Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Table of Contents **Page**

Introduction 2

Methods 3

Results 4

Discussion 8

Conclusions 13

Acknowledgments 14

References 14

Supplementary Figure S1. Imaging findings in vaping-associated acute lung injury. 16

Supplementary Figure S2. Histology of vaping-associated acute lung injury. 17

Supplementary Figure S3. Histology in fatal cases of vaping-associated acute lung injury. 19

Supplementary Figure S4. Cytology of bronchioloalveolar lavage fluid in vaping-associated acute lung injury 20

Supplementary Table S1. Patient demographics and clinical findings. 21

Supplementary Table S2. Radiologic and pathologic summaries. 24

Supplementary Table S3. Frequency of histopathologic features in vaping-associated acute lung injury. 26

INTRODUCTION

Use of electronic cigarette ("e-cigarette") devices has exploded in popularity in recent years. E-cigarettes now represent the most common nicotine-containing products used in the United States, especially among adolescents and young adults[1]. These battery-operated devices heat liquids, oils, or waxes containing nicotine, cannabinoids, synthetic cannabinoids, illicit drugs, and other chemical compounds, producing an aerosolized vapor that can be inhaled, or vaped, by the user[2]. Using e-cigarettes ("vaping") is often viewed and marketed as a safer alternative to smoking combustible tobacco cigarettes. However, there is increasing recognition that vaping is not entirely harmless, and numerous reports of vaping-related pulmonary illness have begun to emerge in the medical literature and also in the lay press, with a spike in reported cases in recent weeks[3-6]. Many, but not all, of the reported cases have been associated with vaping of cannabinoid-containing products and not simply nicotine[3], but the chemical compounds responsible for adverse pulmonary reactions remain obscure in many cases, due to the wide diversity and unregulated nature of vaping devices, products, and practice habits among users. Several pathologic changes in the lung have been described in isolated case reports[7-11] and a cluster of cases recently reported from Wisconsin and Illinois[3], but the spectrum of vaping-induced histopathologic changes that can occur in the lung remains poorly understood.

We have encountered several cases of pulmonary illness clinically suspected to be due to vaping in our clinical and pathology consultation practices in the last few years with a spike in cases in recent weeks, including two cases that culminated in the patient's death. This prompted us to review our experience with this phenomenon. Our aim was to characterize not only the spectrum of clinical and imaging findings in our patients, but also the types of pathologic changes that occur in the lung, to better understand the pathogenesis of this problem and facilitate recognition of the diagnosis in future patients.

METHODS

This study was approved by the Institutional Review Board at Mayo Clinic (IRB# 19-008434). All cases of clinically suspected vaping-associated acute lung injury encountered in our clinical and pathology consultation practices with available lung biopsy material were included from three of our institutional sites (in Scottsdale, Arizona, Rochester, Minnesota, and Jacksonville, Florida). In all cases, vaping was clinically favored to be the culprit prior to the biopsy, and biopsies were performed to confirm this clinical impression and rule out other causes. Two older cases (one from 2016, one from 2017) were identified by searching our laboratory reporting system and extramural consultation files for pathology reports containing the word "vaping". Recent cases (all received in the last 4 weeks) were identified in real time upon receipt in the surgical pathology laboratory or receipt for a consultative opinion. Clinical records were retrospectively reviewed, and relevant elements were tabulated in each case including the patient's age, gender, presenting symptoms, underlying conditions, occupational history, and other inhalational exposures. Results of serologic testing, microbiologic cultures, and other testing for infectious diseases were reviewed whenever available. Tobacco smoking history, vaping history, and history of illicit drug use was also reviewed, including the duration of these exposures and types of substances used whenever available. Imaging studies were reviewed in each case, and relevant radiologic findings were tabulated. Treatments and clinical outcomes were also reviewed.

After reviewing all available clinical information, cases were classified using criteria for vaping-related lung injury recently proposed by the United States Centers for Disease Control and Prevention (CDC).[3] These criteria are subject to change, but at the time of this writing, criteria for a "Confirmed" case include: 1) use of an e-cigarette or vaping device within 90 days of symptom onset; 2) presence of pulmonary infiltrates; 3) absence of clinical and laboratory

evidence of infection; and 4) absence of evidence for alternative diagnoses (e.g. cardiac, rheumatologic, or neoplastic processes). Criteria for a "Probable" case are identical with the exception of the third criterion, where evidence of infection is identified, but the clinical team caring for the patient does not believe infection is the sole cause of respiratory illness, or minimum criteria for ruling out infection have not been met.

Pathology slides from each case were retrieved from the pathology archives at each participating site and reviewed. The type of biopsy and lobe(s) sampled in each case were recorded. All existing hematoxylin- and eosin-stained slides from each case were evaluated for histologic evidence of acute lung injury, including patterns of acute fibrinous pneumonitis, diffuse alveolar damage, and organizing pneumonia, and other histologic features of acute injury including interstitial edema and intraalveolar fibrin. When possible, the distribution of these changes (as airway-centered, peripherally accentuated, random, or diffuse) was characterized. The presence of pneumocyte vacuolization, foamy macrophage accumulation, eosinophilic infiltrates, chronic lymphoplasmacytic interstitial inflammation, bronchiolitis, pigmented macrophages, granulomatous inflammation, airway-centered scarring, peribronchiolar metaplasia, and other relevant findings were noted. Results of special stains for microorganisms were tabulated, including Gomori-methenamine silver stains for fungal organisms, Ziehl-Neelsen stains for acid-fast bacilli, and immunohistochemical studies for viral infection.

RESULTS

Patient Demographics and Clinical Presentation

A total of 17 patients met inclusion criteria, including cases from Arizona (n=11), Minnesota (n=5), and Florida (n=1). Patient demographics and clinical findings are summarized

in Supplementary Table S1. Most patients were young adults (median age 35 years, range 19-67) and most were men (76%). Many patients were previously healthy prior to their presentation and had no significant past medical history. All presented in the acute to subacute time frame. Five patients reported nausea and vomiting in addition to respiratory complaints. Eight patients had a history of tobacco smoking, but only two were actively smoking at the time of presentation. Four patients had a history of marijuana smoking. Eleven patients met CDC criteria for a "confirmed" diagnosis of vaping-related lung injury, with the remaining six patients meeting CDC criteria for a "probable" designation, largely due to incomplete information on the autoimmune and infectious disease workup.

All patients had a history of vaping in the weeks and days prior to presentation. Two patients were vaping in an attempt to quit smoking tobacco. Twelve patients (71%) reported vaping tetrahydrocannabinol (THC), cannabis oils, cannabidiol (CBD), or other non-nicotine products. Both manufactured prepackaged vape pods and open access tank style vaporizers were used by subjects.

