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Vaping, Lung Damage, and Cytopathology: A New Twist in the Medical Mystery

Accumulating evidence has clarified the role of cytopathology in the diagnosis of vaping-associated lung injuries and implicated vitamin E acetate as a likely culprit

In mid-2019, pulmonologists around the country began observing severe lung damage and even respiratory failure occurring mainly in younger, otherwise healthy patients. As of February 4, 2020, the alarming injuries had afflicted more than 2700 individuals in all 50 states, the District of Columbia, and 2 US territories, and killed 64 of them.

Medical sleuths have tied the phenomenon to vaping and dubbed it EVALI (E-cigarette or vaping product use associated lung injury). What is less clear is which substances may be contributing to the damage, and how. Initial cytopathology test results from Oil Red O staining that appeared to hint at exogenous lipoid pneumonia only added to the confusion.

Since then, multiple lines of evidence have implicated a compound called vitamin E acetate, which has been used to dilute tetrahydrocannabinol (THC) oil in vaping cartridges sold illicitly. The emerging picture suggests not only how the additive might trigger the lung injury, perhaps via toxic fumes, but also how cytopathologists might help clinical teams reach a proper diagnosis.

A Cytopathology Test Creates Confusion

In a letter to the editor published in September 2019 in *The New England Journal of Medicine*, researchers at the University of Utah Health turned heads with their report of lipid-laden macrophages within the lungs of 6 patients diagnosed with EVALI who were analyzed at the University of Utah Hospital in Salt Lake City.[1] The cytopathology stain Oil Red O revealed the cells' oily droplets. "We've taken care of close to 40 patients at the University of Utah now, and nearly every single patient has similar findings," says study co-author Sean J. Callahan, MD, assistant professor in the pulmonary and critical care medicine division.

A few other studies have reported similar results, initially fueling suspicion that inhalation of an oil in the vaping mixture might be causing exogenous lipoid pneumonia. However, Dr. Callahan says additional evidence has pointed to a proinflammatory response triggered by an inhaled substance, an assessment that is shared by other clinicians who have called the Oil Red O finding a "red herring."

In a more recent study published in *The New England Journal of Medicine*, pathologists at the Mayo Clinic reviewed lung biopsies from 17 patients with suspected EVALI, 2 of whom subsequently died.[2] In every case, the histopathology demonstrated an acute lung injury such as acute fibrinous pneumonitis, diffuse alveolar damage, or cryptogenic organizing pneumonia.

Corresponding author Brandon Larsen, MD, PhD, a consultant and pulmonary pathologist at the Mayo Clinic in Scottsdale, Arizona, says neither the computed tomography (CT) scans nor the microscopic analyses of lung biopsies agreed with the Oil Red O based suggestions that lipids or oils were accumulating in patients' lungs. Dr. Larsen says the analyses instead suggested that a toxic chemical fume was to blame. "Every single case had that appearance," he says.

In another study, led by Sanjay Mukhopadhyay, MD, director of pulmonary pathology at the Cleveland Clinic, researchers collected lung biopsies and performed CT scans on 8 patients with suspected EVALI. The researchers found no signs of an infection but evidence of acute lung damage in all cases, including diffuse bilateral ground-glass opacities that appeared white and hazy on chest CT scans.[3]

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The biopsies indicated cryptogenic organizing pneumonia, diffuse alveolar damage, or both. However, they did not demonstrate any clear signs of exogenous lipoid pneumonia, which was in agreement with the Mayo Clinic findings but in conflict with the initial conclusions reached by the researchers at the University of Utah Health.

Similar to Dr. Larsen, Dr. Mukhopadhyay contends that Oil Red O staining of lung macrophages has not been helpful in the search for the true EVALI culprit. In a new commentary to be published in an upcoming issue of *Cancer Cytopathology*, both researchers joined Anjali Saqi, MD, MBA, professor of pathology and cell biology at Columbia University in New York City, and other colleagues in trying to clear up some of the confusion.

Dr. Saqi emphasizes that Oil Red O staining readily picks up lipids and oils but is nonspecific regarding whether their source is exogenous or endogenous (after a lung injury, lipids from dead and dying cells can be engulfed by macrophages). In the commentary, she and her coauthors reviewed findings from the lung cells of 6 patients with no apparent risk factors for exogenous lipoid pneumonia. The researchers nevertheless observed Oil Red O staining in macrophages from 5 of these individuals.[4]

"At the same time, we also looked at cases of patients who do have exogenous lipoid pneumonia, and they do have Oil Red O staining," Dr. Saqi says, referring to prior reports and unpublished data. They noted the same for several patients meeting the EVALI criteria. "So basically, it can be seen across the spectrum," she says.

