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Authors

Kelesidis, Theodoros
Sharma, Madhav
Satta, Sandro
et al.

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CORRESPONDENCE

Ectodomain shedding of proteins important for SARS-CoV-2 pathogenesis in plasma of tobacco cigarette smokers compared to electronic cigarette vapers: a cross-sectional study

Theodoros Kelesidis[1] · Madhav Sharma[1] · Sandro Satta[1] · Elizabeth Tran[2] · Rajat Gupta[2] · Jesus A. Araujo[2,3] · Holly R. Middlekauff[2]

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Abstract

The impact of tobacco cigarette (TCIG) smoking and electronic cigarette (ECIG) vaping on the risk of development of severe COVID-19 is controversial. The present study investigated levels of proteins important for SARS-CoV-2 pathogenesis present in plasma because of ectodomain shedding in smokers, ECIG vapers, and non-smokers (NSs). Protein levels of soluble angiotensin-converting enzyme 2 (ACE2), angiotensin (Ang) II (the ligand of ACE2), Ang 1–7 (the main peptide generated from Ang II by ACE2 activity), furin (a protease that increases the affinity of the SARS-CoV-2 spike protein for ACE2), and products of ADAM17 shedding activity that predict morbidity in COVID-19 (IL-6/IL-6R alpha (IL-6/IL-6Rα) complex, soluble CD163 (sCD163), L-selectin) were determined in plasma from 45 NSs, 30 ECIG vapers, and 29 TCIG smokers using ELISA. Baseline characteristics of study participants did not differ among groups. TCIG smokers had increased sCD163, L-selectin compared to NSs and ECIG vapers ($p < 0.001$ for all comparisons). ECIG vapers had higher plasma furin compared to both NSs ($p < 0.001$) and TCIG smokers ($p < 0.05$). ECIG vaping and TCIG smoking did not impact plasma ACE2, Ang 1–7, Ang II, and IL-6 levels compared to NSs ($p > 0.1$ for all comparisons). Further studies are needed to determine if increased furin activity and ADAM17 shedding activity that is associated with increased plasma levels of sCD163 and L-selectin in healthy young TCIG smokers may contribute to the future development of severe COVID-19 and cardiovascular complications of post-acute COVID-19 syndrome.

Keywords Ectodomain shedding · ACE2 activity · ADAM17 activity · Smoking · Oxidative stress · COVID-19 · Inflammation

Introduction

The ongoing COVID-19 pandemic has had a dramatic impact on morbidity and mortality worldwide [1]. The impact of tobacco cigarette (TCIG) smoking on progression and development of COVID-19 has been controversial [2]. Electronic cigarettes (ECIGs), used by smokers often trying to quit, but also among young non-smokers, have also variably been associated with a risk of COVID-19 diagnosis [3]. Although levels of cotinine, the metabolite of nicotine, are comparable in smokers and vapers, emissions from ECIGs contain far fewer non-nicotine toxicants compared to emissions from TCIGs [4]. It has been proposed that nicotine has anti-inflammatory, protective, effects on the pathogenesis of severe COVID-19 [4]. It remains unknown whether vaping, with its exposure to fewer non-nicotine toxicants, carries the same risk as smoking for progression of COVID-19 and cardiovascular complications of post-acute COVID-19 syndrome (PACS), a determination with significant public health implications.

Increased soluble protein levels of receptors or products of sheddase activity of enzymes in accessible plasma are surrogate measures that reflect cleaving of the ectodomain portion of these receptors from the cell and diffusion into the circulation. Thus, soluble plasma levels of proteins

✉ Theodoros Kelesidis
tkelesidis@mednet.ucla.edu

[1] Department of Medicine, Division of Infectious Disease, David Geffen School of Medicine at UCLA, 47-100 CHS, 10833 Le Conte Avenue, Los Angeles, CA 90095, USA

[2] Department of Medicine, Division of Cardiology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

[3] Department of Environmental Health Sciences, Fielding School of Public Health at UCLA, Los Angeles, CA, USA

that are shed may reflect increased cellular activity of proteins in less accessible epithelial cells (that are targets of SARS-CoV-2). Plasma levels of proteins that are shed may also reflect systemic effects of TCIG smoking and ECIG vaping toxicity on the cellular and tissue levels of these proteins. To address this question, a comparison of levels of proteins important for SARS-CoV-2 pathogenesis present in plasma due to ectodomain shedding among smokers, vapers, and non-smokers/vapers may be illuminating.

Angiotensin (Ang)-converting enzyme 2 (ACE2) is the host receptor for the viral spike protein of SARS-COV-2 and is critical for viral entry. Shedding of ACE2 by lung epithelial cells is mediated by tumor necrosis factor-alpha-converting enzyme (TACE, ADAM17 metalloproteinase) that increases soluble ACE2 levels [5]. Thus, although the physiological role of ACE2 ectodomain shedding in COVID-19 remains elusive, the interplay between ACE2 and ADAM17 may be a major regulator in COVID-19 [6]. Plasma levels of soluble ACE2 may reflect the systemic toxicity of ECIG vaping and TCIG smoking, levels of ACE2 among several tissues and protein expression among different cell subtypes such as epithelial and endothelial cells. Angiotensin II is expressed in several cells and tissues, binds to ACE2, and regulates inflammatory responses in tissues during SARS-CoV-2 infection. However, it is unclear whether increased oxidative stress in TCIG smokers induces ectodomain shedding of proteins mediated by ADAM17 activity that may contribute to pathogenesis of COVID-19.

