Specific biomarker comparison in current smokers, e-cigarette users, and non-smokers

Abstract

Introduction This study aims to address the existing gap in the literature, while adding evidence, by comparing tobacco-specific biomarkers (Cotinine and nitrosamines: NNK, NNAL, NNN), and other biomarkers of e-cigarette use (Glycerol, 1,2-PD, 1,3-PD) according to five study groups.

Methods: A pooling analysis including two different studies was conducted. In both analyses, we took saliva samples from smokers (n = 409) and non-smokers (n = 154), dual tobacco and e-cig users (n = 92), exclusive e-cig user with nicotine (n = 158), and exclusive e-cig users without nicotine (n = 38). We analyzed and compared among groups the concentration of tobacco-specific biomarkers, and e-cigarette biomarkers. We calculated geometric means (GM) and geometric standard deviations (GSD), stratifying according to study groups. We used log-linear models adjusted for sex, and age, to model the change percentage in tobacco-specific biomarkers, and e-cigarette biomarkers (after log 10 transformation), and their 95% confidence intervals.

Results: Cotinine was significantly higher in nicotine consumers, independently of the source of nicotine, and in e-cigarette exclusive users without nicotine when compared to non-smokers. TSNAs were generally significantly lower in non-smokers compared with every group.

Conclusions: Although there was a reduction in TSNAs in e-cigarette exclusive users compared to smokers, and a reduction of Cotinine concentrations in e-cigarette exclusive users without nicotine compared to smokers, there are still high levels of these biomarkers when compared to non-smokers, which may be a clue towards the harmful effect of e-cigarettes.

Key words: biomarker, e-cigarette, tobacco, Cotinine, TSNAs, Glycerol, propanediols

Introduction

Nowadays, the research regarding the effects and the use of electronic cigarette (e-cigarette) on health has not achieved a strong consensus. On one hand, most researchers and health professionals dread e-cigarette health consequences and that their use may be a gateway to smoking, especially among the younger population [1, 2]. On the other hand, some researchers, argue that e-cigarettes could contribute to a harm reduction in comparison with tobacco smoking, and can be a useful tool in smoking cessation [3].

Due to the increasing prevalence of the e-cigarette use [4, 5], and the controversy of e-cigarette use, there is the necessity of doing research on the possible e-cigarette's harm. Studies comparing tobacco-specific biomarkers from e-cigarette and tobacco may be useful. Regarding tobacco-specific biomarkers, one of the most prevalent carcinogens in unburned tobacco are N-nitrosamines (TSNAs), including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNK), its main metabolite, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and N′-nitrosonornicotine (NNN) [6]. In addition, Cotinine, the main metabolite of Nicotine, has been extensively used as marker of tobacco exposure in the 3 or 4 days prior to sample collection [7]. Several studies have proved that TSNAs have strong organ-specific carcinogenicity, mutagenicity, and teratogenic action, among others [8, 9]. NNK and NNN have been shown to be the strongest carcinogens among the TSNAs [10]. However, according to previous studies, lower levels of TSNAs are found in e-cigarettes compared to conventional tobacco, and it could be linked to a lower carcinogenic effect as well [11], although no studies that analyze the health effect associated with TSNAs concentration have been found, providing evidence of the dose-toxicity of them.

Few previous studies analyzed Cotinine and/or TSNAs in e-cigarette users in comparison with smokers [12–17]. Although the results obtained were heterogenous, most of them

found lower levels of TSNAs [12, 14, 15, 17] when comparing e-cigarette users vs smokers, and no difference in Cotinine [13–15, 17] between these groups. However, according to the results obtained in these studies, it is acceptable to assume that none of these studies included information regarding e-cigarettes without nicotine users, and just a few of them included information on non-smokers [12, 13]. Moreover, regarding TSNAs analysis, most studies only included information on NNAL [12, 14–17].

Moreover, the main diluents found in the liquids needed to use electronic cigarette (e-liquids), Glycerol and propanediols (PD), also known as propylene glycol, are potentially hazardous [18]. Although both components are used in a wide variety of consumer products, and their exposure via oral and dermal appear to be innocuous, inhalation risks are still not well characterized. In this sense, some research [19–21] suggests that Glycerol and PD are both airway irritants, and that PD causes eye, mouth, throat, mucous membrane, and respiratory irritation, dry cough, constriction of peripheral airways, and inflammatory responses through MAP kinases, JAK/STAT, and NF-kB pathways. Moreover, according to the International Agency for Research on Cancer (IARC) [22] there is sufficient evidence in experimental animals to conclude PD carcinogenicity. Moreover, to the best of our knowledge, no previous research analyzes these components comparing e-cigarette users with or without nicotine.