Radiologic and Histopathologic Findings

The radiologic and histopathologic findings are summarized in Supplementary Table S2. Sixteen of the 17 patients had HRCT data available, with the remaining patient only having chest x-ray data available. All patients had bilateral ground glass and/or consolidative opacities on imaging (Supplementary Figure S1). The imaging in six of the cases showed a distinct bronchocentric distribution of opacities. No consistent upper versus lower lobe distribution of imaging abnormalities was noted.

Histopathologic assessment was performed using transbronchial forceps biopsies (9 patients), transbronchial cryobiopsies (1), and surgical wedge biopsies (7). In the surgical wedge biopsy and cryobiopsy cases that allowed assessment of the distribution of disease, six of the eight cases showed a distinct centrilobular distribution of the histologic abnormalities. Although the changes varied, all cases showed one or more patterns or features of acute lung injury including acute fibrinous pneumonitis, diffuse alveolar damage, organizing pneumonia, interstitial edema, intraalveolar fibrin, and reactive type 2 pneumocyte hyperplasia. Four cases met criteria for a histologic diagnosis of diffuse alveolar damage with hyaline membranes. In cases with bronchioles present for assessment, bronchiolitis with bronchial edema and mucosal ulceration were often encountered. Mild chronic inflammatory infiltrates and/or neutrophilic infiltrates were also common. Airway-centered accumulation of foamy macrophages in peribronchiolar airspaces was universally present, along with similar-appearing vacuolization of the cytoplasm of hyperplastic type 2 pneumocytes. In seven cases, the airspace macrophages were accompanied by a minor population of lightly pigmented (in addition to foamy) macrophages with mixed brown and black particles, reminiscent of "smokers' macrophages". These particles were typical of particles or products of combustion seen in smokers' macrophages (less than 1 μm), and were never refractile. No larger inhaled or aspirated particles were identified in any case. Interestingly, only four of the seven patients with pigmented macrophages reported a history of tobacco and/or marijuana smoking, with the remaining three patients denying these activities. Eosinophils were often encountered, but were always rare. A single case with a recent history of pneumothorax showed organizing fibrinous pleuritis, an expected secondary change in this context, but no other cases showed evidence of pleuritis or pleural fibrosis. None of the cases showed granulomatous inflammation, and none showed

histologic evidence of exogenous lipoid pneumonia. Special stains for microorganisms were negative in all cases tested (n=13). Histologic findings are summarized in Supplementary Table S3, and representative photomicrographs of lung biopsies from patients with non-fatal and fatal pulmonary reactions are shown in Supplementary Figures S2 and S3, respectively.

Bronchioloalveolar lavage specimens were only available for review in two cases, but reported differential results were available in several additional cases, which are summarized in Supplementary Table S2. In the two cases formally reviewed, we observed abundant macrophages with foamy, finely vacuolated cytoplasm in both cases, although both cases also showed rare macrophages with larger vacuoles, and one case also showed a lesser population of macrophages containing brown and black pigment particles, reminiscent of "smokers' macrophages". The macrophages in both of these cases closely resembled the alveolar macrophages seen in both respective lung biopsy specimens. Representative photomicrographs of bronchioloalveolar lavage findings are shown in Supplementary Figure S4.

Outcomes

Treatment information was available in seven patients, three of whom had acute and organizing diffuse alveolar damage in their biopsies, and four of whom had airway-centered acute fibrinous pneumonitis or organizing pneumonia. All were treated with broad-spectrum antibiotics and steroids and supportive care. Two patients (both with diffuse alveolar damage) succumbed to their illness, and the other five were improving or improved at the time of last follow-up.

DISCUSSION

Pathologic Findings and Pathogenesis

To date, histologic findings reported in putative cases of vaping-associated acute lung injury include organizing pneumonia[9,10], lipoid pneumonia[7], diffuse alveolar hemorrhage[8], mild nonspecific inflammation[3], diffuse alveolar damage with foamy macrophages[3], interstitial and peribronchiolar granulomatous pneumonitis[3], and respiratory bronchiolitis[11], although not all of these reports include illustrations of the pathology, and the patient reported to have respiratory bronchiolitis was also a cigarette smoker. Our cases corroborate many but not all of these descriptions, with most of our cases showing airway-centered patterns of acute or subacute lung injury with bronchiolitis, foamy macrophage accumulation, and type 2 pneumocyte hyperplasia with vacuolization. Several of our cases showed more severe injury with a pattern of diffuse alveolar damage, including two patients with fatal outcomes. Eosinophils were occasionally seen, but when present, they were never prominent and did not meet histologic criteria for acute eosinophilic pneumonia. It has also been suggested in one case report that vaping may cause hypersensitivity pneumonitis[12], but this interpretation was based purely on clinical and imaging findings and analysis of bronchioloalveolar lavage fluid, without histologic confirmation. Definitive granulomas were not encountered in any of our cases, and none of our cases showed histologic features of hypersensitivity pneumonitis or giant cell interstitial pneumonia from metal fumes.

The pathogenesis of vaping-associated acute lung injury remains poorly understood, but much attention has been given recently to the possibility that this may represent a form of exogenous lipoid pneumonia. Indeed, lipid-laden macrophages have been found in bronchioloalveolar lavage fluid from patients with vaping-related pulmonary illness[4,13], and one

case was reported to show a histologic pattern of "lipoid pneumonia"[7], although a review of the illustrated histopathology reveals cholesterol clefts but no interstitial lipid droplet accumulation associated with a foreign body-type histiocytic reaction as would be expected in exogenous lipoid pneumonia. Notably, none of our cases showed histologic features of exogenous lipoid pneumonia, calling into question the diagnostic utility of identifying lipid-laden macrophages or performing oil red O staining on bronchioloalveolar lavage fluid as a marker of vaping-associated lung injury, as has been proposed[4,13]. The significance of this observation remains unclear, particularly in patients who already have a known vaping exposure to aerosolized lipid, and until more data accumulates on this finding, our observations suggest that this finding should be interpreted with caution, as it may simply represent a marker of exposure to aerosolized lipid and not necessarily a marker of toxicity related to the exposure.

Although none of the individual histologic findings in our cases were specific, foamy macrophage accumulation and pneumocyte vacuolization were universal findings and could be useful diagnostic clues in an appropriate clinical context. This pattern closely resembles the type of changes that are characteristic of toxic reactions to medications (especially amiodarone) or noxious chemical fumes, suggesting a similar mechanism of injury. While it is difficult to discount the potential role of aerosolized lipid accumulation in this injury, no cases showed coalescence of lipid into large droplets as occurs in exogenous lipoid pneumonia. This is notable, as water-insoluble oil droplets tend to have high interfacial tension that facilitates droplet coalescence, and even fine emulsifications of insoluble oils and water will eventually phase separate with time and form larger oil droplets[14]. Our observation is also concordant with the recently reported absence of typical radiologic findings of fat accumulation in the lung that would be expected in exogenous lipoid pneumonia[5].

