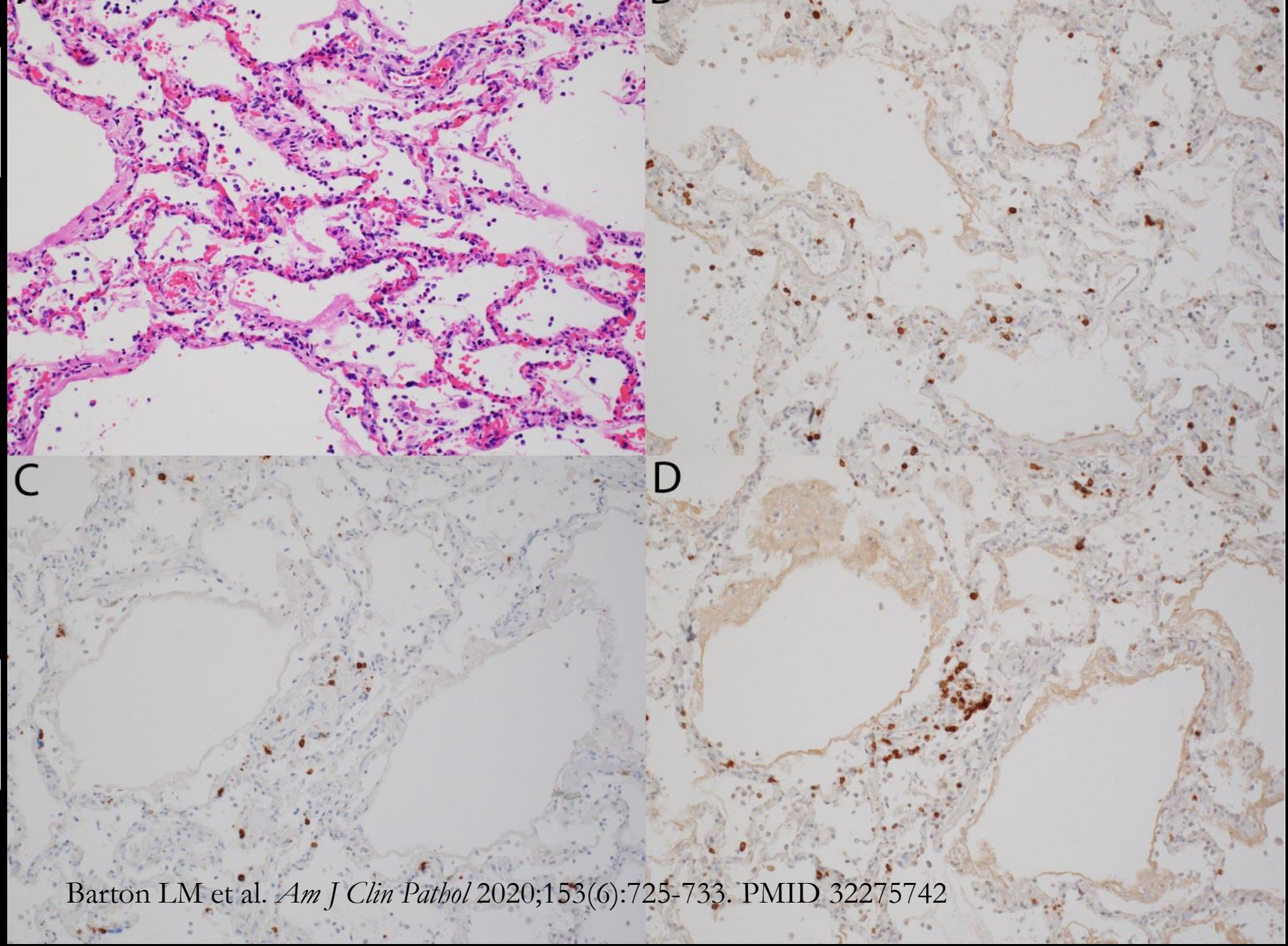




Barton LM et al. *Am J Clin Pathol* 2020;153(6):725-733.

H&E

CD3



CD4

CD8

Barton LM et al. *Am J Clin Pathol* 2020;153(6):725-733. PMID 32275742

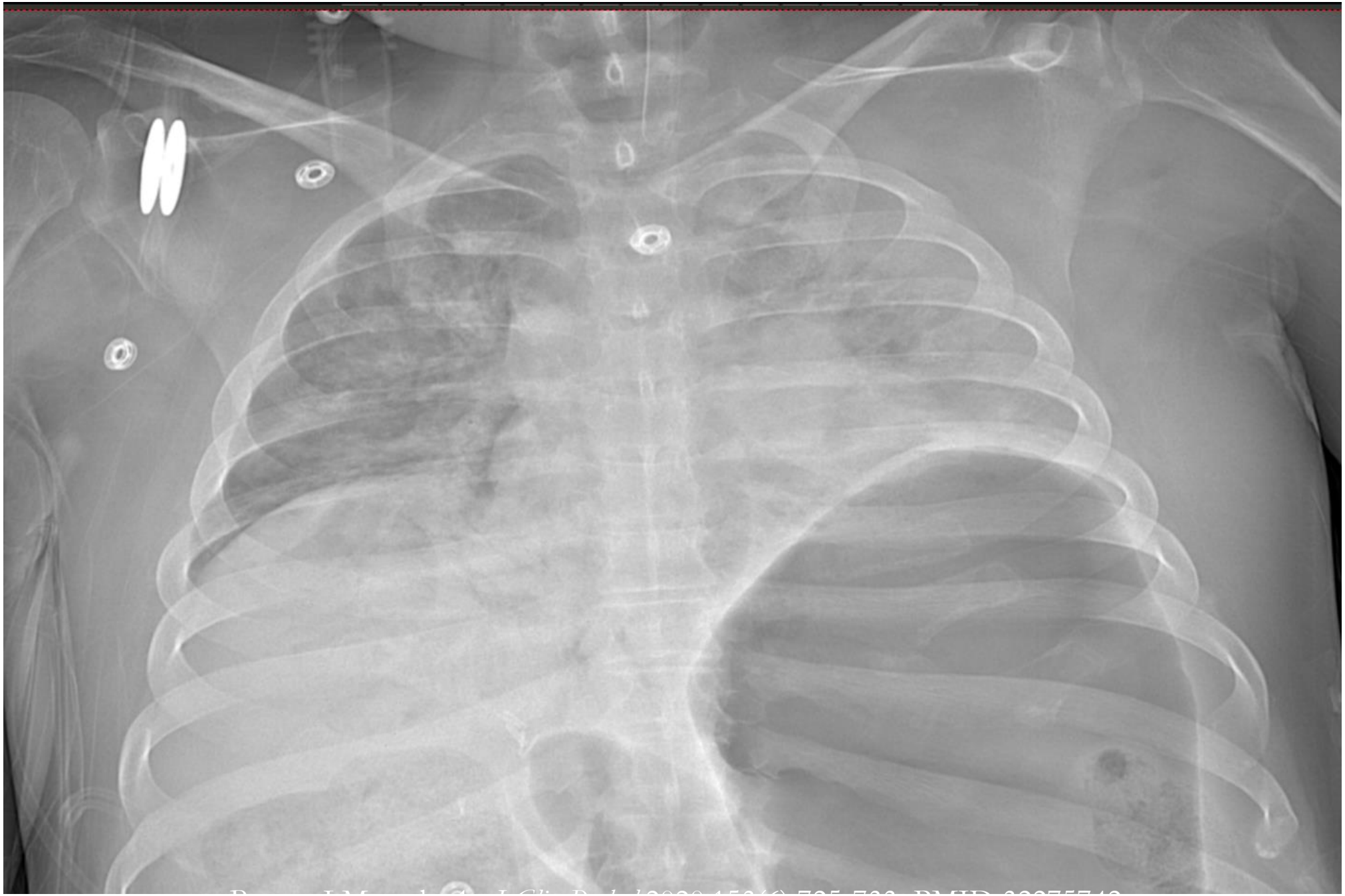
Case 2: 42/M

H/o abdominal pain f/b respiratory symptoms (“CAP”)