This study aims to address the existing gap in the literature, while adding evidence, by comparing tobacco biomarkers (Cotinine and nitrosamines: NNK, NNAL, NNN), and e-cigarette biomarkers (Glycerol, 1,2-PD, 1,3-PD) according to five groups: 1) non-smokers, 2) e-cigarette exclusive users without nicotine, 3) e-cigarette exclusive users with nicotine, 4) smokers, and 5) dual users (e-cigarette and tobacco).

Methods

A pooling analysis including two different studies was conducted. In one of the two studies (dCOT e-cig), we obtained the data of e-cigarettes users. This study was a cross-sectional study of a sample of adult (≥18 years) current e-cigarette users of the city of Barcelona in Spain (n=300) [23]. We recruited the e-cigarette user using the technique known as 'consumer panels' as described previously elsewhere [24]. Briefly, e-cigarette users were recruited in all neighborhoods of the city of Barcelona between January of 2017 and February of 2018 by sensors (specifically trained personnel for the recruitment of e-cigarette users). In the other study, we obtained data of smokers and non-smokers. We used the follow-up of tobacco smoking patterns of the "Determinants of Cotinine project-phase 3 (dCOT3 study)", a cohort study of a representative sample of the adult (≥ 16 years old) general population of the city of Barcelona (Catalonia, Spain). Participants were recruited during the years 2004-2005, and the follow-up was carried out during 2013-2014. Data cleansing details conducted to get the final sample can be found elsewhere [25]. A face-to-face interview was conducted with the individuals who agreed to participate in both studies.

In both studies, we obtained a saliva sample for tobacco biomarkers (Cotinine and nitrosamines: NNK, NNAL, NNN) analysis using the same protocol of previous studies [26]. In addition, in the dCOT e-cig study, Glycerol, 1,2-PD, and 1,3-PD were also included. They were asked to rinse their mouths and then suck a lemon candy (Smint ®) to stimulate saliva production. Then, first they were asked to spit out a small amount of saliva and to provide about 9 ml of saliva by spitting into a funnel placed in a test tube. The sample was separated into about 3 ml aliquots and frozen to -20 ºC for storage. The frozen samples were sent to the Municipal Institute for Medical Research (IMIM-Hospital del Mar) in Barcelona. The concentration of all biomarkers was measured by alkaline single liquid-liquid extraction with dichloromethane/isopropanol. Analytical details can be found elsewhere [27]. We

determined the TSNAs (NNN, NNK, and NNAL) with limits of quantification of 1.0, 2.0 and 0.50 pg/mL respectively, and 0.1 ng/mL for Cotinine. The limits of quantification of Glycerol, and propanediols (1,2-PD and 1,3-PD) were 10 and 3,0 nmol/mL respectively. When concentrations were below the limit of quantification a value of half the level of quantification was assigned. Both studies were approved by the ethics committee of the Bellvitge University Hospital (dCOT3: PR118/11, dCOT e-cig: PR133/15) and all participants signed an informed consent.

Subjects that declared being non-smokers but had a Cotinine concentration above 10 ng/mL were not considered, as those concentrations of Cotinine are not expected to be found in non-smokers [28] and the answers in the survey cannot be reliable.

Study variables

Group definition

We defined five groups of analysis according to self-reported information: 1) non-smokers, 2) e-cigarette exclusive users without nicotine, 3) e-cigarette exclusive users with nicotine, 4), smokers, and dual users (e-cigarette and tobacco). In both studies, subjects are considered non-smokers if they declared it in the survey with the question: “Which of the following sentences best indicated your current relationship with tobacco”, and answered “Currently I do not smoke, but I used to smoke everyday”, “Currently I do not smoke, but I used to occasionally smoke more than 6 months ago” or “I have never smoked”. Subjects are considered smokers if they declared smoking at the moment of the survey with the answers to the previous question: “Currently I smoke everyday” or “Currently I occasionally smoke“. We consider e-cigarette user any person that was currently using an e-cigarette. The dCOT e-cig study surveyed people that was carrying or using an e-cigarette at the