We hypothesized that smoking and, potentially, ECIG vaping are associated with increased plasma levels of (i) soluble ACE2; (ii) Ang II (the ligand of ACE2) and Ang 1–7 (the main peptide generated from Ang II by ACE2 activity; the cross-talk between the ACE2/Ang 1–7 and the ACE/Ang II–I axis Ang II levels regulates tissue inflammation [6]; (iii) furin, a protease that cleaves the spike protein, thereby increasing its affinity for ACE2 [7]; (iv) plasma IL-6/IL-6R alpha (IL-6/IL-6Rα) complex given that sIL-6R is a product of ADAM17 shedding activity and its ligand plasma IL-6 predicts morbidity in COVID-19 [1]; (v) plasma CD163, a product of ADAM17 shedding activity that predicts morbidity in severe COVID-19 [8]; and (vi) plasma L-selectin, a product of ADAM17 shedding activity in activated neutrophils during acute inflammation [9], that is a predictor for thrombosis in hospitalized COVID-19 patients [10]. We determined the impact of TCIG smoking and ECIG vaping on ectodomain shedding of proteins important for SARS-CoV-2 pathogenesis in plasma of otherwise healthy young people without other cardiac risk factors, except smoking or vaping. This research may advance the knowledge of the potential risk of TCIG smokers and ECIG vapers to develop severe COVID-19 and cardiovascular complications of PACS after SARS-CoV-2 infection.

Methods

Study participants

Plasma was collected before the pandemic from 45 non-smokers, 30 ECIG vapers, and 29 TCIG smokers. All participants had refrained from smoking 12 h before the study session. Healthy male and female volunteers with previously described characteristics [11] between the ages of 21 years and 45 years were eligible for enrollment if they were chronic (> 1 year), as follows: (1) active TCIG smokers (regardless of the number of pack-years) or (2) ECIG vapers (no dual users) or (3) non-smokers. Former TCIG smokers were eligible to be included in the non-smoker group, if greater than 1 year had elapsed since quitting. End-tidal CO, elevated > 10 ppm in smokers, was measured in EC vapers and non-smokers to confirm none was surreptitiously smoking TCIGs. Current smoking/vaping burden was estimated by plasma cotinine levels as previously described [11]. The experimental protocols were approved by the UCLA Institutional Review Board (IRB#18–001,147), and all participants provided written informed consent.

Immunoassays

Plasma protein levels of ACE2, Ang II, Ang 1–7, furin, IL-6/IL-6Rα, soluble CD163 (sCD163), and L-selectin were determined in plasma from participants using ELISA kit according to the manufacturer's instructions (ACE2, Ang II, Ang 1–7 [Cloud-Clone Corp, Houston, USA]; furin [Sigma Aldrich®, St. Louis, MO, USA]; IL-6/IL-6R alpha complex, sCD163, and L-selectin [R&D Minneapolis, MN, USA]).

Statistical analysis

Kruskal-Wallis analysis of variance (ANOVA) was used to compare the 3 groups, and if the results were statistically significant ($p < 0.05$), then the Mann-Whitney test was used to compare individual 2 groups. Overall and pairwise p values for comparing categorical covariates (sex, race, and education) across 2 study groups were computed using the Fisher exact test. This is an exploratory, hypothesis-generating small study, and we did not adjust for multiple comparisons.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Results

Study participants

The baseline characteristics of study participants are shown in Table 1. Baseline characteristics of participants including age, sex, race, and educational level, did not differ among groups. Four (8.9%) of the 45 non-users were former TCIG smokers, all quitting at least 1 year before the study. Twenty (66.7%) of the 30 ECIG vapers were former TCIG smokers, all quitting at least 1 year before the study. None was a current TCIG smoker. Importantly, TCIG smokers and ECIG vapers had similar current smoking and vaping burdens based on plasma cotinine levels (Table 1).

Plasma levels of ACE2 activity

There were no differences in plasma ACE2 (Fig. 1A), Ang II (Fig. 1B), and Ang 1–7 (Fig. 1C) among the three groups. These data in healthy young adults suggest that ECIG vaping and TCIG smoking do not impact plasma levels of ACE2 activity compared to non-smokers.

Plasma levels of ADAM17 activity

There were no differences in plasma IL-6/IL-6Rα among the three groups (Fig. 1D). TCIG smokers had increased plasma levels of CD163 compared to NSs ($p < 0.001$) and ECIG vapers ($p = 0.003$) (Fig. 1E). TCIG smokers had increased plasma levels of L-selectin compared to NSs ($p < 0.001$) and ECIG vapers ($p = 0.015$) (Fig. 1F). ECIG vapers had also increased plasma levels of L-selectin compared to NSs ($p = 0.006$) (Fig. 1F). These data in healthy young adults suggest that TCIG smoking increases independent blood products of the ADAM17 activity (sCD163, L-selectin) compared to non-smokers. Our data also suggest that ECIG vaping increases plasma L-selectin compared to non-smokers.

Plasma levels of furin

ECIG vapers had the highest plasma levels of furin compared to both NSs ($p < 0.001$) and TCIG smokers ($p = 0.037$) (Fig. 1G). These data in healthy young adults suggest that ECIG vaping may increase systemic levels of furin compared to non-smokers and TCIG smokers.

Plasma levels of furin and activities of ACE2 and ADAM17 among ECIG vapers with or without prior history of smoking

There were no differences in plasma ACE2 (Fig. 2A), Ang II (Fig. 2B), Ang 1–7 (Fig. 2C), IL-6/IL-6Rα (Fig. 2D), CD163 (Fig. 2E), and L-selectin (Fig. 2F) among ECIG vapers with or without prior history of smoking. These data in healthy young adults suggest that prior remote history of TCIG smoking in ECIG vapers does not impact plasma levels of furin and ADAM17 activity and that the increases in plasma levels of L-selectin, sCD163, and furin observed