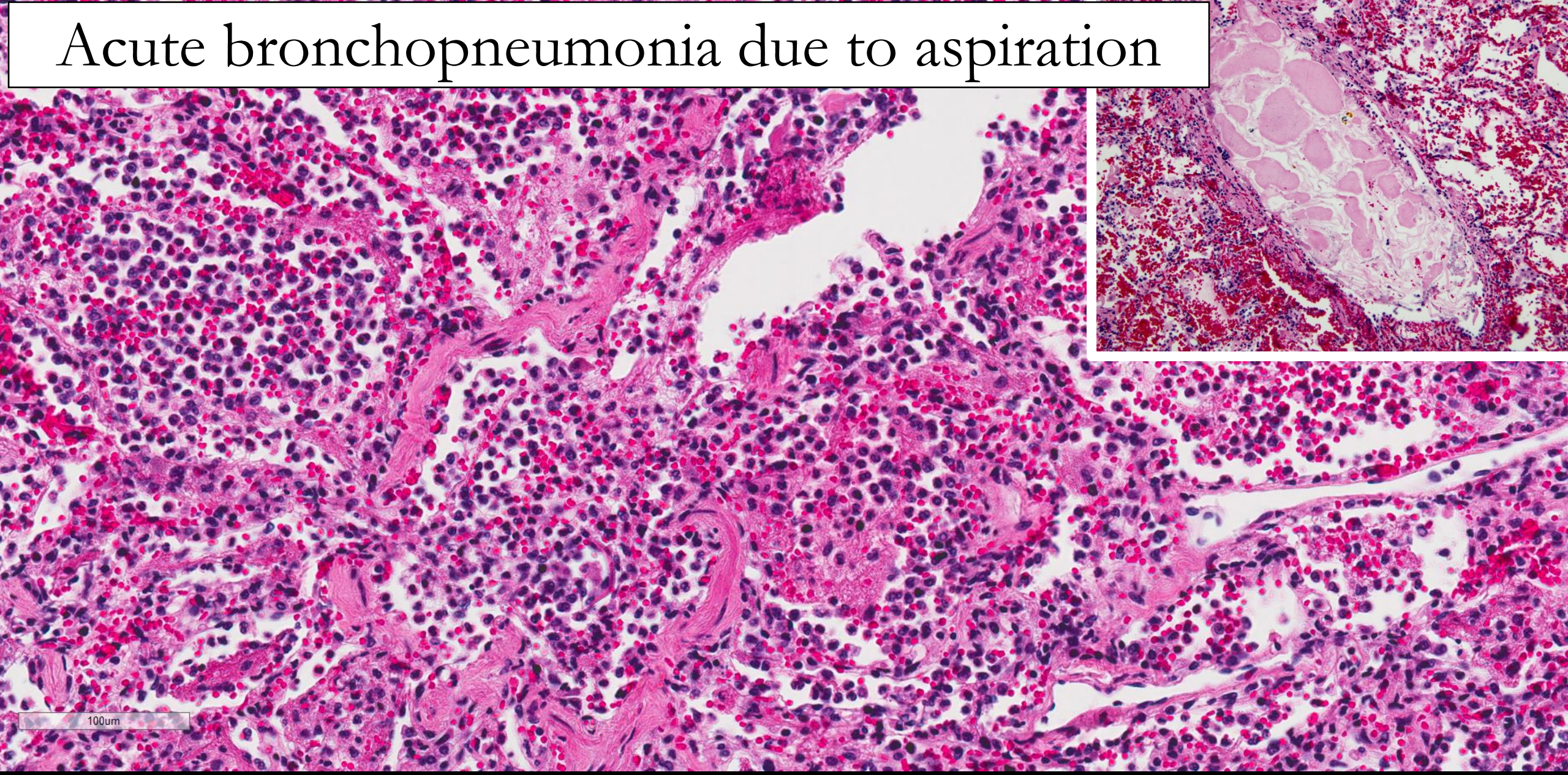
H/o myotonic dystrophy

Never tested for COVID-19 pre-mortem

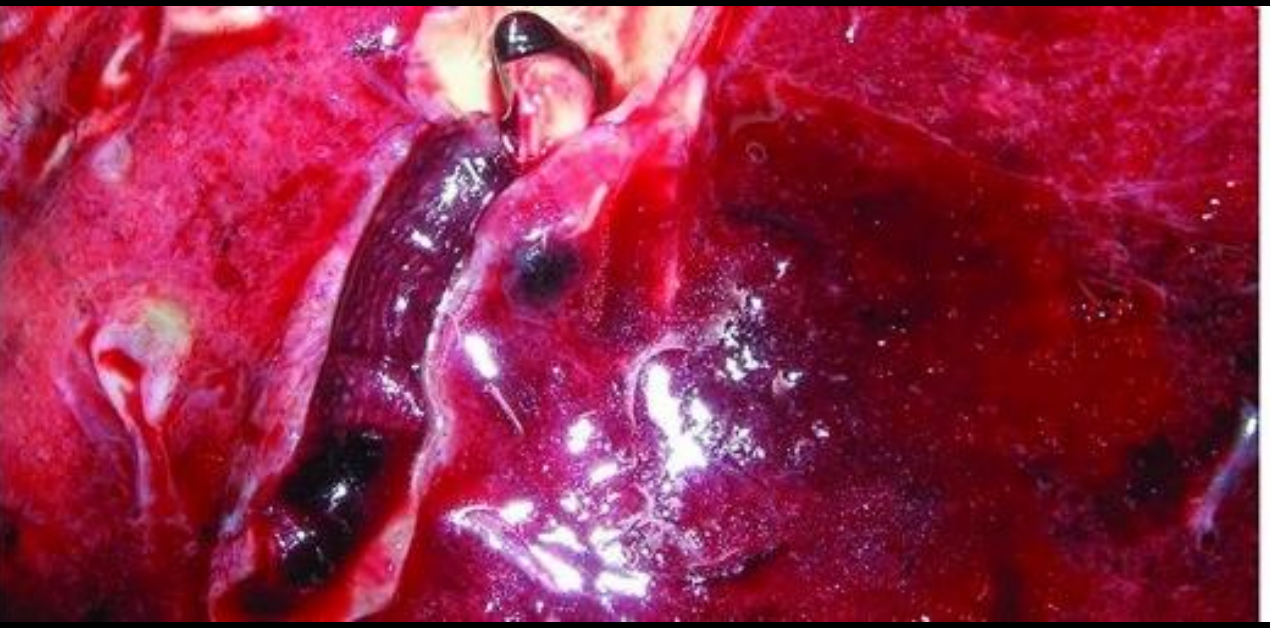
Died after few hours in hospital

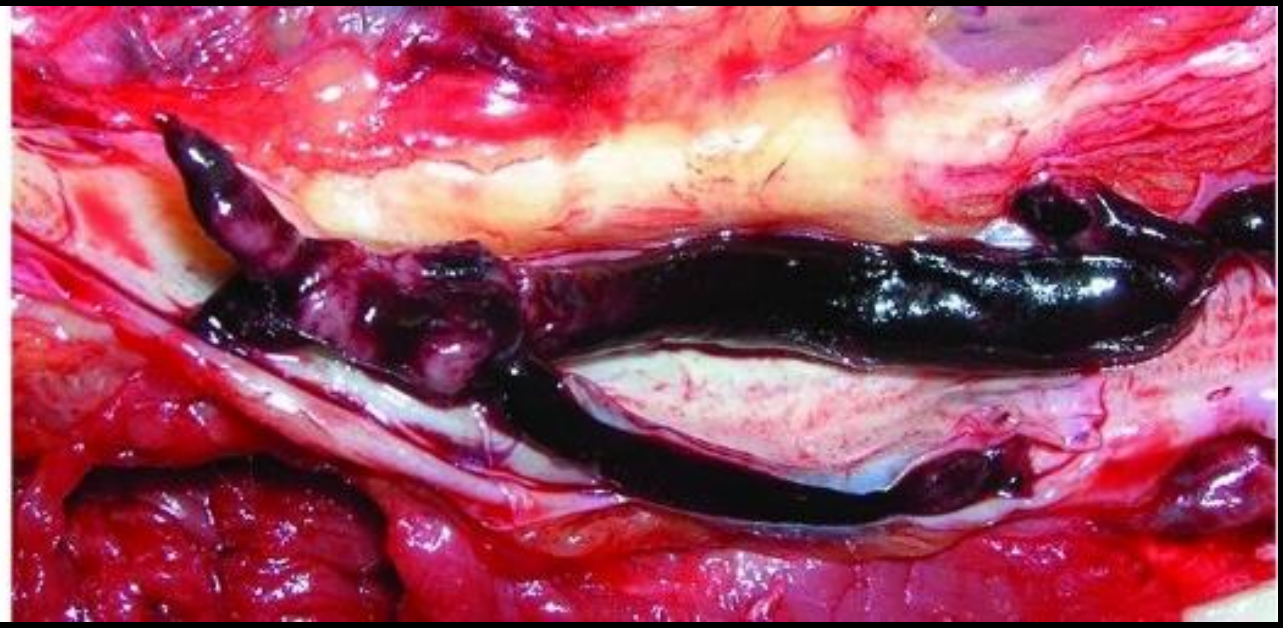


Acute bronchopneumonia due to aspiration



Barton LM et al. *Am J Clin Pathol* 2020;153(6):725-733. PMID 32275742



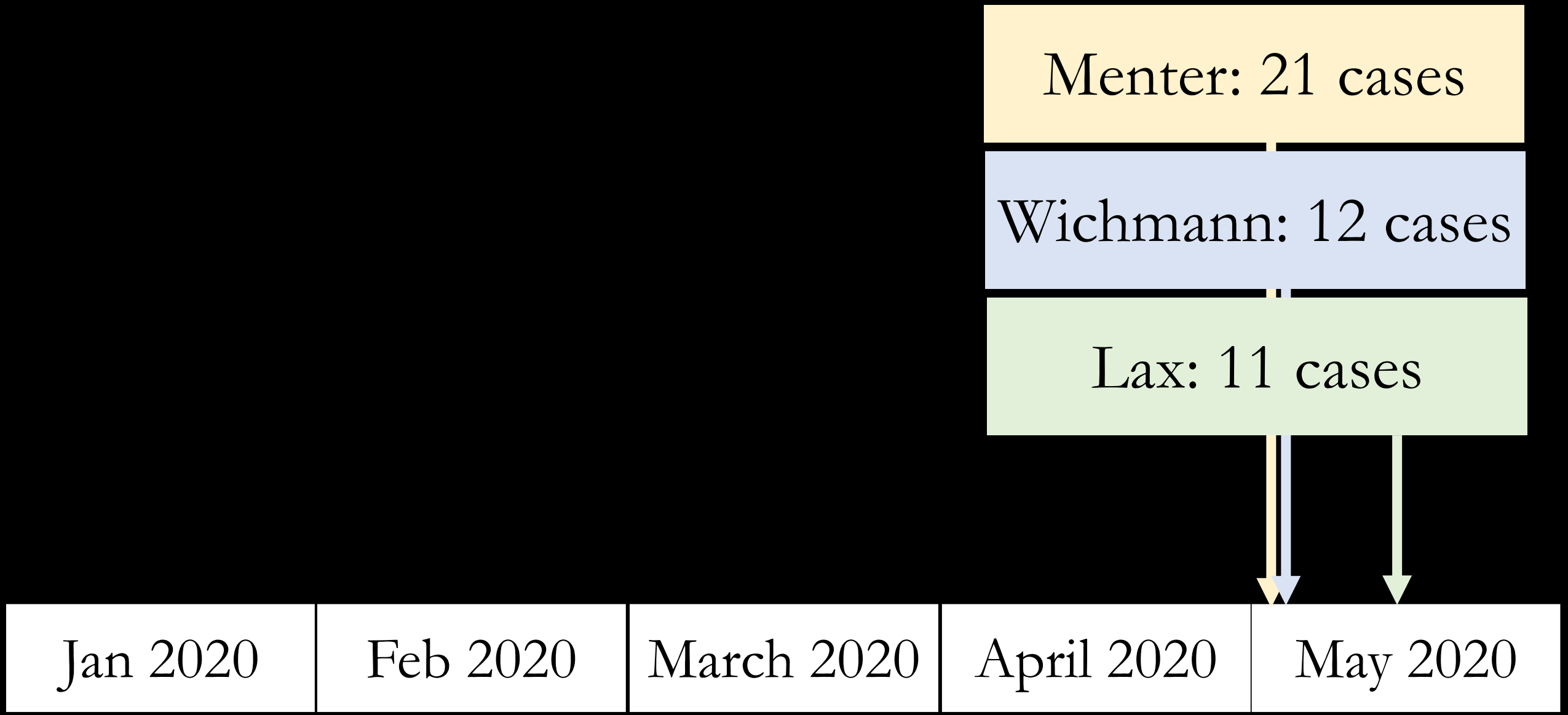


OTHER AUTOPSY STUDIES

DIFFUSE ALVEOLAR DAMAGE

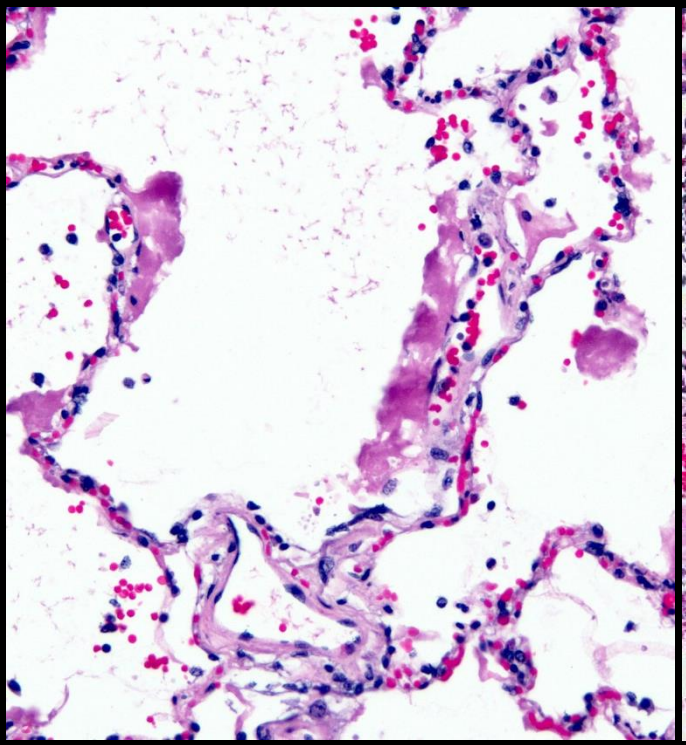
ACUTE BRONCHOPNEUMONIA

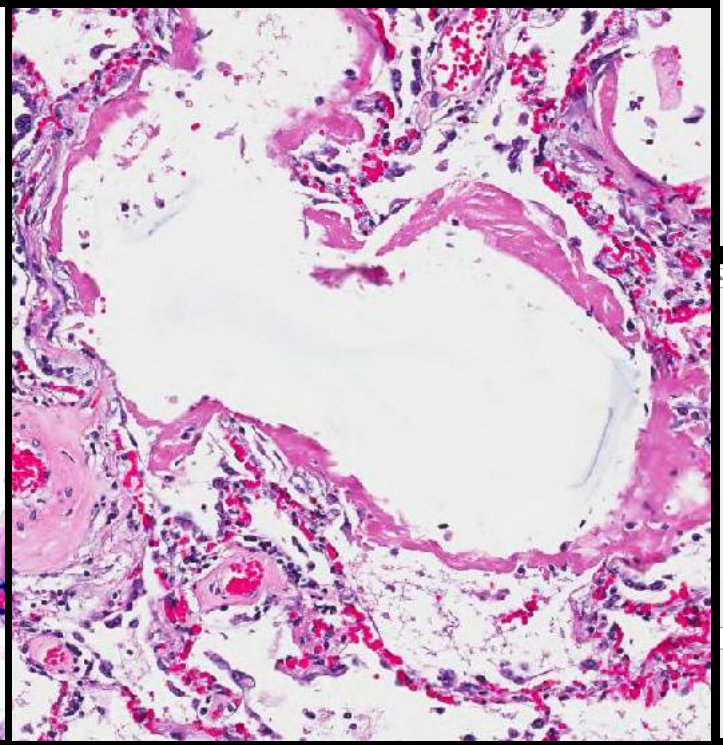
THROMBOSIS



Menter T, et al. *Histopathology* 2020. PMID 32364264.
Wichmann D, et al. *Ann Intern Med* 2020. PMID 32374815.
Lax SF, et al. *Ann Intern Med* 2020. PMID 32422076.

DAD is very common in fatal COVID-19





Histopathology

DAD in 16 (acute)and 8 (org)

ORIGINAL ARTICLE | Open Access

Post-mortem examination of COVID19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings of lungs and other organs suggesting vascular dysfunction

T. Menter, J.D. Haslbauer, R. Nienhold, S. Savic, H. Hopfer, N. Deigendesch, S. Frank, D. Turek, N. Willi, H. Pargger, S. Bassetti, J.D. Leuppi, G. Cathomas, M. Tolnay, K.D. Mertz, A. Tzankov

First published:04 May 2020 | https://doi.org/10.1111/his.14134

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DAD in 8/12

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A Prospective Cohort Study

Dominic Wichmann, MD, Jan-Peter Sperhake, MD, Marc Lütgehetmann, MD, Stefan Steurer, MD, Carolin Edler, MD,

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DAD in 10/11

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Original Research | 14 May 2020

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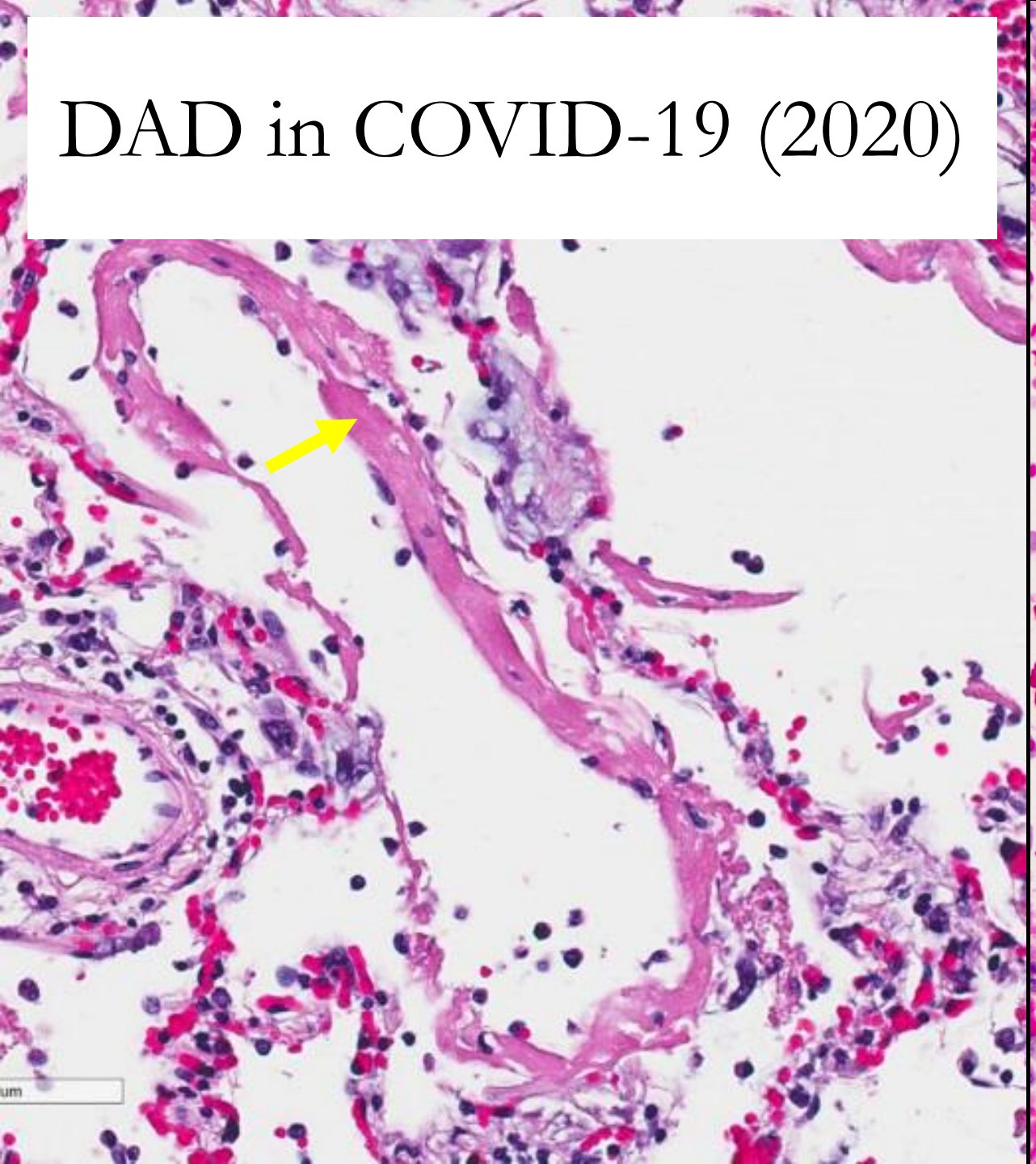
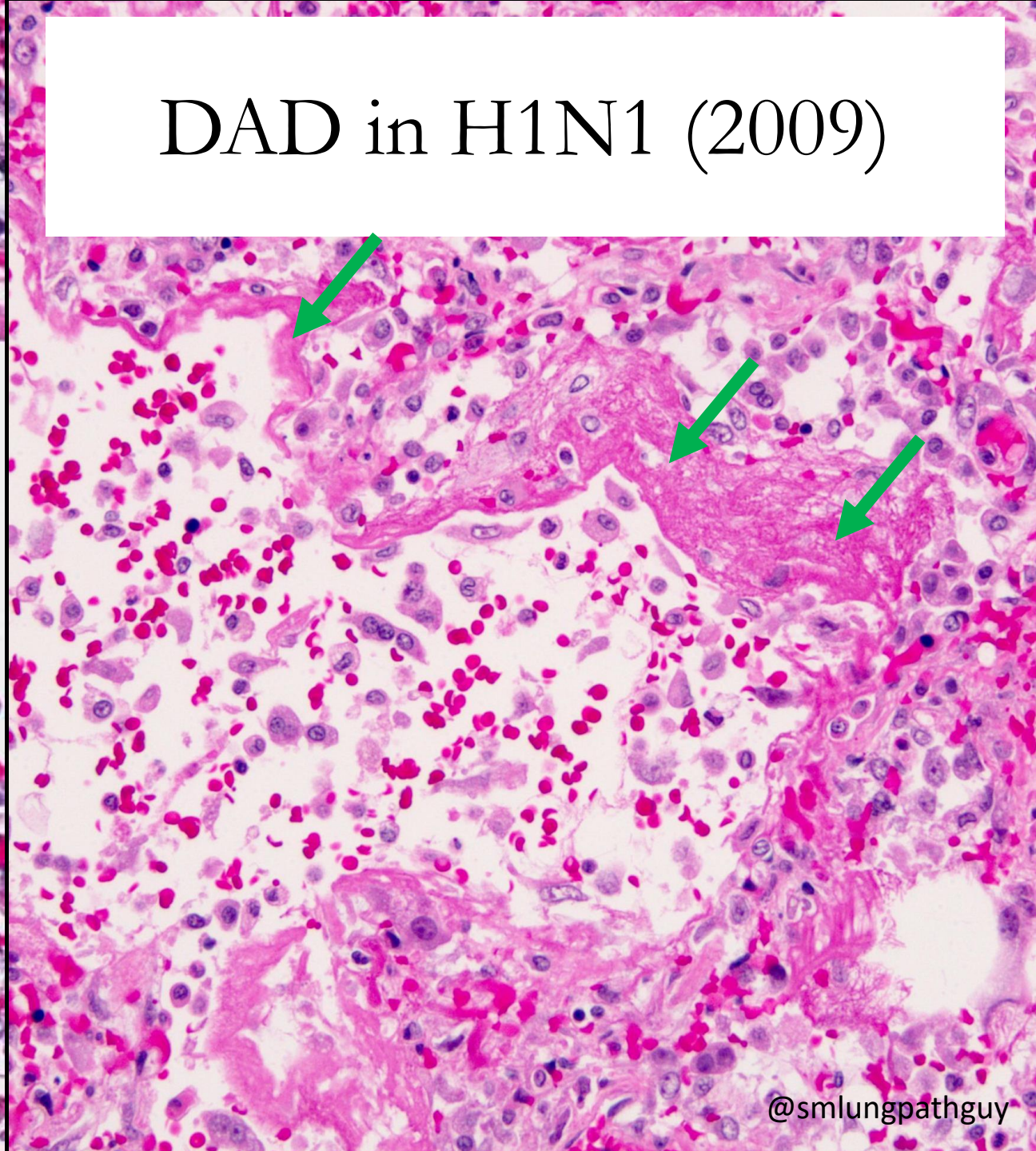
Sigurd F. Lax, MD, PhD, Kristijan Skok, MD, Peter Zechner, MD, Harald H. Kessler, MD, PhD, ... View all authors +

Author, Article and Disclosure Information

DAD in COVID-19 (2020)



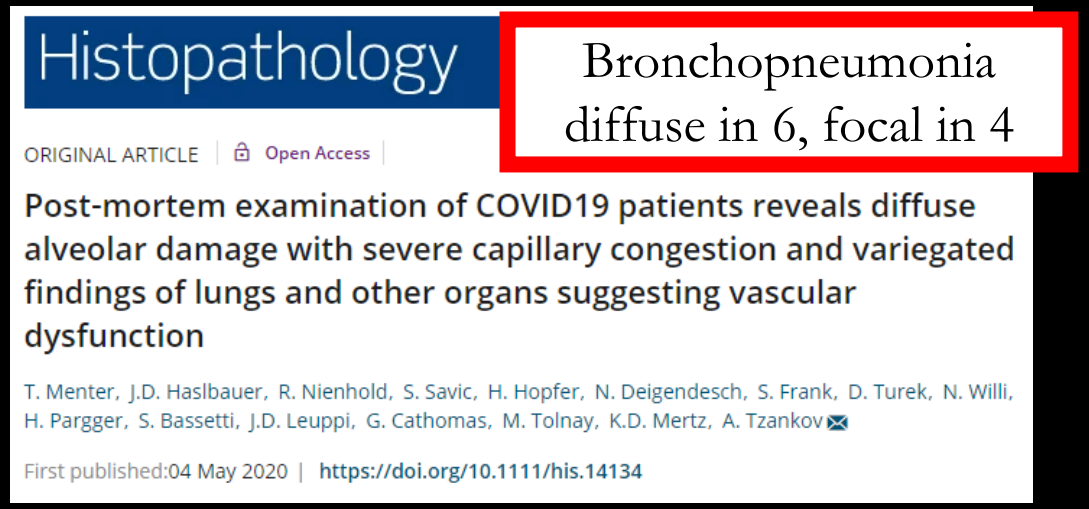
DAD in H1N1 (2009)



Bronchopneumonia is common in COVID-19

Focal bronchopneumonia in 4/12

Bronchopneumonia in 6 patients, focal to confluent



Histopathology

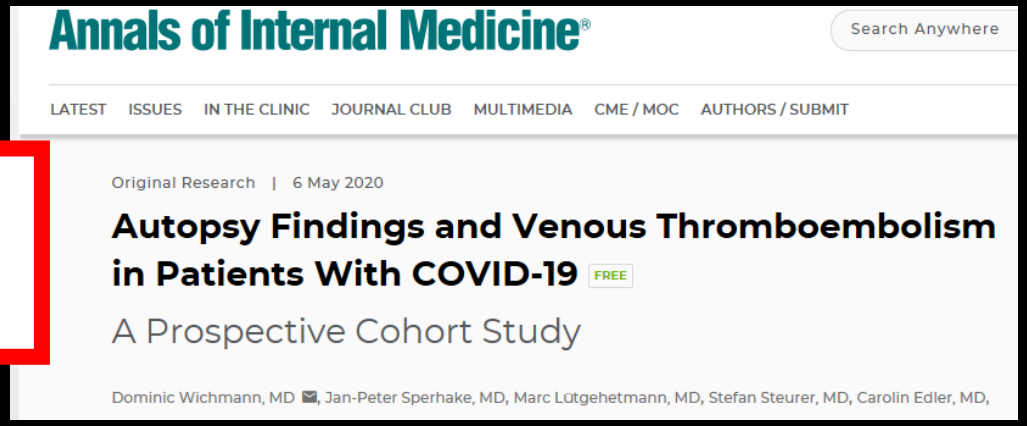
Bronchopneumonia diffuse in 6, focal in 4

