



Determinants of self-reported smoking and misclassification during pregnancy, and analysis of optimal cut-off points of urinary cotinine: a cross sectional study

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Complete List of Authors:	Aurrekoetxea, Juan; University of Basque Country UPV/EHU, Public Health Department; Health Research Institute (BIODONOSTIA), Murcia-Hinarejos, Mario; Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), ; Center for Public Health Research (CSISP), Rebagliato, Marisa; Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Lopez, Maria; Agència de Salut Pública, ; Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Castilla, Ane; Public Health Laboratory of Bilbao, ; Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Santa Marina, Loreto; Basque Government, Public Health Department; Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Guxens, M; Center for Research in Environmental Epidemiology (CREAL), ; Hospital del Mar Research Institute (IMIM), Fernández-Somoano, Ana; Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), ; Universidad de Oviedo, Medicina Preventiva y Salud Pública Espada, Mercedes; Public Health Laboratory of Bilbao, Lertxundi, Aitana; University of Basque Country UPV/EHU, Public Health Department; Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Tardón, Adonina; University of Oviedo, Preventive Medicine and Public Health; Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Ballester, Ferran; University of Valencia, Nursing; CSISP, Environment and Health
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10 JJ Aurrekoetxea ^{1,2,3}, M Murcia ^{4,5}, M Rebagliato ^{4,5}, MJ López ^{4,6,7}, AM Castilla ^{1,4}, L Santa Marina ^{1,3,4}, M
11 Guxens ^{4,8,9}, A Fernández-Somoano ^{4,10}, M Espada ¹, A Lertxundi ^{2,3,4}, A Tardón ^{4,10}, F Ballester ^{4,5,11}
12
13
14 (Juan J Aurrekoetxea ^{1,2,3}, Mario Murcia ^{4,5}, Marisa Rebagliato ^{4,5}, María José López ^{4,6,7}, Ane Miren Castilla
15 ^{1,4}, Loreto Santa Marina ^{1,3,4}, Mónica Guxens ^{4,8,9}, Ana Fernández-Somoano ^{4,10}, Mercedes Espada ¹, Aitana
16 Lertxundi ^{2,3,4}, Adonina Tardón ^{4,10}, Ferran Ballester ^{4,5,11})
17
18

19
20
21 1 *Public Health Department, Basque Government, Spain*

22 2 *University of Basque Country UPV/EHU, Spain*

23 3 *Health Research Institute (BIODONOSTIA), Spain*

24 4 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP)*

25 5 *Center for Public Health Research (CSISP), Valencia, Spain*

26 6 *Public Health Agency of Barcelona, Spain*

27 7 *Institute of Biomedical Research (IIB Sant Pau), Spain*

28 8 *Center for Research in Environmental Epidemiology (CREAL), Spain*

29 9 *Hospital del Mar Research Institute (IMIM), Barcelona, Spain*

30 10 *University of Oviedo, Spain*

31 11 *University of Valencia, Spain*

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40
41
42
43 **Juan J Aurrekoetxea**, *Public Health Department, Basque Government, Spain;*

44 *University of Basque Country UPV/EHU, Spain;*

45 *Health Research Institute (BIODONOSTIA), Spain.*

46 jj.aurreko@gmail.com

47
48
49 **Mario Murcia**: *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

50 *Center for Public Health Research (CSISP); Valencia, Spain.*

51 murcia_mar@gva.es

52
53
54 **Marisa Rebagliato**, *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

55 *Center for Public Health Research (CSISP); Valencia, Spain.*

56 rebagliato_mar@gva.es

57
58
59 **Maria José López**, *Public Health Agency of Barcelona, Spain;*

1 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

2 *Institute of Biomedical Research (IIB Sant Pau), Spain.*

3 mjlopez@aspb.cat

4 **Ane Miren Castilla**, *Public Health Laboratory of Bilbao, Spain.*

5 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

6 ANE_M2002@yahoo.es

7 **Loreto Santa Marina**, *Public Health Department, Basque Government, Spain;*

8 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

9 *Health Research Institute (BIODONOSTIA), Spain.*

10 ambien4ss-san@ej-gv.es

11 **Mònica Guxens**, *Center for Research in Environmental Epidemiology (CREAL), Spain;*

12 *Hospital del Mar Research Institute (IMIM), Spain;*

13 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

14 mguxens@creal.cat

15 **Ana Fernández-Somoano**, *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

