Effects of Short- and Long-Term Exposure to E-Cigarette Vapour

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1. Background

In recent years, a substantial increase in the development and use of electronic cigarettes (e-cigarettes, electronic nicotine delivery devices) has been observed, with

rising popularity, particularly in young adults in Western countries. In Germany, the frequency of e-cigarette use has been assessed in a number of studies, including the ongoing DEBRA (Deutsche Befragung zum Rauchverhalten) study (1). In this population-based assessment, about 10% of participants reported to have ever used e-cigarettes, and the overall percentage of current e-cigarette users was about 2%. While these data indicate that a significant proportion of the general population is exposed to e-cigarettes, large cohorts enabling an examination of potential health effects, especially regarding relationships with respiratory status including measures of pulmonary function, are lacking.

E-cigarettes are heavily advertised as a healthier alternative to conventional cigarettes and are further described as an aid to support smoking cessation (2). This strategy also targets highly vulnerable populations such as pregnant women (3) and adolescents. E-cigarettes are frequently perceived as being safer than regular cigarettes both by consumers and health care professionals (4, 5). As opposed to conventional cigarettes and the recently introduced heated tobacco products, these devices are based on the vaporization of liquids or e-liquids as the main constituent instead of burning or heating tobacco. The primary compounds of these liquids are humectants such as propylene glycol, flavourings, and in most cases nicotine. Health effects of e-cigarettes can be either direct, i.e., based on toxicity and risks of the device and liquids on their own, or indirect by providing a gateway, especially for young e-cigarette users to start smoking and later possibly switch to conventional cigarettes, or undermining efforts regarding smoking cessation in adults by contributing to the social normalization of smoking (6).

Recently, concerns regarding the safety of e-cigarettes have been fuelled by several cases of severe lung affection in young adults across various US states, including fatalities by respiratory failure after the use of e-cigarettes. The exact cause and pathophysiologic mechanisms of these events are far from being understood (30). As of October 8, 2019, more than 1,000 lung injury cases associated with the use of e-cigarettes or vaping products have been reported to the CDC (Centers for Disease Control and Prevention) with twenty-six confirmed deaths by respiratory failure. So far, no single product or substance could be linked to all lung injury cases, which raised the question of illicit modifications of e-cigarettes. On the other hand, affected subjects might be carriers of risk alleles for respiratory diseases and hence be particularly vulnerable towards exposure to e-cigarettes.

While existing data indicate that the toxicity of e-cigarettes is generally lower compared to conventional cigarettes, the evidence base regarding potential health effects, including short-term alterations and especially with respect to long-term use, is scarce (7). Beyond known effects of nicotine per se, this particularly applies to the contained flavourings that usually have been approved for oral consumption, but in most cases were not examined regarding possible toxicity when administered via inhalation. In addition, a variety of further potentially toxic substances has been detected in e-cigarette aerosols, including metals and chemicals possibly causing mutagenesis (8).

Discussed direct health effects of e-cigarettes comprise a spectrum of impairments, including effects on respiratory function and symptoms (9, 10), alterations of the microbiome in the oral cavity and airways (11), periodontal disease (12), effects on immune cells and subsequent impairment of immunity (13), carcinogenic effects (14), effects on the cardiovascular system either due to the contained nicotine or further flavouring components (15), and impact on pregnancy outcomes (3) as well as foetal development (16). Furthermore, poisoning by ingestion of e-liquids has been described (18). Beyond these direct health effects, the evidence for the usefulness of e-cigarettes with respect to smoking cessation, particularly in view of common dual use of electronic and conventional cigarettes, is heterogeneous, and major international respiratory associations have recently published statements against the recommendation of such devices in any therapeutic context (6, 19-21). At the population level, the German National Cohort (GNC, NAKO Gesundheitsstudie) (22, 23) provides a unique opportunity to investigate potential health effects, especially regarding relationships with respiratory status, including measures of pulmonary function, on a nationwide basis in Germany. It is of particular value for the examination of even subtle e-cigarette effects since it is based on the general population and thus includes the spectrum of early and subclinical alterations that are not yet diagnosed and therefore often not available in clinical cohorts.

