Changes in the salivary cotinine cut-offs to discriminate smokers and non-smokers before and after Spanish smoke-free legislation

Hipólito Pérez-Martín[1]; Cristina Lidón-Moyano[1*]; Adrián González-Marrón[1*]; Marcela Fu[2,3,4,5]; Raúl Pérez-Ortuño[6]; Montse Ballbè[2,3,5,7]; Juan Carlos Martín-Sánchez[1]; José A. Pascual[6,8***]; Esteve Fernández[2,3,4,5***]; Jose M. Martínez-Sánchez[1***]

[1]*Group of Evaluation of Health Determinants and Health Policies, Department of Basic Sciences, Universitat Internacional de Catalunya, Sant Cugat del Vallès, Spain.*

[2]*Tobacco Control Unit, Cancer Prevention and Control Program, Institut Català d'Oncologia, L'Hospitalet de Llobregat, Barcelona, Spain*

[3]*Tobacco Control Research Group, Epidemiology, public health, cancer prevention and palliative care program, Institut d'Investigació Biomèdica de Bellvitge - IDIBELL, L'Hospitalet de Llobregat, Spain*

[4]*School of Medicine and Health Sciences, Universitat de Barcelona, Barcelona, Spain*

[5]*CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain*

[6]*Group of Integrative Pharmacology and Systems Neuroscience, Neurosciences Programme, IMIM (Hospital del Mar Medical Research Institute), Parc de Recerca Biomèdica de Barcelona, Barcelona, Spain*

[7]*Addictions Unit, Institute of Neurosciences, Hospital Clínic de Barcelona, Barcelona, Spain*

[8]*Department of Medicine and Life Sciences, Universitat Pompeu Fabra, Parc de Recerca Biomèdica de Barcelona, Barcelona, Spain*

**Equally contribution and corresponding authors.*

**** The three authors can be considered senior authors.*

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CORRESPONDING AUTHORS

C. Author 1: Cristina Lidón-Moyano E-mail address 1: clidon@uic.es

C. Author 2: Adrián Gónzalez-Marrón E-mail address 2: agonzalezm@uic.es

Group of Evaluation of Health Determinants and Health Policies, Departament de Ciències Bàsiques, Universitat Internacional de Catalunya, Carrer de Josep Trueta s/n, 08195 Sant Cugat del Vallès, Barcelona, Spain.

DECLARATION OF INTEREST

The authors declare no potential conflicts of interest.

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ABSTRACT

Introduction: High levels of cotinine in non-smokers indicate passive exposure to tobacco smoke. This study aims to evaluate variations in salivary cotinine cut-offs to discriminate smokers and non-smokers before and after the implementation of smoke-free legislation (Law 28/2005 and Law 42/2010) in a sample of the adult population of Barcelona, Spain.

Methods: This longitudinal study analyzes salivary cotinine samples and self-reported information from a representative sample (n = 676) of the adult population from Barcelona before and after the approval of smoke-free legislation. We calculated the receiver operating characteristic (ROC) curves, to obtain optimal cotinine cut-off points to discriminate between smokers and non-smokers overall, by sex and age and their corresponding sensitivity, specificity, and area under the curve. We used linear mixed-effects models, with individuals as random effects, to model the percentage change of cotinine concentration before and after the implementation of both laws.

Results: The cut-off point was significantly lower post-2010 law (-85.8%, $p < 0.001$). The ROC curves determined that the optimal cotinine cut-off points for discriminating non-smokers and smokers were 10.8 ng/mL (pre-2005 law) and 5.6 ng/mL (post-2010 law), with a post-2010 law sensitivity of 92.6%, specificity of 98.4%, and an area under the curve of 97.0%. The post-2010 law cotinine cut-off points were 5.6 ng/mL for males and 1.9 ng/mL for females.

Conclusion: The implementation of Spanish smoke-free legislation was effective in reducing secondhand smoke exposure and, therefore, also in reducing the cut-off point for salivary cotinine concentration. This value should be used to better assess tobacco smoke exposure in this population.