16 *University of Oviedo, Spain.*

17 capua.uo@uniovi.es

18 **Mercedes Espada**, *Public Health Department, Basque Government, Spain;*

19 metabobi-san@ej-gv.es

20 **Aitana Lertxundi**, *University of Basque Country UPV/EHU, Spain;*

21 *Health Research Institute (BIODONOSTIA), Spain;*

22 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

23 aitana.lertxundi@gmail.com

24 **Adonina Tardón**, *University of Oviedo, Spain;*

25 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

26 atardon@uniovi.es

27 **Ferran Ballester**, *University of Valencia, Spain;*

28 *Center for Public Health Research (CSISP), Valencia, Spain;*

29 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

30 ballester_fer@gva.es

31 Corresponding author: Juan J Aurrekoetxea Ph.D; Departamento de Sanidad del Gobierno Vasco; C/ Sancho el Sabio 35; 20.010, San

32 Sebastián; Telephone: 34.943.023033; Fax: 34.943.023093; E-mail: jj.aurreko@gmail.com

33 **Key words:** Pregnancy; Smoking; Validation studies; Cut-off; Urinary cotinine.

ABSTRACT

Objectives: To estimate the prevalence and factors associated with smoking and misclassification in pregnant women from INMA project, Spain, and to assess the optimal cut-offs for urinary cotinine (UC) that best distinguishes smokers from non-smokers.

Design: We used logistic regression models for study the relationship between sociodemographic variables and self-reported smoking and misclassification. ROC curves were used to calculate the optimal cut-off point for discriminating smokers from non-smokers. The cut-off points were also calculated after stratification among non smokers for SHS exposure.

Participants: At third trimester of pregnancy 2263 pregnant women of the INMA cohort were interviewed and a urine sample was collected for the quantification of UC.

Results: Prevalence of self-reported smokers at third trimester of pregnancy was 18.5%, and other 3.9% misreported their smoking status. Variables associated with self-reported smoking and misreporting were similar, including born in Europe, educational level, and exposure to second-hand smoke (SHS). The optimal cut-off for smoking discrimination was 82ng/ml (95% CI 42 to 136); sensitivity: 95.2% and specificity: 99.4%. The area under the ROC curve was 0.996 (95% CI 0.993 to 0.998). The cut-offs varied according to the SHS exposure level being 42 (95% CI 27 to 57), 82 (95% CI 55 to 136) and 106 ng/ml (95% CI 79 to 201) for not SHS exposed, exposed to one and to two or more sources of SHS, respectively. The optimal cut-off for discriminating occasional smokers from non-smokers was 27 ng/ml (95% CI 11 to 43); sensitivity: 89.2% and specificity: 90.6%.

Conclusions: Current efforts made to prevent smoking in pregnant women are insufficient as its high prevalence shows. UC is a reliable biomarker for classifying pregnant women according their tobacco consumption. However, cut-offs would differ based on baseline exposure to SHS in highly exposed populations.

ARTICLE SUMMARY

Article focus

The focus of this study is on:

- There is no current consensus regarding the cut-off point for urinary cotinine in pregnant women able to discriminate regular or occasional smokers from non-smokers,
- These cut-offs would also differ according to baseline exposure to second hand smoke (SHS)
- This study assess the maternal factors influencing both self-reported and misclassification of smoking; and evaluate the optimal cut-off point for urinary cotinine that best distinguishes smokers from non-smokers according to frequency of smoking and SHS exposure.

Key messages

- The prevalence of both smoking (18.5%) and SHS exposure (45.9%) was high in a population based sample of pregnant women in Spain.
- Factors associated with self-reported smoking and misreporting were similar, including lower level of education and living in a smoking environment, which highlights the need of reinforcing the preventive interventions and policies.
- The optimal cut-off point to discriminate smokers from non-smokers varied according to the frequency of smoking (occasional or daily smokers) and to SHS exposure levels.
- This study highlights the importance of SHS exposure for selecting reference cut-offs to discriminate smoking status, especially in high SHS exposed populations.

Strengths and limitations of this study

- This study has the ability to assess the role of baseline exposures to SHS in the estimate of cut-offs, given the detailed information collected on SHS exposure and its elevated prevalence.
- This study uses population based samples of pregnant women from the INMA birth cohort, which might not be fully representative of all pregnant women in the study areas,

INTRODUCTION

Risks for mother and foetus has been widely related to smoking during pregnancy.¹ Several studies have indicated that pregnant women tend to under-report their consumption of tobacco,²⁻⁸ due to social pressure⁹ or to avoid criticism from health professionals.³ Indeed, it is known to be a higher rate of misreporting of smoking among the groups in which it is not considered as acceptable, such as pregnant women and patients with smoking-related diseases.⁹

Cotinine is the main metabolite of nicotine and the biomarker of choice for distinguishing smokers from non-smokers and for assessing exposure to second-hand smoke (SHS).¹⁰ The women's clearance of cotinine is faster during pregnancy¹¹ and its plasma half-life is a little less than 9h.¹² For this reason, urinary cotinine (UC) tests may give false negatives in pregnant women who have not recently smoked.