With respect to potential mechanisms underlying the observed effects, a number of factors have been described, including the formation of reactive oxygen species and inflammatory mediator production (24), DNA damage (14), epigenetic alterations (25), endothelial cell dysfunction (26) and impairment of ciliary function (27). Studies in mice have revealed that short-term exposure to the inhalation of nebulized, nicotine-containing liquid from e-cigarettes was associated with lung inflammation, oxidative stress and alterations of the endothelial barrier of the lung (28). Furthermore,

e-cigarette liquid containing nicotine enhanced airway hyper-responsiveness in an allergic airway model and the risk of respiratory infection after *Streptococcus pneumonia* or influenza A virus infection [summarized in (28)]. While exposure to four months of e-cigarette vapour did not result in development of emphysema, lung inflammation and tissue damage increased after influenza A virus infection (29). However, the overall mechanistic understanding of vaping-associated health impairments, particularly in terms of long-term and indirect effects, is currently not more than fragmentary.

The proposed cross-disciplinary, multicentre project will bundle the expertise of all DZL sites to comprehensively investigate potential health effects of e-cigarettes, using a synergistic approach at methodologies spanning from early altered signalling pathways in *Drosophila* airways over effects on signal transduction and influences on late immunity in mice models to the quantification of the impact on respiratory status, including functional and structural measures in population-based and patient cohorts.

The fruit fly *Drosophila* has a very simple airway system, and thus permits the rapid morphological and functional investigation of the airway epithelial early responses to toxicants as well as parameters of survival. Early changes in the fly's airway gene expression due to e-cigarettes and their components will be validated in murine lung material from short- and long-term exposed mice and in endothelial in vitro models. Those that are jointly regulated in non-vertebrate and mammalian models are likely to be of central importance as they are evolutionarily conserved. The in vitro setting enables us to experimentally regulate the expression of certain identified RNA candidates, thereby directly testing its potential contribution to a pathological e-cigarette response. Based on the level of (joint) regulation and contribution to central pathways, molecules will be prioritized for future investigation in human biomaterials from the GNC.

2. Early Responses to E-Cigarettes and Their Components: Airway Structural Changes, Signalling Pathways and Survival in *Drosophila Melanogaster* in the Context of Risk Alleles

2.1 *Aims*

To identify the early morphological and transcriptional changes in the airways of *Drosophila melanogaster*, eventually leading to decreased survival in the context of known risk alleles for airway disease.

2.2 *Preliminary Results*

All necessary techniques for this project are established in our laboratory:

1) Exposure of female flies to e-nicotine reduced the size and weight of their offspring larvae. During pupation larvae undergo a profound organismal reorganization. Despite this intense restructuring, hatching flies were still smaller with reduced body size and weight indicating prolonged effects of e-nicotine at least across one generation.
2) Exposing Drosophila larvae to conventional cigarettes increased airway Cyp18a1 (marker for exposure to xenobiotics) and increased heat shock protein levels.
3) The airway-specific deregulation of the asthma risk alleles modified the airway morphology in *Drosophila* larvae.

2.3 *Working Plan*

1) Analysis of the early airway response to e-cigarettes and their ingredients, and impact on future health in wild-type flies

Drosophila 2nd instar larvae will be exposed to single ingredients of commercially available e-cigarette liquids (vegetable glycerine, propylene glycol, nicotine flavourings). The impact of the different ingredients will be screened on the basis of airway remodelling processes, respiratory gene expression and parameters of survival and fitness. Conventional cigarettes will be used as a control. To make conventional and e-cigarettes comparable, the exposure to nicotine will be evaluated by 1) negative geotaxis assay, where the climbing ability of adult flies is quantified and 2) by Cyp18A1 gene expression levels in isolated larval airways.

After exposure, the tracheal development, structure and airway remodelling will be assessed in 3rd instar larvae using microscopy techniques. In addition, RNA sequencing will be done in isolated larval airways followed by *in silico* pathway analyses to detect potential target genes and pathways. This will allow us a) to investigate which pathways are differentially regulated between conventional and e-cigarettes, and b) to obtain insight into the contribution of the single ingredients of e-cigarettes.