To us, the histologic findings would instead seem to suggest that vaping-associated lung injury represents a form of airway-centered chemical pneumonitis induced by one or more inhaled toxic substances in the aerosolized vapor, rather than an exogenous lipoid pneumonia *per se*. Indeed, it is well known that e-cigarette liquids contain not only propylene glycol and glycerin but may also contain numerous contaminants, including polycyclic aromatic hydrocarbons, nitrosamines, endotoxins, diacetyl, and a wide variety of other organic and inorganic chemicals and flavoring compounds that may not be entirely inert[15]. In many cases, we also observed a lesser population of lightly pigmented macrophages that were often subtle and easily missed unless specifically sought. These macrophages contained minute, mixed brown and black particles resembling particulate matter that is characteristically present in macrophages from smokers. This finding often but not always correlated with a history of smoking, and it is possible that some of these particles may have originated from the vaping liquids or devices and could represent products of combustion, particularly in those patients with no history of smoking. If this observation is validated by other studies, this could also represent an additional useful diagnostic clue, at least in a nonsmoker.

Differential Diagnoses

Adverse reactions to drugs and toxic agents are challenging to diagnose to a reasonable degree of clinical certainty, and this has certainly been true with recent reports of suspected vaping-related lung injury. In his classic monograph on drug reactions[16], Irey defined criteria that must be satisfied when an adverse reaction to an agent is suspected. There must be proof of exposure to the agent, temporal eligibility (i.e. exposure to the agent before symptom onset), and an appropriate latency period between exposure to the agent and symptom onset. In addition,

empiric correlation between the agent and suspected reaction is supported when other possibilities have been eliminated by appropriate investigations (e.g., a negative laboratory workup for infections and rheumatologic disorders). In reality, proving causality with absolute certainty can be difficult, even after all alternative etiologies have been excluded. To address these inherent uncertainties in the diagnostic process yet provide a meaningful definition for surveillance, the CDC established criteria for "confirmed" and "probable" cases of vaping-related lung injury, following Irey's fundamental principles[3]. Eleven of our patients met the CDC's definition of a "confirmed" case of vaping-related lung injury, with the remaining cases meeting criteria for a "probable" designation, a ratio similar to that reported in the recent cluster of cases in Wisconsin and Illinois[3].

As with other adverse reactions to drugs and toxic agents in the lung, the histopathologic manifestations of vaping-related acute lung injury are nonspecific, and this is a diagnosis of exclusion. Whether diffuse alveolar damage, acute fibrinous pneumonitis, organizing pneumonia, or other patterns of injury are seen, the differential diagnosis for vaping-related acute lung injury is similar and primarily includes acute infection, autoimmune disease, reactions to other drugs, and other inhalational injuries. Clinically, distinction between infection and steroid-responsive causes of acute lung injury is critical, and more than half of cases of vaping-related pulmonary illness have been reported to be steroid-responsive[3]. Special stains, cultures, and other laboratory studies should be utilized liberally to evaluate for infection. Distinguishing vaping-related lung injury from autoimmune disease can be challenging, but a thorough clinical and laboratory workup with serologic testing should enable this distinction in most cases. Distinguishing vaping-related lung injury from pneumonitis induced by other drugs or inhaled toxins may be particularly challenging, especially in patients who may be reluctant to admit use

of e-cigarettes or the types of substances vaped. Many medications and illicit drugs have been associated with foamy macrophage accumulation and pneumocyte vacuolization, and to date, no specific histologic features have been noted with any one agent. Furthermore, these features are not specific to drug reactions, and can be seen with acute lung injury from other causes[17-19]. Based on our observations in our cases, this histopathologic nonspecificity is also characteristic of acute lung injury from vaping, and it would seem that the diagnosis of vaping-related pulmonary illness cannot be made on histologic grounds alone, and is only possible with careful clinicopathologic correlation.

Study Limitations

This study has several limitations. First, only patients subjected to a lung biopsy were included. This approach is useful for characterizing pathologic changes in lung tissue related to vaping, but there is an inherent selection bias in our study and it remains unknown whether these findings are generalizable to all patients with vaping-related lung injury, including those who did not require a lung biopsy as part of their clinical management or those with subclinical disease. Second, all lung biopsies were obtained from patients presenting with acute pulmonary illness, and the long-term consequences of vaping remain unknown and warrant further study. Constrictive (obliterative) bronchiolitis is a well-recognized long-term consequence of severe acute airway injury from chemical fume exposures and other toxic inhalational exposures, and the histologic similarities observed with vaping-associated acute lung injury raise concern that small airways disease or other respiratory problems may materialize over time in patients who experience vaping-related acute lung injury or perhaps even subclinical airway injury from vaping. Third, specific details of the vaping devices, products, and vaping habits were lacking in

many of the subjects and the chemical composition of the vaping product(s) used by the subjects were unknown. However, it is notable that most of our patients were using marijuana-containing products or cannabis oils, as has been reported by others[3,13], and these compounds could be playing an important role. Finally, the concentrations of chemical exposure to the terminal airways and alveoli are unknown in our study, although at least three patients reported "heavy" or "significant" vaping use prior to presentation. Vaping products, particularly the open access tank style vaporizers, allow the user to aerosolize essentially limitless combinations of chemicals at concentrations far higher and durations far longer than with traditional tobacco cigarettes. For these reasons, we cannot exclude a dose-dependent toxicity of one or more compounds.

CONCLUSIONS

Histopathologically, vaping-associated acute lung injury shows nonspecific injury patterns that are usually airway-centered, including acute fibrinous pneumonitis, diffuse alveolar damage, organizing pneumonia, interstitial edema, and intraalveolar fibrin accumulation, often accompanied by bronchiolitis. Regardless of the fundamental pattern of injury seen, foamy macrophages and foamy pneumocytes are universally present, sometimes with a lesser number of pigmented macrophages, and neutrophils are often numerous. Although the pathogenesis and the chemical agent(s) responsible for this problem remain unknown, this constellation of histologic changes suggests the possibility of direct lung toxicity from an inhaled noxious agent or agents. Although nonspecific, foamy macrophages and foamy pneumocytes may be a useful diagnostic clue in an appropriate clinical context.