There is no current consensus regarding the cut-off point for UC in pregnant women. Several thresholds have been proposed being 50 ng/ml, the most widely used.¹³⁻¹⁶ On the other hand, Higgins *et al*¹⁷ proposed 25 ng/ml as the cut-off point, while Gorber *et al*⁹ underlined the need to decide on a suitable threshold for pregnant women in particular, for whom the sensitivity of the test may be different, and also suggested that a new cut-off point should be established for occasional smokers. Spierto *et al*¹⁸ found 79 ng/ml as the cut-off between non-smoker and smoker pregnant women.

The aims of our study were: 1) to assess the prevalence of self-reported smoking and the UC levels in a cohort of pregnant women; 2) to assess the prevalence of misclassification of maternal smoking status according to the most widely accepted cut-off point in the literature of 50 ng/ml, and to study maternal factors associated with both self-reported and misclassification of maternal smoking; and 3) to identify the optimal cut-off point for UC that best distinguishes smokers from non-smokers in our study sample, according to frequency of smoking (occasional or daily smokers) and SHS exposure.

METHODS

Study population

The INMA [*Infancia y Medio Ambiente*, Environment and Childhood] project is a Spanish multi-centre prospective birth cohort study which aims to evaluate the impact of exposure to the most prevalent environmental pollutants, and the role of diet, on foetal and infant growth, health and development.¹⁹ From eligible pregnant women recruited between 2003 and 2008, a 56% agreed to participate. The inclusion criteria were at least 16 years of age, singleton pregnancy, enrolment at 10 to 13 weeks of gestation, no

1 assisted conception, delivery scheduled at the reference hospital, and no communication handicap. Of the
2
3 2644 women who agreed to participate in the study, 119 (4.5%) were lost (59 miscarriages, 8 foetal death, 47
4
5 withdrew and 5 lost to follow-up). Around week 32 their pregnancy 2263 of the 2525 remaining women
6
7 completed a questionnaire on smoking and other variables and 2290 provided urine samples for
8
9 determination of UC. The hospital ethics committee of each centre approved the research protocol and all
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11 pregnant women gave written informed consent before inclusion at the first trimester of pregnancy.
12

13 **Information concerning smoking**

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15 Questionnaire on tobacco consumption included smoking history, patterns of consumption (occasional or
16
17 regular) and exposure to SHS. We considered the women who, at this interview, reported smoking
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19 occasionally or daily to be smokers, regardless of their UC levels. Women who had UC levels higher than
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21 the widely used level of 50 ng/ml to distinguish smokers from non-smokers,¹³⁻¹⁶ but who did not report
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23 smoking, were classed as misclassified. It was considered that the participants were exposed to SHS when
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25 they reported exposure at least twice a week in any of the following environments: at work, at home, or in
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27 leisure time outside the home (e.g. bars/restaurants, or other homes). We analysed whether women had any
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29 passive exposure to tobacco smoke (yes or no), and also the number of exposure sources, between 0 and 3,
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31 according to the reported places of exposure: at work, at home and/or elsewhere in leisure time.
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34 **Urinary cotinine**

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36 The urine samples were collected in the same interview during the third trimester of pregnancy. Urine was
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38 collected in 100 ml polyethylene containers and stored at -20°C. One aliquot of the sample from each of the
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40 participants was sent to the Public Health Laboratory of Bilbao (Spain) to be analysed. All urine samples
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42 were stored for a minimum of one year and a maximum of 5 years before analysis. The analysis of the UC
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44 was performed by competitive enzyme immunoassay (EIA) using commercial EIA microplate test kits
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46 (OraSure Technologies, Inc., Bio-Rad) for determining salivary cotinine adapted for urine samples using
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48 urine controls (0, 2.5, 10 and 50 ng/ml, Bio-Rad). Samples with UC levels above 50 ng/ml were diluted.
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50 Before testing the urine samples the method was validated; a certified reference material was used
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52 (EPA/NIST Reference Material 8444) to evaluate the repeatability and reproducibility. The quantification
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54 limit was 4.0 ng/ml, the coefficient of repeatability 7% and the reproducibility 10%.
55

56 **Other variables**

1 The women were interviewed twice during pregnancy (first and third trimester of gestation) to obtain
2 information about their sociodemographic characteristics and life-style variables. Social status of the women
3 (or her partner, if she had never worked outside the home) was defined using Spanish adaptation of British
4 classification system.²⁰
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9 **Statistical analysis**