To assess the effect of larval e-cigarette exposure on future health, survival parameters such as developmental times, fertility, animal size, and lifetime will be investigated in adult flies. Furthermore, physiological parameters such as body fat, metabolic rate, behaviour (circadian rhythm), and stress perception will be assessed.

2) Evaluate the risk of e-cigarette consumption in carriers of asthma associated risk alleles

The interaction of different *Drosophila* models targeting risk alleles for asthma development (ORMDL3, SERBINB3, SPINK5) with e-cigarettes will be investigated. These risk genes are known to influence airway development, morphology and function (see also preliminary work). *Drosophila* larvae, with airway specific deregulation of risk genes, will be exposed to defined concentration of e-nicotine and harmful ingredients at concentrations as identified in part 1, followed by the measurements described above.

3) Analyse the impact of e-cigarette vapour on early events in airway development and future health in a transgenerational model

Beneath being carrier of risk alleles exposures during pregnancy confers risk to mothers and offspring. The fast reproduction rate of *Drosophila melanogaster* makes it possible to study the influence of a maternal e-cigarette on offspring health. Females will be exposed to e-cigarettes with prior defined nicotine concentrations and ingredients daily for one week before mating and egg deposition. In the following generation, the impact on early events in the development of the respiratory system will be assessed by analysing airway development and morphology. Furthermore, survival and physiological (body fat, etc.) parameters will be measured as described above.

3. Effect of Short- and Long-Term Exposure to E-Cigarette Vapour on Inflammation, Endothelial Barrier Function and Structural Alteration in Murine Lungs

3.1 *Aims*

To identify the mechanisms of short-term e-cigarette exposure leading to acute respiratory failure, and define effects of long-term e-cigarette exposure on pulmonary integrity.

3.2 *Preliminary Results*

This research has shown that short-term in vivo exposure to e-cigarette vapour with or without nicotine induces an inflammatory lung response. In vitro exposure of pulmonary arterial smooth muscle cells and alveolar type II cells to e-cigarette vapour extract (ECVE) with and without nicotine induces a distinct expression profile of various genes.

3.3 *Working Plan*

1) Identify the effect of e-cigarette vapour on baseline pulmonary inflammation, endothelial function and lung function

a) A mouse model of in vivo e-cigarette exposure (two weeks to three months) will be used to investigate the effect of e-cigarette vapour with different concentrations of nicotine on pulmonary inflammation (by FACs, cytokine release), endothelial barrier function (by BAL protein concentration, Evans blue permeability assay, wet-to-dry ratio, haematocrit measurements) and lung function (by FlexiVent). Lungs will be harvested to assess specific RNAs identified in the earlier described *drosophila* RNA sequencing to confirm the findings from our ARCN collaborator in a second species. Laser microdissection will be used for compartmental specific analysis.
b) Endothelial integrity will be further investigated in isolated ventilated and perfused lungs of e-cigarette smoke exposed mice and after inhalative application of e-cigarette vapour in the isolated lung setup (by quantification of the capillary filtration coefficient). The latter setup allows application of short-term, very high concentrations of e-cigarette vapour as it may occur in human e-cigarette consumers. Furthermore, the effect of single ingredients of commercially available e-cigarette liquids (vegetable glycerine, propylene glycol, nicotine in a concentration from 0 to 24 mg per millilitre, flavourings) can be examined separate from each other.
c) The effect of e-cigarette vapour on isolated endothelial cells will be investigated in vitro by exposure to ECVE with different concentrations of nicotine and analysis of gene expression by microarray. Functional in vitro assays of endothelial permeability upon ECVE include classical transwell experiment as well as real-time measurements of transendothelial electrical resistance (TER) by electric cell-substrate impedance sensing (ECIS). To analyse the interaction between circulation inflammatory cells and the endothelium transmigration, assays will be performed with either EC exposed to ECVE co-incubated with naïve monocytes or vice versa.
All responses will be compared to cigarette smoke exposure at similar nicotine concentrations (in vivo plasma levels or in vitro applied concentration, respectively). Specific RNA candidates identified in the *Drosophila* screening will be downregulated in ECs by RNAi in the above described scenarios of ECVE exposure. Promising targets will be modified *in vivo* by application of adeno-associated viral gene transfer.