1. INTRODUCTION

Smoking is considered a major risk factor for the health of both smokers and people exposed to smoke[1, 2]. According to the World Health Organization (WHO), eight million people died from tobacco use in 2020 and around one million of those deaths were of non-smokers exposed to second-hand smoke (SHS)[3]. It is estimated that around 15% of the global population were smokers in 2018[4]. To attenuate the tobacco epidemic, the WHO Framework Convention on Tobacco Control (WHO FCTC) reaffirmed the necessity of a consistent tobacco control legislation aiming at reducing demand, in addition to tax increase and regulation of tobacco producers and sellers, which several parties have already adopted[5]. In Spain, two laws came into effect. The first one (Law 28/2005) that came into effect on the 1st of January 2006[6] supposed a great improvement in public health since this law decreased the prevalence of smokers in the young population, as well as the acute myocardial infarction morbidity and the prevalence of SHS exposure in non-smokers[7, 8], but it still allowed smoking in some hospitality sectors. Hence, this law did not fully protect the population against passive smoking[5]. The second one (Law 42/2010) came into effect on the 2nd of January 2011, extending the prohibition of smoking in all public indoor areas without exceptions and in some outdoor areas[9]. With both laws in place, secondhand smoke exposure has since been significantly reduced[10].

Passive exposure and tobacco consumption can be estimated with the information obtained from various standardized questionnaires, a particularly useful tool when monitoring the evolution of the tobacco epidemic in the population[5, 11]. However, self-reported tobacco consumption is not always a reliable source of information and subjects may underreport it

due to the increasing population awareness of tobacco's negative health consequences[11]. To avoid this issue, biomarkers of tobacco exposure (e.g., nicotine, cotinine, trans-3-hydroxy-cotinine) have been broadly used as an objective measure to differentiate between smokers and non-smokers[11-13]. Nicotine is an addictive substance used as a biomarker that is present in a variety of tobacco products. Once a person is exposed to tobacco smoke, nicotine is mostly metabolized into cotinine within a few hours[14]. Cotinine has a much longer in vivo half-life (16-20 hours) than nicotine (2 hours) and can be measured in a variety of human fluids (e.g., saliva, plasma, blood, and urine)[15, 16], turning cotinine into a tobacco consumption biomarker widely studied. As cotinine concentration provides an objective measure of passive smoking in non-smokers and of tobacco use in smokers, cotinine cut-offs are a great resource for differentiating smoking status[11-13]. Based on our study conducted in 2009, the optimal cut-off point to discriminate smoking status in the adult population from Barcelona, Spain, was found to be 9.2 ng/mL[11]. However, this study was conducted between the implementation of both Spanish smoke-free legislations. Since passive exposure to tobacco smoke has decreased from 2009, we believe that this cut-off point needs to be reassessed. Our research group evaluated the impact of smoke-free legislation using cotinine from a general population cohort to validate the results[5, 10], but we did not evaluate if cotinine cut-offs changed after the implementation of both laws. Thus, this study aims to assess the changes in the salivary cotinine cut-offs in an adult sample of cigarette smokers and non-smokers before and after the implementation of the Spanish smoke-free legislation.

2. METHODS

We used data from a follow-up study "Determinants of Cotinine project-phase 3 (dCOT3 study)". This cohort study included data of the adult (>16 years) population in Barcelona (Catalonia, Spain). The baseline was carried out during the years 2004-2005 (n = 1,245) and one follow-up was realized during 2013-2014 (n = 736). Saliva samples were collected by trained staff in both interviews employing the same protocol that prevents contamination from recent smoking, and analyzed in the same lab using same validated procedures, which can be found elsewhere[11,17]. After rinsing their mouths and sucking a lemon candy (Smint ®) to stimulate saliva production, participants provided 9 mL of saliva by spitting it into a funnel placed in a test tube. Each sample was separated into 3 mL aliquots, and frozen to -20ºC for storage. The frozen samples were sent to the Municipal Institute for Medical Research (IMIM-Hospital del Mar) in Barcelona. Cotinine was measured by alkaline single liquid-liquid extraction with dichloromethane/isopropanol[18]. This method has a quantification limit of 0.10 ng/mL and a detection limit of 0.03 ng/mL.