10 The chi-square test was used to test hypotheses for categorical variables, while the distribution of UC was
11 assessed using the Mann-Whitney U and the Kruskal Wallis tests for variables with two or more categories,
12 respectively. In order to identify the variables independently associated with being either a smoker or a
13 misclassified, and both, logistic regression models were built including geographical area and the variables
14 related with the outcome at $p < 0.10$ in the univariate analysis, and sequentially excluding those variables not
15 related at $p < 0.10$ in the adjusted model using the likelihood ratio test. For ordinal categorical variables, the p
16 for a linear trend was also calculated. We used the receiver operating characteristic (ROC) curve to analyse
17 the relationship between the sensitivity and false positive cases for various different cut-off points that
18 dichotomize UC to distinguish smokers from non-smokers, using self-reported cigarette smoking status as
19 the reference value. Overall accuracy was evaluated by means of the area under the curve (AUC). The
20 Youden's index was calculated as the optimal cut-off point that maximizes sensitivity+specificity-1.
21 Confidence intervals for the optimal cut-off point were established using bootstrap resampling procedures.
22 Specifically, the data for each of the cohorts and the overall results were analysed with the level of UC given
23 by the Youden's Index and for the most widely used cut-off points, namely 50 and 100 ng/ml, or 25 and 50
24 ng/ml when analyses were restricted to occasional smokers. Women who declared that they did not smoke
25 but with UC levels above 200 ng/ml were excluded from the analyses. The cut-off points were also
26 calculated after stratification among non smokers for SHS exposure. Additional sensitivity analysis excluded
27 self reported non-smokers who claimed to stop smoking during pregnancy, since this group is more likely to
28 misreport their smoking status. Statistical analysis was carried out using SPSS (version 17.0) and R (2.10.0)
29 statistical software.
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52 **RESULTS**

53 **Study setting and characteristics of the sample**

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Overall, 61.2% of women reported to have smoked at least once in their life, while 32.4% were occasional or regular smokers when they became pregnant, falling to 19.7% at first trimester and 18.5% at third trimester of their pregnancy (Table 1).

Table 1: Description of the sample and variables of interest.

	N ^a	%
Cohort		
Asturias	416	18.4
Gipuzkoa	545	24.1
Sabadell	591	26.1
Valencia	711	31.4
Age		
≤ 24	154	6.8
25-29	717	31.7
30-34	973	43.0
≥ 35	418	18.5
Social class		
I-II (more affluent)	492	21.8
III	584	25.8
IV-V (less affluent)	1186	52.4
Level of education		
Primary or no education	547	24.2
Secondary	936	41.4
University	776	34.4
BMI (pre-pregnancy)		
<18.5	100	4.4
18.5 – 25	1568	69.3
25 – 30	420	18.6
≥ 30	175	7.7
Previous parity	957	43.1
Birth In Europe	2130	94.3
Reported having smoked in their life		
No	879	38.8
Occasional	146	6.5
Regular	1238	54.7
Reported smoking at the start of pregnancy		
No	1529	67.6

Occasional	28	1.2
Regular	706	31.2
Reported smoking at first trimester of pregnancy		
No	1813	80.3
Occasional	35	1.6
Regular	410	18.2
Reported smoking at third trimester of pregnancy		
No	1845	81.5
Occasional	37	1.6
Regular	381	16.8
Cigarettes/day at third trimester of pregnancy		
0	1845	81.5
Occasional	37	1.6
1-4	149	6.6
5-9	141	6.2
≥ 10	91	4.0
Exposed to SHS in non-smoking women:		
At home (partner or others)	479	26.0
At work	186	10.1
Elsewhere in leisure time ^b	715	38.8
Number of sources of exposure to SHS ^c		
0	798	43.5
1	735	40.0
2	271	14.8
3	32	1.7
Cotinine (ng/ml) all the women		
< 50	1773	78.3
50-99	31	1.4
100-199	19	0.8
200-499	52	2.3
500-999	70	3.1
≥ 1000	318	14.1

a: The numbers and rates that do not match the total are due to missing data

b: Other homes or public places, e.g. pubs or restaurants

c: Work, home and elsewhere in leisure time among non smokers

Smoking and SHS exposure

The median UC level in women who did not refer to smoke and were not exposed to SHS was below the quantification level of 4.0 ng/ml while in non-smokers exposed to SHS it was 7.6 ng/ml. Among all smokers the UC median level was 1744.3 ng/ml (Table 2). Occasional smokers had a median level of 260.7 ng/ml. Among daily smokers a clear trend was observed between UC concentration and the number of cigarettes smoked per day ($p < 0.001$). In the same way, in non-smokers there was also a trend between UC levels and the number of sources of exposure to SHS; that are, work, home and elsewhere in leisure time ($p < 0.001$). Figure 1 shows the different distribution patterns of UC among non-smokers, exposed or not to SHS, and occasional and daily smokers.