ORIGINAL ARTICLE | Open Access

Post-mortem examination of COVID19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings of lungs and other organs suggesting vascular dysfunction

T. Menter, J.D. Haslbauer, R. Nienhold, S. Savic, H. Hopfer, N. Deigendesch, S. Frank, D. Turek, N. Willi, H. Pargger, S. Bassetti, J.D. Leuppi, G. Cathomas, M. Tolnay, K.D. Mertz, A. Tzankov

First published:04 May 2020 | https://doi.org/10.1111/his.14134



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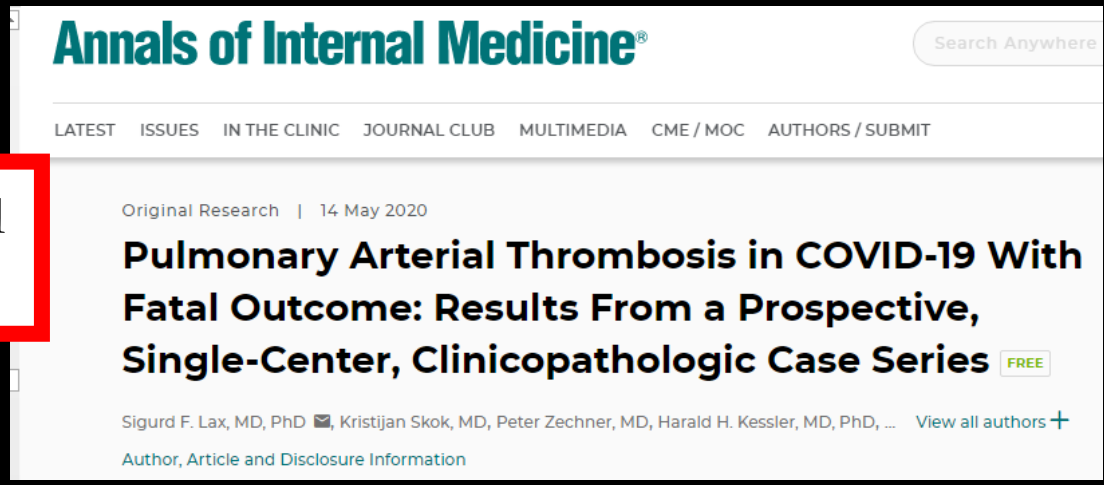
Original Research | 6 May 2020

Autopsy Findings and Venous Thromboembolism in Patients With COVID-19 FREE

A Prospective Cohort Study

Dominic Wichmann, MD, Jan-Peter Sperhake, MD, Marc Lütgehetmann, MD, Stefan Steurer, MD, Carolin Edler, MD,



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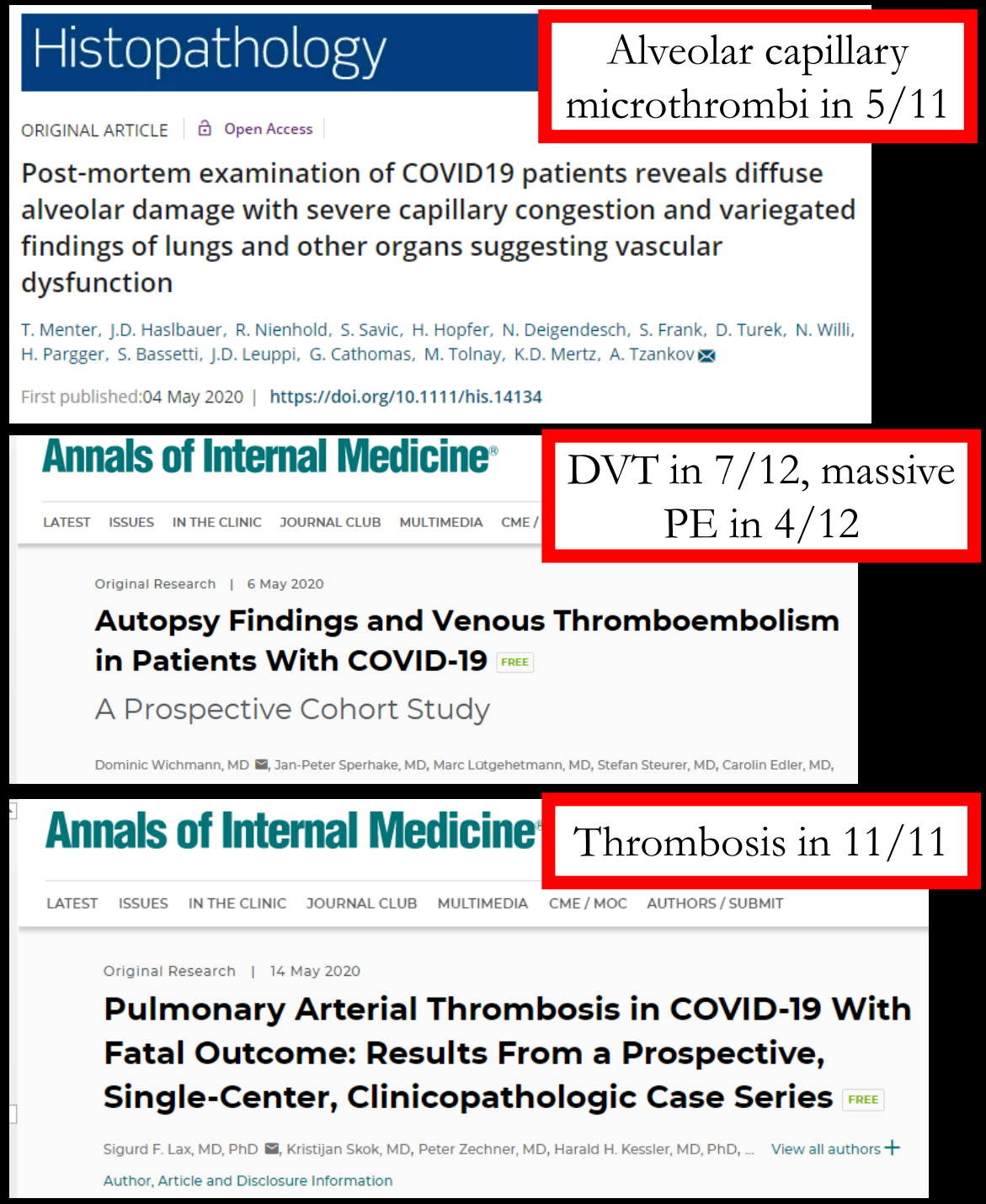
Original Research | 14 May 2020

Pulmonary Arterial Thrombosis in COVID-19 With Fatal Outcome: Results From a Prospective, Single-Center, Clinicopathologic Case Series FREE

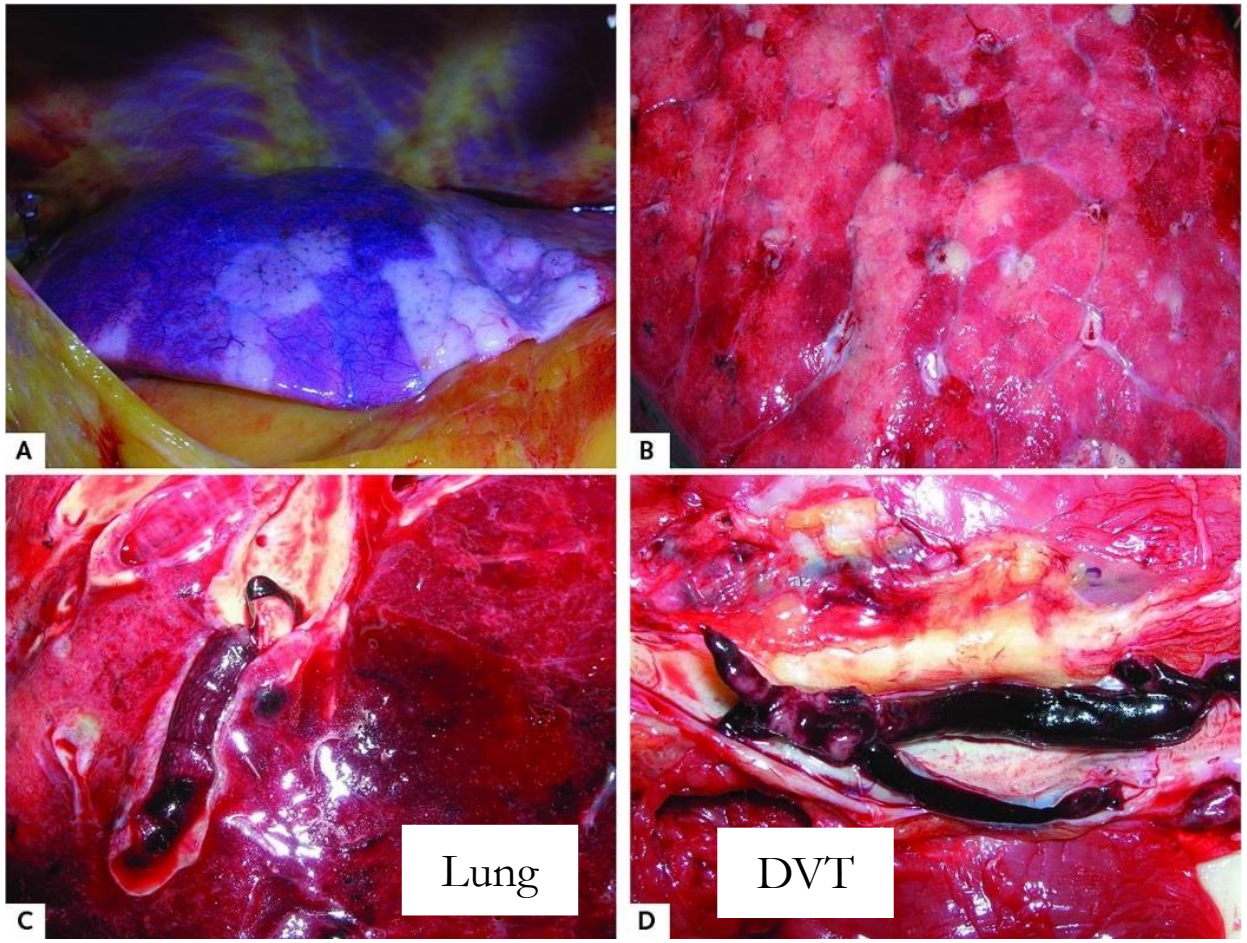
Sigurd F. Lax, MD, PhD, Kristijan Skok, MD, Peter Zechner, MD, Harald H. Kessler, MD, PhD, ... View all authors +

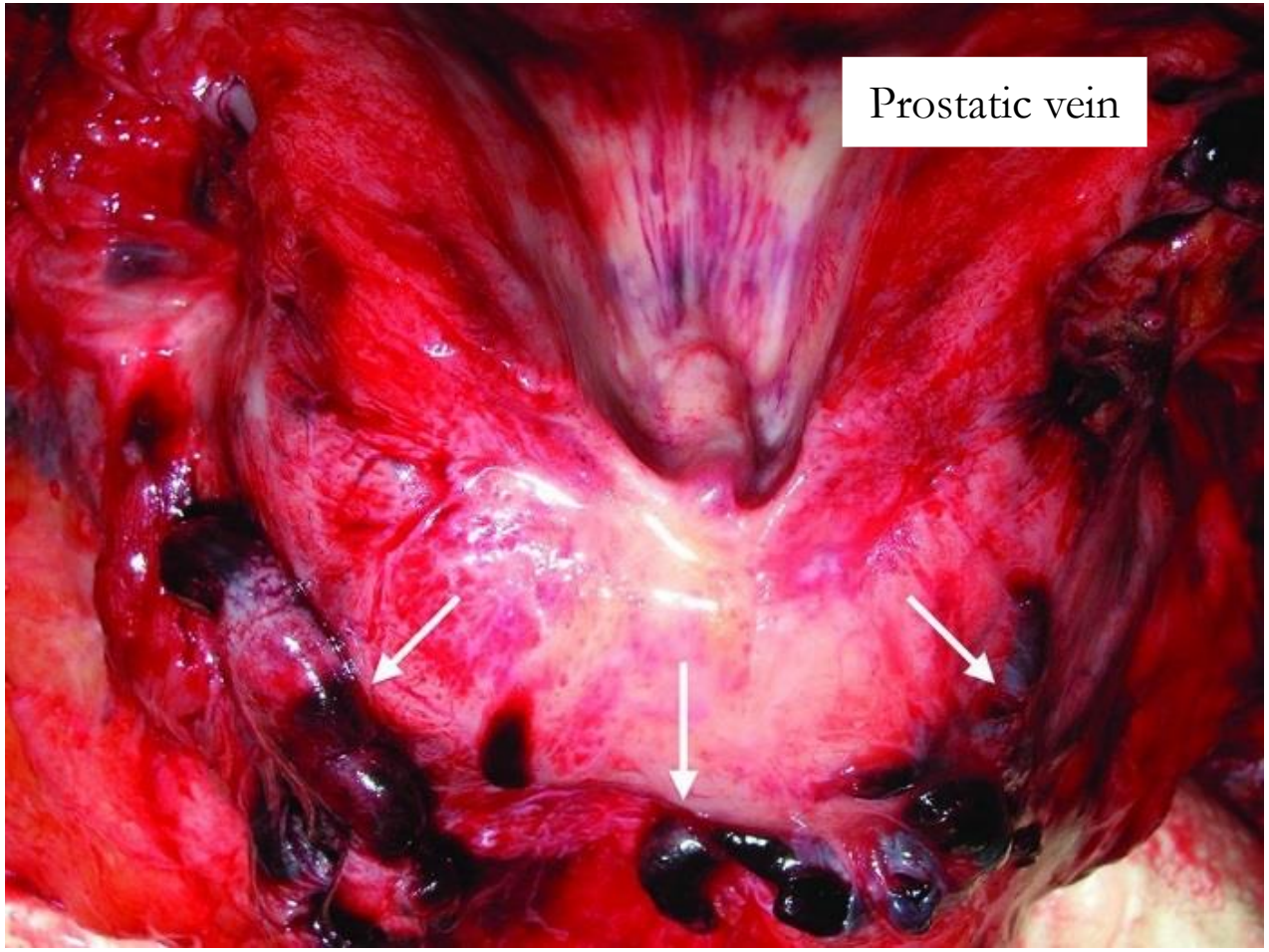
Author, Article and Disclosure Information

Thrombosis is common in fatal COVID-19



Thrombosis





Wichmann D, et al. *Ann Intern Med* 2020;173(4): 268-277. PMID 32374815.

Microthrombi in COVID-19

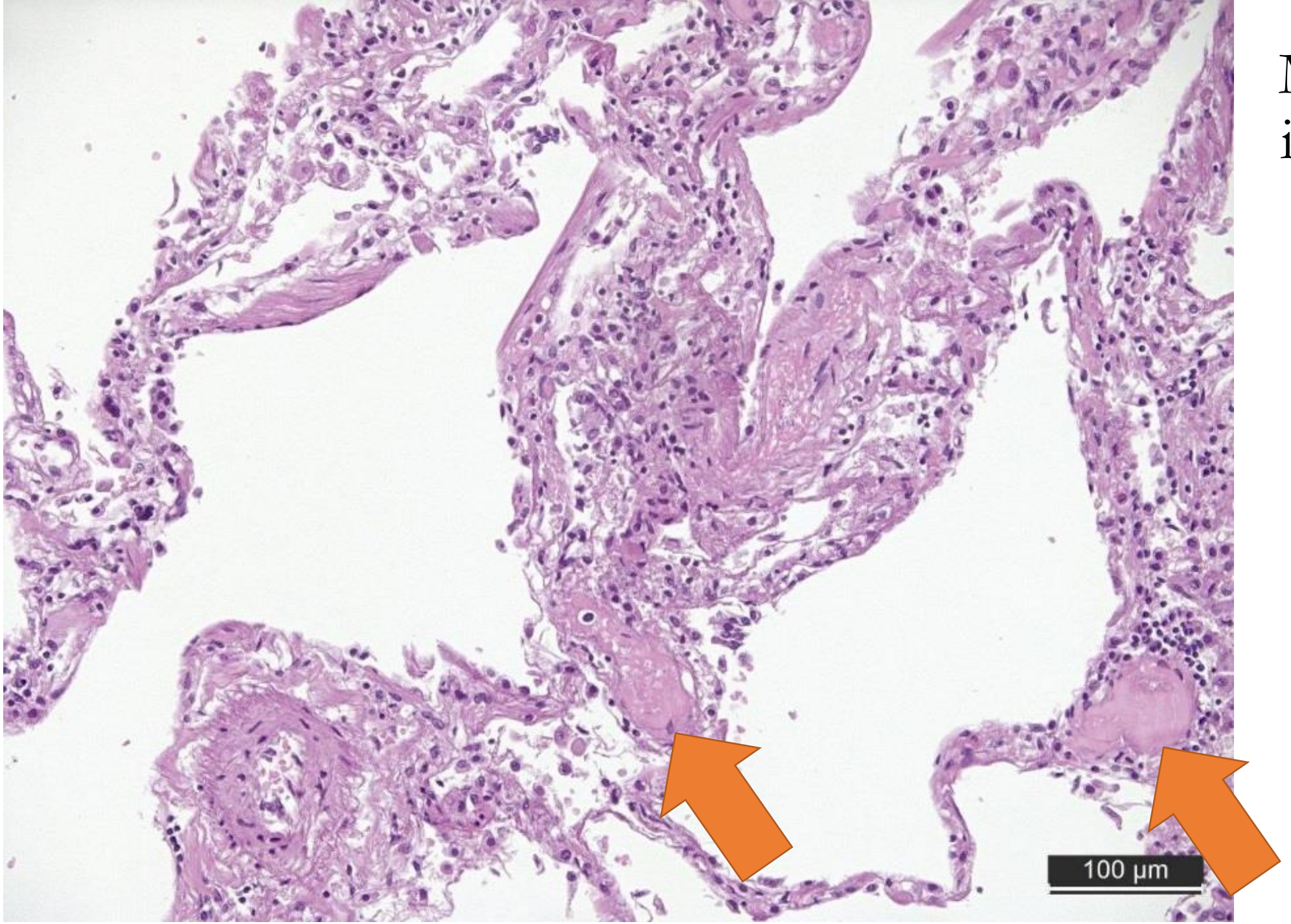
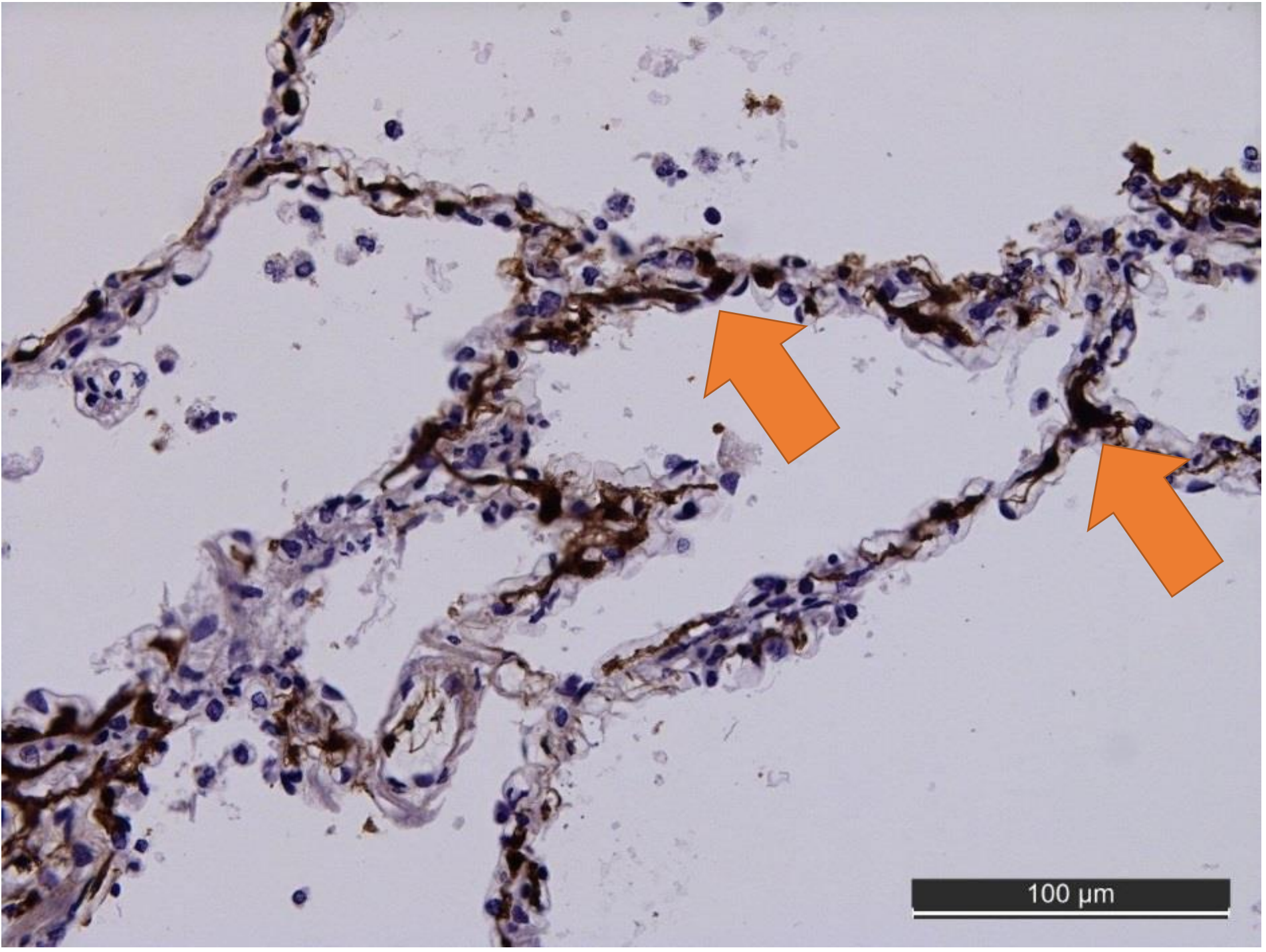