2) Characterize the response of e-cigarette exposed lungs to inflammatory and infectious stimuli

Mice will be stimulated by lipopolysaccharide (LPS, to mimic a canonical gram-

negative bacterial response) or poly I:C (to mimic viral infection) after different time periods of chronic exposure to e-cigarette vapour (with or without nicotine). Afterwards, the inflammatory response, endothelial integrity and lung function will be determined as outlined above. Moreover, the isolated lung setup will be used to stimulate in vivo or in vitro e-cigarette exposed lungs with LPS or poly I:C and determine endothelial integrity, cytokine release, etc.

In parallel in vitro experiments on isolated endothelial cells (ECs) will be performed analogously to the above described assays. In brief, ECs exposed / or unexposed to ECVE will be co-stimulated with LPS (+LBP,CD14), and functional read out of endothelial integrity will be assessed (TER etc.). The interaction between ECs and monocytes will be analysed upon LPS stimulation both ways in naïve or ECVE exposed ECs or monocytes.

This set-up could be very useful to test potential therapeutic candidates. Two approaches in this context are proposed:

1. Specific targets: Modulate a well-known vascular protective pathway (i.e., Angiopoietin/Tie2) by pharmacological intervention with a 7-mer peptide (HHRHSF) that potently activates Tie2, thereby globally protecting vascular barrier dysfunction.
2. Drug repurposing: Given the high-throughput potential of the above described in vitro experiments, aim to screen potential off-target effects of approved drugs (using an FDA-library screening) for protective effects in the LPS stimulated and ECVP exposed endothelium.

3) Characterize the response of cigarette-smoke exposed lungs to e-cigarette exposure

As e-cigarette consumers often use conventional tobacco products concomitantly or prior to the use of e-cigarettes, we will investigate the effect of e-cigarette exposure on mouse lungs after chronic cigarette smoke exposure. To this end, we will expose mice to cigarette smoke for three and eight months (time periods when they develop structural alterations of the lung) and subsequently for two weeks to e-cigarette vapour. We will analyse the inflammatory response, endothelial integrity, lung function and histological alterations as outlined above.

4) Investigate long term effects of e-cigarette vapour on lung function

Mice will be exposed to e-cigarettes with and without nicotine for up to twelve months, and effects on lung function and structure determined.

4. Association of E-Cigarette Use with Respiratory Status in Lung-Healthy Subjects and Pulmonary Patients

4.1 *Aims*

a) Examine associations between e-cigarette use and respiratory status within the population-based German National Cohort (GNC, NAKO Gesundheitsstudie).
b) Assess structural alterations of the cardiorespiratory unit associated with e-cigarette use based on whole-body magnetic resonance imaging (MRI) scans within the GNC and their relationship with parameters of respiratory function.

4.2 *Data Basis*

Within the GNC, more than 200,000 men and women aged 20-69 years, randomly selected in a population-based manner from different regions all over Germany, are examined regularly over an envisaged period of 30 years in 18 study centres located in different geographic regions in Germany, and the baseline assessment started in 2014 was finished in 2019. The highly standardized GNC assessments target a variety of diseases and health-related issues, including lung health and respiratory allergies. Moreover, a comprehensive set of biological samples is retrieved from participants and stored in a central biorepository (Munich) comprising blood (plasma, serum), saliva, nasal swab, urine, and stool (31).

With respect to respiratory health, the core of the assessments are standardized interviews and questionnaires and, most importantly, spirometries performed in all 200,000 participants as well as the measurement of exhaled nitric oxide performed in a subsample of more than 40,000 participants. The presence of a number of respiratory diseases or impairments as well as risk factors are assessed using standardized questionnaires and interviews. These include physician-diagnosed chronic bronchitis or COPD, bronchial asthma, hay fever, cancer of the mouth, throat or lung, infections of the upper or lower airways within the past year as well as a number of respiratory symptoms indicative of hay fever, asthma or chronic bronchitis. In particular, the GNC assessments comprise a detailed questionnaire on the use of e-cigarettes including duration, intensity and patterns of use and concomitant smoking of conventional cigarettes, including smoking history.