Table 1 Study population characteristics

Cohort	Non-users	ECIG users	TCIG users	*p* value
Sample size	$n = 45$	$n = 30$	$n = 29$	
Age (mean ± SD)	27.4 ± 5.71	28.7 ± 5.36	27.6 ± 5.99	0.387
Sex (F/M)	19/26	9/21	9/20	0.531
BMI (mean ± SD)	23.0 ± 3.02	24.6 ± 3.51	24.4 ± 2.73	0.191
Race				
White	28	18	17	0.782
Asian	8	7	9	
African American	1	2	2	
Hispanic	5	2	1	
American Indian/Alaskan	0	0	0	
Native Hawaiian	2	0	0	
Unknown	1	1	0	
Ex-smokers (*n*, %) in non-users and ECIG users	4 (8.9%)	20 (66.7%)	–	< 0.001*
Pack-years, median (Q1, Q3)	0.32 (0.05, 1.54)	1.63 (0.24, 3.88)	3.75 (1.39, 7.13)	0.451
Base cotinine (nL/dL), median (Q1,Q3)	0	83.0 (25.1, 140.0)	98.0 (50.7, 205.0)	0.172**

BMI body mass index (expressed in kg/m^2)

*p value of ECIG cohort vs non-user cohort

**p value of ECIG cohort vs TCIG cohort

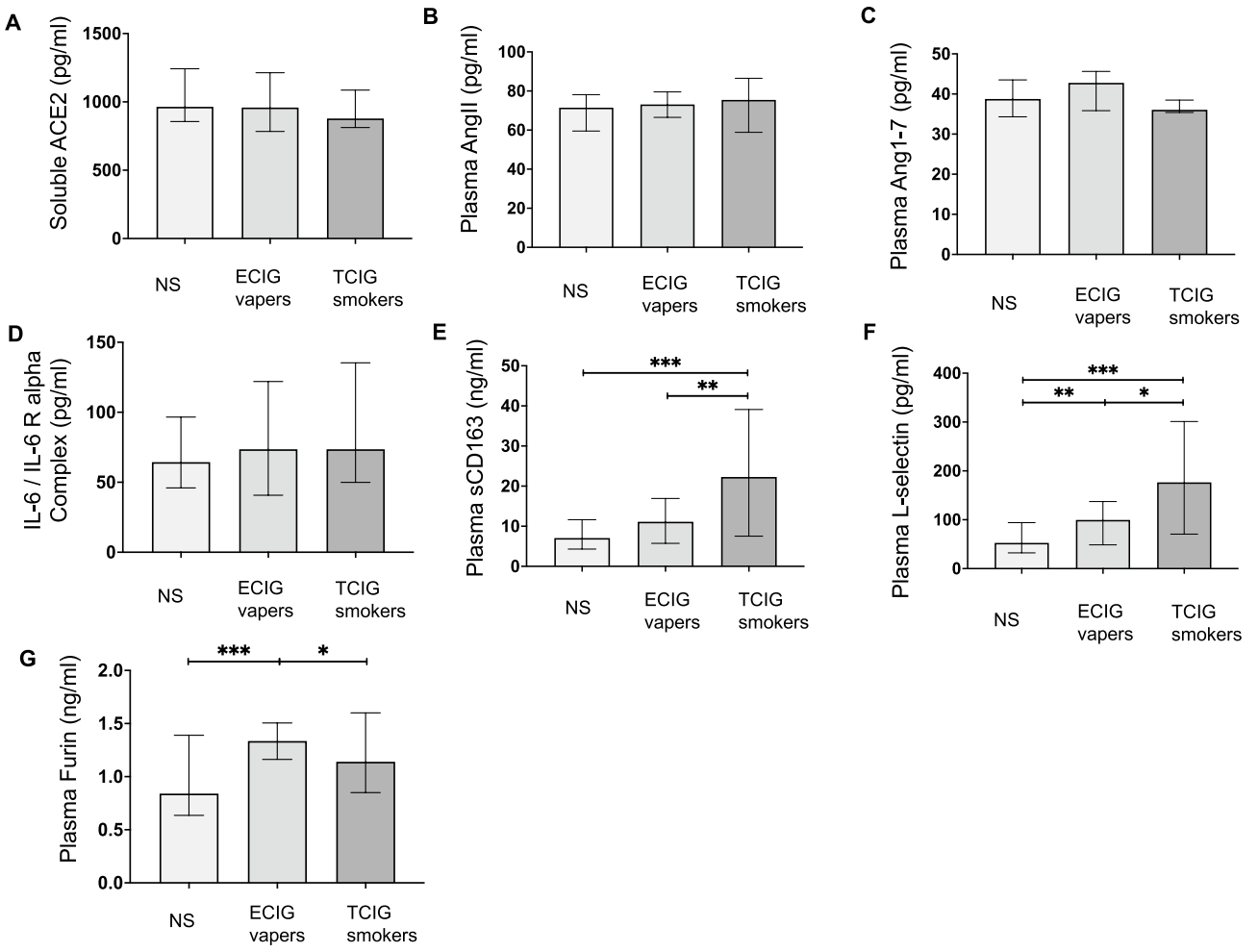

Fig. 1 Plasma levels of proteins involved in ACE2 and ADAM17 activities among smoker groups in study participants. Levels of soluble ACE2 (**A**), angiotensin (Ang) II (**B**), Ang 1–7 (**C**), IL-6/IL-6R alpha (IL-6/IL-6Rα) complex (**D**), sCD163 (**E**), L-selectin (**F**), and furin (**G**) were determined by ELISA immunoassays. Data are shown as median and interquartile range (IQR). The Kruskal–Wallis analysis of variance (ANOVA) was used to compare the 3 groups, and if the results were statistically significant ($p < 0.05$), then the Mann–Whitney test was used to compare individual 2 groups (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

in ECIG vapers compared to non-smokers were associated with ECIG vaping per se.