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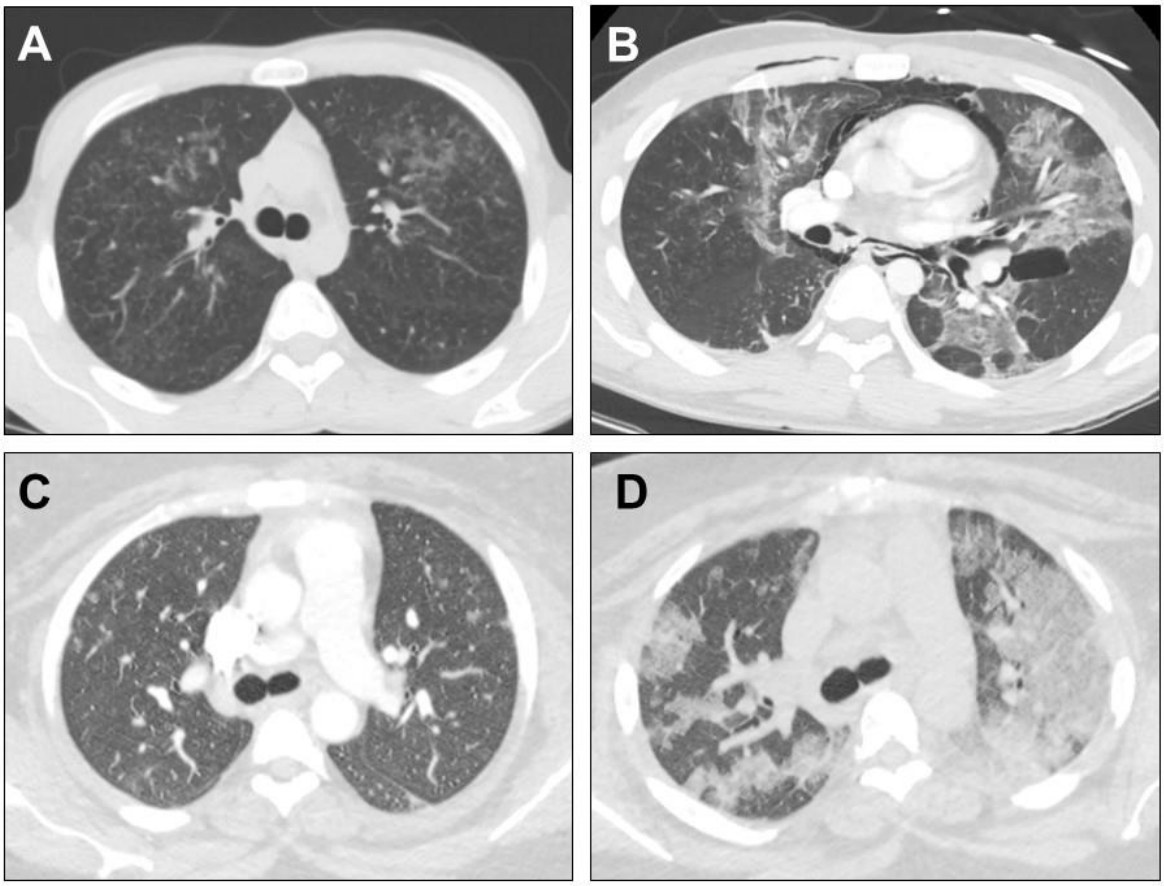
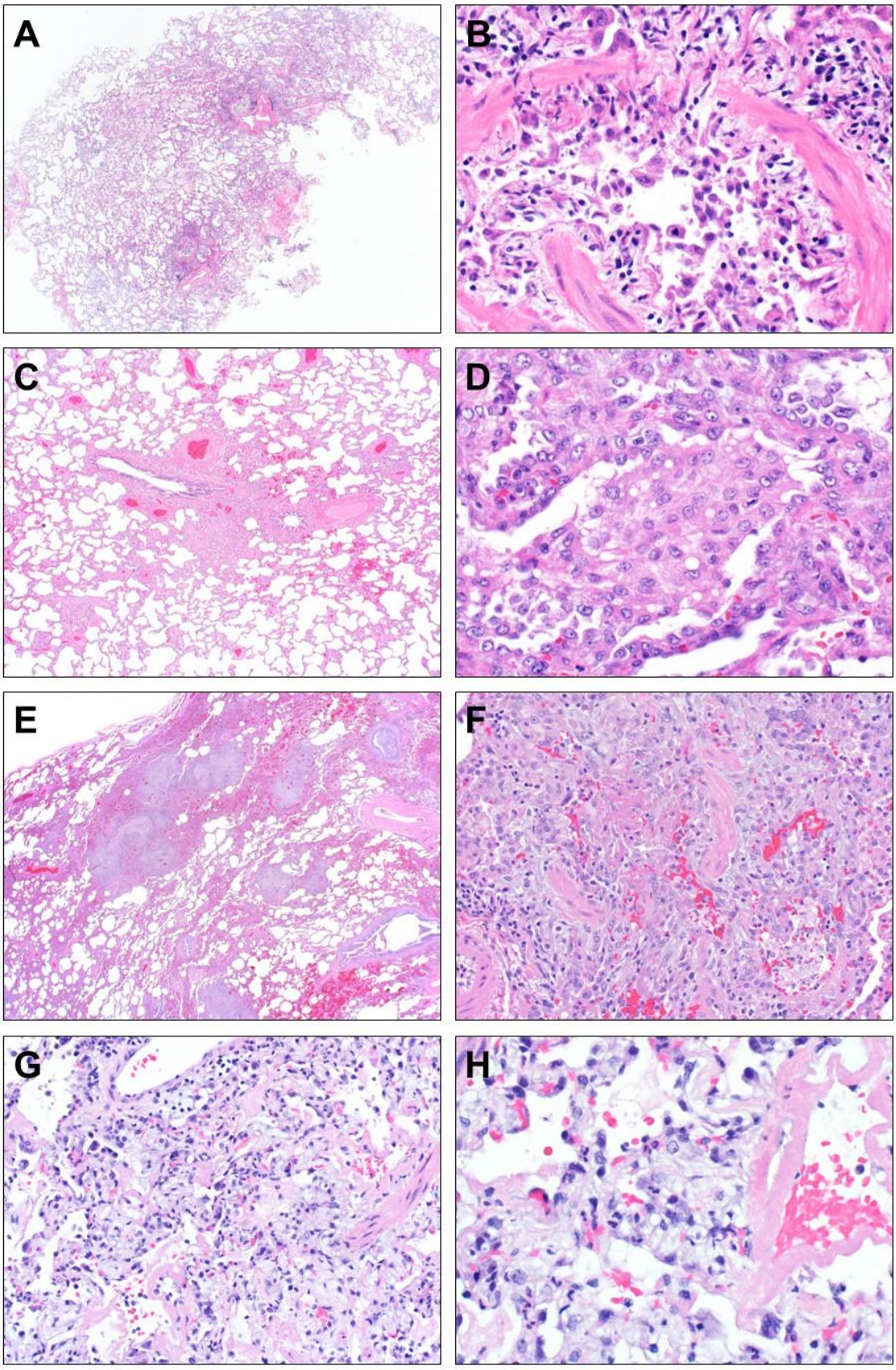
REFERENCES