Furthermore, a whole-body MRI scan is performed in a subgroup of 30,000 participants in 5 of the 18 GNC study centres as part of the GNC assessments (32). Although not specifically addressing the respiratory system, the availability of these images offers

a unique opportunity to investigate the relationship between structural and functional alterations of the cardiorespiratory unit, including novel evaluation approaches based on radiomics analyses of MRI data which may be particularly sensitive for detection of early and potentially reversible alterations in the lung periphery and vasculature.

4.3 *Working Plan*

1) *Characterize patterns of e-cigarette use within the GNC*

The first step will be an assessment of the frequency and patterns of e-cigarette use and their self-reported motivation within the GNC including type(s) of e-cigarette used, nicotine content of liquid, duration and frequency of use as well as amount of e-liquid consumed. Findings will be compared to existing data on e-cigarette use from smaller cohorts, and the dependence on age, sex, socioeconomic status, geographical region and level of urbanization as well as the relationships with current smoking and smoking history of conventional tobacco products such as cigarettes, cigars and pipes will be evaluated.

2) *Quantify associations of e-cigarette use with obstructive or restrictive ventilatory patterns as well as chronic bronchitis, COPD, respiratory infections and respective symptoms*

Potential associations between e-cigarette use and spirometric indices of lung volume and airflow limitation as well as symptoms or self-reported diagnoses of chronic bronchitis and COPD will be examined. The analysis will specifically compare participant characteristics and potential health impact between subgroups of users smoking only e-cigarettes, those with concomitant smoking of conventional cigarettes or tobacco products (dual users), and lifelong never-smokers. Furthermore, potential relationships with infections of the upper or lower airways will be assessed.

3) *Examine associations of e-cigarette use with FeNO levels and bronchial asthma, respiratory allergies as well as respective symptoms and healthcare utilization*

Associations between the frequency and intensity of e-cigarette use with levels of FeNO as a measure of Th2 (T helper cell type 2)-driven inflammation in the airways will be examined taking account of known influencing factors including respiratory or systemic medication. Potential associations with symptoms indicative of bronchial asthma as well as asthma attacks will be assessed, and relationships with diagnoses or symptoms of (seasonal) respiratory allergies, e.g., hay fever, will be evaluated. Furthermore, the health care utilization of e-cigarette users will be assessed based on

self-reported information regarding physician visits and current medication intake compared to smokers using conventional cigarettes or tobacco products as well as lifelong never-smokers.

4) *Identify whole-body MRI-based imaging patterns associated with e-cigarette use*

In parallel, whole-body MRI data, including non-contrast-enhanced thoracic MR angiographies, will be analysed using morphologic, shape-based analyses as well as novel radiomics approaches to derive quantitative parameters and identify imaging patterns associated with e-cigarette use. This includes training and validation of algorithms for pulmonary and vascular segmentation as well as radiomics feature extraction. In a next step, the association of imaging features based on aforementioned approaches with functional parameters, especially lung status, will be evaluated. Observations from the population-based GNC data will be further compared to findings in patients with moderate to severe COPD based on analogous radiomics analyses of MRI data from the nationwide Chronic Obstructive Pulmonary Disease (COPD) cohort COSYCONET.

4.4 *Perspective*

In perspective, cross-sectional findings from the analyses outlined above will form the basis for the investigation of e-cigarette effects on the development of respiratory status over time. This question will be addressed based on the first GNC follow-up survey (follow-up time five years) that was started in 2019 and is projected to be finished in 2023. These data will enable much needed insights into possible long-term effects of e-cigarette use, including their combined use with conventional cigarettes, on respiratory function, symptoms, and possibly disease as well as healthcare utilization.