Discussion

To our knowledge, this is the first study in humans to study the impact of TCIG smoking and ECIG vaping on ectodomain shedding of proteins important for SARS-CoV-2 pathogenesis (ACE2, furin, Ang II, Ang 1–7, IL-6R, sCD163, L-selectin) in plasma collected before the pandemic (Fig. 3). Thus, our study suggests unique immunomodulatory mechanisms in TCIG smokers that could predispose them to a higher risk of severe COVID-19 compared to non-smokers. Importantly, the plasma cotinine levels were not different between ECIG vapers and TCIG smokers implicating the non-nicotine pro-oxidant toxicants in TCIG smoke or ECIG vapor as instigators of increased levels of these proteins.

Our data that the plasma levels of sCD163 and L-selectin were increased in TCIG smokers compared to non-smokers are consistent with prior evidence that ADAM17 may be upregulated by TCIG smoking [12–14]. Importantly, smoking triggers the generation of oxygen radicals, which, in turn, stimulate ADAM17 through the activation of src kinase [15]. Increased ADAM17 sheddase activity may contribute to the pathogenesis of severe COVID-19. ADAM17 shedding seems to be involved in SARS-CoV cellular entry and replication, and an ADAM17 inhibitor suppresses SARS-CoV replication in vitro [16]. Consistent with this evidence, we have previously shown that ADAM17, a protein that is important for pathogenesis of COVID-19, was increased in blood immune cells of TCIG smokers but not in ECIG

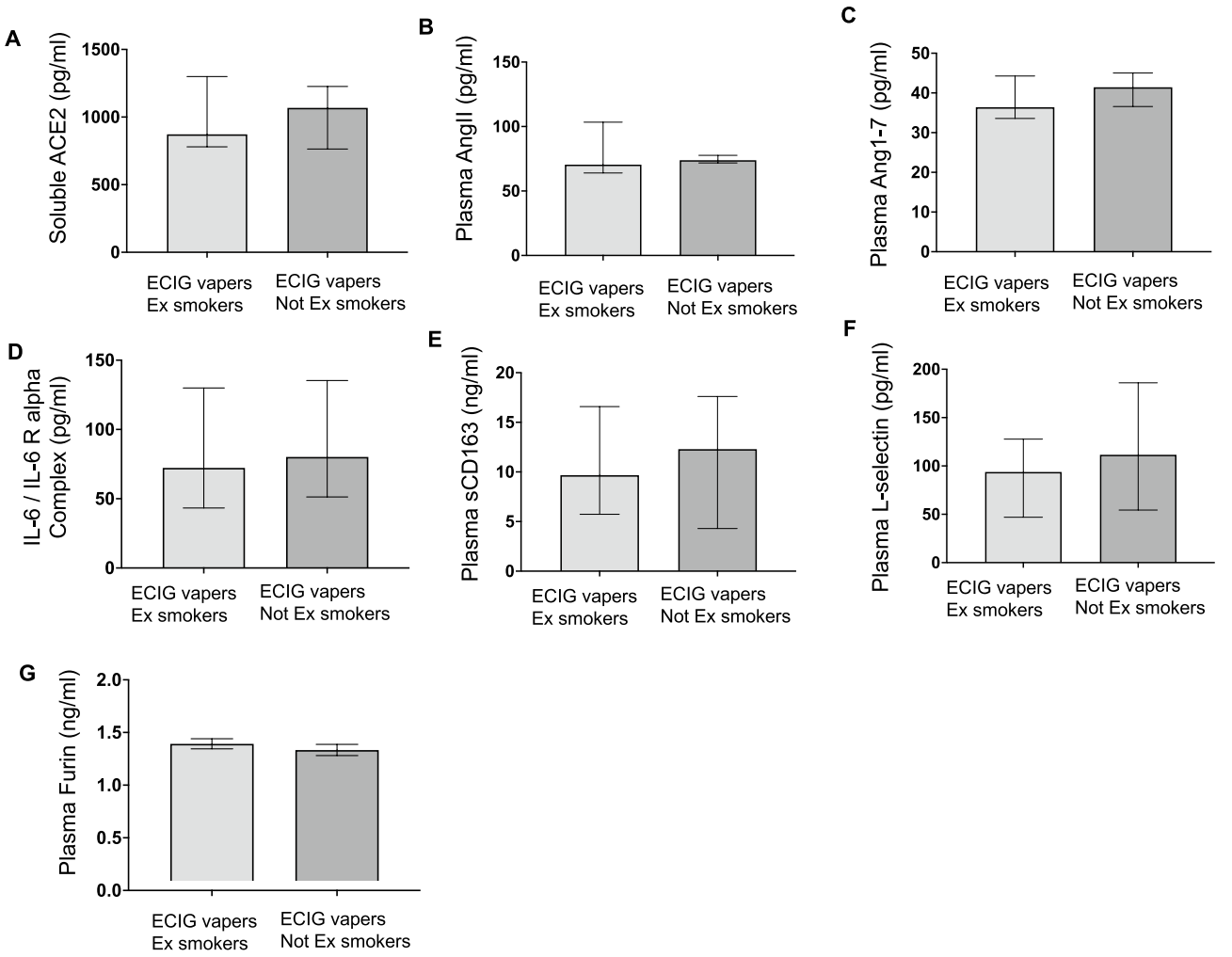

Fig. 2 Plasma levels of proteins involved in ACE2 and ADAM17 activities among electronic cigarettes (ECIG) users ($n=30$) with ($n=20$) or without ($n=10$) prior history of tobacco cigarette (TCIG) smoking. Levels of soluble ACE2 (**A**), angiotensin (Ang) II (**B**), Ang 1–7 (**C**), IL-6/IL-6R alpha (IL-6/IL-6Rα) complex (**D**), sCD163 (**E**), L-selectin (**F**), and furin (**G**) were determined by ELISA immunoassays. Data are shown as median and interquartile range (IQR). The Mann–Whitney test was used to compare individual 2 groups

vapers by at least twofold compared to non-smokers [11]. However, our prior study [11] was small and was done in total heterogeneous peripheral blood immune cells of healthy young adults. No blood immune cells were available for the participants of this study. Further larger studies in ECIG vapers that also measure protein levels of ADAM17 in monocytes/macrophages among different groups of ECIG vapers with higher burden of exposure to ECIG may better determine the effect of ECIG use on ADAM17 levels.