We used the self-reported information on the smoking status and salivary cotinine concentration from the baseline and the follow-up[5, 11]. Subjects that did not have available salivary cotinine at baseline (n = 24) or the follow-up (n = 36) were excluded. The final sample included 676 individuals (Figure 1). The variable smoking status was self-reported with five possible options: 1) smoker of at least one cigarette a day; 2) occasional smoker (they smoke, but not every day); 3) former daily smoker (at least one year without smoking), but used to smoke at least one cigarette a day; 4) former non-daily smoker (at least one year without smoking), but used to smoke occasionally; 5) never smoker. For the purpose of our

analysis, former smokers (categories 3 and 4) and never smokers were all recategorized as non-smokers.

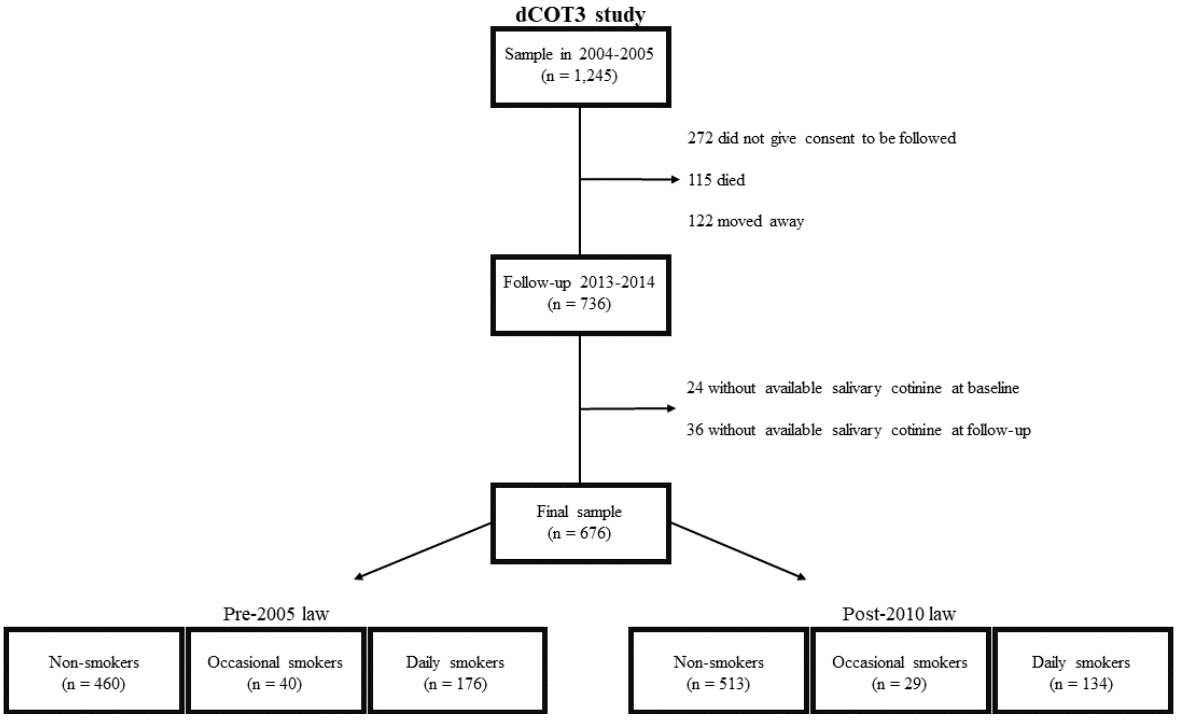

Figure 1: Flow chart of Samples for dCOT3 Pre-2005 law and Post-2010 law.

We used receiver operating characteristic (ROC) curves to obtain the areas under the curve (AUCs) and the optimal cotinine cut-off values to discriminate between smokers and non-smokers, with an approach to maximize the sum of sensitivity and specificity. In total, six ROC curves were obtained according to the smoking status, three corresponding to the data before the first law and three using the data after the law (pre-2005 law and post-2010 law, respectively). These ROC curves were calculated for: 1) smokers (daily and occasional) versus non-smokers; 2) daily smokers versus non-smokers, and 3) occasional smokers versus non-smokers.