Table 2: Active smoking and exposure to SHS in pregnant women in the INMA cohort. Median levels of urinary cotinine (ng/ml) at third trimester of pregnancy.

	N	%	Urinary cotinine ^a
Total	2263	100	7.4
Non smokers ^b	1845	81.5	4.4
No SHS exposure	798	35.3	< 4
SHS exposure	1038	45.9	7.6
1 source ^{c, d}	735	32.5	5.8
2 sources	271	12.0	11.7
3 sources	32	1.4	16.9
Smokers ^d	418	18.5	1744.3
Occasional	37	1.6	260.7
1-2 cigarettes/day	76	3.4	1036.4
3-4 cigarettes/day	73	3.2	1330.7
5-9 cigarettes/day	141	6.2	1848.5
≥ 10 cigarettes/day	91	4.0	3033.0

a: Median level of urinary cotinine ng/ml.

b: Exposed and not exposed to SHS; Mann-Whitney test: $p < 0.001$ for smoking and urinary cotinine

c: Sources of exposure to SHS at work/at home/in leisure time outside the home

d: Kruskal Wallis test < 0.001

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Figure 1: Distribution of urinary cotinine (ng/ml) according to active or passive tobacco exposure in pregnant women from the INMA cohort. (Me: median).

SHS: Second-hand smoking
SRS Occas: Self reported smoking, occasional
SRS Daily: Self reported smoking, daily

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Self-reported smoking and misclassification

Of the 2263 women studied, 1755 (77.6%) reported that they did not smoke and had UC levels below 50 ng/ml (true negative). A further 18 (0.8%) also had UC levels under 50 ng/ml despite claiming to smoke, though 13 of these claimed to be occasional smokers. On the other hand, 90 women (3.9%) reported that they did not smoke but were found to have UC levels above 50 ng/ml and were considered as misclassified and, finally, 400 women (17.7%) were true positive. Table 3 shows the ORs of the variables associated with smoking and misclassification, before and after adjusting. In the adjusted model, the risk of smoking and misclassification were associated with low educational level, country of birth, and exposure to SHS. Age was related only with misclassification risk. In relation with smoking history, only smoking at the beginning of pregnancy was associated with misclassification. Adding women misclassified to self-reported smokers did not vary the pattern of the association found with self-reported smoking.

Table 3: Unadjusted and adjusted odds ratios (ORs) and variables associated with smoking, self-reported and misclassification of smoking status.

	Unadjusted analysis							Adjusted analysis ^a					
	Non-smokers ^b		Self-reported smokers ^c			Misclassification ^d		Self-reported smokers ^c		Misclassification ^d		Both ^e	
	N	OR	95% CI	N	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	
Total	1755	418	-	90	-	-	-	-	-	-	-	-	
Cohort													
Asturias	326	75	1	-	15	1	-	1	-	1	-	-	
Gipuzkoa	467	65	0.60	0.42 to 0.88	13	0.60	0.27 to 1.36	0.74	0.50 to 1.11	0.62	0.28 to 1.37	0.72	0.50 to 1.05
Sabadell	466	99	0.92	0.66 to 1.29	26	1.21	0.61 to 2.44	0.67	0.47 to 0.98	0.80	0.40 to 1.61	0.72	0.51 to 1.01
Valencia	496	179	1.57	1.15 to 2.15	36	1.58	0.82 to 3.07	0.94	0.67 to 1.33	0.85	0.43 to 1.66	0.95	0.69 to 1.32
Social class													
I+II (highest)	438	41	1	-	13	1	-	-	-	-	-	-	
III	469	96	2.19	1.48 to 3.22	19	1.36	0.67 to 2.80	-	-	-	-	-	
IV+V (lowest)	847	281	3.54	2.50 to 5.02	58	2.31	1.25 to 4.26	-	-	-	-	-	
				(p ^f < 0.001)			(p ^f = 0.002)						
Level of education													
University	695	59	1	-	22	1	-	1	-	-	-	1	-
Secondary	703	192	3.22	2.36 to 4.39	41	1.84	1.09 to 3.17	2.52	1.81 to 3.50	-	-	2.22	1.66 to 2.98
Primary or less	355	165	5.47	3.96 to 7.57	27	2.40	1.34 to 4.32	3.52	2.47 to 5.02	-	-	2.95	2.14 to 4.05
				(p ^f < 0.001)			(p ^f = 0.002)						
Age													
≤ 24	93	43	1	-	18	1	-	-	-	1	-	-	-
25-29	537	152	0.62	0.40 to 0.94	28	0.27	0.14 to 0.53	-	-	0.38	0.19 to 0.75	-	-
30-34	792	153	0.42	0.27 to 0.64	28	0.18	0.09 to 0.36	-	-	0.27	0.13 to 0.53	-	-
≥ 35	332	70	0.46	0.29 to 0.73	16	0.25	0.12 to 0.54	-	-	0.45	0.21 to 0.98	-	-
				(p ^f < 0.001)			(p ^f = 0.001)						