5. Outreach

Given the high public health relevance of the use of e-cigarettes, the project aims – next to publications in journals for basic-translational lung research and epidemiology – to produce systematic reviews and position papers. Results will be regularly presented at the national (e.g., DGP [German National Respiratory Society], GPP [German National Pediatric Respiratory Society], etc.) and international conferences (e.g., ERS [European Respiratory Society], ATS [American Thoracic Society], EAACI [European Academy of Allergy and Clinical Immunology], keystone symposia) and the DZL Annual Conferences and workshops. In addition, dissemination will be

sought via the operating national and international information systems, including the DZL website, the websites of the single participating institutes, the Lung Information Service, and the European Lung Foundation, which target patients, journalists, multipliers from the health sector, and non-professionals. The Balzan Foundation will be explicitly acknowledged in all activities described above.

References

1. Kotz D, Kastaun S. [E-cigarettes and heat-not-burn products: representative data on consumer behaviour and associated factors in the German population (the DEBRA study)]. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2018;61(11):1407-14.
2. Fairchild AL, Lee JS, Bayer R, Curran J. E-Cigarettes and the Harm-Reduction Continuum. N Engl J Med. 2018;378(3):216-9.
3. Cardenas V, Fischbach L, Chowdhury P. The use of electronic nicotine delivery systems during pregnancy and the reproductive outcomes: A systematic review of the literature. Tobacco Induced Diseases. 2019;17(July).
4. Van Gucht D, Baeyens F. Health professionals in Flanders perceive the potential health risks of vaping as lower than those of smoking but do not recommend using e-cigarettes to their smoking patients. Harm Reduct J. 2016;13(1):22.
5. Pepper JK, Emery SL, Ribisl KM, Rini CM, Brewer NT. How risky is it to use e-cigarettes? Smokers' beliefs about their health risks from using novel and traditional tobacco products. J Behav Med. 2015;38(2):318-26.
6. Nowak D, Gohlke H, Hering T, Herth FJ, Jany B, Raupach T, et al. [Position paper of the German Respiratory Society (DGP) on electronic cigarettes (E-cigarettes) in cooperation with the following scientific societies and organisations: BVKJ, BdP, DGAUM, DGG, DGIM, DGK, DKG, DGSMP, GPP]. Pneumologie. 2015;69(3):131-4.
7. Bals R, Boyd J, Esposito S, Foronjy R, Hiemstra PS, Jimenez-Ruiz CA, et al. Electronic cigarettes: a task force report from the European Respiratory Society. Eur Respir J. 2019;53(2).
8. Hess CA, Olmedo P, Navas-Acien A, Goessler W, Cohen JE, Rule AM. E-cigarettes as a source of toxic and potentially carcinogenic metals. Environ Res. 2017;152:221-5.
9. McConnell R, Barrington-Trimis JL, Wang K, Urman R, Hong H, Unger J, et al. Electronic Cigarette Use and Respiratory Symptoms in Adolescents. Am J Respir Crit Care Med. 2017;195(8):1043-9.