In healthy young adults without comorbidities, we found that sCD163 was increased in TCIG smokers compared to ECIG vapers and non-smokers. Increased plasma CD163 reflects overall ADAM17 cellular activity since CD163 is known to be shed exclusively by cellular activity of ADAM17 acting on cells such as macrophages and epithelial cells [17]. Our data is consistent with prior evidence that TCIG smoking reduces membrane levels of CD163 on myeloid cells [18–20] that reflect increased cellular activity of ADAM17 on macrophages and increased levels of shed sCD163 in plasma [17]. CD163 is an important biomarker not only of hyperinflammation and innate immune activation in severe COVID-19 [8] but also of atherosclerotic cardiovascular disease [21]. Herein, we found that sCD163 was increased in TCIG smokers compared to ECIG vapers and non-smokers even in healthy young participants. Increased levels of CD163 in smokers may be a biomarker of predisposition of this vulnerable population to increased morbidity and mortality not only from severe COVID-19 [8] but also from atherosclerotic heart disease [21]. Increased innate immune responses may also contribute to the adverse sequelae of PACS [22]. Our findings are consistent with data from others that TCIG use is associated with increased

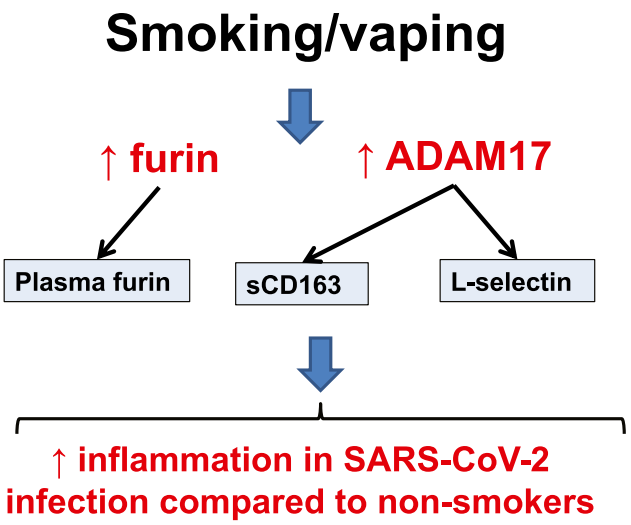

Fig. 3 Overall hypothesis. Epithelial and endothelial cells are targets of SARS-CoV-2 infection and express ADAM17 sheddase and furin, a protease that increases the affinity of the SARS-CoV-2 spike protein for ACE2. Shedding of cellular levels of furin and increased ADAM17 activity increase the circulating blood levels of furin, soluble CD163 (sCD163), and L-selectin. The increased baseline blood levels of furin, sCD163, and L-selectin in healthy young people who smoke tobacco cigarette and/or vape electronic cigarettes compared to non-smokers reflect the increased cellular activity of furin and ADAM17. Increased cellular activity of furin and ADAM17 may contribute to increased inflammation and future development of severe COVID-19 and cardiovascular complications of post-acute COVID-19 syndrome

activation of innate immune responses that may predispose to severe COVID-19 [23]. This novel finding is consistent with our prior data, in which we reported that expression of key proteins of the Toll-like receptor 4–inflammasome–IL-6 signaling axis was increased in blood immune cells from TCIG smokers compared to ECIG vapers and non-smokers [24]. Just as important as the elevation of CD163 in TCIG smokers is the absence of CD163 elevation in ECIG vapers.

In healthy young adults without comorbidities, we also found that L-selectin was increased in TCIG smokers compared to ECIG vapers and non-smokers. ECIG vapers had also increased plasma levels of L-selectin compared to NSs. These data are consistent with prior evidence that ECIG use causes a pro-inflammatory response from human neutrophils and induces matrix metalloproteinases [25–27]. Notably, L-selectin induces neutrophil activation and transmigration of neutrophils across endothelial cells [28, 29] and is a product of ADAM17 shedding activity in activated neutrophils during acute inflammation [9]. Thus, our data support the hypothesis that chronic ECIG use induces activation of ADAM17 and L-selectin activity in neutrophils and may contribute to inflammation in airways of ECIG users.

ADAM17 also induces shedding of IL-6R, the receptor for IL-6 that is an important mediator of hyperinflammation and innate immune activation in severe COVID-19 [1] and atherosclerotic cardiovascular disease [30]. We have previously shown that IL-6Rα, the receptor for IL-6 that contributes to lung inflammation and morbidity in COVID-19, was increased in blood immune cells of TCIG smokers but not in ECIG vapers compared to non-smokers [11]. However, our data suggest that plasma levels of the IL-6/IL-6Rα complex did not differ among different smoker groups in healthy adults and these levels should be further assessed in smoker groups with COVID-19.

Furin is a protease that regulates binding of the SARS-CoV-2 spike protein to ACE2 in epithelial cells during SARS-CoV-2 infection, but it is also expressed in blood and immune cells and other tissues. Furin can be shed into the extracellular space as an active enzyme, and furin-mediated protein processing perpetuates many infections including COVID-19 [1, 31]. In this study, plasma furin was increased in ECIG vapers compared to non-smokers and TCIG smokers. These data are consistent with our recently reported data that in ECIG vapers, furin was increased in blood immune cells compared to NSs [11]. Thus, the small increase in furin levels in plasma and blood immune cells of ECIG vapers compared to non-smokers is a consistent finding and is concerning. Differential immunomodulatory effects of ECIG use on T cells [32] and other non-immune cells such as endothelial [33] and epithelial [34] cells compared to TCIG use may explain the differential effect of ECIG versus TCIG use on furin, but further studies are needed to show this. Our data are consistent with evidence from preclinical experimental studies in mice that chronic inhalation of ECIG aerosols alters the inflammatory state of the lungs [35].