Country of birth														
In Europe	1637	410	1	-	83	1	-	1	-	-	1	-		
Outside Europe	114	8	0.28	0.13 to 0.60	7	1.21	0.50 to 2.79	0.26	0.12 to 0.56	-	0.41	0.23 to 0.74		
Exposure to SHS at home														
No	1328	144	1	-	34	1	-	1	-	1	-	1	-	
Yes	423	274	5.97	4.75 to 7.51	56	5.17	3.33 to 8.03	4.35	3.40 to 5.57	3.19	1.99 to 5.12	4.37	3.48 to 5.49	
Exposure to SHS at work														
No	1582	343	1	-	76	1	-	1	-	-	1	-		
Yes	172	74	1.98	1.48 to 2.67	14	1.69	0.94 to 3.06	1.53	1.09 to 2.14	-	1.55	1.13 to 2.12		
Exposure to SHS elsewhere in leisure time														
No	1082	175	1	-	44	1	-	1	-	1	-	1	-	
Yes	669	243	2.25	1.81 to 2.79	46	1.69	1.11 to 2.59	1.88	1.47 to 2.40	1.51	0.95 to 2.39	1.84	1.47 to 2.31	
Reported having smoked in their life ^g														
No	862	0	-	-	17	1	-	-	-	-	-	-	-	
Yes	893	418	-	-	73	4.15	2.43 to 7.09	-	-	-	-	-	-	
Reported smoking at the start of pregnancy ^g														
No	1489	1	-	-	39	1	-	-	-	1	-	-	-	
Yes	266	417	-	-	51	7.32	4.73 to 11.33	-	-	5.48	3.46 to 8.68	-	-	

a: Only variables showed in the table were entered in the logistic equation

b: Non-smokers: women who reported that they did not smoke and were found to have urinary cotinine levels of less than 50 ng/ml. the reference group

c: Smokers: those who reported smoking

d: Misclassification: those who claimed that they did not smoke but were found to have urinary cotinine levels above 50 ng/ml

e: Both: c+d

f: p for trend

g: Only analysed with regards to misclassification. given the extremely strong association with smoking at third trimester of pregnancy

Cut-off points of UC for smoking

Optimal cut-off points for distinguishing non-smokers from smokers (daily and occasional) calculated by the Youden's index (excluding self reported non-smokers with UC values above 200 ng/ml), was 82 ng/ml, with a sensitivity of 95.2%, specificity of 99.4% and AUC 0.996 (95% CI 0.993 to 0.998) (Table 4). Sensitivity and specificity for the cut-off points of 50 and 100 ng/ml were quite close to that of 82 ng/ml. The exclusion from the analysis of 277 women who declared quitting smoking during pregnancy as possible group at risk of misclassification, did not improve the validation parameters of the test (data not shown).

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Table 4: Parameters for assessing the optimal cut-off point for urinary cotinine, ng/ml, obtained by the Youden's index, as well as the levels of 25, 50, and 100 ng/ml, for classifying pregnant women as regular or occasional smokers.^a

Youden's index ^b	Cut-off point (95% CI) ^c	Sensitivity	Specificity	Positive PV ^d	Negative PV ^d	AUC of the ROC (95% CI) ^e
Regular and occasional smokers: 1792 non-smokers. 418 smokers						
-	50	0.957	0.979	0.915	0.99	
-	100	0.950	0.995	0.978	0.988	0.996 (0.993 to 0.998)
0.947	82 (42 to 136)	0.952	0.994	0.975	0.989	
Results stratified by frequency of smoking						
Occasional smokers: 1792 non-smokers. 37 smokers						
-	25	0.892	0.896	0.150	0.998	
-	50	0.649	0.979	0.393	0.993	0.966 (0.942 to 0.986)
0.798	27 (11 to 43)	0.892	0.906	0.164	0.998	
Daily smokers: 1792 non-smokers. 381 smokers						
-	50	0.987	0.979	0.910	0.997	
-	100	0.982	0.995	0.977	0.996	0.998 (0.997 to 1)
0.978	115 (57 to 189)	0.982	0.996	0.982	0.996	
Results stratified by SHS exposure among non-smokers						
Non exposed to SHS: 791 non-smokers. 418 active smokers						
-	50	0.957	0.996	0.993	0.978	
-	100	0.950	0.999	0.997	0.974	0.998 (0.997 to 1)
0.962	42 (27 to 57)	0.971	0.991	0.983	0.985	
Exposed to SHS (1 source^f): 707 non-smokers. 418 active smokers						
-	50	0.957	0.972	0.952	0.974	
-	100	0.950	0.996	0.993	0.971	0.995 (0.991 to 0.998)
0.948	82 (55 to 136)	0.952	0.996	0.993	0.972	
Exposed to SHS (2-3 sources^f): 285 non-smokers. 418 active smokers						
-	50	0.957	0.951	0.966	0.938	
-	100	0.950	0.982	0.988	0.930	0.991 (0.985 to 0.996)
0.936	106 (79 to 201)	0.950	0.986	0.990	0.930	