Image courtesy of
Dr. Alexandar Tzankov
and Dr. Sambit Mohanty

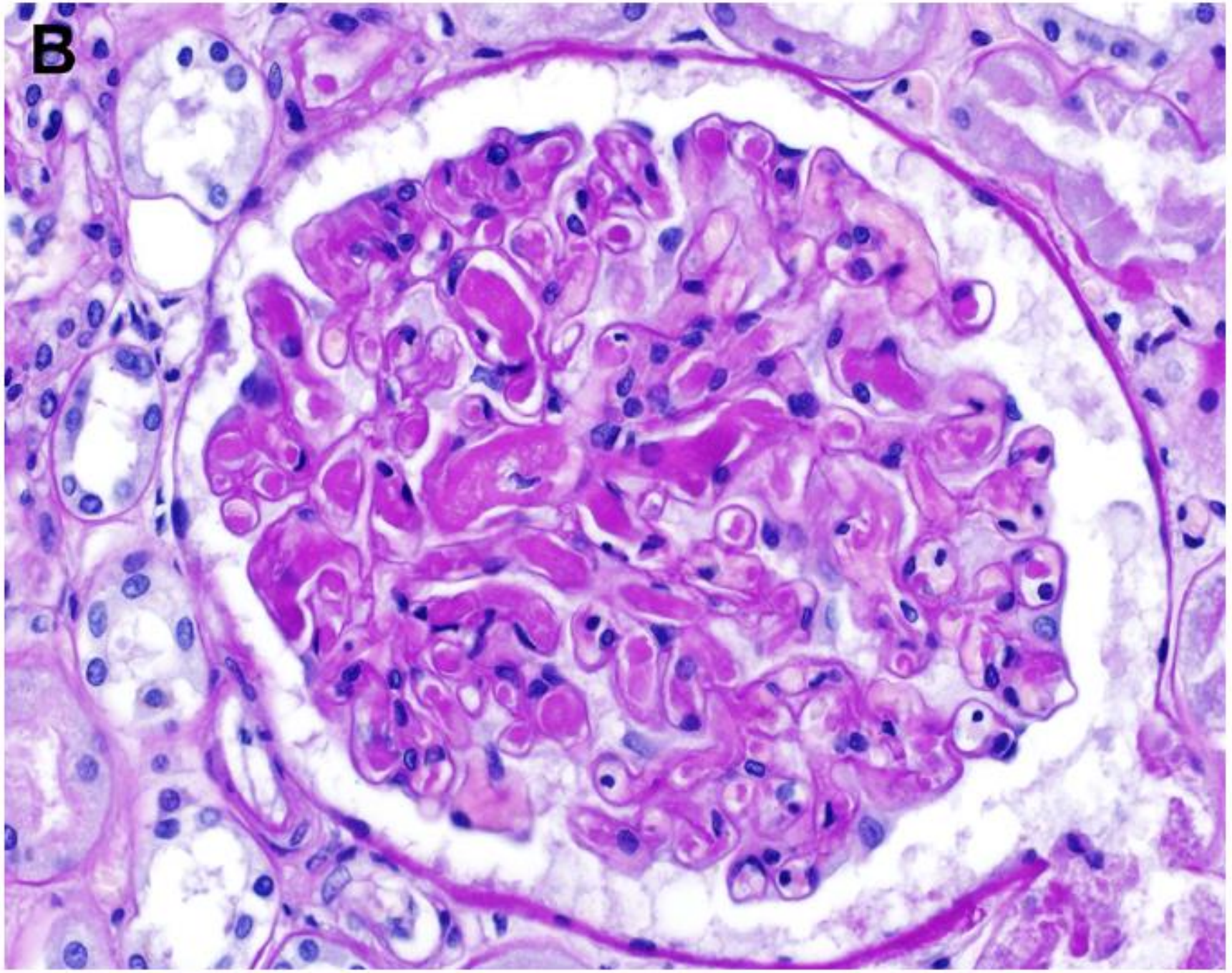
Menter T, et al.
Histopathology 2020.
PMID 32364264.

Fibrin microthrombi



Immunostain for fibrin.
Image courtesy of
Dr. Alexandar Tzankov

Menter T, et al.
Histopathology 2020.
PMID 32364264.



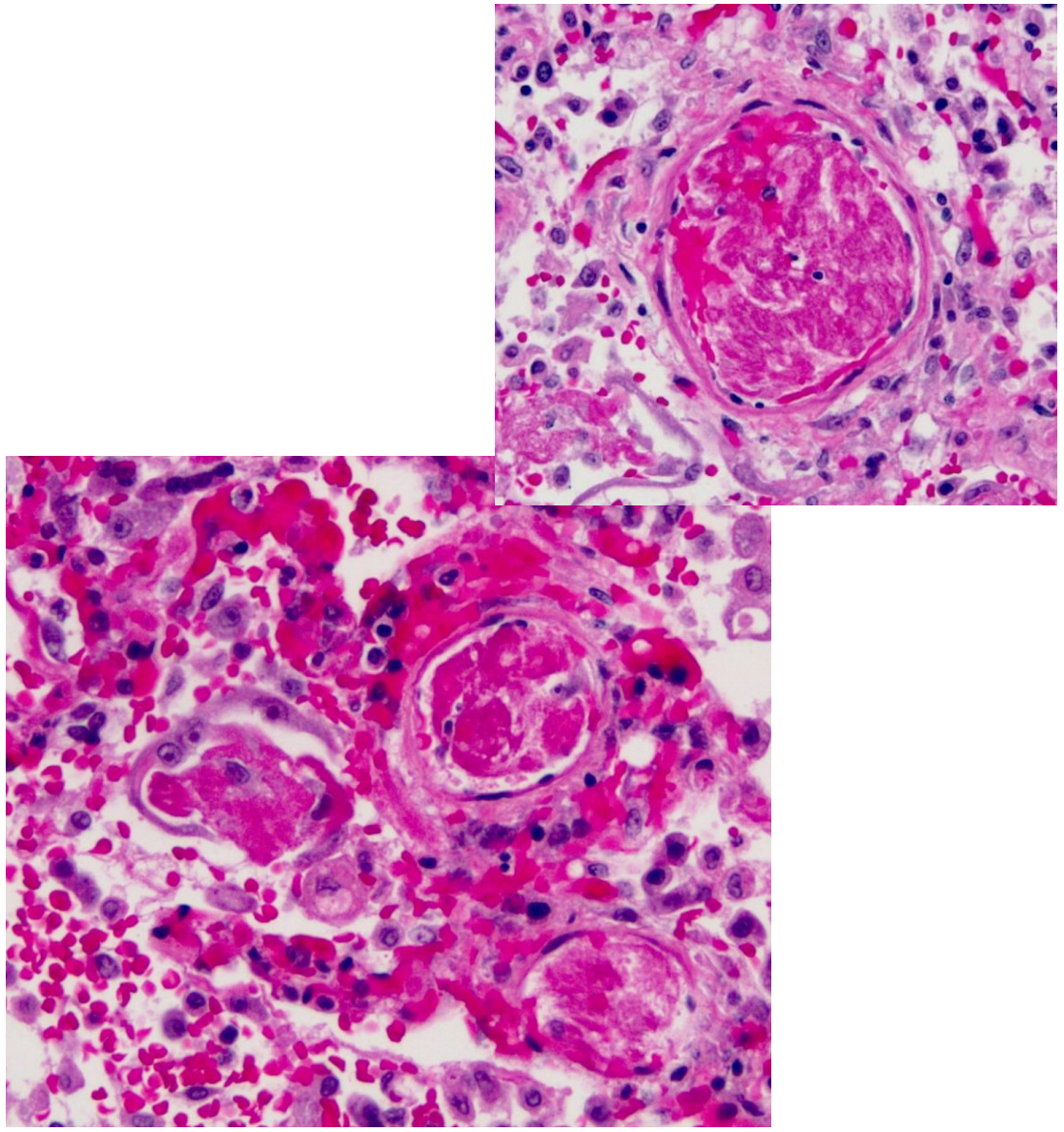
Microthrombi in glomerular capillaries

Pic courtesy of Dr. Alexandar Tzankov.

Thrombi in small pulmonary arteries are common in DAD

"Fibrin thrombi in various stages of organization are often present in small pulmonary arteries and they may be numerous. They are thought to occur secondary to endothelial damage"

Katzenstein AL. Diffuse alveolar damage. In: Surgical pathology of non-neoplastic lung disease, 4th edition. Page 20.



Thrombi in DAD: "everything old is new again"

"Thromboemboli, were the most consistently observed vascular feature, present in 21 of 22 patients."

"Microthrombi, also present in 19 patients…"

Tomashefski JF, et al. *Am J Pathol* 1983;112:112-126

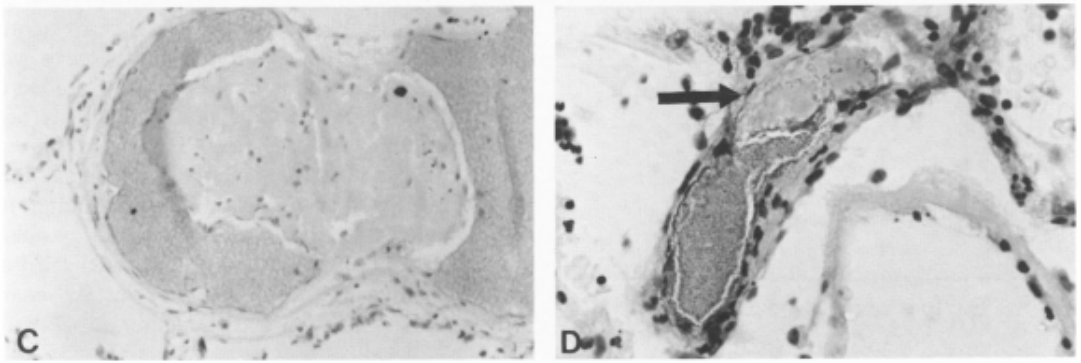


Figure 2—Microthrombosis. **A**—Postmortem arteriogram of normal adult lung. The pleural surface is at the bottom. (×2.4) **B**—Arteriogram of a patient with early ARDS (6 days after aspiration). There are reduced filling of small arteries and prominent, edematous interlobular septa. (×2.4) **C**—Organizing microthrombus adherent to the wall of an alveolar duct artery (17 days after inhalation of toxic fumes). (H&E, ×100) **D**—Platelet fibrin thrombus (*arrow*) obstructing the flow of contrast medium in an alveolar wall artery. A hyaline membrane is below and extravasated red blood cells to the right of the thrombosed vessel (same patient as in **B**). (H&E, ×250) The arteriograms of tissue blocks illustrated in Figures 2, 10, 13, and 14 were taken under the same X-ray exposure.

chiolar spaces. The three patient groups roughly corresponded, respectively, to the exudative, proliferative, and fibrotic stages of diffuse lung injury.[12-14]

Thromboembolic Vascular Disease

Thromboemboli, macroscopically or microscopically identified, were the most consistently observed vascular feature, present in 21 of 22 patients. In the arteriograms, they were present as intravascular filling defects with distal nonfilling. Intravascular linear streaks were due to contrast medium in the recanalized lumens of thrombi (Figure 1).

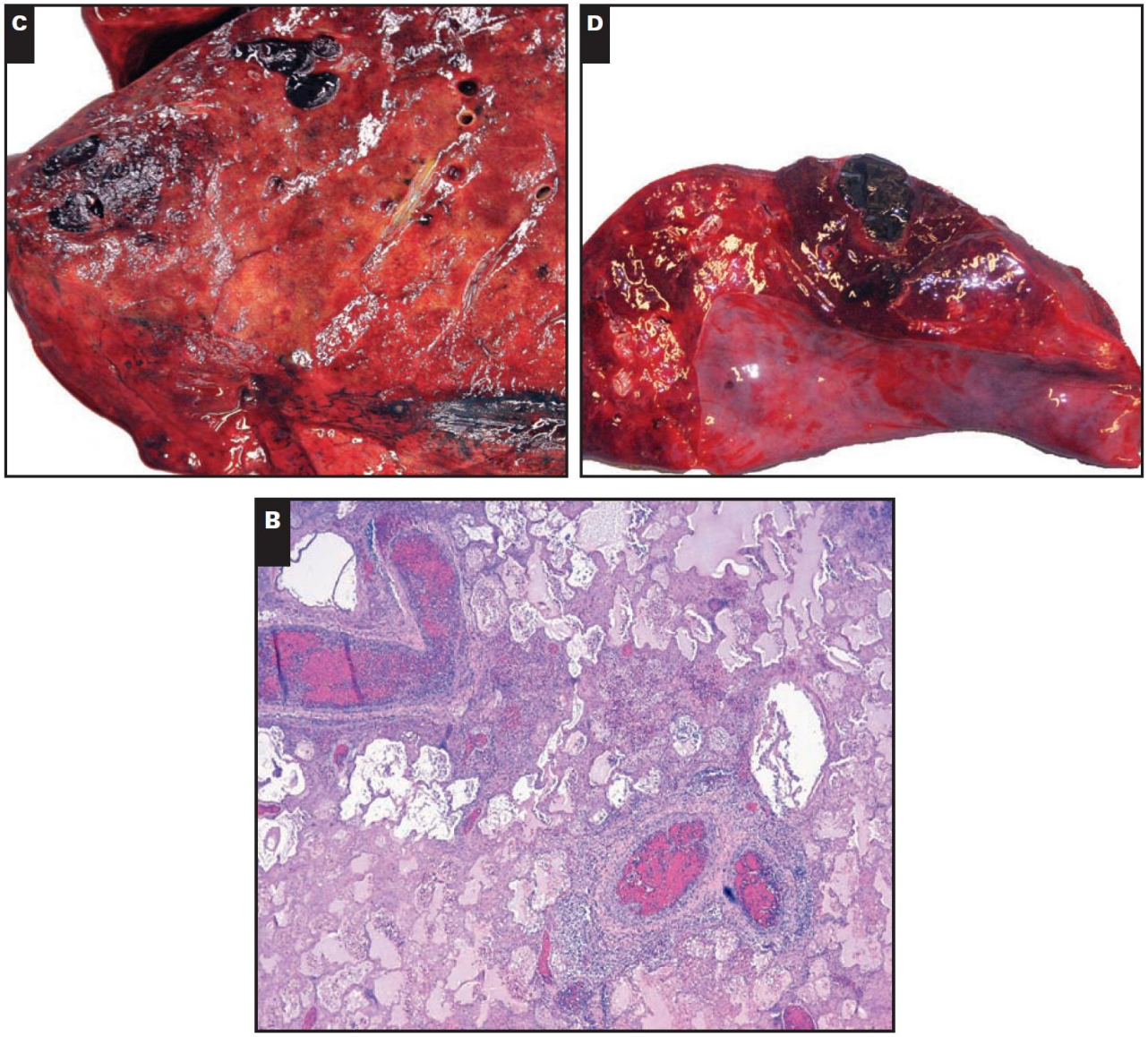
Macrothrombi were present in 19 patients (86.4%) and were less frequent in those with a prolonged course (Figure 3). Microthrombi, also present in 19 patients (of these, 2 did not have macrothrombi) were of two types (Figure 2). The first type, found in capillaries and small alveolar wall arteries, was a dense, hyaline clot formed of platelets and fibrin. These were numerous in only 3 patients, all in the early group. The second type of microthrombus was found in small preacinar and large intraacinar arteries and often included red and white cells and layered fibrin in addition to hyaline regions. This was the more common type, found in all patient groups as well as in the 3 patients with capillary thrombi (Figure 3). In the postmortem arteriogram, the presence of microthrombi was reflected in reduced filling of small arteries (Figure 2).