In our young population, we did not find any differences in plasma levels of ACE2 and Ang II among the compared groups. In contrast, we recently described that TCIG smokers compared to NSs had significantly increased cellular expression of ACE2 in blood immune cells compared to NSs [11]. Collectively, these data suggest that cellular and tissue levels of ACE2 and its ligands, rather than circulating levels of ACE2, Ang II, and Ang 1–7, may better reflect the impact of TCIG smoking versus ECIG vaping on the ACE2 signaling pathway and the risk of development of severe COVID-19.

Overall, some (L-selectin, sCD163), but not all (such as sACE2), targets of ADAM17 were increased in the plasma of TCIG smokers compared to non-smokers. These data suggest that cellular and tissue levels of ADAM17 and its activity on its targets do not fully reflect circulating levels of products of ADAM17 shedding activity. Other mechanisms other than ADAM17 may also regulate circulating levels of L-selectin and sCD163. Notably, both L-selectin and CD163 are mainly expressed in immune cells while ACE2 is mostly expressed in tissue parenchymal cells (such as epithelial cells) and is not abundant in blood immune cells [36].

Both macrophages [37] and neutrophils [38] express redox mechanisms that are essential for their function and may be more prone to systemic redox effects of smoking and ECIG use that also regulate ADAM17 activity [15]. Consistent with this hypothesis and our data in this report, we have previously shown that the cellular oxidative stress in blood immune cells is lower in non-smokers and ECIG users compared to TCIG smokers [39]. These redox mechanisms may be less relevant in certain parenchymal cells (such as epithelial cells) [40]. A differential regulation of ADAM17 in immune cells compared to epithelial cells (a more important source of sACE2 compared to blood immune cells) would also explain the discrepant results between the current study that TCIG smoking did not impact sACE2 levels compared to NSs while TCIG smokers had significantly increased cellular expression of ACE2 in blood immune cells compared to NSs [11]. Further larger studies with single cell analysis of ACE2, redox pathways, CD163, L-selectin, and ADAM17 levels among cell subsets (immune, epithelial, and endothelial cells) in smoking groups are needed to fully elucidate the impact of TCIG smoking and ECIG use on the differential regulation of ADAM17 activity.

Our study has limitations. Our study is small. We studied readily accessible plasma instead of less accessible tissues such as lung epithelial tissues. Soluble blood levels of a protein may reflect the sheddase activity of ADAM17 in several heterogeneous tissues and cells. Thus, no normalization of soluble levels of each protein against the total cell-associated levels of each protein could be performed and the cell-specific enzymatic activity of ADAM17 could not be assessed. Instead, soluble levels of each protein were normalized by the volume of plasma (pg/μl) and reflect systemic levels and ectodomain shedding. We did not measure the studied soluble proteins in TCIG smokers and ECIG vapers with acute COVID-19 to compare levels to those of uninfected TCIG smokers and ECIG vapers. Despite the established role of ADAM17 sheddase in COVID-19 pathogenesis, there is lack of published evidence on the clinical relevance of sCD163, s-L-selectin, and furin in SARS-CoV-2-uninfected persons for future risk of severe COVID-19. Our data need to be validated in larger longitudinal studies of TCIG smokers and ECIG vapers who developed different levels of severity of COVID-19. Nonetheless, the data herein complement our prior study where we showed a consistent increase in the expression of key proteins in COVID-19 pathogenesis in blood immune cells from TCIG smokers compared to ECIG vapers and non-smokers [11]. Confirmation of these findings in specific tissues that are targets for SARS-CoV-2 infection, including lung and/or gastrointestinal, is warranted.

In conclusion, the finding that plasma levels of CD163 and L-selectin, independent products of ADAM17 sheddase activity, are lower in non-smokers and chronic ECIG vapers compared to TCIG smokers, is intriguing and warrants additional investigation. ECIG vapers had increased plasma levels of furin and L-selectin compared to non-smokers. E-cigarette vapers may also be at higher risk of developing infections and inflammatory disorders of the lungs. ECIGs are not harmless and should be used for only the shortest time possible in smoking cessation, and not at all by non-smokers. Further studies are needed to demonstrate whether increased innate immune responses and ADAM17 activity in healthy young TCIG smokers may explain why these young patients can develop severe COVID-19 even with no apparent pre-existing medical conditions.

Author contribution TK: design of the study, flow cytometry experiments, supervision of the experiments, data analysis, interpretation of the data, writing of the manuscript (with HRM), revision of the manuscript, and financial support. MS: data collection, processing of the biospecimens, and performance of the immunoassays. ET: data collection and revision of the manuscript. GS: data collection and revision of the manuscript. RG: processing of the biospecimens and revision of the manuscript. JA: processing of the biospecimens and revision of the manuscript. HRM: design of the study, interpretation of the data, writing of the manuscript (with TK), revision of the manuscript, and financial support.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate The experimental protocols were approved by the UCLA Institutional Review Board (IRB#18–001147), and all participants provided written informed consent.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

1. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC (2020) Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. JAMA 324:782–793. https://doi.org/10.1001/jama.2020.12839
2. Umnuaypornlert A, Kanchanasurakit S, Lucero-Prisno DEI, Saokaew S (2021) Smoking and risk of negative outcomes among COVID-19 patients: a systematic review and meta-analysis. Tob Induc Dis 19:09. https://doi.org/10.18332/tid/132411
3. Jose T, Croghan IT, Hays JT, Schroeder DR, Warner DO (2021) Electronic cigarette use is not associated with COVID-19 diagnosis. J Prim Care Community Health 12:21501327211024391. https://doi.org/10.1177/21501327211024391