To assess significant changes in cotinine geometric means, we used linear mixed-effects models, with individuals as random effects, adjusted to model the percentage change (and

95% confidence intervals) of salivary cotinine concentrations (after log 10 transformation) for the baseline and follow-up. Each analysis was stratified by sex and age. Age was categorized into two levels (17-44 years old and >44 years old) to ensure an equitable distribution of the sample and the quality of the ROC curves. Data were analyzed using R-4.0.4.

The data generated in this study are not publicly available as information could compromise participants consent but are available upon reasonable request from the corresponding authors.

RESULTS

The baseline sample for this study consisted of 460 (68.1%) non-smokers, 176 (26.0%) daily smokers, and 40 (5.9%) occasional smokers. In the follow-up after the implementation of the latest Spanish smoke-free legislation these numbers changed to: 513 (75.9%) non-smokers, 134 (19.8%) daily smokers, and 29 (4.3%) occasional smokers. The count and percentage at baseline of males in the sample was 310 (46%), and there were 365 (54%) people over 44 years old.

Table 1 shows the optimal post-2010 law cut-off points, sensitivity, specificity, and the area under the ROC curve, overall and stratified according to sex and age. The optimal cut-off point of salivary cotinine concentration that discriminates between smokers (daily and occasional) and non-smokers was 5.6 ng/mL, with a sensitivity of 92.6% and a specificity of 98.4% (AUC = 97.0%). The cut-off point was higher in males than in females (5.6 vs 1.9 ng/mL, with sensitivities and specificities higher than 93.0%). According to groups of age,

the optimal cut-off point was higher in individuals older than 44 years than in the group of 17 – 44 years (5.6 vs 1.3 ng/mL, with sensitivities and specificities higher than 90.0%).

Table 1: Optimal cut-off points of salivary cotinine concentration Post-2010 law (2013-2014), sensitivity, specificity and area under the curve for different comparisons, overall and according to sex and age.

	Post-2010 law					
	n (%)	% smokers	CP-Post (ng/mL)	Se (%)	Sp (%)	% AUC (95% CI)
Smokers (daily and occasional) vs. non-smokers						
Overall	676	24.1	5.6	92.6	98.4	97.0 (95.0; 99.0)
Sex						
Male	310 (45.9)	27.4	5.6	95.3	97.9	98.0 (96.0; 100.0)
Female	366 (54.1)	21.3	1.9	93.6	96.9	96.0 (92.0; 99.0)
Age (years old)						
17 - 44	311 (46.0)	32.8	1.3	97.1	94.3	98.0 (96.0; 100.0)
> 44	365 (54.0)	16.7	5.6	91.8	99.3	96.0 (92.0; 99.0)
Daily smokers vs. Non-smokers						
Overall	647	20.7	18.0	99.3	98.6	99.0 (98.0; 100.0)
Sex						
Male	295 (45.6)	18.2	18.0	99.9	97.8	100.0 (99.0; 100.0)
Female	352 (54.4)	23.7	26.0	98.4	99.3	99.0 (96.0; 1.0)
Age (years old)						
17 - 44	294 (45.4)	28.9	18.0	99.9	97.6	100.0 (99.0; 100.0)
> 44	353 (54.6)	13.9	26.0	98.0	99.3	98.0 (94.0; 100.0)
Occasional smokers vs. Non-smokers						
Overall	542	5.4	0.9	79.3	94.0	88.0 (79.0; 96.0)
Sex						
Male	240 (44.3)	6.3	0.9	86.7	93.3	92.0 (82.0; 99.0)
Female	302 (55.7)	4.6	1.9	71.4	96.9	84.0 (67.0; 98.0)
Age (years old)						
17 - 44	226 (41.7)	7.5	1.3	82.4	94.3	89.0 (77.0; 98.0)
> 44	316 (58.3)	3.8	0.9	75.0	94.8	87.0 (69.0; 99.0)

n: sample size; CP-Post: cut-off point post- 2010 law; Se: sensitivity; Sp: specificity; AUC: areas under the curves; CI: confidence interval

The optimal cut-off point that discriminates between daily smokers and non-smokers was 18.0 ng/mL, with a sensitivity of 99.3% and a specificity of 98.6% (AUC = 99.0%). The cut-off point was lower in males than in females (18.0 vs 26.0 ng/mL with sensitivities and

specificities higher than 97.0%). According to groups of age, the optimal cut-off point was higher in older individuals (18.0 vs 26.0 ng/mL with sensitivities and specificities higher than 97.0%).