1. Cullen KA, Ambrose BK, Gentzke AS, Apelberg BJ, Jamal A, King BA. Notes from the Field: Use of Electronic Cigarettes and Any Tobacco Product Among Middle and High School Students - United States, 2011-2018. MMWR Morb Mortal Wkly Rep 2018;67:1276-7.
2. Breitbarth AK, Morgan J, Jones AL. E-cigarettes-An unintended illicit drug delivery system. Drug Alcohol Depend 2018;192:98-111.
3. Layden JE, Ghinai I, Pray I, et al. Pulmonary Illness Related to E-Cigarette Use in Illinois and Wisconsin - Preliminary Report. N Engl J Med 2019.
4. Maddock SD, Cirulis MM, Callahan SJ, et al. Pulmonary Lipid-Laden Macrophages and Vaping. N Engl J Med 2019.
5. Henry TS, Kanne JP, Kligerman SJ. Imaging of Vaping-Associated Lung Disease. N Engl J Med 2019.
6. Arter ZL, Wiggins A, Hudspath C, Kisling A, Hostler DC, Hostler JM. Acute eosinophilic pneumonia following electronic cigarette use. Respir Med Case Rep 2019;27:100825.
7. Viswam D, Trotter S, Burge PS, Walters GI. Respiratory failure caused by lipoid pneumonia from vaping e-cigarettes. BMJ Case Rep 2018;2018.
8. Agustin M, Yamamoto M, Cabrera F, Eusebio R. Diffuse Alveolar Hemorrhage Induced by Vaping. Case Rep Pulmonol 2018;2018:9724530.
9. He T, Oks M, Esposito M, Steinberg H, Makaryus M. "Tree-in-Bloom": Severe Acute Lung Injury Induced by Vaping Cannabis Oil. Ann Am Thorac Soc 2017;14:468-70.
10. Khan MS, Khateeb F, Akhtar J, et al. Organizing pneumonia related to electronic cigarette use: A case report and review of literature. Clin Respir J 2018;12:1295-9.
11. Flower M, Nandakumar L, Singh M, Wyld D, Windsor M, Fielding D. Respiratory bronchiolitis-associated interstitial lung disease secondary to electronic nicotine delivery system use confirmed with open lung biopsy. Respirol Case Rep 2017;5:e00230.
12. Sommerfeld CG, Weiner DJ, Nowalk A, Larkin A. Hypersensitivity Pneumonitis and Acute Respiratory Distress Syndrome From E-Cigarette Use. Pediatrics 2018;141.
13. Davidson K, Brancato A, Heetderks P, et al. Outbreak of Electronic-Cigarette-Associated Acute Lipoid Pneumonia - North Carolina, July-August 2019. MMWR Morb Mortal Wkly Rep 2019;68:784-6.
14. Boyson TK, Pashley RM. A study of oil droplet coalescence. J Colloid Interface Sci 2007;316:59-65.
15. Clapp PW, Jaspers I. Electronic Cigarettes: Their Constituents and Potential Links to Asthma. Curr Allergy Asthma Rep 2017;17:79.
16. Irey NS. Teaching monograph. Tissue reactions to drugs. Am J Pathol 1976;82:613-47.
17. Colby TV. Pathologic aspects of bronchiolitis obliterans organizing pneumonia. Chest 1992;102:38S-43S.
18. Katzenstein AL, Myers JL, Prophet WD, Corley LS, 3rd, Shin MS. Bronchiolitis obliterans and usual interstitial pneumonia. A comparative clinicopathologic study. Am J Surg Pathol 1986;10:373-81.

19. Stanley MW, Henry-Stanley MJ, Gajl-Peczalska KJ, Bitterman PB. Hyperplasia of type II pneumocytes in acute lung injury. Cytologic findings of sequential bronchoalveolar lavage. Am J Clin Pathol 1992;97:669-77.