The macrothrombus score was highest in patients with multiple pulmonary arterial filling defects on balloon occlusion pulmonary angiography and low in those without these defects (Figure 4). Most patients with filling defects on antemortem angiography had microthrombi, but the number of thrombi varied from patient to patient (Figure 4). The group of 13 patients with a clinical diagnosis of DIC had no distinctive pattern of thrombosis. Eight of these had numerous (+ + – + + +) and five had sparse (+) microthrombi.

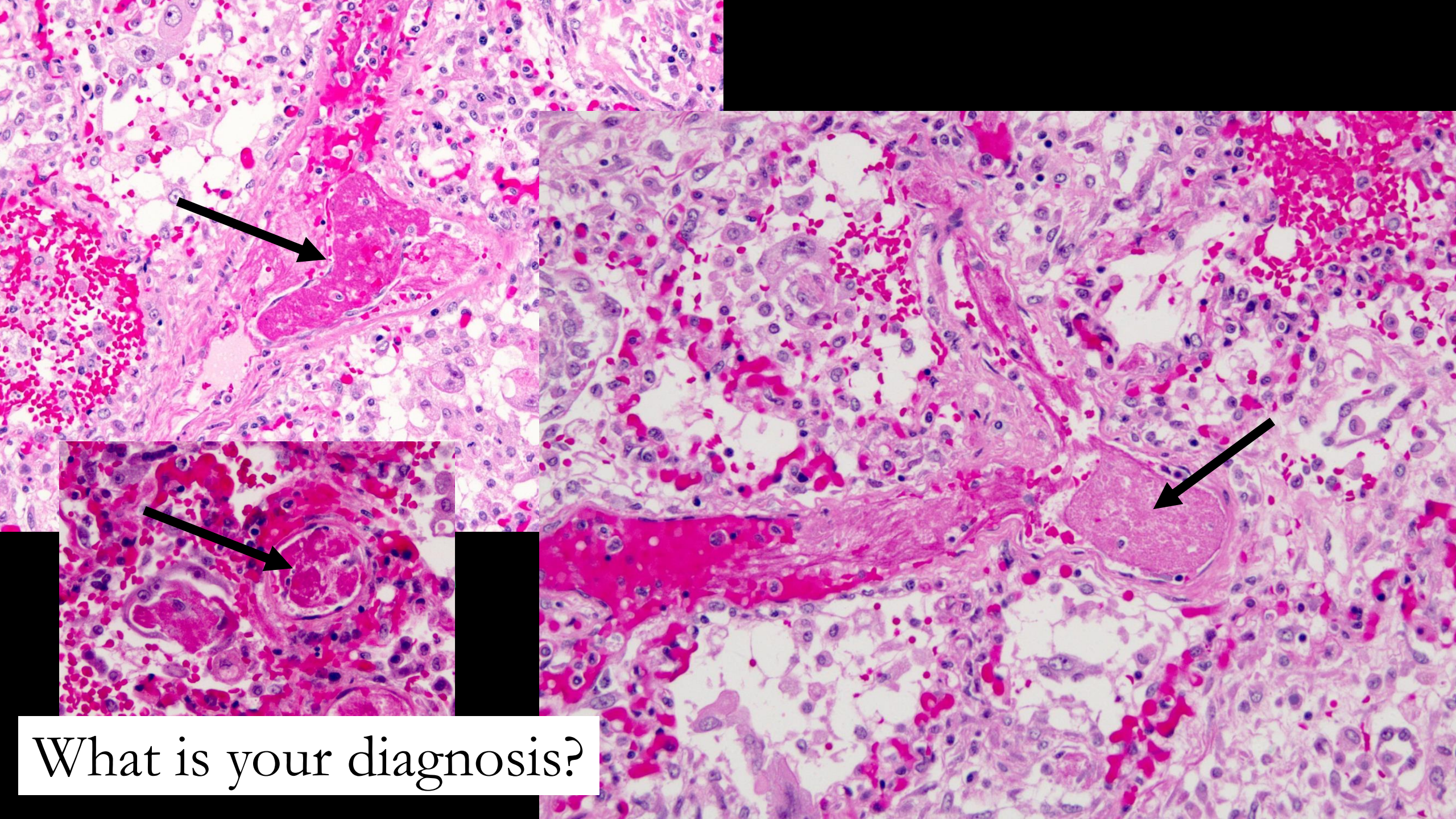
In 2 of the 3 patients with numerous capillary

Déjà vu

"Although microscopic thrombi within the pulmonary arterial system have been described in association with influenza and DAD, the number, size and peripheral location of the thrombi observed in our study may be unique to novel H1N1 influenza virus infection."

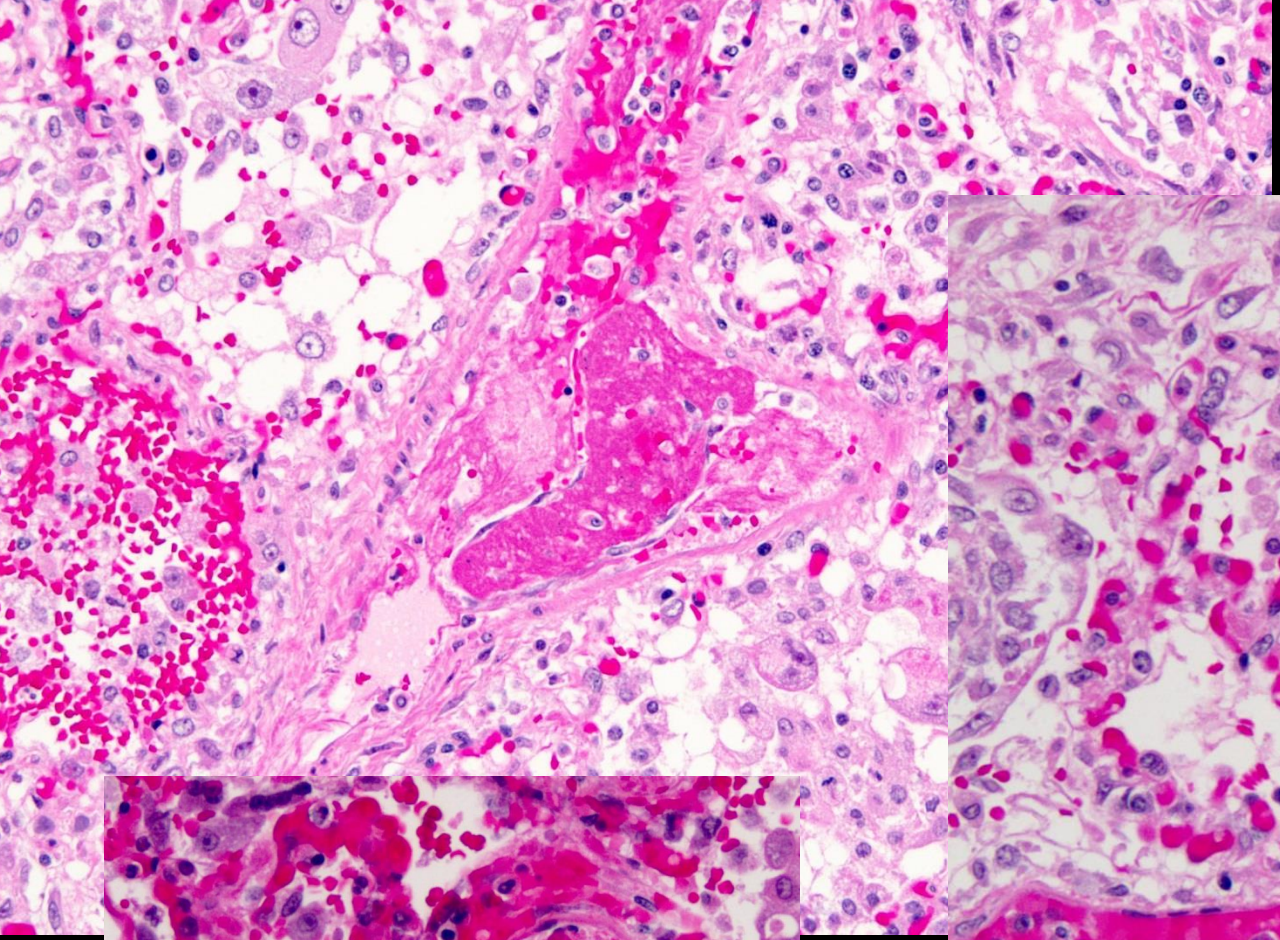


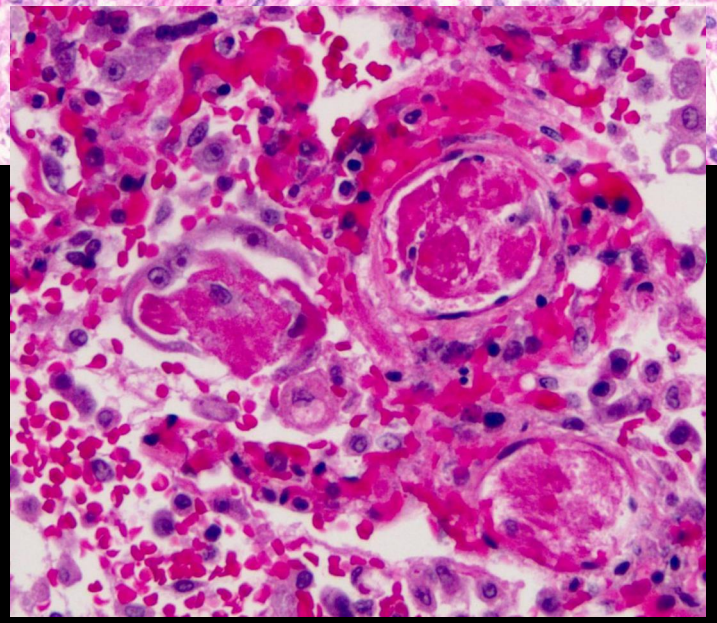
Harms PW et al. *Am J Clin Pathol* 2010;134(1):27-35

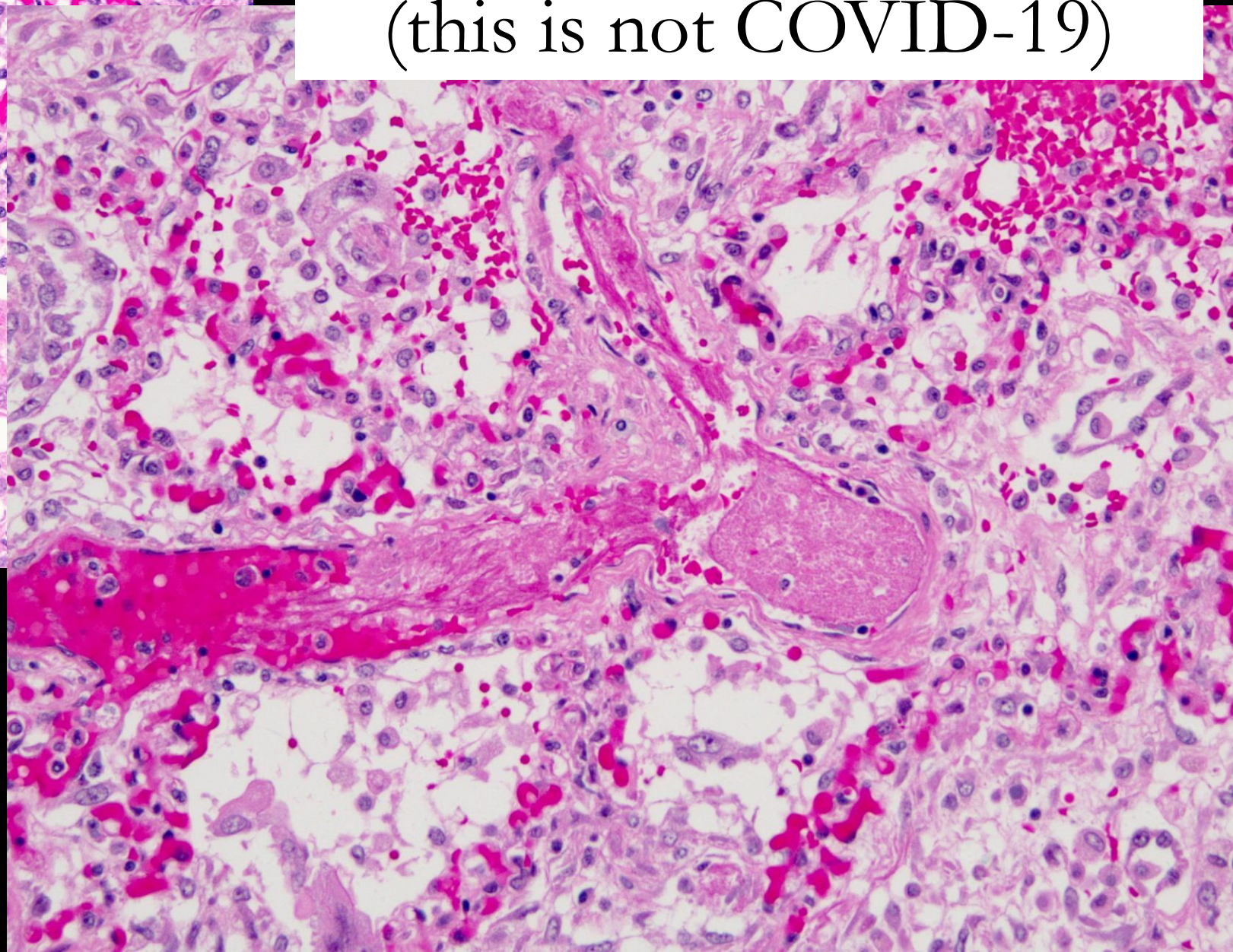


What is your diagnosis?

Thrombi in H1N1 (2009) (this is not COVID-19)







COVID-19 pulmonary pathology: a multi-institutional autopsy cohort from Italy and New York City

Alain C. Borczuk[1] · Steven P. Salvatore[1] · Surya V. Seshan[1] · Sanjay S. Patel[1] · James B. Bussel[2] · Maria Mostyka[1] · Sarah Elsoukkary[1] · Bing He[1] · Claudia Del Vecchio[3] · Francesco Fortarezza[4] · Federica Pezzuto[4] · Paolo Navalesi[5] · Andrea Crisanti[3] · Mary E. Fowkes[6] · Clare H. Bryce[6] · Fiorella Calabrese[4] · Mary Beth Beasley[6]