moment the sensor found the subject, so everybody in the first study is considered a current e-cigarette user. In the dCOT3, which was focused on smokers and non-smokers, there was a question that asked if the subject was using or had ever used an e-cigarette. Those people that answered that were using e-cigarette at the moment of sample extraction were considered dual users (n=9) and removed from the study to increase internal validity. Exclusive e-cigarette users were defined as those subjects that declared using e-cigarette but not smoking other tobacco products. On the other hand, e-cigarette users that declared smoking at the same time are considered dual users (e-cigarette and tobacco). From exclusive e-cigarette users, we used a dichotomic question to differentiate subjects according to nicotine e-liquid usage, indicating whether subjects use e-liquid containing some concentration of nicotine “e-cigarette exclusive users with nicotine”, or e-liquid without containing any concentration of nicotine “e-cigarette exclusive users without nicotine”.

Sociodemographic variables, such as sex and age, were also included in the analysis.

Statistical analysis

Due to the skewness in TSNAs and Cotinine concentration distribution, we calculated their geometric mean (GM) and geometric standard deviation (GSD), as well as the Median and the Interquartile range (IQR). The analysis was conducted stratifying according to study groups in addition to the overall analysis including all the individuals. To perform the multiple test analysis, we did a pairwise Wilcoxon test for independent samples. To adjust for the false-discovery error rate for multiple comparisons we used the Bonferroni’s correction, setting the significance level to 0.001. Finally, we fitted a Linear regression Model (LM) with logarithmic response [29] to estimate the differences (percentage change) in the geometric mean of TSNAs and Cotinine concentration, focusing on the comparison between the study

groups, and adjusting for sociodemographic variables (sex and age). The statistical program used was R-3.4.2.

Results

The final sample included 409 (48.06%) non-smoker subjects, 154 (18.10%) exclusive tobacco smoker subjects, 38 (4.47%) exclusive e-cigarette users without nicotine, 158 (18.57%) exclusive e-cigarette users with nicotine, and 92 (10.81%) dual users. Cotinine concentration showed the most evident group separation (Figure 1 (panel A)), Table 1). On the one hand, nicotine consumers (tobacco smokers (GM: 150.61; GSD: 9.30), dual users (GM: 137.10; GSD: 6.13) and e-cigarette exclusive users with nicotine (GM: 174.04; GSD: 3.91)) had a geometric mean above 135 ng/mL, with no significant difference between these groups. On the other hand, no nicotine consumers (e-cigarette exclusive users without nicotine (GM: 2.13; GSD: 13.57) and non-smokers (GM: 0.12; GSD: 2.45)) had a geometric mean concentration below 2.5 ng/mL. However, significant differences were found between these two groups, having e-cigarette exclusive users without nicotine a higher concentration of Cotinine. Moreover, the percentage of change resulted significantly in each group when comparing with non-smokers (Table 2). In this sense, the lower percentage of change was obtained in e-cigarette exclusive users (without nicotine) comparison (% change: 1403.14 (783.08, 2452.70)) while the highest percentage of change was obtained in the e-cigarette exclusive users (with nicotine) comparison (% change: 122643 (88820, 169333)) (Table 2).

Regarding nitrosamines, concentrations were significantly higher in tobacco smokers, either smokers or dual users compared with non-smokers or exclusive e-cigarette users (Figure 1 (panel B, C, and D), Table 1). However, NNAL concentrations were significantly higher among smokers when comparing with dual users. Concentrations of NNAL and NNN, were significantly lower among e-cigarette exclusive users (with nicotine) when comparing with

smokers, and when comparing with dual users only for NNN. The most significant changes are found when comparing every group with non-smokers, except in the case of NNK and NNAL concentrations for e-cigarette exclusive users without nicotine which are non-significant (Figure 1 (panel B, C, and D), Table 1). This pattern is also observed in the percentage of change, resulting significant when comparing every group with non-smokers, except for NNK and NNAL concentrations for e-cigarette exclusive users without nicotine, which is non-significant (Table 2). For NNN and NNAL, the highest percentage of change were obtained in the dual smokers' comparison (% change NNN: 1164.74, 95% CI: 904.62 - 1492.21); % change NNAL: 345.66, 95% CI: 292.64, 405.82) and in the dual user's comparison for NNK (% of change: 116.77, 95% CI: 84.93 - 154.10) (Table 2).