Supplementary Figure S1. Imaging findings in vaping-associated acute lung injury. Representative images from high-resolution computed tomography of the chest. (A) A 21-year-old man (Case 7) with a 5-year history of vaping nicotine products presented with cough, chest tightness, fevers, and nausea for several days after switching to a product containing both nicotine and marijuana, and had bilateral tree-in-bud opacities and bronchocentric ground glass opacities with upper lobe predominance. (B) A 25-year-old man (Case 6) with a history of vaping nicotine products mixed with cannabis oils presented acutely with a fever and cough, and had bilateral perihilar ground-glass opacities with areas of lobular sparing and pneumomediastinum. (C) A 31-year-old woman (Case 5) with a history of vaping presented with a cough and increasing shortness of breath for several weeks, and was found to have bilateral ground-glass nodules that (D) rapidly progressed to more extensive ground-glass opacities and consolidation. Despite treatment with high-dose corticosteroids and maximum supportive care including extracorporeal membrane oxygenation therapy, the patient eventually died of her acute lung injury.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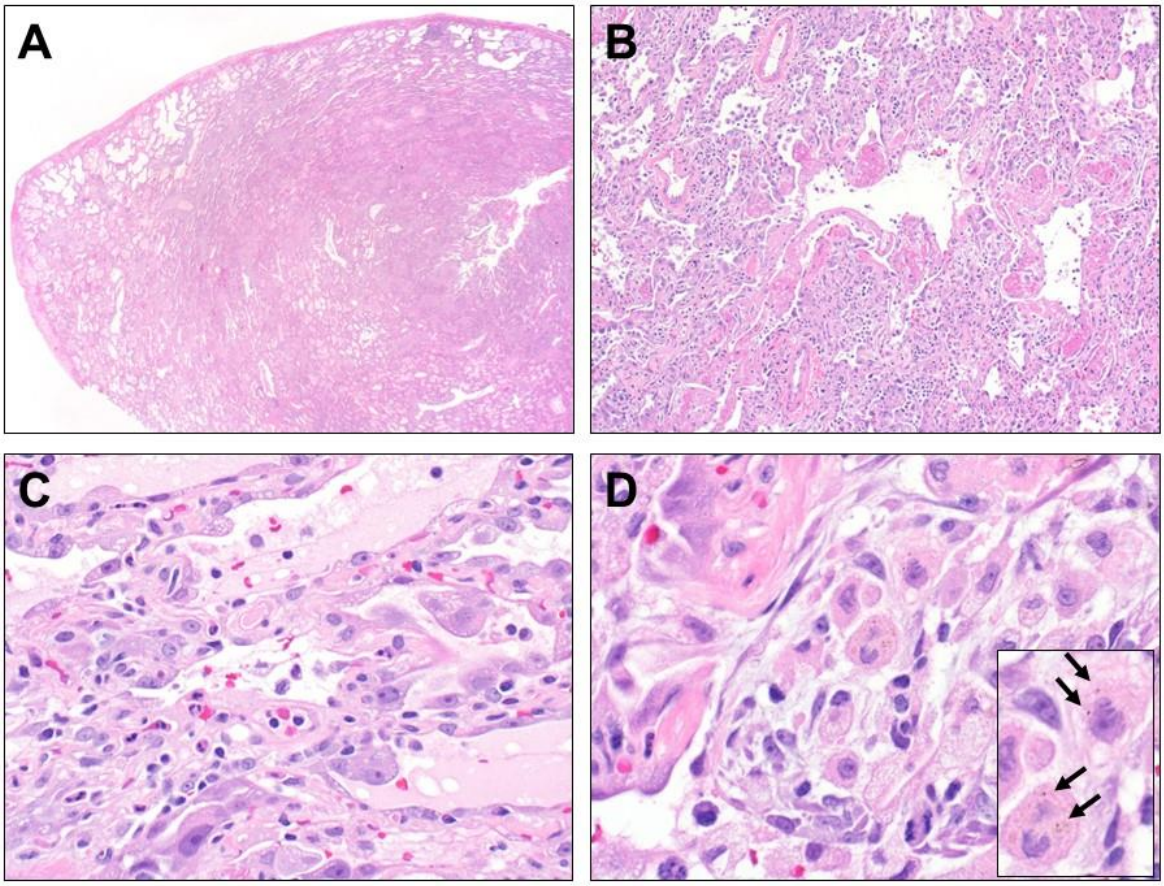
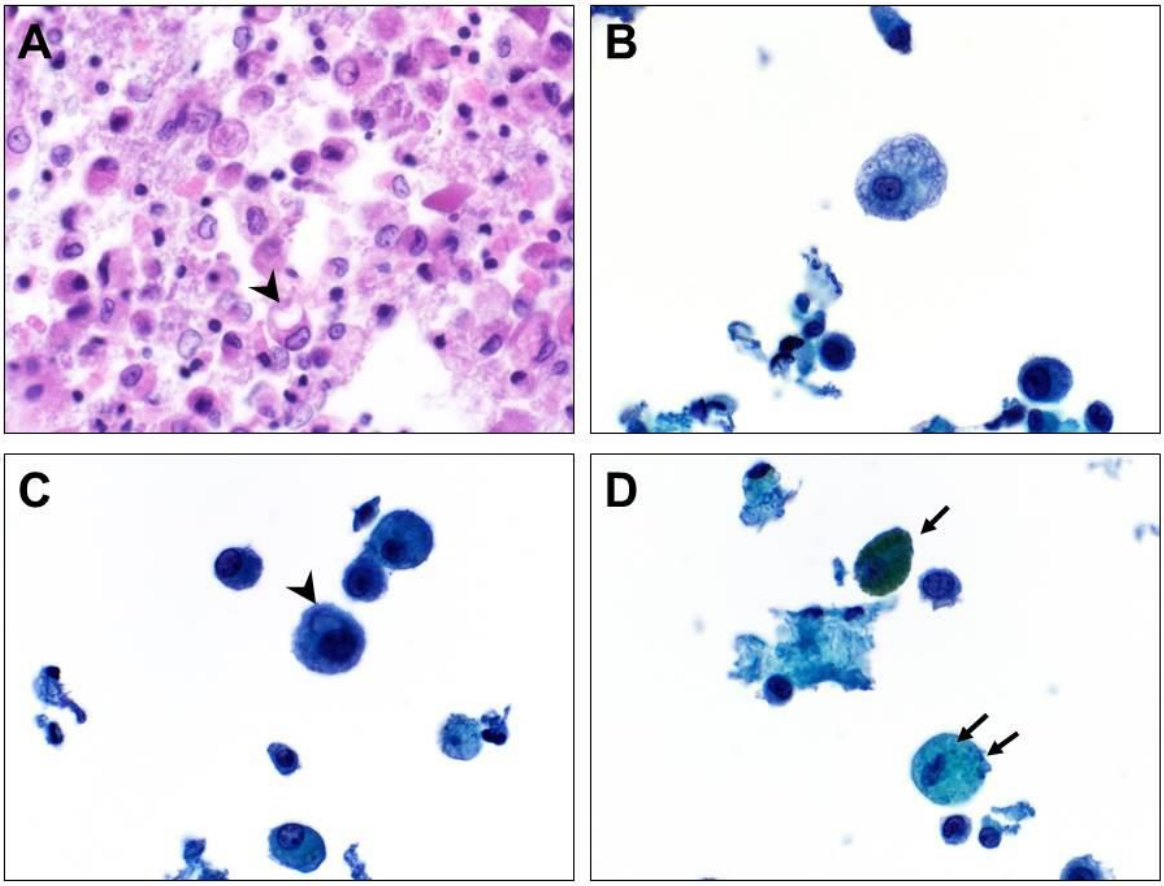
Supplementary Figure S2 (previous page). Histology of vaping-associated acute lung injury.
Representative photomicrographs of vaping-associated acute lung injury. (A-B) A transbronchial cryobiopsy from a 21-year-old man (Case 7) vaping nicotine and marijuana showed an exquisitely airway-centered organizing acute lung injury process, with severe bronchiolitis accompanied by marked mucosal edema, fine vacuolization and sloughing of bronchiolar epithelium, and peribronchiolar organization. (C-D) A surgical lung biopsy from a 27-year-old man (Case 8) vaping cannabis daily showed airway-centered acute lung injury with bronchiolitis, organization, and accumulation of innumerable finely and coarsely vacuolated macrophages in peribronchiolar airspaces. (E-F) Surgical lung biopsies from a 51-year-old man (Case 3) vaping marijuana showed a vaguely nodular, airway-centered acute lung injury pattern with severe bronchiolitis, abundant fibrin within bronchioles and peribronchiolar airspaces, vacuolization of the bronchiolar mucosa and pneumocytes, foamy macrophage accumulation, and airway-centered organization. (G-H) Transbronchial forceps biopsies from a 23-year-old woman (Case 4) showed acute and organizing diffuse alveolar damage with hyaline membranes, fibrin, interstitial edema, coarsely vacuolated pneumocytes and macrophages, and scattered neutrophils.



Supplementary Figure S3. Histology in fatal cases of vaping-associated acute lung injury. Representative photomicrographs of surgical lung biopsies from a 31-year-old woman (Case 5) with vaping-associated acute lung injury that culminated in her death. (A) At scanning magnification, the process is quite diffuse, but still vaguely accentuated in centrilobular regions. (B) Closer examination reveals characteristic features of diffuse alveolar damage in a subacute, organizing phase with well-formed hyaline membranes lining respiratory bronchioles and alveolar ducts. (C) At higher magnification, striking vacuolization and reactive atypia of pneumocytes is apparent, with edematous alveolar septa and scattered inflammatory cells including a few neutrophils and rare eosinophils. (D) In centrilobular regions, abundant macrophages filled airspaces and were intimately admixed with proliferating fibroblasts and organization. Most macrophages were foamy, but scattered individual macrophages contained light brown pigmented particles (inset, arrows).