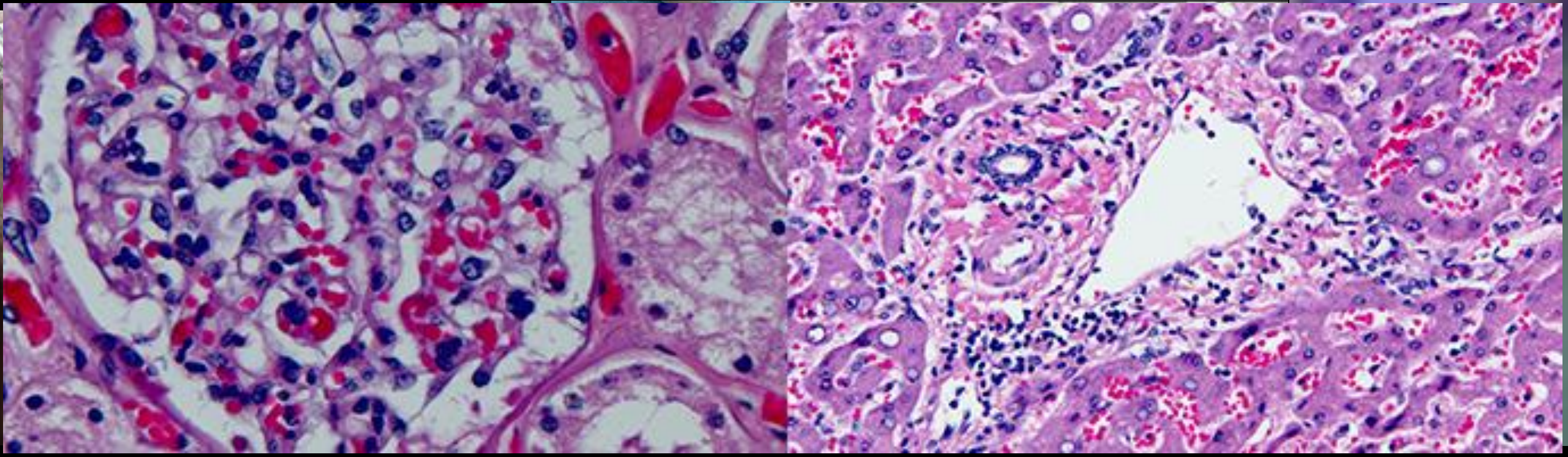
68 autopsies; Sept 2020

DAD = 87%

Acute inflammation = 43%

Large thrombi = 32%

Microthrombi = 65%

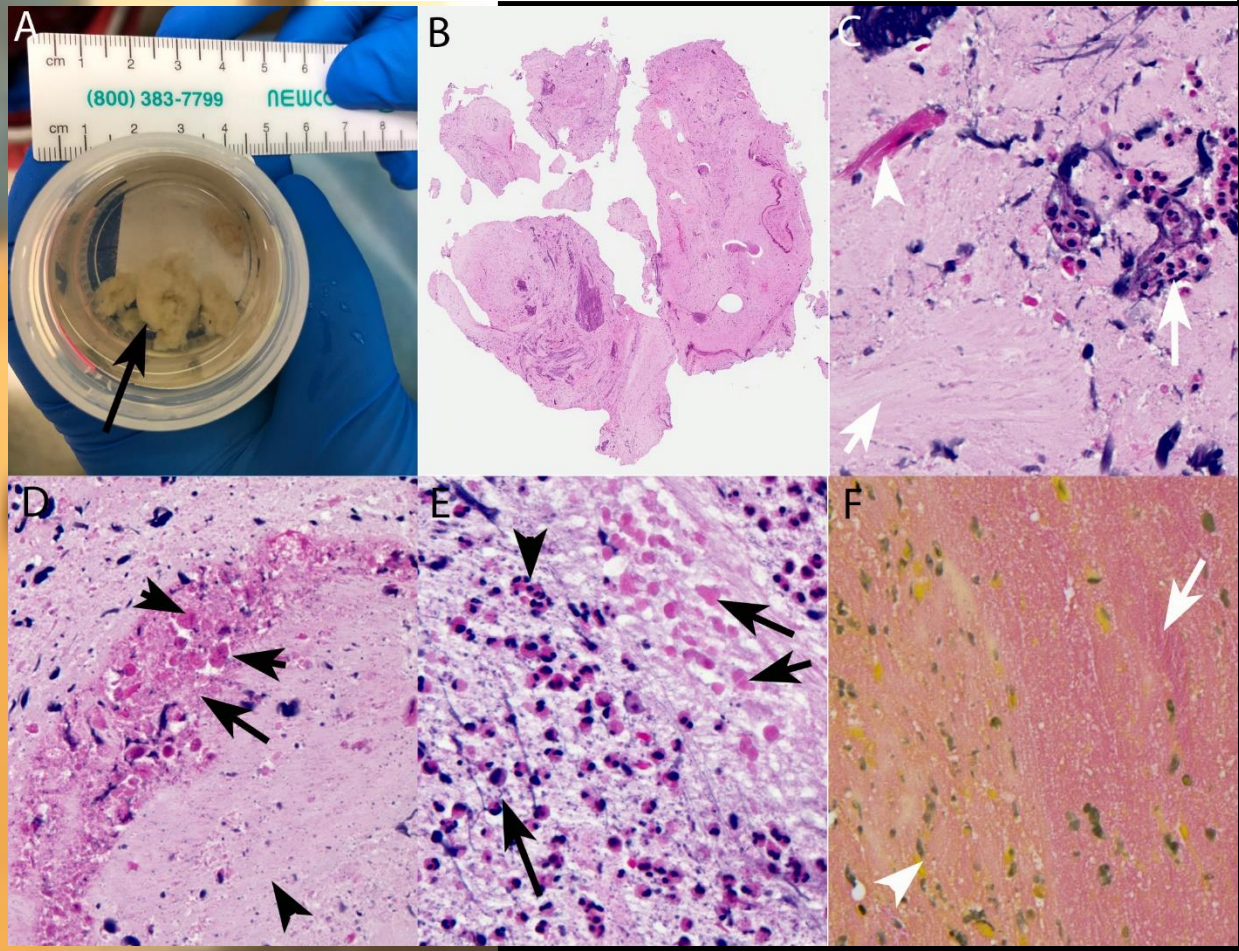


BEYOND AUTOPSIES

OBSERVATIONS

Endotracheal Tube Obstruction Among Patients Mechanically Ventilated for ARDS Due to COVID-19: A Case Series

Samuel Wiles, MD[1], Eduardo Mireles-Cabodevila, MD[1], Scott Neuhofs, RRT, MBA[1], Sanjay Mukhopadhyay, MD[2], Jordan P. Reynolds, MD[2], and Umur Hatipoğlu, MD, MBA[1]



Wiles S, et al. *J Intensive Care Med* 2020. PMID 33375893

Pulmonary Pathology of COVID-19 Following 8 Weeks to 4 Months of Severe Disease

A Report of Three Cases, Including One With Bilateral Lung Transplantation

Scott W. Aesif, MD, PhD,[1] Alejandro C. Bribriesco, MD,[2] Ruchi Yadav, MD,[3] Summer L. Nugent, MD,[4] Dmitriy Zubkus, MD,[5] Carmela D. Tan, MD,[1] Atul C. Mehta, MD,[6] and Sanjay Mukhopadhyay, MD[1]

Table 1

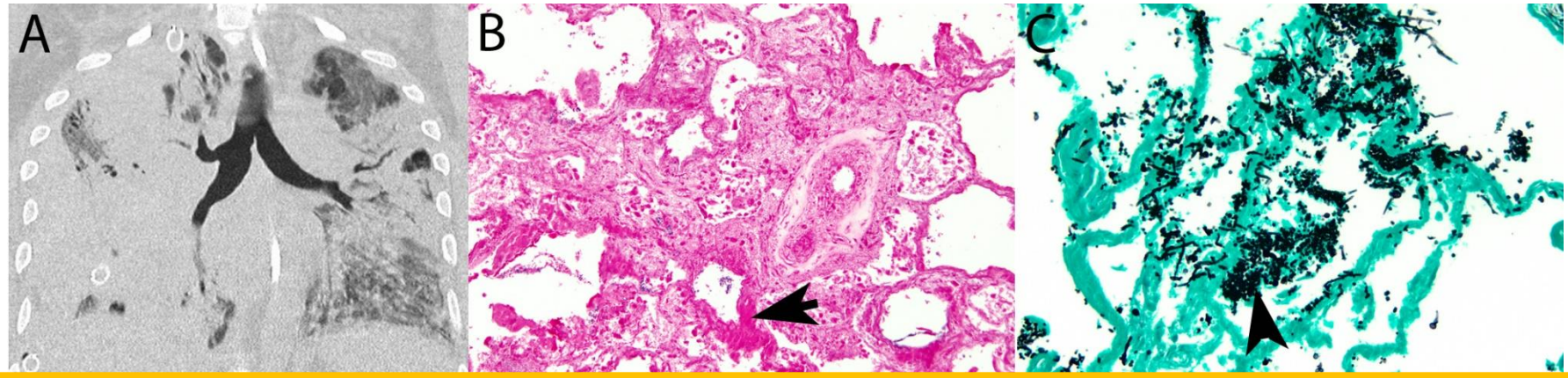
Timeline of Major Events in Three Patients With Severe COVID-19[a]

Event	Case 1 (46 y/M)	Case 2 (57 y/F)	Case 3 (57 y/M)
Test for COVID-19 positive	Day 1	Day 1	Day 1
ECMO	Day 21	Day 12	Day 54
Mechanical ventilation	Day 14	Day 5	Day 15
Pathology	Lung debridement on day 38, 5 weeks after positive test; autopsy on day 57	Open lung biopsy on day 74, 10 weeks after positive test	Bilateral lung transplantation on day 126, 4 months after positive test
Last follow-up or death	Died on day 57, 8 weeks after positive test	Died on day 74, 10 weeks after positive test	Alive, 7 months after positive test

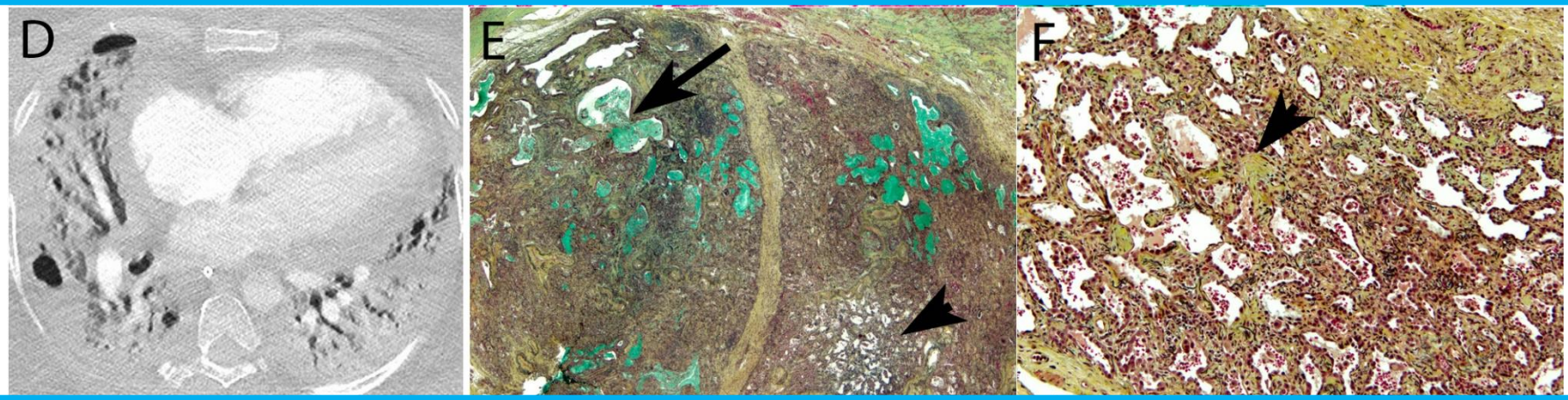
COVID-19, coronavirus disease 2019; ECMO, extracorporeal membrane oxygenation.

[a]Day 1 in this table is the day of the first positive severe acute respiratory syndrome coronavirus 2 test.

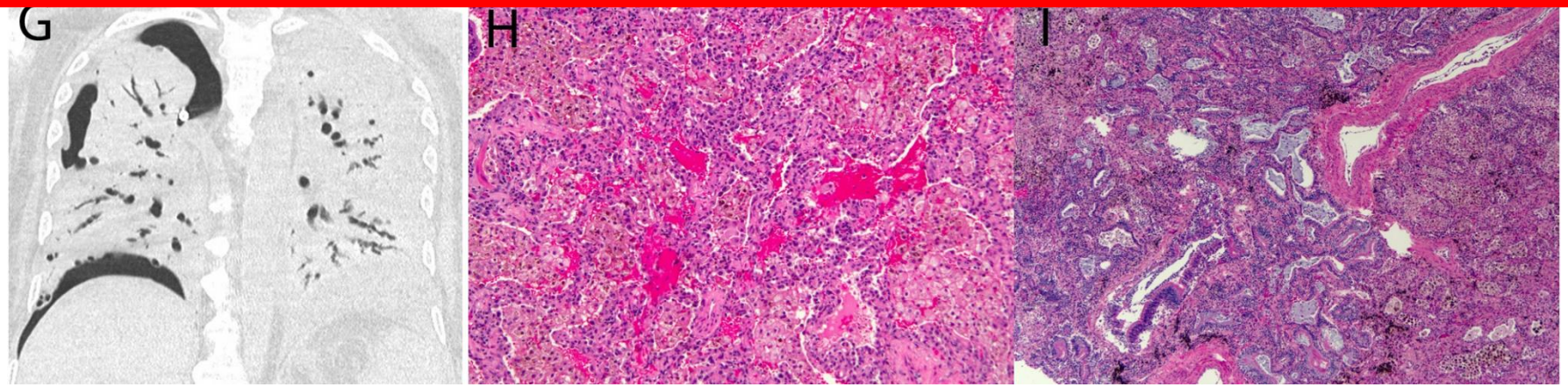
Aesif SW et al. *Am J Clin Pathol* 2020;Online ahead of print. PMID 33316056



Massive unilateral infarction + DAD
Day 38: debridement
Day 57: autopsy



Diffuse interstitial fibrosis
Day 74: surgical bx



Diffuse interstitial fibrosis
Day 126: bilateral lung transplant

Aesif SW, et al. *Am J Clin Pathol* 2020

Laboratory Investigation
https://doi.org/10.1038/s41374-020-0464-x

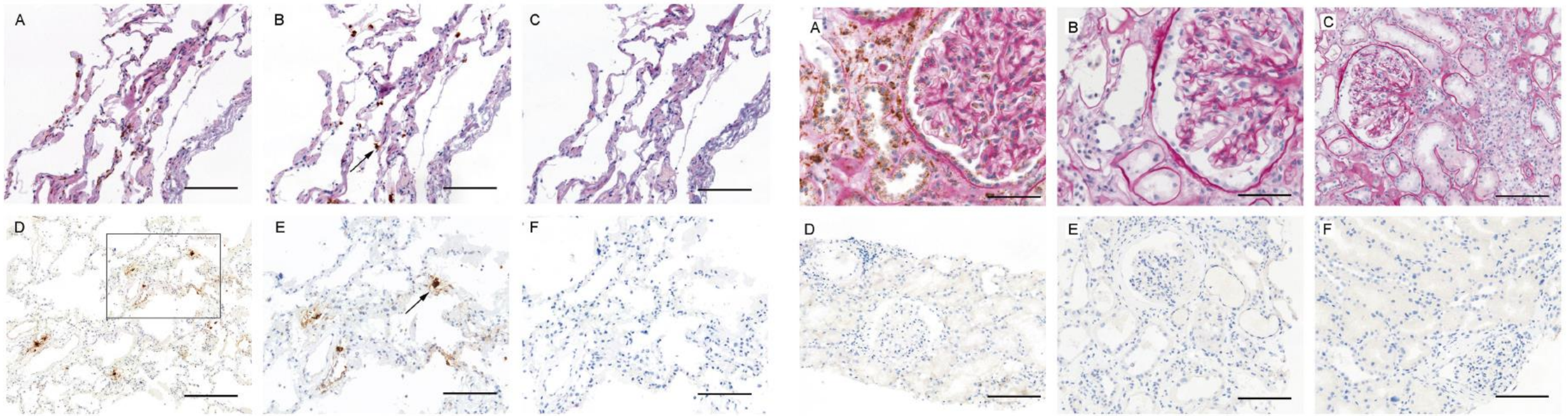
TECHNICAL REPORT

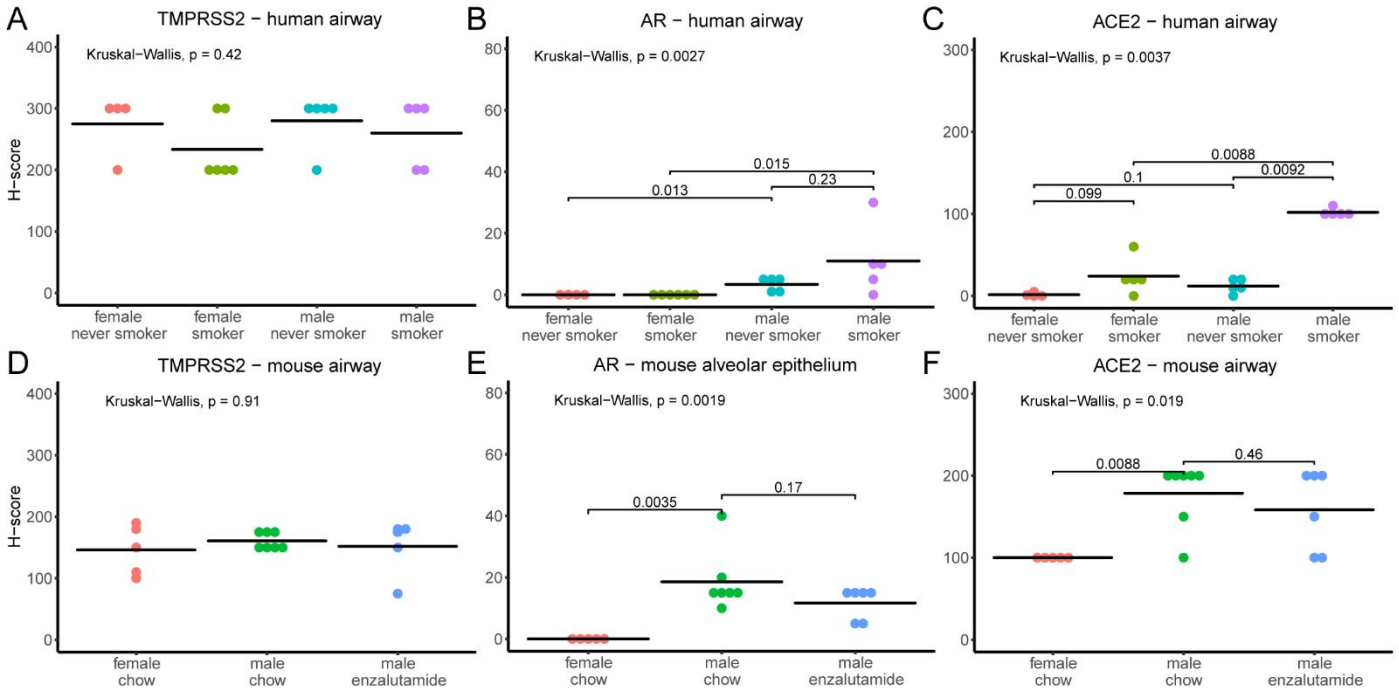
Table 1 Commercial antibodies and probes for SARS-CoV-2 detection.