a: Excluding cases with cotinine > 200 ng/ml in self-reported non-smokers (n=53).

b: Youden's index = max (Sensitivity+Specificity-1).

c: 95% bootstrap confidence interval for the cut-off point associated with the Youden's index.

d: Predictive value of a positive or negative result for the prevalence of the study. 18.5%.

e: Area under the receiver operating characteristic curve and 95% confidence interval.

f: Number of sources of exposure among: work, home and elsewhere in leisure time.

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4 The Youden's index and AUC for daily smoking were higher, with a cut-off point of 115 ng/ml. Occasional
5 smoking was analysed, by excluding from the analysis the 381 women who admitted that they smoked
6 regularly at third trimester of pregnancy. The optimal cut-off point for discriminating occasional smokers
7 from non-smokers was 27 ng/ml (95% CI 11 to 43), with a sensitivity and specificity of 89.2% and 90.6%,
8 respectively. The exclusion of women who declared to quit during pregnancy, improved the specificity to
9 92.1%, but did not almost change the Youden's Index or the sensitivity.

10 Not exposed women to SHS compared with all smokers, daily or occasional, had a lower cut-off point of 42
11 ng/ml (95% CI 27 to 57), while for exposed to one or to two or more sources of SHS, cut-off points were 82
12 (95% CI 55 to 136) and 106 ng/ml (95% CI 79 to 201), respectively.
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14

15 **DISCUSSION**

16 **Main findings in relation to the literature**

17 The prevalence of smoking in pregnant women at third trimester was 18.5%. In this later stage, the
18 prevalence of active smoking increased up to 22.5% if women who did not report smoking but had UC levels
19 above 50 ng/ml were reclassified as smokers, assuming that false positives were due to maternal
20 misreporting of smoking status. Prevalence of self reported smokers and misclassified in our study is close to
21 the referred by Kendrick *et al*⁶ and Lindqvist *et al*⁷, and smoking rate and UC levels lower than that showed
22 by Pickett *et al*¹⁵. Our study had, nevertheless, a lower rate of smoking misreporting than other studies.²⁻⁸

23 There was a clear relationship between UC and smoking dose among smokers, and with the number of
24 sources of exposure to SHS among non-smokers. Specifically, those who smoked 10 or more cigarettes per
25 day had median UC levels of 3033 ng/ml, while the levels were 260 ng/ml for occasional smokers and less
26 than 17 ng/ml for non-smokers, increasing with the number of sources of exposure to SHS. This data
27 reinforces the validity of UC also as an indicator of exposure to SHS.¹⁰

28 England *et al*¹³ indicated that few studies have identified differences between misclassified and self-reported
29 smokers and the way in which this would affect epidemiological studies. Our study shows similar patterns of
30 association and both self-reported smoking and misclassification were strongly associated with various
31 predictive variables. In particular, we found a higher risk of smoking and misclassification among women
32 with low education level. These results are consistent with those reported by other authors.^{6 14 21} We also
33 found a higher risk of smoking and misclassification in women from Europe, and women exposed to SHS in
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1 different places. Women younger than 24 years had an increased risk of misreport her smoking, as indicated
2 by Dietz *et al.*⁴ In this study, exposure to SHS was associated with smoking. In other words, there were more
3 smoking people around pregnant women who smoked. In addition, misclassification was significantly
4 associated with exposure to SHS at home. Jhun *et al.*¹⁴ and Orr *et al.*²² also showed higher prevalence of
5 smoking among pregnant women whose partners smoked at home. Having smoked previously was
6 associated with a higher probability of misreporting the habit, as observed by England *et al.*¹³