The optimal cut-off point that discriminates between occasional smokers and non-smokers was 0.9 ng/mL, with a sensitivity of 79.3% and a specificity of 94.0% (AUC = 88.0%). The cut-off point was lower in males than in females (0.9 vs 1.9 ng/mL with sensitivities and specificities higher than 71.0%). According to groups of age, the optimal cut-off point was higher in individuals from 17 to 44 years than in those older than 44 years (1.3 vs 0.9 ng/mL with sensitivities and specificities higher than 75%). We found a leftward shift in the salivary cotinine concentration cut-off point after the implementation of the two laws (Figure 2).

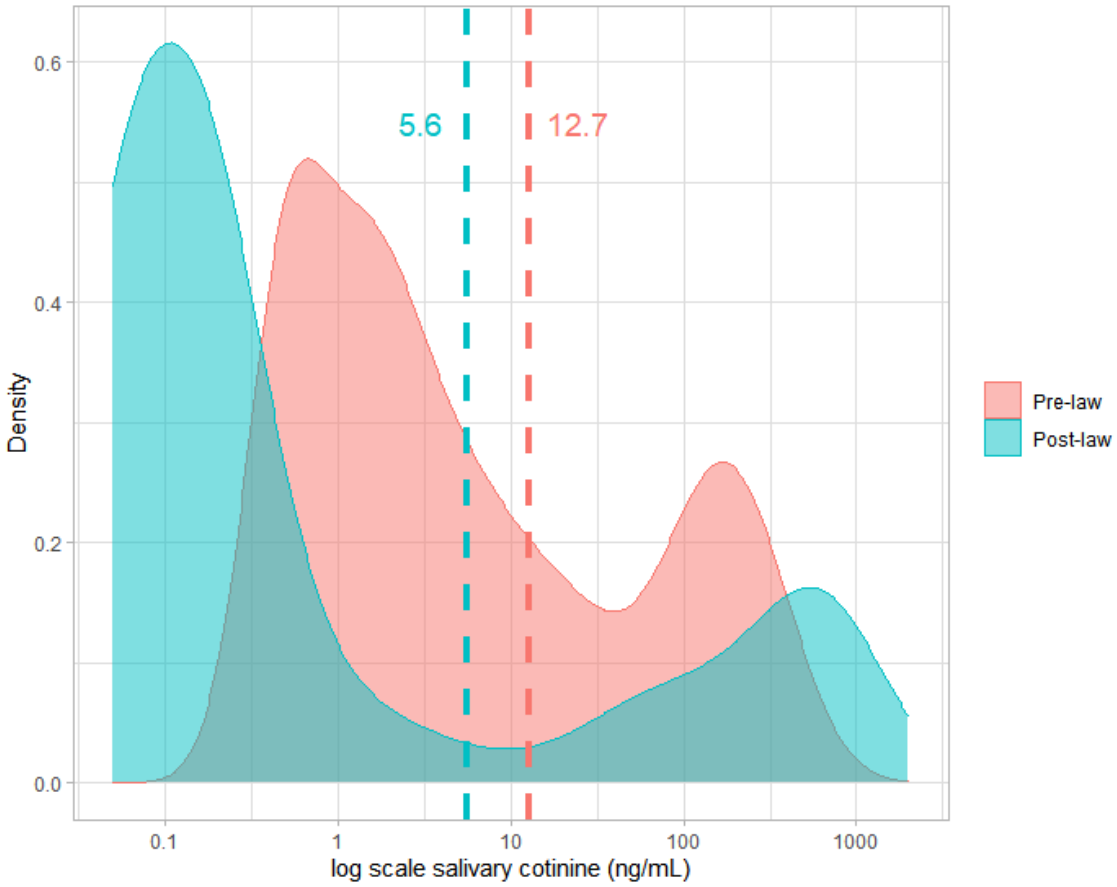

Figure 2: Distribution (histogram) of log cotinine in saliva (ng/mL) of individuals from the sample from the general population of Barcelona, Spain, before and after the implementation of Spanish smoke-free legislation, with cut-off points to distinguish smokers from non-smokers in both periods.