4. Nayeri A, Middlekauff H (2021) Vaping instead of cigarette smoking: a panacea or just another form of cardiovascular risk? Can J Cardiol 37:690–698. https://doi.org/10.1016/j.cjca.2020.12.008
5. Lambert DW, Yarski M, Warner FJ, Thornhill P, Parkin ET, Smith AI, Hooper NM, Turner AJ (2005) Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). J Biol Chem 280:30113–30119. https://doi.org/10.1074/jbc.M505111200
6. Zipeto D, Palmeira JDF, Arganaraz GA, Arganaraz ER (2020) ACE2/ADAM17/TMPRSS2 interplay may be the main risk factor for COVID-19. Front Immunol 11:576745. https://doi.org/10.3389/fimmu.2020.576745
7. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W et al (2005) A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med 11:875–879. https://doi.org/10.1038/nm1267
8. Zingaropoli MA, Nijhawan P, Carraro A, Pasculli P, Zuccala P, Perri V, Marocco R, Kertusha B, Siccardi G, Del Borgo C et al (2021) Increased sCD163 and sCD14 plasmatic levels and depletion of peripheral blood pro-inflammatory monocytes, myeloid and plasmacytoid dendritic cells in patients with severe COVID-19 pneumonia. Front Immunol 12:627548. https://doi.org/10.3389/fimmu.2021.627548
9. Wang Y, Herrera AH, Li Y, Belani KK, Walcheck B (2009) Regulation of mature ADAM17 by redox agents for L-selectin shedding. J Immunol 182:2449–2457. https://doi.org/10.4049/jimmunol.0802770
10. Watany MM, Abdou S, Elkolaly R, Elgharbawy N, Hodeib H (2022) Evaluation of admission levels of P, E and L selectins as predictors for thrombosis in hospitalized COVID-19 patients. Clin Exp Med. https://doi.org/10.1007/s10238-021-00787-9
11. Kelesidis T, Zhang Y, Tran E, Sosa G, Middlekauff HR (2021) Instigators of COVID-19 in immune cells are increased in tobacco cigarette smokers and electronic cigarette vapers compared to non-smokers. Nicotine Tob Res. https://doi.org/10.1093/ntr/ntab168
12. Saad MI, McLeod L, Yu L, Ebi H, Ruwanpura S, Sagi I, Rose-John S, Jenkins BJ (2020) The ADAM17 protease promotes tobacco smoke carcinogen-induced lung tumorigenesis. Carcinogenesis 41:527–538. https://doi.org/10.1093/carcin/bgz123
13. Stolarczyk M, Amatngalim GD, Yu X, Veltman M, Hiemstra PS, Scholte BJ (2016) ADAM17 and EGFR regulate IL-6 receptor and amphiregulin mRNA expression and release in cigarette smoke-exposed primary bronchial epithelial cells from patients with chronic obstructive pulmonary disease (COPD). Physiol Rep 4. https://doi.org/10.14814/phy2.12878
14. Lemjabbar-Alaoui H, Sidhu SS, Mengistab A, Gallup M, Basbaum C (2011) TACE/ADAM-17 phosphorylation by PKC-epsilon mediates premalignant changes in tobacco smoke-exposed lung cells. PLoS One 6:e17489. https://doi.org/10.1371/journal.pone.0017489
15. Lemjabbar H, Li D, Gallup M, Sidhu S, Drori E, Basbaum C (2003) Tobacco smoke-induced lung cell proliferation mediated by tumor necrosis factor alpha-converting enzyme and amphiregulin. J Biol Chem 278:26202–26207. https://doi.org/10.1074/jbc.M207018200
16. Haga S, Yamamoto N, Nakai-Murakami C, Osawa Y, Tokunaga K, Sata T, Yamamoto N, Sasazuki T, Ishizaka Y (2008) Modulation of TNF-alpha-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-alpha production and facilitates viral entry. Proc Natl Acad Sci USA 105:7809–7814. https://doi.org/10.1073/pnas.0711241105
17. Gooz M (2010) ADAM-17: the enzyme that does it all. Crit Rev Biochem Mol Biol 45:146–169. https://doi.org/10.3109/10409231003628015
18. Dewhurst JA, Lea S, Hardaker E, Dungwa JV, Ravi AK, Singh D (2017) Characterisation of lung macrophage subpopulations in COPD patients and controls. Sci Rep 7:7143. https://doi.org/10.1038/s41598-017-07101-2
19. Kunz LI, Lapperre TS, Snoeck-Stroband JB, Budulac SE, Timens W, van Wijngaarden S, Schrumpf JA, Rabe KF, Postma DS, Sterk PJ et al (2011) Smoking status and anti-inflammatory macrophages in bronchoalveolar lavage and induced sputum in COPD. Respir Res 12:34. https://doi.org/10.1186/1465-9921-12-34
20. Higham A, Baker JM, Jackson N, Shah R, Lea S, Singh D (2021) Dysregulation of the CD163-haptoglobin axis in the airways of COPD patients. Cells 11. https://doi.org/10.3390/cells11010002
21. Aristoteli LP, Moller HJ, Bailey B, Moestrup SK, Kritharides L (2006) The monocytic lineage specific soluble CD163 is a plasma marker of coronary atherosclerosis. Atherosclerosis 184:342–347. https://doi.org/10.1016/j.atherosclerosis.2005.05.004
22. Nalbandian A, Sehgal K, Gupta A, Madhavan MV, McGroder C, Stevens JS, Cook JR, Nordvig AS, Shalev D, Sehrawat TS et al (2021) Post-acute COVID-19 syndrome. Nat Med 27:601–615. https://doi.org/10.1038/s41591-021-01283-z
23. Lee AC, Chakladar J, Li WT, Chen C, Chang EY, Wang-Rodriguez J, Ongkeko WM (2020) Tobacco, but not nicotine and flavor-less electronic cigarettes, induces ACE2 and immune dysregulation. Int J Mol Sci 21. https://doi.org/10.3390/ijms21155513
24. Kelesidis T, Zhang Y, Tran E, Sosa G, Middlekauff HR (2021) Expression of key inflammatory proteins is increased in immune cells from tobacco cigarette smokers but not electronic cigarette vapers: implications for atherosclerosis. J Am Heart Assoc 10:e019324. https://doi.org/10.1161/JAHA.120.019324
25. Higham A, Rattray NJ, Dewhurst JA, Trivedi DK, Fowler SJ, Goodacre R, Singh D (2016) Electronic cigarette exposure triggers neutrophil inflammatory responses. Respir Res 17:56. https://doi.org/10.1186/s12931-016-0368-x
26. Reidel B, Radicioni G, Clapp PW, Ford AA, Abdelwahab S, Rebuli ME, Haridass P, Alexis NE, Jaspers I, Kesimer M (2018) E-cigarette use causes a unique innate immune response in the lung, involving increased neutrophilic activation and altered mucin secretion. Am J Respir Crit Care Med 197:492–501. https://doi.org/10.1164/rccm.201708-1590OC
27. Ghosh A, Coakley RD, Ghio AJ, Muhlebach MS, Esther CR Jr, Alexis NE, Tarran R (2019) Chronic e-cigarette use increases neutrophil elastase and matrix metalloprotease levels in the lung. Am J Respir Crit Care Med 200:1392–1401. https://doi.org/10.1164/rccm.201903-0615OC
28. Simon SI, Burns AR, Taylor AD, Gopalan PK, Lynam EB, Sklar LA, Smith CW (1995) L-selectin (CD62L) cross-linking signals neutrophil adhesive functions via the Mac-1 (CD11b/CD18) beta 2-integrin. J Immunol 155:1502–1514
29. Smolen JE, Petersen TK, Koch C, O'Keefe SJ, Hanlon WA, Seo S, Pearson D, Fossett MC, Simon SI (2000) L-selectin signaling of neutrophil adhesion and degranulation involves p38 mitogen-activated protein kinase. J Biol Chem 275:15876–15884. https://doi.org/10.1074/jbc.M906232199
30. Narazaki M, Kishimoto T (2018) The two-faced cytokine IL-6 in host defense and diseases. Int J Mol Sci 19. https://doi.org/10.3390/ijms19113528
31. Braun E, Sauter D (2019) Furin-mediated protein processing in infectious diseases and cancer. Clin Transl Immunology 8:e1073. https://doi.org/10.1002/cti2.1073
32. Pesu M, Watford WT, Wei L, Xu L, Fuss I, Strober W, Andersson J, Shevach EM, Quezado M, Bouladoux N et al (2008) T-cell-expressed proprotein convertase furin is essential for maintenance of peripheral immune tolerance. Nature 455:246–250. https://doi.org/10.1038/nature07210
33. Negishi M, Lu D, Zhang YQ, Sawada Y, Sasaki T, Kayo T, Ando J, Izumi T, Kurabayashi M, Kojima I et al (2001) Upregulatory expression of furin and transforming growth factor-beta by fluid