More telling, she says, is a cell morphology marker that pathologists rarely have commented on in EVALI case reports to date. Larger cytoplasmic vacuoles in lung macrophage cells that can resemble soap bubbles are more closely associated with exogenous lipoid pneumonia, whereas the smaller, finer vacuoles noted in endogenous lipoid pneumonia cause macrophages to appear "foamy." If the vacuole size and Oil Red O stain in a bronchoalveolar lavage (BAL) fluid sample align with biopsy evidence, she says, "Then we can say, 'OK, there are features of exogenous lipoid pneumonia.'" However, so far pathologists have not observed a clear match of all parameters in patients with known or suspected EVALI.

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Growing Suspicions Point to a Diluting Agent

Many researchers now suspect that the true cause of EVALI may be an inflammatory condition caused by the inhalation of toxic chemicals in vaping devices. Vitamin E acetate has emerged as one of the most likely culprits.

In November 2019, researchers from the Centers for Disease Control and Prevention in Atlanta, Georgia, reported finding vitamin E acetate in all 29 BAL samples that they analyzed from patients in 10 states who were diagnosed with EVALI.[5] They also detected THC or its metabolites in 23 of 28 samples tested, including 3 from patients who previously had denied using THC. Nicotine metabolites were detected in 16 of 26 BAL samples tested. A follow-up study by the Centers for Disease Control and Prevention found vitamin E acetate in BAL fluid collected from 48 of 51 patients with confirmed or suspected EVALI, but not from any healthy volunteers.[6]

Vitamin E acetate oil is cheap and resembles THC oil. Multiple observers have compared its use as a cutting agent with how drug dealers commonly dilute cocaine with look-alike powders to increase their profits. However, clarifying the confusion over Oil Red O staining has led to a new question: if vitamin E acetate does not cause exogenous lipoid pneumonia, how might it contribute to the observed lung injuries?

Although the research has not proven a causal link or ruled out contributions from other toxic substances, Dr. Larsen points to a forthcoming study by chemists in Dublin, Ireland, that could tie multiple observations together. The report suggests that vitamin E acetate, when heated to temperatures similar to those noted in many vaping devices, may produce "exceptionally toxic ketene gas."[7] Animal studies have suggested that ketene toxicity has a clinical effect and mode of action similar to those of phosgene gas, a chemical weapon blamed for killing more than 75,000 individuals during World War I.

At least one point is certain: continued research and vigilance will be critical. Although no single cell feature or test result can definitively point to EVALI, Dr. Saqi says cytopathologists can help to rule out infections or other types of injuries. In addition, they can alert physicians to observations, such as lipid-laden macrophages or large vacuoles, that can at least suggest the need to ask patients about their vaping history.

"THC is going to continue to be the most expensive component of these things that young people are vaping, and there's always going to be an incentive for people to cut it," Dr. Callahan says. Although the outbreak potentially linked to vitamin E acetate may be waning, other additives could cause similar injuries in the future. "We've spotted one, I think, obvious problem," Dr. Larsen says, "but I don't think this is the end of this story." ■

References

1. Maddock SD, Cirulis MM, Callahan SJ, et al. Pulmonary lipid-laden macrophages and vaping. *N Engl J Med*. 2019;381:1488-1489. doi:10.1056/NEJMc1912038
2. Butt YM, Smith ML, Tazelaar HD, et al. Pathology of vaping-associated lung injury. *N Engl J Med*. 2019;381:1780-1781. doi:10.1056/NEJMc1913069
3. Mukhopadhyay S, Mehrad M, Dammert P, et al. Lung biopsy findings in severe pulmonary illness associated with e-cigarette use (vaping). *Am J Clin Pathol*. 2020;153:30-39. doi:10.1093/ajcp/aqz182
4. Saqi A, Butt Y, Doxtader E, et al. E-cigarette or vaping product use associated lung injury: what is the role of cytologic assessment? *Cancer Cytopathol*. In press.
5. Blount BC, Karwowski MP, Morel-Espinosa M, et al. Evaluation of bronchoalveolar lavage fluid from patients in an outbreak of e-cigarette, or vaping, product use–associated lung injury—10 States, August-October 2019. *MMWR Morb Mortal Wkly Rep*. 2019;68:1040-1041. doi:10.15585/mmwr.mm6845e2
6. Blount BC, Karwowski MP, Shields PG, et al; Lung Injury Response Laboratory Working Group. Vitamin E acetate in bronchoalveolar-lavage fluid associated with EVALI. *N Engl J Med*. Published online December 20, 2019. doi:10.1056/NEJMoa1916433
7. Wu D, O'Shea D. Potential for release of pulmonary toxic ketene from vaping pyrolysis of vitamin E acetate. Posted June 12, 2019. Preprint revised November 11, 2019. Accessed January 24, 2020. https://chemrxiv.org/articles/Potential_for_Release_of_Pulmonary_Toxic_Ketene_from_Vaping_Pyrolysis_of_Vitamin_E_Acetate/10058168

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