Finally, overall, no differences were found regarding e-cigarette biomarkers concentrations (Figure 2, Table 1, Table 2).

Discussion

Cotinine was significantly higher in nicotine consumers independently of the source of nicotine, and in e-cigarette exclusive users without nicotine when compared to non-smokers. TSNAs were also significantly higher in tobacco smokers in comparison with the other groups. However, no differences were found in Glycerol, 1,2-PD, and 1,3-PD.

E-cigarette exclusive users without nicotine had significantly increased levels of Cotinine compared to non-smokers. The source of Nicotine or Cotinine could come from a passive exposure to tobacco, as e-cigarette users may be more prone to interact with smokers, or from unreported nicotine consumption. Alternatively, this result may be due to, in considerably low concentrations, Nicotine being present in e-liquids labelled as nicotine-

free, as some research suggests [30]. To the best of our knowledge, there are no previous studies comparing biomarker information in e-cigarette exclusive users without nicotine and non-smokers. However, these results are especially worrisome for several reasons. First, e-cigarettes are generally perceived not as harmful products [31, 32]. Second, the prevalence of e-cigarette use has proved to be higher among young population[33]. Therefore, if nicotine would be present in e-liquids labeled as nicotine-free [30], its use not only would pose a health hazard, but could also cause dependence, making the e-cigarette a possible gateaway to smoking [33].

In addition, as Cotinine is a metabolic product from Nicotine [34], as expected, Nicotine consumers were distinctly identified according to Cotinine concentrations, with no significant differences in Cotinine concentration between Nicotine consumers. These results counteract the idea that replacing tobacco with e-cigarettes may be a useful tool against addictive behaviours, emphasizing that similar concentrations of Cotinine are found between e-cigarette exclusive users with nicotine, smokers, and dual users. Moreover, this is in line with previous research showing similar concentrations of Cotinine among e-cigarette exclusive users with nicotine, tobacco smokers, or dual users in urine [13–15, 17, 35, 36], serum [37], and saliva [17, 38]. However, other studies, analyzing potentially harmful constituents specifically found on e-liquids, showed lower concentrations of tobacco-specific biomarkers on e-cigarette compositions when compared with conventional tobacco as measured with TSNAs [39].

The concentration of all TSNAs were, in general, distinctly higher between tobacco smokers (either smokers or dual users) when comparing with the other groups. This was expected, as these compounds are specifically found in tobacco products, and is in line with previous research [12, 14–17, 36, 40]. However, TSNAs were also found in e-cigarette exclusive users with and without nicotine in significantly higher concentrations when compared with

non-smokers (except for NNK, which was non-significant between e-cigarette exclusive users without nicotine and non-smokers). In addition, there were some exceptions in which non-significant differences in TSNA concentrations were found between e-cigarette exclusive users when comparing with smokers and dual users, which may counteract again the harm reduction hypothesis. Moreover, according to our results, NNAL concentrations were significantly higher among smokers when comparing with dual users. This could be explained by differences in the NNAL concentrations in comparison with NNN and NNK. NNAL concentrations were lower, with higher variation, and with higher number of missing values in comparison to other TSNAs (NNN: 8 and NNK: 3 vs NNAL: 25). Moreover, these lower levels in dual users might also be due to lower levels of NNAL in e-cigarettes, or due to dual users smoke less cigarettes than smokers, as e-cigarette are in some cases used as a tool to quit smoking. In this regard, in this sample, dual users smoke 9.8 cigarettes daily, while smokers smoke 16.5, being this difference significant (p-value < 0.0001, CI 95% [4.1642, 9.4268]). This is in line with previous studies that also showed that dual users smoked less cigarettes than cigarette-only smokers [36].

Regarding Glycerol, 1,2-PD, and 1,3-PD, no differences in their concentrations were found when comparing e-cigarette exclusive users with nicotine, without nicotine, or dual users. As the concentration of these biomarkers correlates with the consumption of e-cigarettes, this might suggest that, independently on the presence of nicotine in the e-liquid, or whether the e-cigarette user smokes or not, there are no differences in e-cigarette use. However, future studies should include this information to analyze differences in these biomarkers between e-cigarette users with nicotine, without nicotine, and dual users.