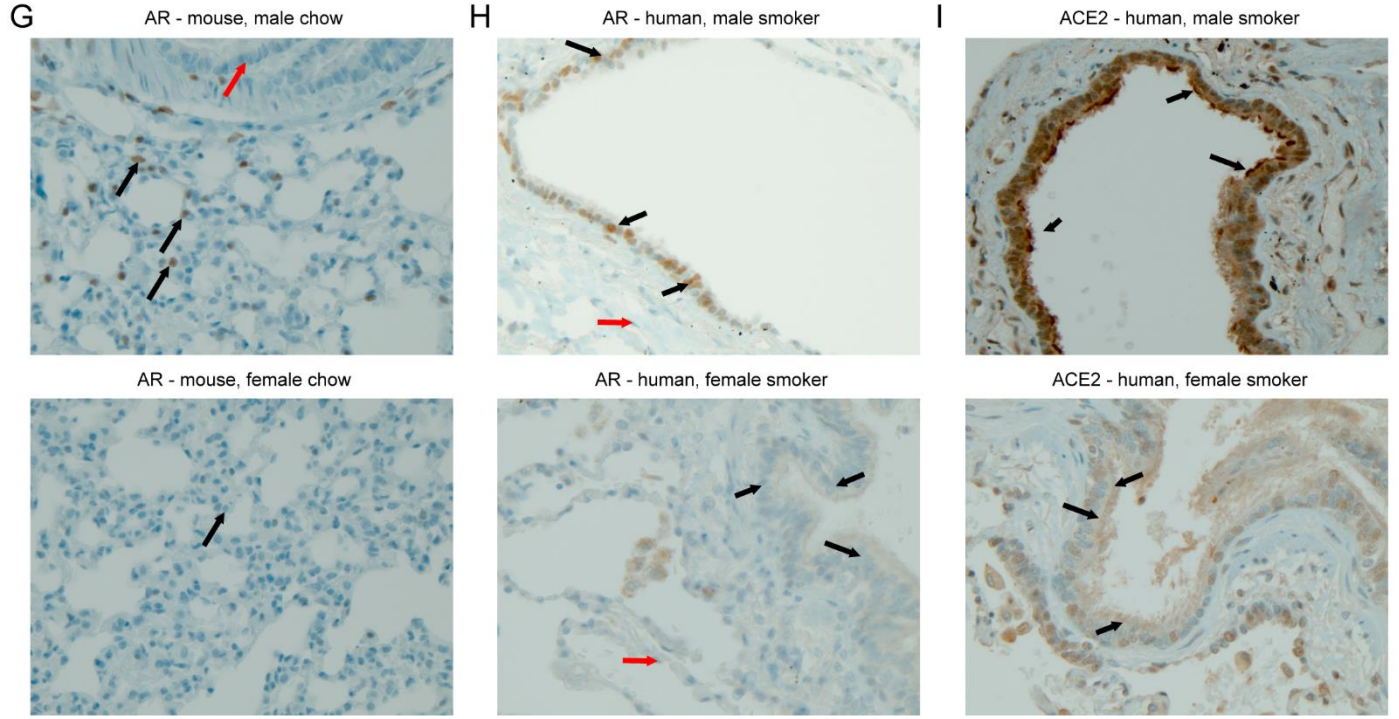
Company	Product #	Listed target	Dilution
ACDBio	848568	SARS-CoV-2 RNA (21631–23303)	Ready to use
Bioss	BSM-41411M	Recombinant SARS-CoV-2 Nucleocapsid protein (His-tag)	1:100
Bioss	BSM-49131M	Recombinant SARS Nucleocapsid protein (no tag)	1:400
Thermo	MA1-7404	SARS nucleoprotein preparation	1:100

Detection of SARS-CoV-2 in formalin-fixed paraffin-embedded tissue sections using commercially available reagents

Alejandro Best Rocha[1] · Edana Stroberg[2] · Lisa M. Barton[2] · Eric J. Duval[2] · Sanjay Mukhopadhyay[3] · Nicole Yarid[4] · Tiffany Caza[1] · Jon D. Wilson[1] · Daniel J. Kenan[1] · Michael Kuperman[1] · Shree G. Sharma[1] · Christopher P. Larsen[1]







Sex differences in COVID-19 outcomes are independent of TMPRSS2

› bioRxiv. 2020 Oct 14;2020.04.21.051201. doi: 10.1101/2020.04.21.051201. Preprint

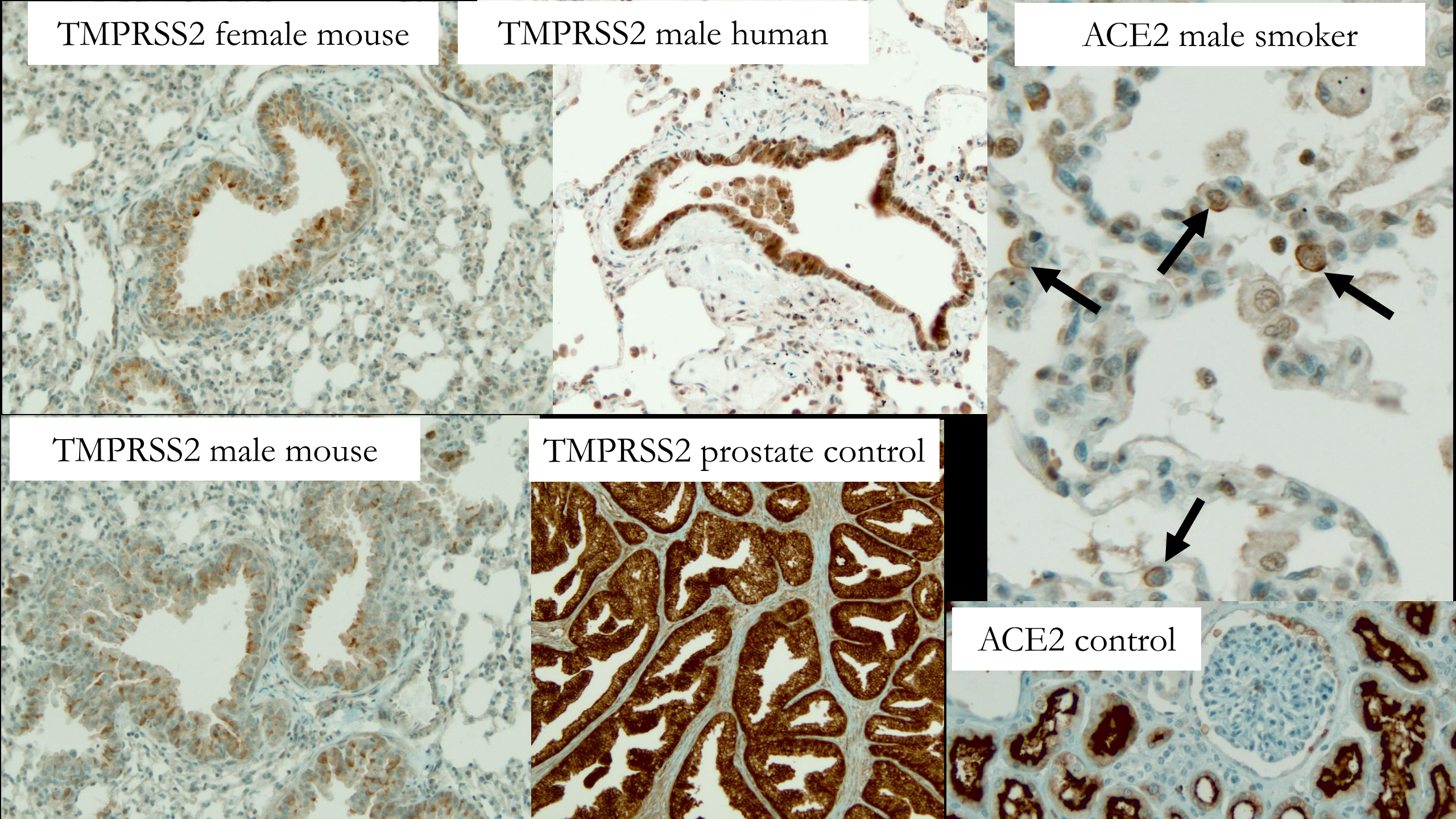
Sex, androgens and regulation of pulmonary AR, TMPRSS2 and ACE2

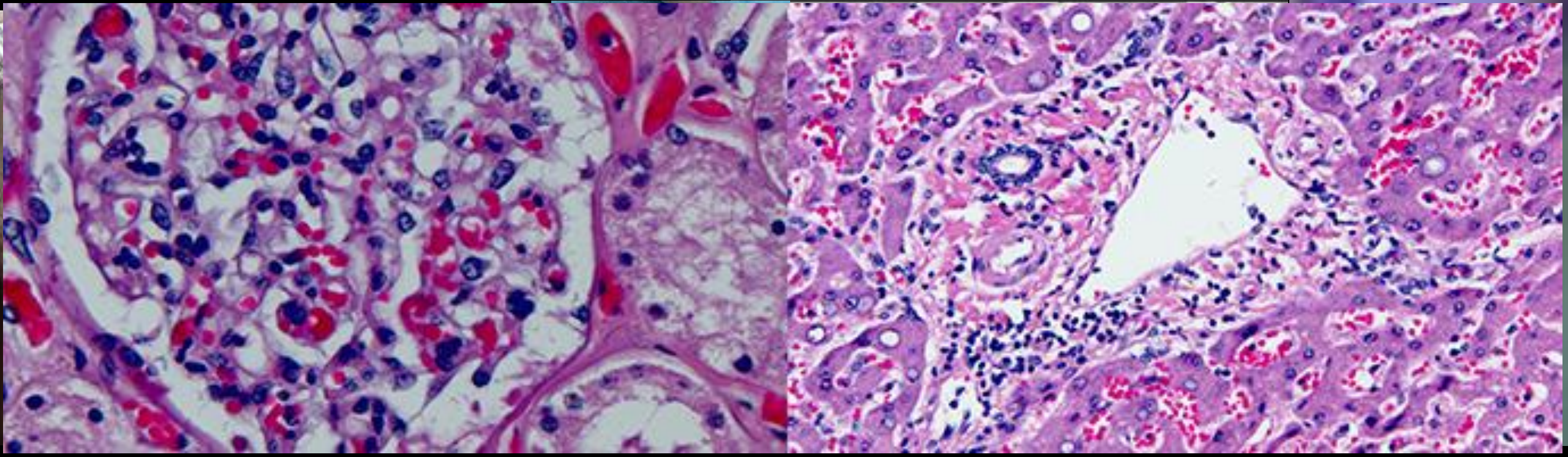
Mehdi Baratchian, Jeffrey M McManus, Mike Berk, Fumihiko Nakamura, Sanjay Mukhopadhyay, Weiling Xu, Serpil Erzurum, Judy Drazba, John Peterson, Eric A Klein, Ben Gaston, Nima Sharifi

PMID: 33083800 PMCID: PMC7574256 DOI: 10.1101/2020.04.21.051201
Free PMC article

Abstract

The sex discordance in COVID-19 outcomes has been widely recognized, with males generally faring worse than females and a potential link to sex steroids. A plausible mechanism is androgen-induced expression of TMPRSS2 and/or ACE2 in pulmonary tissues that may increase susceptibility or severity in males. This hypothesis is the subject of several clinical trials of anti-androgen therapies around the world. Here, we investigated the sex-associated TMPRSS2 and ACE2 expression in human and mouse lungs and interrogated the possibility of pharmacologic modification of their expression with anti-androgens. We found no evidence for increased *TMPRSS2* expression in the lungs of males compared to females in humans or mice. Furthermore, in male mice, treatment with the androgen receptor antagonist enzalutamide did not decrease pulmonary TMPRSS2. On the other hand, ACE2 and AR expression was sexually dimorphic and higher in males than females. ACE2 was moderately suppressible with enzalutamide therapy. Our work suggests that sex differences in COVID-19 outcomes attributable to viral entry are independent of TMPRSS2. Modest changes in ACE2 could account for some of the sex discordance.


TMPRSS2 female mouse
TMPRSS2 male human
ACE2 male smoker
TMPRSS2 male mouse
TMPRSS2 prostate control
ACE2 control



FACT OR FICTION?

CONTROVERSIES

Myocarditis in COVID-19?

JAMA Cardiology | Original Investigation

Outcomes of Cardiovascular Magnetic Resonance Imaging in Patients Recently Recovered From Coronavirus Disease 2019 (COVID-19)

Valentina O. Puntmann, MD, PhD; M. Ludovica Carerj, MD; Imke Wieters, MD; Masia Fahim; Christophe Arendt, MD; Jedrzej Hoffmann, MD; Anastasia Shchendrygina, MD, PhD; Felicitas Escher, MD; Mariuca Vasa-Nicotera, MD; Andreas M. Zeiher, MD; Maria Vehreschild, MD; Eike Nagel, MD

78% had abnormal cardiovascular MR findings (only 3 with pathology)

Letters

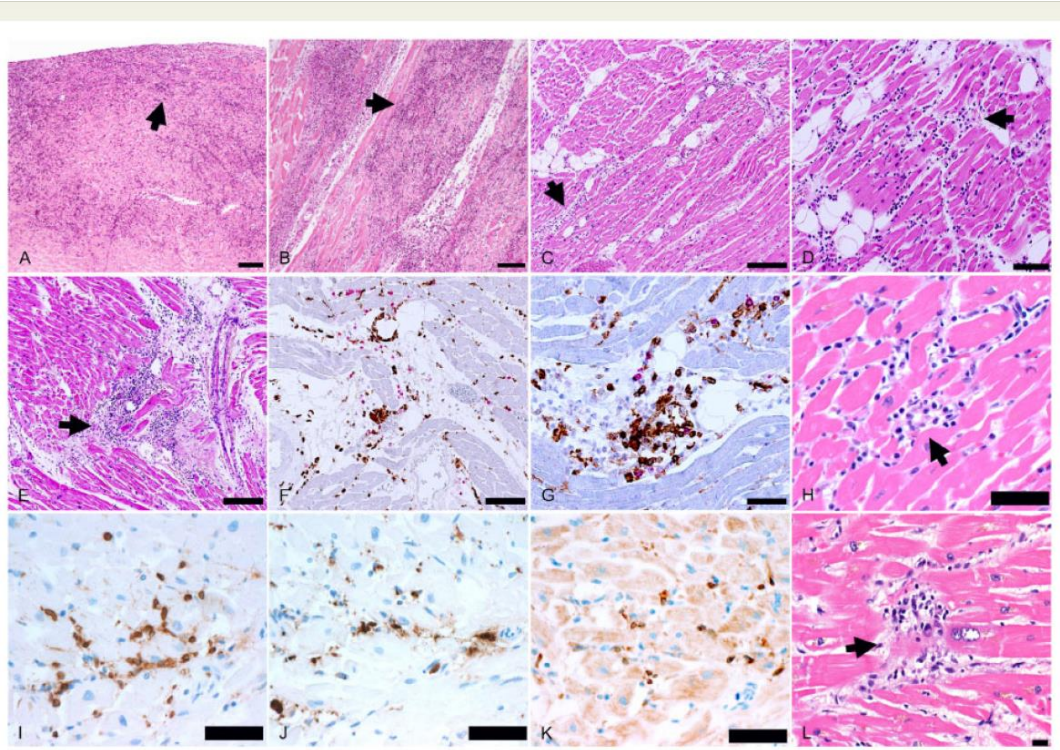
RESEARCH LETTER

Cardiovascular Magnetic Resonance Findings in Competitive Athletes Recovering From COVID-19 Infection

Myocarditis is a significant cause of sudden cardiac death in competitive athletes and can occur with normal ventricular function.[1] Recent studies have raised concerns of myocardial inflammation after recovery from coronavirus disease 2019 (COVID-19), even in asymptomatic or mildly symptomatic patients.[2] Our objective was to investigate the use of cardiac magnetic resonance (CMR) imaging in competitive athletes recovered from COVID-19 to detect myocardial inflammation that would identify high-risk athletes for return to competitive play.

Methods | We performed a comprehensive CMR examination including cine, T1 and T2 mapping, extracellular volume fraction, and late gadolinium enhancement (LGE), on a 1.5-T scanner (Magnetom Sola; Siemens Healthineers) using standardized protocols,[3] in all competitive athletes referred to the sports medicine clinic after testing positive for COVID-19 (reverse transcriptase-polymerase chain reaction) between June and August 2020. The Ohio State University institutional review board approved the study, and informed consent in writing was obtained from participating athletes. Cardiac magnetic resonance imaging was performed after recommended quarantine (11-53 days). Electrocardiogram, serum troponin I, and transthoracic echocardiogram were performed on day of CMR imaging.

4/26 (15%) competitive athletes had CMR findings suggestive of myocarditis



Lymphocytic myocarditis in 14%
Interstitial macrophage infiltration in 86%
Non-occlusive fibrin microthrombi in microvasculature most (12/15)*

Image from Basso C, et al. *Eur Heart J* 2020. PMID 32968776
*Bois MC, et al. *Circulation* 2020. PMID 33197204

Virus in endothelial cells?

Endothelial cell infection and endotheliitis in COVID-19

Cardiovascular complications are rapidly emerging as a key threat in coronavirus disease 2019 (COVID-19) in addition to respiratory disease. The mechanisms underlying the disproportionate effect of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection on patients with cardiovascular comorbidities, however, remain incompletely understood.[1,2]

SARS-CoV-2 infects the host using the angiotensin converting enzyme 2 (ACE2) receptor, which is expressed in several organs, including the lung, heart, kidney, and intestine. ACE2 receptors are 'so expressed by endothelial cells.[3] ..hether vascular derangements in COVID-19 are due to endothelial cell involvement by the virus is currently

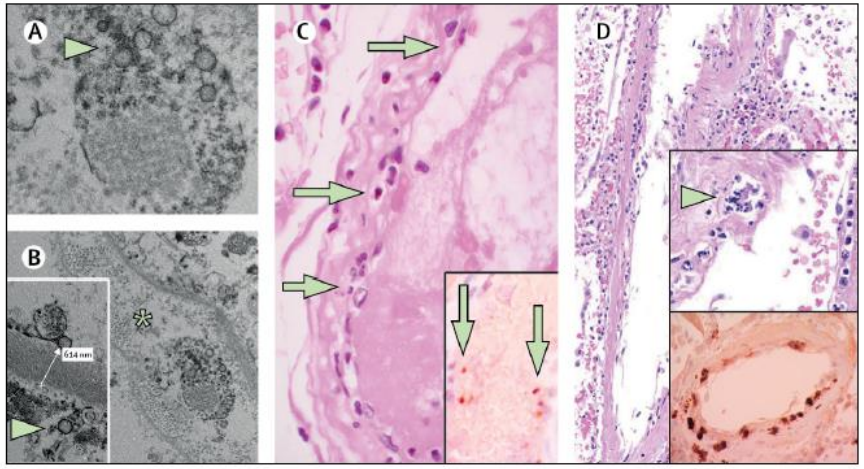


Figure: **Pathology of endothelial cell dysfunction in COVID-19**
(A, B) Electron microscopy of kidney tissue shows viral inclusion bodies in a peritubular space and viral particles in endothelial cells of the glomerular capillary loops. Aggregates of viral particles (arrow) appear with dense circular surface and lucid centre. The asterisk in panel B marks peritubular space consistent with capillary containing viral particles. The inset in panel B shows the glomerular basement membrane with endothelial cell and a viral particle (arrow; about 150 nm in diameter). (C) Small bowel resection specimen of patient 3, stained with haematoxylin and eosin. Arrows point to dominant mononuclear cell infiltrates within the intima along the lumen of many vessels. The inset of panel C shows an immunohistochemical staining of caspase 3 in small bowel specimens from serial section of tissue described in panel D. Staining patterns were consistent with apoptosis of endothelial cells and mononuclear cells observed in the haematoxylin-eosin-stained sections, indicating that apoptosis is induced in a substantial proportion of these cells. (D) Post-mortem lung specimen stained with haematoxylin and eosin showed thickened lung septa, including a large arterial vessel with mononuclear and neutrophilic infiltration (arrow in upper inset). The lower inset shows an immunohistochemical staining of caspase 3 on the same lung specimen; these staining patterns were consistent with apoptosis of endothelial cells and mononuclear cells observed in the haematoxylin-eosin-stained sections. COVID-19=coronavirus disease 2019.