Supplementary Figure S4. Cytology of bronchioloalveolar lavage fluid in vaping-associated acute lung injury.
Representative photomicrographs of bronchioloalveolar lavage fluid. (A) A cell block preparation of lavage fluid from a 40-year-old man (Case 17) with vaping-associated acute lung injury showed abundant macrophages, most of which showed markedly foamy cytoplasm with fine vacuolization, although rare macrophages with larger vacuoles (arrowhead) were also present. Papanicolaou stains of lavage fluid from a 21-year-old man (Case 7) with vaping-associated acute lung injury showed (B) numerous macrophages with markedly foamy, finely vacuolated cytoplasm, although (C) rare macrophages contained larger vacuoles (arrowhead), and (D) a few scattered macrophages also contained a variable number of brown and black pigmented particles (arrows). Interestingly, this latter patient denied a history of smoking and had no alternative explanation for pigmented macrophages.

Supplementary Table S1. Patient Demographics and Clinical Findings

Age/Sex (Case #)	Year	Presenting Symptoms	Duration of Symptoms At Time of Biopsy	Medical History	Occupational Exposures	Smoking / Drug Use History	Vaping History	Serology/ Microbiology	CDC Case Designation
44 M (#1)	2016	Acute presentation with 2-day history of hemoptysis and dyspnea	3 days	None	None	Former tobacco smoker, 2.5 pack-years	Began vaping at the time of smoking cessation, 2 years before presentation	Extensive serologic and infectious workup negative, ESR low	Confirmed
42 M (#2)	2017	Acute hypoxic respiratory failure	Approximately 1 week	GERD, HTN	Possible exposure to mold	Former tobacco smoker, 20 pack-years	Started vaping to stop smoking approximately 1 year prior to presentation	Unknown	Probable
51 M (#3)	2019	Progressive subacute dyspnea and cough	Approximately 4 weeks	Obesity, asthma	None	Denied	Vaping marijuana	Infectious work-up negative except for positive IgM for Mycoplasma pneumonia	Probable
23 F (#4)	2019	Acute presentation with cough, myalgias, fever, nausea, vomiting, and respiratory failure eventually requiring intubation	Acute, but further details unknown	Anxiety, depression	None	Unknown	Vaping THC for several months with tank style vaping device	Infectious workup negative; positive ANA, elevated procalcitonin, and remaining serologic testing negative	Confirmed
34 F (#5)	2019	Dyspnea and cough, progressing to acute respiratory failure requiring intubation	1 month	Obesity	None	Unknown	History of vaping, no details known	Extensive serologic and infectious workup negative	Confirmed
25 M (#6)	2019	Acute presentation with fever and cough, developed pneumomediastinum, high-grade fever, and respiratory failure requiring intubation.	Acute, but further details unknown	None	Unknown	Remote history of tobacco smoking	History of vaping nicotine and mixing with cannabis oils	Extensive serologic and infectious workup negative	Confirmed
21 M (#7)	2019	Acute presentation with nausea, vomiting, fevers, night sweats, weight loss, progressive cough, pleuritic pain, and chest tightness	15 days	IBD	None	Denied	Vaping nicotine for 5 years; on weekend prior to presentation, started vaping nicotine with marijuana for the first time	Extensive infectious work-up negative; CRP 274, ESR 90, procalcitonin slightly elevated	Confirmed
27 M (#8)	2019	Acute presentation with flu-like symptoms, fever of 40.6ºC,	2 days	None	None	Former tobacco smoker	Vaping cannabis daily	Extensive serologic and	Confirmed

		nausea, vomiting, cough, and dyspnea progressing to respiratory failure requiring intubation						infectious workup negative	
38 M (#9)	2019	Acute respiratory failure, fevers, diarrhea, nausea, and vomiting	Acute, but further details unknown	Anemia, OSA	None	Denied	Multiyear history of vaping with "heavy" use of CBD oil	Extensive serologic and infectious workup negative	Confirmed
34 F (#10)	2019	Acute presentation with dyspnea, fever, and suspected pneumonia treated with antibiotics without improvement; developed hypoxic respiratory failure requiring oxygen	Acute, but further details unknown	Anxiety	None	Denied	"Heavy" vaping use over the past few months; vaping with cannabis	Extensive serologic and infectious workup negative	Confirmed
28 M (#11)	2019	Acute presentation with dyspnea	Acute, but further details unknown	Generalized anxiety disorder, opioid and ethanol use	Unknown	Denied tobacco smoking, but current marijuana smoker	1-year history of vaping 20-30 cartridges per day, also vaping THC	Extensive serologic and infectious workup negative except for positive Aspergillus antibody in serum	Probable
35 M (#12)	2019	Acute presentation with dyspnea and cough, with subsequent pneumothorax	Acute, but further details unknown	Previous admission months prior for respiratory failure, treated with steroids	Unknown	Former tobacco smoker, occasional marijuana smoking	History of vaping for months previously and prior to present admission	Unknown	Probable
54 F (#13)	2019	Acute hypoxic respiratory failure with worsening dyspnea	2 weeks	ADHD, anxiety, bipolar disorder, COPD, depression, diabetes, HTN, hepatitis C with cirrhosis	Unknown	Current tobacco smoker, 45 pack-years	History of vaping prior to presentation with nicotine and cannabis oil	Negative respiratory viral panel, but other details unavailable	Probable
67 M (#14)	2019	Subacute presentation with dyspnea and cough, rapidly progressing and requiring intubation.	Acute, approximately 9 days	None	Unknown	Current tobacco smoker	History of vaping prior to presentation	Extensive serologic and infectious workup negative	Confirmed
19 M (#15)	2019	Acute presentation with abdominal pain, nausea, vomiting, and fever	4 days	None	Unknown	Current marijuana smoker	Vaping several times per week for several months, including cannabis oils	Extensive serologic and infectious workup negative	Confirmed
39 M (#16)	2019	Cough, dyspnea, night sweats, intermittent fevers, and fatigue	2 months	Asthma	None	Remote tobacco smoker for 2 years;	"Significant" vaping of non-nicotine	ANA titer 1:40, remaining	Confirmed

		for 6 weeks				current marijuana smoker	products at time of presentation, further details unavailable	serologic and infectious workup negative	
40 M (#17)	2019	Worsening cough, myalgias, dyspnea, and fever for 1 week	1 week	None	Unknown	Unknown	Vaping for last 6 years, and vaping synthetic cannabinoids from "black market" for last 6½ months	Cultures negative, serologic testing unavailable	Probable

ADHD, attention deficit hyperactivity disorder; ANA, Anti-Neutrophil Antibody; ANCA, Anti-Neutrophilic Cytoplasmic Antibody; CBD, cannabidiol; CDC, Centers for Disease Control and Prevention; COPD, chronic obstructive pulmonary disease; CRP, C-Reactive Protein; ESR, Erythrocyte Sedimentation Rate; GERD, Gastroesophageal Reflux Disease; HTN, Hypertension; IBD, Inflammatory Bowel Disease; OSA, obstructive sleep apnea; THC, tetrahydrocannabinol