Our study has some limitations. First, regarding biomarker concentrations, we assume that TSNAs, Cotinine, 1,3-PD, 1,2-PD, and Glycerol come exclusively from tobacco or the use of e-cigarettes. This might not be the case, as there are multiple sources, including passive

exposure of the participants to smoke. Next, when talking about the study design, we are combining two different studies that, although it proved stronger conclusions regarding the sample size, the sample extractions are done in different time and there might be a batch effect when pooling the values of the concentrations. Hence, the comparisons between samples from the first study (e-cigarette and dual users) and the second study (smokers and non-smokers) might be slightly biased. Another limitation of the study is the fact that the information was obtained using surveys, leading to self-reported data that we were not able to validate in some cases. For instance, samples of the e-liquid were not obtained in the first study. Therefore, information regarding use of nicotine, or non-nicotine, e-liquids was only self-reported, and we were not able to obtain and analyze a sample of the e-liquids. On the other hand, we collected the data using a face-to-face questionnaire. However, we checked plausible biomarker concentrations regarding tobacco smoke, removing individuals with discrepancies between their report in the questionnaire and the biomarker concentrations, thus increasing the internal validity of the study. Moreover, both studies included a sample with enough statistical power to undertake the current analysis. The combination of the two studies allowed us to compare not only with different e-cigarette types, but also with tobacco consumption, which is the actual strong point of the whole study. Our findings are important to describe the TSNAs and Cotinine profile in smokers, non-smokers, and different e-cigarette users and to compare all of them, shedding some light on the uncertainties about the health effect of e-cigarettes when compared to smokers and non-smokers.

In conclusion, main differences have been found between smokers and non-smokers, between smokers and e-cigarette exclusive users (especially without nicotine), and between e-cigarette exclusive users (especially with nicotine) and non-smokers. Although the concentration of TSNAs was lower in e-cigarette exclusive users compared to smokers, and a reduction of Cotinine concentrations in e-cigarette exclusive users without nicotine

compared to smokers, there are still high levels of these biomarkers when compared to non-smokers, which may be a clue towards the harmful effect of e-cigarettes. A deeper analysis on the liquids should be considered to make sure that they do not contain harmful agents, other than those labeled.

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DECLARATION OF INTERESTS

Authors declare that they have no conflicts of interest.

DATA AVAILABILITY

Data not publically available.

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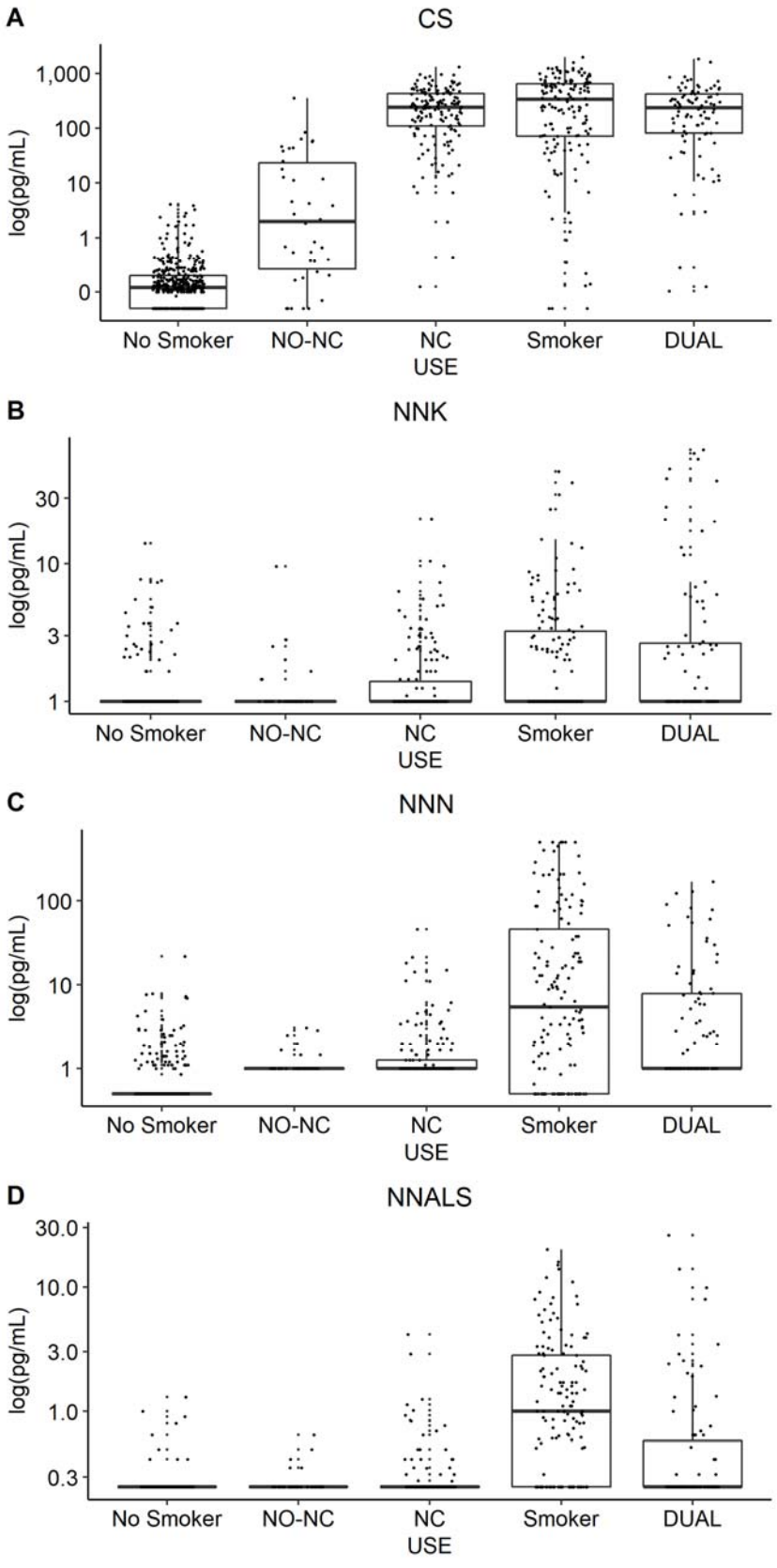