shear stress in vascular endothelial cells. Arterioscler Thromb Vasc Biol 21:785–790. https://doi.org/10.1161/01.atv.21.5.785
34. Dong S, Lu Y, Peng G, Li J, Li W, Li M, Wang H, Liu L, Zhao Q (2021) Furin inhibits epithelial cell injury and alleviates experimental colitis by activating the Nrf2-Gpx4 signaling pathway. Dig Liver Dis 53:1276–1285. https://doi.org/10.1016/j.dld.2021.02.011
35. Masso-Silva JA, Moshensky A, Shin J, Olay J, Nilaad S, Advani I, Bojanowski CM, Crotty S, Li WT, Ongkeko WM et al (2021) Chronic e-cigarette aerosol inhalation alters the immune state of the lungs and increases ACE2 expression, raising concern for altered response and susceptibility to SARS-CoV-2. Front Physiol 12:649604. https://doi.org/10.3389/fphys.2021.649604
36. Song X, Hu W, Yu H, Zhao L, Zhao Y, Zhao X, Xue HH, Zhao Y (2020) Little to no expression of angiotensin-converting enzyme-2 on most human peripheral blood immune cells but highly expressed on tissue macrophages. Cytometry A. https://doi.org/10.1002/cyto.a.24285
37. Canton M, Sanchez-Rodriguez R, Spera I, Venegas FC, Favia M, Viola A, Castegna A (2021) Reactive oxygen species in macrophages: sources and targets. Front Immunol 12:734229. https://doi.org/10.3389/fimmu.2021.734229
38. Nguyen GT, Green ER, Mecsas J (2017) Neutrophils to the ROScue: mechanisms of NADPH oxidase activation and bacterial resistance. Front Cell Infect Microbiol 7:373. https://doi.org/10.3389/fcimb.2017.00373
39. Kelesidis T, Tran E, Arastoo S, Lakhani K, Heymans R, Gornbein J, Middlekauff HR (2020) Elevated cellular oxidative stress in circulating immune cells in otherwise healthy young people who use electronic cigarettes in a cross-sectional single-center study: implications for future cardiovascular risk. J Am Heart Assoc 9:e016983. https://doi.org/10.1161/JAHA.120.016983
40. Leiva-Juarez MM, Kolls JK, Evans SE (2018) Lung epithelial cells: therapeutically inducible effectors of antimicrobial defense. Mucosal Immunol 11:21–34. https://doi.org/10.1038/mi.2017.71