Significant differences in the cut-off points were found before and after the implementation of both Spanish laws. In all cases, the adjusted mean percentage change decreased by more than 80.0% (Table 2).

Table 2: Comparison of salivary cotinine concentration cut-off points and before the implementation of Law 28/2005 and after the implementation of Law 42/2010 in Spain and change percentage of cotinine geometric mean (with 95% confidence intervals) between different smoking status groups, overall and according to sex and age.

	n	CP – Pre (ng/mL)	CP – Post (ng/mL)	% change (95% CI)	p-value

Smokers (daily and occasional) vs. non-smokers					
Overall	676	10.8	5.6	-85.8 (-88.3; -82.8)	<0.001
Sex					
Male	310	25.1	5.6	-85.8 (-89.6; -80.7)	<0.001
Female	366	10.8	1.9	-85.8 (-88.9; -81.9)	<0.001
Age (years old)					
17 - 44	311	12.4	1.3	-81.2 (-86.2; -74.3)	<0.001
> 44	365	10.8	5.6	-88.8 (-91.2; -85.8)	<0.001
Daily smokers vs. Non-smokers					
Overall	647	22.6	18.0	-86.8 (-89.2; -84.0)	<0.001
Sex					
Male	295	25.1	18.0	-87.0 (-90.5; -82.2)	<0.001
Female	352	10.8	26.0	-86.7 (-89.6; -83.0)	<0.001
Age (years old)					
17 - 44	294	22.6	18.0	-83.1 (-87.7; -76.7)	<0.001
> 44	353	25.1	26.0	-89.3 (-91.6; -86.4)	<0.001
Occasional smokers vs. Non-smokers					
Overall	542	2.1	0.9	-93.8 (-94.8; -92.6)	<0.001
Sex					
Male	240	2.7	0.9	-94.7 (-96.0; -92.9)	<0.001
Female	302	1.8	1.9	-93.0 (-94.3; -91.3)	<0.001
Age (years old)					
17 - 44	226	3.5	1.3	-94.4 (-95.8; -92.6)	<0.001
> 44	316	2.1	0.9	-93.3 (-94.6; -91.7)	<0.001

CP-Pre: Cut-off point pre-2005 law; CP-Post: Cut-off point post- 2010 law; 95% CI: 95% confidence interval; % change (95% CI): Change percentage of cotinine geometric mean.

3. DISCUSSION

We found a significant decrease in the salivary cotinine concentration cut-off point to discriminate between smokers and non-smokers in Barcelona after the implementation of the Law 42/2010. The results were similar for the stratified models by sex and age; in all cases, the decrease was higher than 80% compared to 2005 values. There were also significant reductions in salivary cotinine concentration cut-off points when we compared non-smokers with daily smokers (86.8%) and non-smokers with occasional smokers (93.8%).

Similar studies indicate that the cut-off point for distinguishing between smokers and non-smokers have decreased in the last decades, coinciding with the implementation of tobacco

control legislation[12, 19-24]. We had previously estimated the salivary cotinine cut-off in 2005 to be 9.2 ng/mL, much lower than the ones estimated in the general population of other countries[11, 12, 19-24]; however, this cut-off point was calculated before the implementation of the new smoke-free legislation. In this study, we show that the general cotinine cut-off point in the population of Barcelona has decreased from 9.2 to 5.6 ng/mL after the implementation of the new legislation. The aforementioned decrease in the cotinine cut-off point is reflected in Figure 2, which shows a reduction in SHS exposure in nonsmokers after the update of the law (previously described by Fernández et al.[25] and Lidón-Moyano et al.[5]). This is sufficient proof of the effectiveness of this type of measures to reduce passive exposure to tobacco smoke. In addition, an increase in the mean salivary cotinine concentration of smokers is also observable, as described in a previous study by Lidón-Moyano et al.[26].