Figure 1. Boxplot (and individual data) of tobacco-specific biomarkers (Cotinine and nitrosamines: NNK, NNAL, NNN) according to studied groups: 1) non-smokers, 2) e-cigarette exclusive users without nicotine, 3) e-cigarette exclusive users with nicotine, 4), smokers, and 5) dual users (e-cigarette and tobacco).

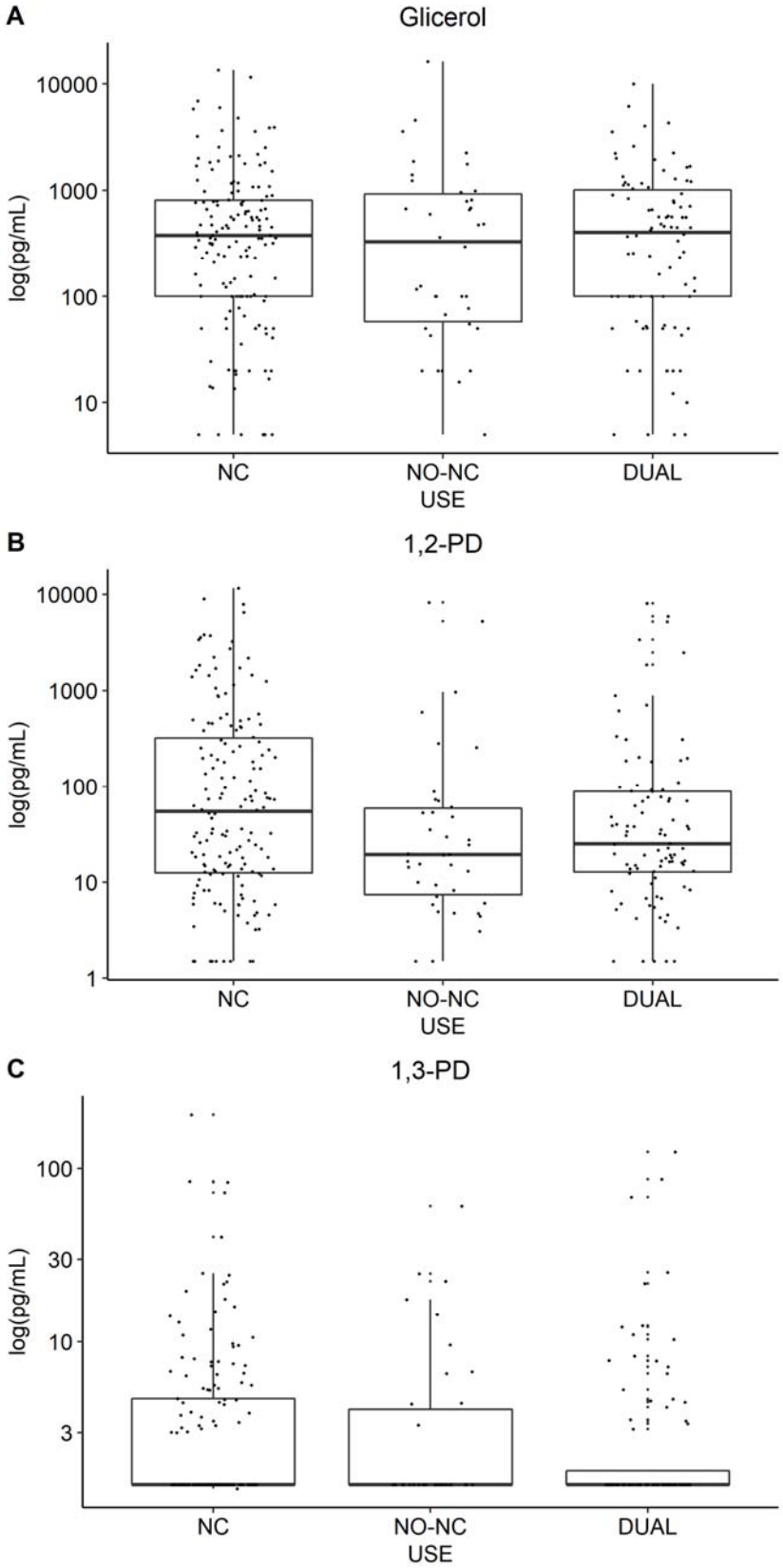

Figure 2. Boxplot (and individual data) of e-cigarette biomarkers (Glycerol, 1,2-PD, 1,3-PD) according to following studied groups: NC: e-cigarette exclusive users with nicotine, NO-NC: e-cigarette exclusive users without nicotine, DUAL: dual users (e-cigarette and tobacco).

Table 1. Questions defining group exposure.

dCOT3 questions	e-cig dCOT3 questions
A. From the following sentences, please indicate the one that describes the best your behaviour regarding tobacco: A1. Currently I smoke everyday (at least one cigarette a day) A2. Currently I ocasionally smoke A3. Currently I do not smoke, but I used to smoke everyday (at least one cigarette a day) A4. Currently I do not smoke, but I used to smoke ocasionally A5. I have never smoked,	B. From the following sentences, please indicate the one that describes the best your behaviour regarding tobacco: B1. Currently I smoke everyday (at least one cigarette a day) B2. Currently I ocasionally smoke B3. Currently I do not smoke, but I used to smoke everyday (at least one cigarette a day) B4. Currently I do not smoke, but I used to smoke ocasionally B5. I have never smoked,
	C. Are you currently using refill liquit with nicotine? C1. Yes C2. Currently I do not, but I did in the past C3. No, never
Group definition: 1) non-smokers (A3, A4, A5), 2) e-cigarette exclusive users without nicotine (B3, B4, B5, C2, C3), 3) e-cigarette exclusive users with nicotine (B3, B4, B5, C1), 4) smokers (A1, A2), and 5) dual users (e-cigarette and tobacco) (B1, B2).	

Table 2. Geometric mean (GM) and Geometric Standard Deviation (GSD) of tobacco biomarkers (Cotinine and nitrosamines: NNK, NNAL, NNN), and e-cigarette biomarkers (Glycerol, 1,2-PD, 1,3-PD) in oral fluid samples according to studied groups: 1) non-smokers, 2) e-cigarette exclusive users without nicotine, 3) e-cigarette exclusive users with nicotine, 4), smokers, and 5) dual users (e-cigarette and tobacco).