10. Lappas AS, Tzortzi AS, Konstantinidi EM, Teloniatis SI, Tzavara CK, Gennimata SA, et al. Short-term respiratory effects of e-cigarettes in healthy individuals and smokers with asthma. Respirology. 2018;23(3):291-7.
11. Gotts JE, Jordt SE, McConnell R, Tarran R. What are the respiratory effects of e-cigarettes? BMJ. 2019;366:l5275.
12. Kumar PS, Clark P, Brinkman MC, Saxena D. Novel Nicotine Delivery Systems. Adv Dent Res. 2019;30(1):11-5.
13. Martin EM, Clapp PW, Rebuli ME, Pawlak EA, Glista-Baker E, Benowitz NL, et al. E-cigarette use results in suppression of immune and inflammatory-response genes in nasal epithelial cells similar to cigarette smoke. Am J Physiol Lung Cell Mol Physiol. 2016;311(1):L135-44.
14. Yu V, Rahimy M, Korrapati A, Xuan Y, Zou AE, Krishnan AR, et al. Electronic cigarettes induce DNA strand breaks and cell death independently of nicotine in cell lines. Oral Oncol. 2016;52:58-65.
15. MacDonald A, Middlekauff HR. Electronic cigarettes and cardiovascular health: what do we know so far? Vasc Health Risk Manag. 2019;15:159-74.
16. Cardenas V, Cen R, Clemens M, Moody H, Ekanem U, Policherla A, et al. Use of Electronic Nicotine Delivery Systems (ENDS) by pregnant women I: Risk of small-for-gestational-age birth. Tobacco Induced Diseases. 2019;17(May).
17. Jones CD, Ho W, Gunn E, Widdowson D, Bahia H. E-cigarette burn injuries: Comprehensive review and management guidelines proposal. Burns. 2019;45(4):763-71.
18. Wang B, Liu S, Persoskie A. Poisoning exposure cases involving e-cigarettes and e-liquid in the United States, 2010-2018. Clin Toxicol (Phila). 2019:1-7.
19. ERS Tobacco Control Committee. ERS Position Paper on Tobacco Harm Reduction. 2019.
20. Ferkol TW, Farber HJ, La Grutta S, Leone FT, Marshall HM, Neptune E, et al. Electronic cigarette use in youths: a position statement of the Forum of International Respiratory Societies. Eur Respir J. 2018;51(5).
21. Schraufnagel DE, Blasi F, Drummond MB, Lam DC, Latif E, Rosen MJ, et al. Electronic cigarettes. A position statement of the forum of international respiratory societies. Am J Respir Crit Care Med. 2014;190(6):611-8.
22. German National Cohort C. The German National Cohort: aims, study design and organization. Eur J Epidemiol. 2014;29(5):371-82.
23. Karrasch S, Peters A, Meisinger C, Ferland M, Jaeschke L, Pischon T et al. [Lung function in the German National Cohort: methods and initial results]. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2020;63(3): 322-331. doi: 10.1007/s00103-020-03102-1.

24. Scott A, Lugg ST, Aldridge K, Lewis KE, Bowden A, Mahida RY, et al. Pro-inflammatory effects of e-cigarette vapour condensate on human alveolar macrophages. Thorax. 2018;73(12):1161-9.
25. Zakarya R, Adcock I, Oliver BG. Epigenetic impacts of maternal tobacco and e-vapour exposure on the offspring lung. Clinical Epigenetics. 2019;11(1).
26. Fetterman JL, Weisbrod RM, Feng B, Bastin R, Tuttle ST, Holbrook M, et al. Flavorings in Tobacco Products Induce Endothelial Cell Dysfunction. Arterioscler Thromb Vasc Biol. 2018;38(7):1607-15.
27. Clapp PW, Lavrich KS, van Heusden CA, Lazarowski ER, Carson JL, Jaspers I. Cinnamaldehyde in flavored e-cigarette liquids temporarily suppresses bronchial epithelial cell ciliary motility by dysregulation of mitochondrial function. Am J Physiol Lung Cell Mol Physiol. 2019;316(3):L470-L86.
28. Dinakar C, O'Connor GT. The Health Effects of Electronic Cigarettes. New England Journal of Medicine. 2016;375(14):1372-81.
29. Madison MC, Landers CT, Gu B-H, Chang C-Y, Tung H-Y, You R, et al. Electronic cigarettes disrupt lung lipid homeostasis and innate immunity independent of nicotine. Journal of Clinical Investigation. 2019;129(10):4290-304.
30. Layden JE, Ghinai I, Pray I, Kimball A, Layer M, Tenforde M, et al. Pulmonary Illness Related to E-Cigarette Use in Illinois and Wisconsin – Preliminary Report. New England Journal of Medicine. 2019.
31. Wichmann HE, Horlein A, Ahrens W, Nauck M. [The biobank of the German National Cohort as a resource for epidemiologic research]. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2016;59(3):351-60.
32. Schlett CL, Hendel T, Weckbach S, Reiser M, Kauczor HU, Nikolaou K, et al. Population-Based Imaging and Radiomics: Rationale and Perspective of the German National Cohort MRI Study. Rofo. 2016;188(7):652-61.