Supplementary Table S2. Radiologic and Pathologic Summaries

Case #*	HRCT Imaging Findings	BAL Fluid Findings	Biopsy Type	Biopsy Location	Histopathologic Patterns of Injury	Distribution	Special Stains	Outcome
1	Diffuse bilateral GGOs with areas of more dense consolidative opacities	Unknown	Surgical wedge biopsies	RLL	Acute fibrinous pneumonitis with organization	Airway-centered	None	Unknown
2	Diffuse GGOs with air trapping and some subpleural cysts, micronodular opacities in lung bases	Unknown	TBBX	RML	Acute fibrinous pneumonitis with organization	Indeterminate	AFB, GMS, HSV, CMV negative	Unknown
3	Diffuse bilateral centrilobular GGOs	Unknown	Surgical wedge biopsies	RUL and RLL	Acute fibrinous pneumonitis with organization and prominent bronchiolitis	Airway-centered	GMS negative	Unknown
4	Diffuse panlobular GGOs more prominent in the dependent lower lobes	Unknown	TBBX	LLL	Acute and organizing diffuse alveolar damage	Indeterminate	GMS stain negative	Improving with steroids
5	Progressive bilateral GGOs	Unknown	Surgical wedge biopsies	RML and RLL	Acute and organizing diffuse alveolar damage	Diffuse	GMS negative	Death despite antibiotics, steroids, ECMO
6	Bilateral perihilar GGOs with pneumomediastinum	Unknown	Surgical wedge biopsies	LUL	Acute fibrinous pneumonitis with organization	Airway-centered	AFB, PAS, and GMS negative	Unknown
7	Diffuse tree-in-bud opacities and centrilobular GGOs bilaterally in upper lobe predominant pattern	Macs 93%, PMNs 3%, Lymphs 4%, Eos 0%; foamy cytoplasmic vacuolization of macs	Transbronchial cryobiopsy	RUL	Acute fibrinous pneumonitis with organization and prominent bronchiolitis	Airway-centered	AFB, GMS Negative	Discharged from hospital, unknown outcome
8	Bilateral pulmonary infiltrates (CXR only, no HRCT)	Unknown	TBBX	Lingula	Acute and organizing diffuse alveolar damage	Indeterminate	AFB, GMS, CMV, HSV negative	Unknown
9	Bilateral GGOs more confluent in lower lobes, enlarged lymph nodes, small pleural effusion	Unknown	Surgical wedge biopsy	RML	Organizing pneumonia	Airway-centered	None	Discharged from hospital, outcome unknown
10	Extensive bilateral GGOs involving upper and lower lobes with central predominance and sparing of subpleural regions and more significant consolidation in posterior lower lobes	Benign mixture of macrophages and mixed inflammatory cells (cell count not available); no characterization of macs	TBBX	RML	Acute fibrinous pneumonitis with organization	Indeterminate	AFB and GMS negative	Unknown
11	Bilateral diffuse GGOs	PMNs 40%, Lymphs 30%, Macs 27%, Eos 1%, Other 2%; no characterization of macs	TBBX	RML	Acute fibrinous pneumonitis with organization	Indeterminate	Unknown	Unknown
12	Right-sided pneumothorax with bilateral central parenchymal opacities including some cavitation	PMNs 50%, Lymphs 50%; no macs noted	Surgical wedge biopsy	RML	Organizing pneumonia	Airway-centered	Unknown	Unknown
13	Bilateral GGOs	First BAL: PMNs 78%,	TBBX	RML and	Acute fibrinous	Indeterminate	GMS	Unknown

		Lymphs 14%, Macs 7%, Eos 1%; Second BAL: PMNs 92%, Lymphs 7%, Macs 1%, Eos 0%; pigmented macs noted		Lingula	pneumonitis with organization		negative	
14	Widespread airspace disease with bilateral GGOs and relative sparing of lung bases	Unknown	Surgical wedge biopsies	LUL	Acute and organizing diffuse alveolar damage	Diffuse	GMS, AFB, HSV, CMV, Gram stain negative	Death despite antibiotics and steroids
15	Diffuse micronodular bronchocentric GGOs	Unknown	TBBX	RML	Acute fibrinous pneumonitis	Indeterminate	AFB, GMS negative	Improved with antibiotics and oral steroids
16	Bilateral reticulonodular opacities with subpleural sparing	Unknown	TBBX	RUL	Organizing pneumonia	Indeterminate	GMS negative	Improving with cessation of vaping
17	Diffuse bilateral opacities	Abundant foamy macrophages; differential unavailable	TBBX	LUL and LLL	Acute fibrinous pneumonitis with organization	Indeterminate	AFB, GMS negative	Unknown

*Case # corresponds to case # in Table 1. AFB, acid-fast bacilli; BAL, bronchioloalveolar lavage; CMV, cytomegalovirus; CXR, Chest X-Ray; DAD, diffuse alveolar damage; ECMO, extracorporeal membrane oxygenation; Eos, eosinophils; GGOs, ground glass opacities; GMS, Gomori Methenamine silver; HRCT, High-resolution computed tomography; HSV, herpes simplex virus; LLL, left lower lobe; LUL, left upper lobe; Lymphs, lymphocytes; Macs, macrophages; OP, organizing pneumonia; PAS, periodic acid-Schiff; PMNs, polymorphonuclear leukocytes (neutrophils); RML, right middle lobe; RLL, right lower lobe; RUL, right upper lobe; TBBX, Transbronchial forceps biopsy

Supplementary Table S3. Frequency of Histopathologic Features in Vaping-Associated Acute Lung Injury

Histologic Feature	Number of cases with feature / Number of cases with relevant structures present for evaluation (%)
Foamy / Vacuolated Macrophages	17 / 17 (100)
Foamy / Vacuolated Pneumocytes	17 / 17 (100)
Intraalveolar Fibrin	16 / 17 (94)
Organizing Pneumonia	14 / 17 (82)
Bronchiolitis	7 / 9 (78)
Bronchiolar Mucosal Ulceration	6 / 9 (67)
Interstitial Edema	11 / 17 (65)
Neutrophils	9 / 17 (53)
Chronic Interstitial Inflammation	9 / 17 (53)
Pigmented Macrophages	7 / 17 (41)
Eosinophils	5 / 17 (29)
Hyaline Membranes	4 / 17 (24)
Peribronchiolar Metaplasia	2 / 9 (22)
Organizing Fibrinous Pleuritis	1* / 7 (14)
Interstitial Fibrosis	1 / 17 (6)
Pleural Fibrosis	0 / 7 (0)
Granulomas	0 / 17 (0)
Exogenous Lipoid Pneumonia	0 / 17 (0)

*Organizing fibrinous pleuritis was present in one patient with a recent pneumothorax.