		Tobacco biomarkers				E-cigarette biomarkers		
	N*	Cotinine (ng/mL) GM (GSD)	NNK (PD/mL) GM (GSD)	NNN (PD/mL) GM (GSD)	NNAL (PD/mL) GM (GSD)	Glycerol (ng/mL) GM (GSD)	1,2-PD (ng/mL) GM (GSD)	1,3-PD (ng/mL) GM (GSD)
Non-smokers	409	0.12(2.45)	1.08(1.38)	0.61(1.73)	0.26(1.17)	-	-	-
E-cigarettes exclusive users (without nicotine)	38	2.13(13.57)	1.18(1.54)	1.14(1.36)	0.27(1.24)	244.62 (6.48)	28.42 (7.21)	2.65 (2.77)
E-cigarettes exclusive users (with nicotine)	158	174.04 (3.91)	1.34(1.78)	1.38 (2.01)	0.29(1.54)	288.02 (5.62)	63.29 (9.16)	2.83 (2.74)
Smokers	154	150.61 (9.30)	1.95(2.51)	7.31 (9.90)	1.09(3.38)	-	-	-
Dual users	92	137.1(6.13)	2.13(3.30)	3.15(4.69)	0.44(2.85)	261.71 (5.85)	36.01 (6.54)	2.39 (2.61)
Comparisons								
Non-smokers vs e-cigarette exclusive users (without nicotine)[1]		<0.001	0.16	<0.001	0.003	-	-	-
Non-smokers vs e-cigarettes exclusive users (with nicotine)[1]		<0.001	<0.001	<0.001	<0.001	-	-	-
Non-smokers vs smokers[1]		<0.001	<0.001	<0.001	<0.001	-	-	-
Non-smokers vs dual users[1]		<0.001	<0.001	<0.001	<0.001	-	-	-
E-cigarette exclusive users (without nicotine) vs e-cigarette exclusive users (with nicotine)[1]		<0.001	1	1	1	1	0.093	1
E-cigarette exclusive users (without nicotine) vs smokers[1]		<0.001	0.017	0.001	<0.001	-	-	-
E-cigarettes exclusive users (without nicotine) vs dual users[1]		<0.001	0.047	0.003	0.013	1	1	1
E-cigarette exclusive users (with nicotine) vs smokers		0.69	0.068	<0.001	<0.001	-	-	-
E-cigarette exclusive users (with nicotine) vs dual users[1]		1	0.06	<0.001	0.04	1	0.187	0.25
Dual users vs smokers[1]		0.53	1	0.41	<0.001	-	-	-

* NNK:1 missing value in Non-Smoker, 2 missing values in Smoker. NNN: 7 missing values in Non Smoker, 1 missing value in Smokers. NNAL: 1 missing value in E-cigarette users with nicotine, 1 missing value in E-cigarette dual users, 9 missing values in non-smokers, 14 missing values in smokers.

[1] P-value obtained by Wilcoxon test for independent samples, and adjusted by Bonferroni: Significant level 0.001

Table 3. Percentage of difference (% difference) and it 95% confidence interval (95% IC), obtained through the log-linear regression models for tobacco biomarkers (Cotinine and nitrosamines: NNK, NNAL, NNN), and e-cigarette biomarkers (Glycerol, 1,2-PD, 1,3-PD), adjusted by sex and age.

	Cotinine % difference (95% CI)	NNK % difference (95% CI)	NNN % difference (95% CI)	NNAL % difference (95% CI)	Glycerol % difference (95% CI)	1,2-PD % difference (95% CI)	1,3-PD % difference (95% CI)
Non-smokers	Ref	Ref	Ref	Ref	-	-	-
E-cigarette exclusive users (without nicotine)	1403.14 (783.08, 2452.70) ***	18.30 (-5.59, 48.25)	112.91 (39.32,224.34) ***	13.50 (-0.943, 42.23)	Ref	Ref	Ref
E-cigarette exclusive users (with nicotine)	122643 (88820, 169333) ***	33.97 (16.68, 53.82) ***	154.68 (96.79, 229.61) ***	22.18 (6.41, 40.28) **	17.76 (-36.90, 120.29)	119.76 (4.96, 361.31) *	3.17 (-25.18, 51.01)
Smokers	116312 (87197, 155138) ***	84.93 (63.68, 109.41) ***	1164.74 (904.62, 1492.21) ***	345.66 (292.64, 405.82) ***	-	-	-
Dual users	103176 (71021, 149523) ***	116.77 (84.93, 154.10) ***	490.20 (338.53, 694.32) ***	86.21 (58.85, 117.77) ***	15.88 (-40.84, 126.98)	23.31 (-43.64, 117.33)	-5.16 (-35.14, 38.37)

*** p-value < 0.001, ** p-value < 0.01, * p-value < 0.05; Obtained by the log-linear model adjusted for sex and age.