Despite this, the salivary cotinine cut-off point calculated post-2010 for adult non-smokers in Barcelona is still higher than other populations in advanced stages of the tobacco epidemic. In the U.S., previous studies of the adult population reported cut-off points around 3-4 ng/mL[12, 13, 25]. Although the cut-off point has lowered in Barcelona, passive exposure is still prevalent and tobacco control measures must continue to be implemented in order to diminish the tobacco epidemic in our population.

Optimal cut-off points varied between different types of smokers and non-smokers, being the cut-off point between non-smokers and daily smokers higher than that between non-smokers and occasional smokers. Furthermore, males have a higher cut-off point than females when comparing non-smokers and smokers, which is consistent with previously reported results with these same data before the law 42/2010 was approved[11]. However, in contrast with

these results, females have higher cut-off points than males when comparing non-smokers versus daily smokers and versus occasional smokers. In addition to this, in the particular case of daily smokers versus non-smokers, the cut-off calculated for females is higher after the implementation of the legislation. This may be a direct consequence of increased smoking prevalence and cigarette consumption among women between 40 and 64 years in the last 20 years as a result of their latest incorporation to the tobacco epidemic, resulting in an increase of their cotinine levels[27–29]. When comparing smokers (daily and occasional) vs. non-smokers, salivary cotinine cut-offs overall and stratified by sex in our sample have similar values to the ones reported in the U.S.A.[13,27]. Other aspect that could affect the reduction of the cut-off is the intensity (number of cigarettes per day) and duration of smoking (time of smoking). In this sense, we have self-reported information about the number of cigarettes smoked during the last 24 and 48 hours just before interview and saliva collection. We observed a statistically significant reduction in the number of cigarettes smoked in the last 24 and 48 hours before and after the coming into effect of Spanish smoking legislations (data not shown).

The overall optimal cut-off point post-2010 has sensitivity, specificity, and AUC values all greater than 90%, much higher values than the ones observed in the pre-2005 cut-off points —which are between 70% and 80%, but do not reach 90% (data not shown)—. It should be noted that salivary cotinine cut-offs for occasional smokers have lower sensitivity and specificity than for daily smokers. As salivary cotinine has a half-life of approx. 17 hours, the differences between the cut-offs observed suggest that cotinine may not be a good biomarker for occasional smokers as other biomarkers of long-term exposure (up to weeks

later) to tobacco smoke, like tobacco-specific nitrosamines[30–32]. Further studies should assess other biomarkers potentially more suitable for occasional smokers.

One limitation of our study is that we analyzed self-reported data. This kind of data may be subdued to information bias affecting the information on smoking status, and therefore, cotinine cut-off points. However, the participants agreed to provide a saliva sample for cotinine analysis, and thus this bias is almost negligible. Also, our results tally with the cut-off points reported in other studies after the implementation of tobacco control legislation[12,27]. In addition, the prevalence of users of other tobacco products and electronic cigarettes was very low in our sample (1.18%) so we could not control for type of product. However, due to the small sample the impact expected is limited. Another limitation of the study is that working with cohort data overestimates the elderly representation. Another limitation of the study is that working with cohort data overestimates the elderly representation. Accordingly, the sample was weighted to minimize limitations and the baseline sample was representative of the city of Barcelona, a full description of the methodology can be found elsewhere[26,30,33,34]. On the other hand, a strength of our study is to obtain updated cut-off values to distinguish between smokers and non-smokers, with different cut-off values by sex and age groups, being able to describe changes over time. Another strong point of this study is that it is the first study that includes data of cotinine in saliva (a matrix widely used to determine cotinine) in the general population which was collected before and after the implementation of Spanish smoke-free legislation.

There was a large reduction in salivary cotinine cut-off points to distinguish between smokers and non-smokers, non-smokers and daily smokers, and non-smokers and occasional smokers after the implementation of Spanish smoke-free legislation. The updated cut-off point to

discriminate between smokers and non-smokers is around 5.6 ng/mL in the adult population, but it differs according to sex and age. When possible, more specific cut-off points according to sex and age should be used.

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