Electron microscopy of SARS-CoV-2: a challenging task

We read with interest the Correspondence by Zsuzsanna Varga and colleagues[1] on the possible infection of endothelial cells by SARS-CoV-2 using electron microscopic (EM) images as evidence. However, we believe the EM images in the Correspondence do not show coronavirus particles but instead show cross-sections of the rough endoplasmic reticulum (RER). These spherical structures are surrounded by dark dots, which might have been interpreted as spikes on coronavirus particles but are instead ribosomes. The purported particles are free within the cytoplasm, whereas within a coronavirus-infected cell, accumulations of virus particles would be found in membrane-bound areas in the cisternae of the RER–Golgi area, where the spikes would be located on the inside of the cisternal space.[2] In addition, cross-sections through the viral nucleocapsid are not seen in the interior of these structures as would be found with coronavirus particles (figure).

Just recently, there have been two additional reports[3,4] in which structures that can normally be found in the cytoplasm of a cell have been misinterpreted as viral particles.[5] EM can be a powerful tool to show evidence of infection by a virus, but care must be taken when interpreting cytoplasmic structures to correctly identify virus particles.

We declare no competing interests. The findings and conclusions are those of the authors and do not necessarily represent the position of the US Centers for Disease Control and Prevention.

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1 Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 2020; **395:** 1417–18.
2 Goldsmith CS, Tatti KM, Ksiazek TG, et al. Ultrastructural characterization of SARS coronavirus. *Emerg Infect Dis* 2004; **10:** 320–26.
3 Su H, Yang M, Wan C, et al. Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China. *Kidney Int* 2020; published online April 9. DOI:10.1016/j.kint.2020.04.003.
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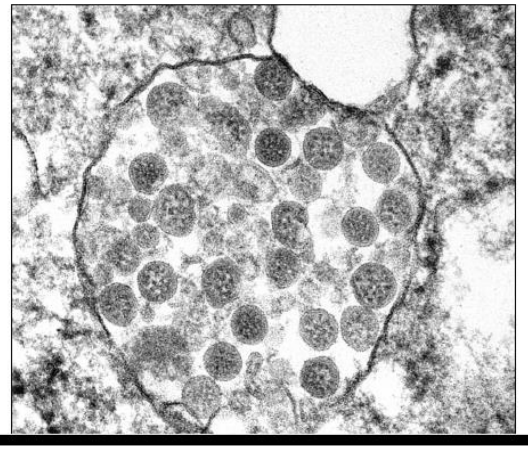

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Misinterpretation of clathrin-coated vesicles as viral particles

Ultrastructural Evidence for Direct Renal Infection with SARS-CoV-2

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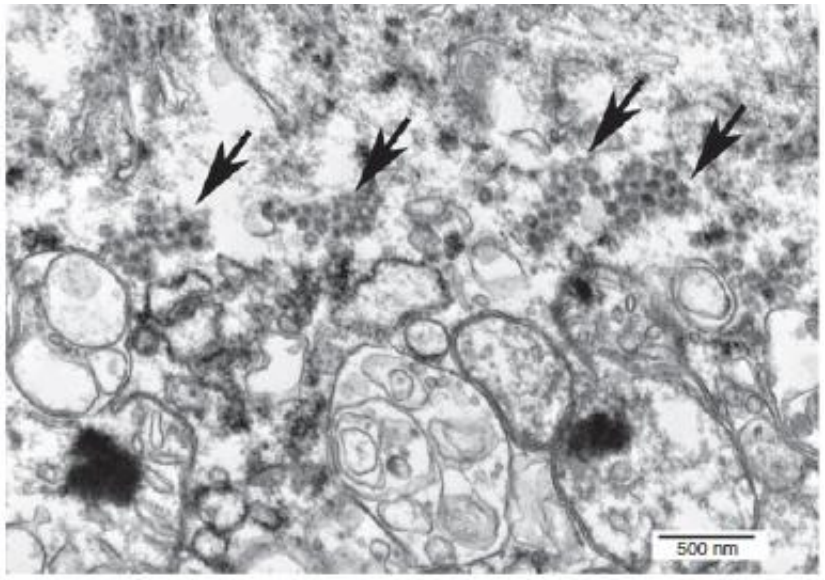


Menter T, et al. *Histopathology* 2020. PMID 32364264.
Larsen CP et al. *Kidney Int Rep* 2020. PMID 32292867
Farkash EA, et al. *J Am Soc Nephrol* 2020. PMID 32371536

Caution in Identifying Coronaviruses by Electron Microscopy

We are concerned about the erroneous identification of coronavirus directly in tissues by authors using electron microscopy. Several recent articles have been published that purport to have identified severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) directly in tissue.[1–4] Most describe particles that resemble, but do not have the appearance of, coronaviruses.[5–7]

The evidence provided in the article by Farkash *et al.*[8] in *JASN* likewise does not confirm the presence of SARS-CoV-2 in kidney tissue. Coronaviruses have been carefully described in electron microscopic images of thin sections.[9–12] In these images of thin sections, coronaviruses appear as spherical structures containing black dots on the inside, which are cross-sections through the helical viral nucleocapsid (Figure 1). Coronaviruses receive their outer covering by budding into cellular membranes of the rough endoplasmic reticulum and Golgi complex forming a vacuole and are found in the intracisternal space. The spikes are seen with difficulty in thin sections of infected cells, but a "fuzz" is sometimes visible. However, note that these spikes face the inside of the vacuole and do not touch the cytoplasm of the cell. Complete virus particles can also be found at the cell surface, having been extruded when the vacuolar membrane fuses with the plasma membrane and exocytoses them; in this case, the spikes on the virions face the extracellular space, again, not the cytoplasm.

In the article by Farkash *et al.*,[8] the electron microscopic images in their Figure 3, A–C do not demonstrate coronaviruses. Rather, the structures described as virus are clathrin-coated vesicles (CCVs), normal subcellular organelles involved in intracellular transport. Figure 3A[8] is a low magnification of a dying cell with nonspecific disorganized cytoplasm with an arrow pointing to an aggregation of CCVs. Panels B and C in their Figure 3[8] show clusters of CCVs, and the inset for Figure 3C[8] shows a higher magnification. None of these spherical structures contain

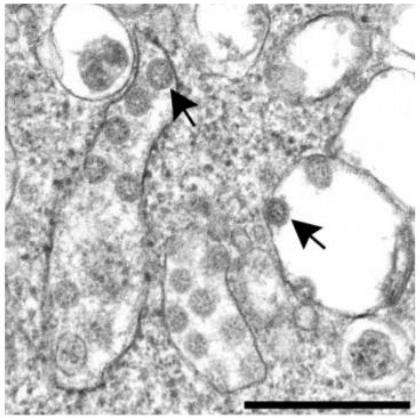


Figure 1. Severe acute respiratory syndrome coronavirus 2 isolate grown in cell culture showing numerous spherical viral particles (arrows) that are in the cisternae of the rough endoplasmic reticulum/Golgi complex area of the cell. Note the black dots on the interior of the particles, which are cross-sections through the viral nucleocapsid. Scale bar: 400 nm.

(MVB), which they have likened to double-membrane vesicles, the replication complex for coronaviruses. The structure shown in the manuscript by Farkash *et al.*[8] does not have the two tightly opposed membranes seen in double-membrane vesicles and does not have the appearance of what is shown in the reference they cite.[13] In addition, MVBs can be found in kidney tissues observed historically.[6] Moreover, MVBs are formed by invaginations of endosomes and are intermediates in trafficking for lysosomes.

Additionally, Farkash *et al.*[8] document their findings by

Goldsmith CS, et al. *J Am Soc Nephrol* 2020. PMID 32651224

“4 times as large as SARS-CoV-2, not located within cell membranes, lack viral nucleocapsids, not within vesicles”

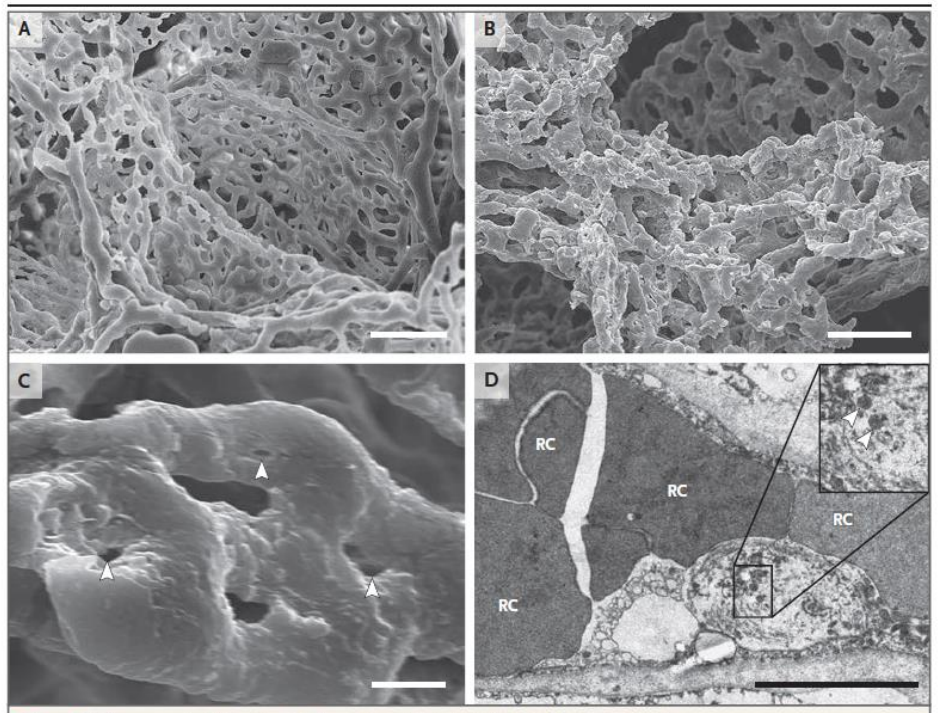


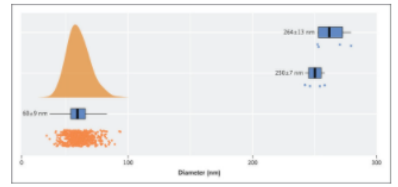
Figure 3. Microvascular Alterations in Lungs from Patients Who Died from Covid-19.
Panels A and B show scanning electron micrographs of microvascular corrosion casts from the thin-walled alveolar plexus of a healthy lung (Panel A) and the substantial architectural distortion seen in lungs injured by Covid-19 (Panel B). The loss of a clearly visible vessel hierarchy in the alveolar plexus is the result of new blood-vessel formation by intussusceptive angiogenesis. Panel C shows the intussusceptive pillar localizations (arrowheads) at higher magnification. Panel D is a transmission electron micrograph showing ultrastructural features of endothelial cell destruction and SARS-CoV-2 visible within the cell membrane (arrowheads) (the scale bar corresponds to 5 μm). RC denotes red cell.

Ackermann M, et al. N Engl *J Med* 2020. 383(2):120-128.

TO THE EDITOR

Ackermann et al. published an image obtained by transmission electron microscopy that depicted ultrastructural features of endothelial cell destruction. We measured the diameters of the two structures pinpointed in their Figure 3D as 250±7 nm in one and 264±13 nm in the other (mean [±SD] of four measurements). We also conducted a meta-analysis of published studies of SARS-CoV-2 (Figure 1), in which we found a mean virion size of 60±9 nm in images from thin-section transmission electron microscopy. The two dark spots in the image provided by Ackermann et al. are thus approximately 4 times as large as expected for SARS-CoV-2 virions, and they are not located within a cell membrane as described. They also lack viral nucleocapsids and are not within vesicles. Since the possibility of confusing SARS-CoV-2 with other subcellular structures is known,[1,2] we ask the authors whether they have other data to support the identification of the particles as SARS-CoV-2.

Figure 1.



Comparison of SARS-CoV-2 Virion Sizes.

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Scholkmann F, Nichols J. N Engl *J Med* 2020. PMID 32678533

Cytokine storm?

Editorial

June 30, 2020

Is a "Cytokine Storm" Relevant to COVID-19?

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» Author Affiliations | Article Information

JAMA Intern Med. 2020;180(9):1152-1154. doi:10.1001/jamainternmed.2020.3313

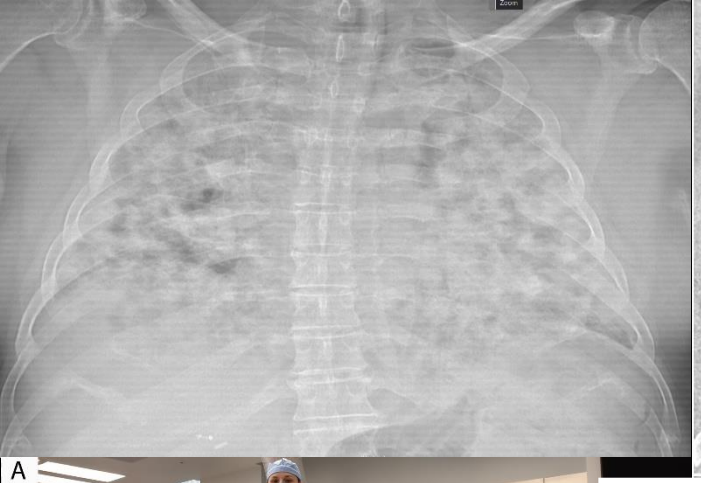
Table. Plasma Levels of Interleukin-6 Reported in COVID-19 Compared With Levels Previously Reported in ARDS[a]

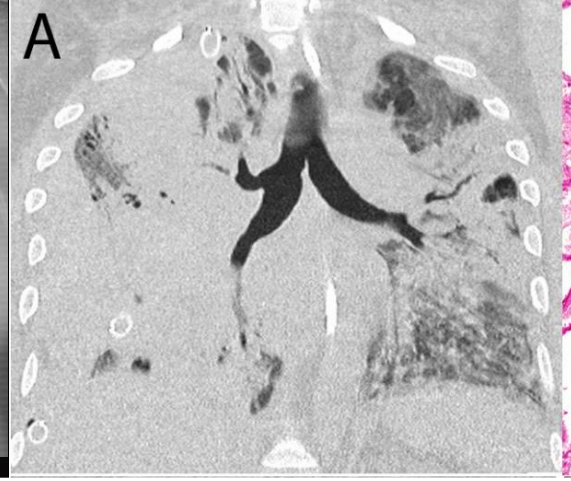
	Total population			Severe disease		
COVID-19	No.	IL-6 levels, pg/mL		No.	IL-6 levels, pg/mL	Measurement platform
Zhou et al[4]	191	7 (5-11)		54[b]	11 (8-14)	CL
Wu et al[1]	123	7 (6-9)		84[c]	7 (6-11)	CL
Mo et al[5]	155	45 (17-96)		85[d]	64 (31-165)	CL
Qin et al[2]	452	21 (6-47)		286[e]	25 (10-55)	CL
Cummings et al[6]	NR	NR		237[f]	26 (11-69)	CL
ARDS	**Total population**		**Hypoinflammatory**		**Hyperinflammatory**	**Measurement platform**
	No.	IL-6 levels, pg/mL	No.	IL-6 levels, pg/mL	No.	IL-6 levels, pg/mL	
ALVEOLI[7]	521	238 (94-741)[f]	386	154 (67-344)	135	1525 (584-3802)	ELISA
FACTT[8]	884	130 (46-411)[f]	638	86 (34-216)	246	578 (181-2621)	ELISA
SAILS[9]	720	443 (173-1513)[f]	451	282 (115-600)	269	1618 (517-3205)	ELISA

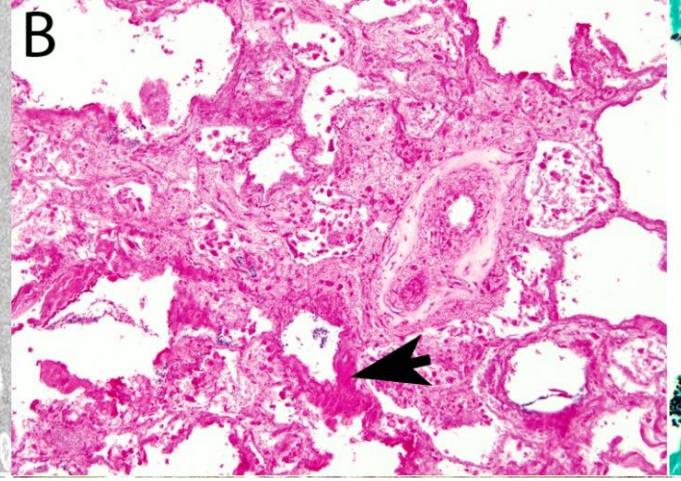
Sinha P, et al. *JAMA Intern Med* 2020. PMID 32602883

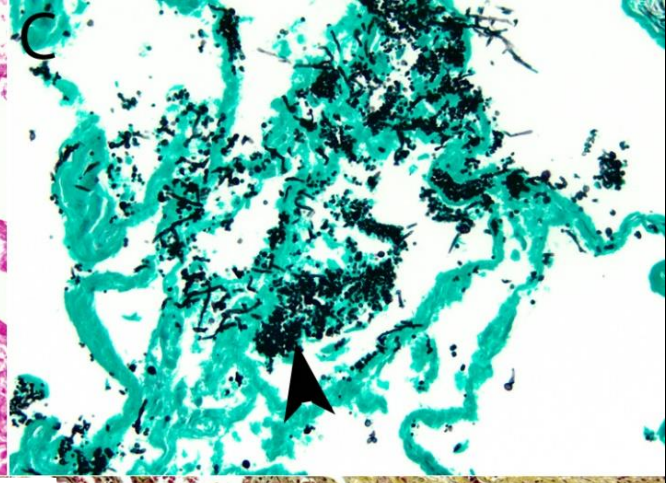
COVID-19 facts:
Lung involvement
Diffuse alveolar damage (DAD)
Bronchopneumonia
Thrombi

COVID-19 controversies:
Degree of extrapulmonary involvement
“Viral particles” in endothelium
“Cytokine storm”









Thank you for your attention