7
8 This work showed an optimal cut-off point for discriminate pregnant women smokers from non-smokers of
9 82 ng/ml, with a confidence interval of 50 and 100 ng/ml. Some studies proposed a cut-off of 50 ng/ml,¹³⁻¹⁶
10 coherent with the women not SHS exposed in this study. Other studies proposed cut-off points of 79 ng/ml¹⁸
11 and 85 ng/ml,⁶ closer to the smoking dose and SHS exposure in our study sample. In our study population
12 both prevalence of smoking and of exposure do SHS are high and this can explain in part why our optimal
13 cut-off point is higher than those reported in other studies.¹³⁻¹⁷ This is also supported by the fact that the
14 optimal cut-off point decreased to 42 ng/ml (27-57) when the analysis was restricted to women who reported
15 no SHS exposure, and increased according to the number of sources reported.. The validity of 27 ng/ml as
16 cut-off point for differentiating occasional smokers from non-smokers was lower than that for differentiating
17 daily smokers, and it could depend on SHS exposure and on the time spent from the last cigarette smoked
18 given the faster elimination of cotinine in pregnant women.¹⁰⁻¹²

36 **Limitations of the study**

37
38 The current study has several limitations. From the eligible population, the participation rate was 56%, and
39 85.6% of the women who agreed to participate completed the study. Therefore, the final study sample might
40 not be fully representative of all pregnant women in the study areas, but its internal validity is not necessarily
41 affected. There were other likely sources of misclassification in addition to maternal misreporting of
42 smoking status, as misclassification of non-smokers as smokers because of high exposure degree to SHS. On
43 the other hand, women who smoked occasionally but report to be non-smokers might have low UC
44 concentrations if they had not smoked recently, and their self-report and UC levels would be in agreement
45
46 Since the optimal cut-off point for UC is determined using self-reported smoking status as the gold standard,
47 the validity of this assumption is important. On the one hand, it is improbable that a non-smoking woman
48 declared to be a smoker, because a battery of items should be completed detailing smoking habits in this
49 case. On the other hand, however, it is possible that some smokers did not reveal their habit. In order to
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1 minimize this type of bias, we excluded in the main analysis self-reported non-smokers with implausible
2 high UC levels. In additional analysis, we excluded self-reported non-smokers who claimed to stop smoking
3 during pregnancy, since these cases are at higher risk of misclassification as reported in table 3; the optimal
4 cut-off point did not change after this exclusion. In general terms, the AUC shows a good overall accuracy,
5 and we think that self-reported smoking is a reliable measure in this study. If some kind of misclassification
6 occurs, it would lead to a shift towards the right in both distributions, and a slight overestimation of the
7 optimal cut-off point as a result.

8 One of the main strengths of this study was the possibility of assessing the role of baseline exposures to SHS
9 in the estimate of cut-offs, given the detailed information collected on SHS exposure and its elevated
10 prevalence. The confirmation that the cut-offs would differ according to the level of exposure to SHS
11 emphasizes the need of taking it into account, especially in countries with elevated SHS exposure.

12 **Implications for practice**

13 This study shows that the efforts made to encourage women to give up smoking before or during pregnancy
14 are not sufficient or particularly effective, given that at least 18.5% of the pregnant women smoked in the
15 third trimester. The results of this study indicate that the groups to which the most effort should be directed
16 are young women, those of a European origin and those from a low social class. Further, the association
17 observed in this study between active smoking of pregnant women and the presence of smokers in their close
18 environment supports the hypothesis that this factor makes it more difficult to stop smoking.²³ It is necessary
19 to undertake effective programmes for reducing smoking before and during pregnancy, reaching also
20 misclassified, and to reduce SHS exposure, in order to prevent risks for women and foetus.

21 **CONCLUSION**

22 Smoking is an important risk factor for health and development and should be taken into account as
23 confounder when analysing the potential effects of environmental contaminants in studies like the INMA
24 project. To have a reliable marker like UC and a valid a cut-off point able to discriminate regular or
25 occasional smokers from non-smokers is a critical issue. The cut-off point of 82 ng/ml showed a good
26 validity for discriminating smokers from non-smokers in our study sample while 27 ng/ml is the optimal
27 point for discriminating occasional smokers from non-smokers. It should be emphasized that cut-offs would
28 differ based on baseline exposure to SHS, and this should be taken into account when selecting reference
29 cut-offs for specific populations.

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4 **Contributors:** All authors contributed to various aspects of this paper. JJA, MM and MR designed the study
5 and analysed the data. AMC and ME analysed cotinine in urine samples. MJL, AMC, LSM, MG, AF-S, ME,
6 AL, AT and FB revised the design of the study and the results. JJA redacted the manuscript and the other
7 authors participated in the review of the different drafts and approved the final version.
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10
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19

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21

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23

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31 participantes/en_entidades-colaboradoras/](http://www.proyectoinma.org/instituciones-participantes/en_entidades-colaboradoras/)
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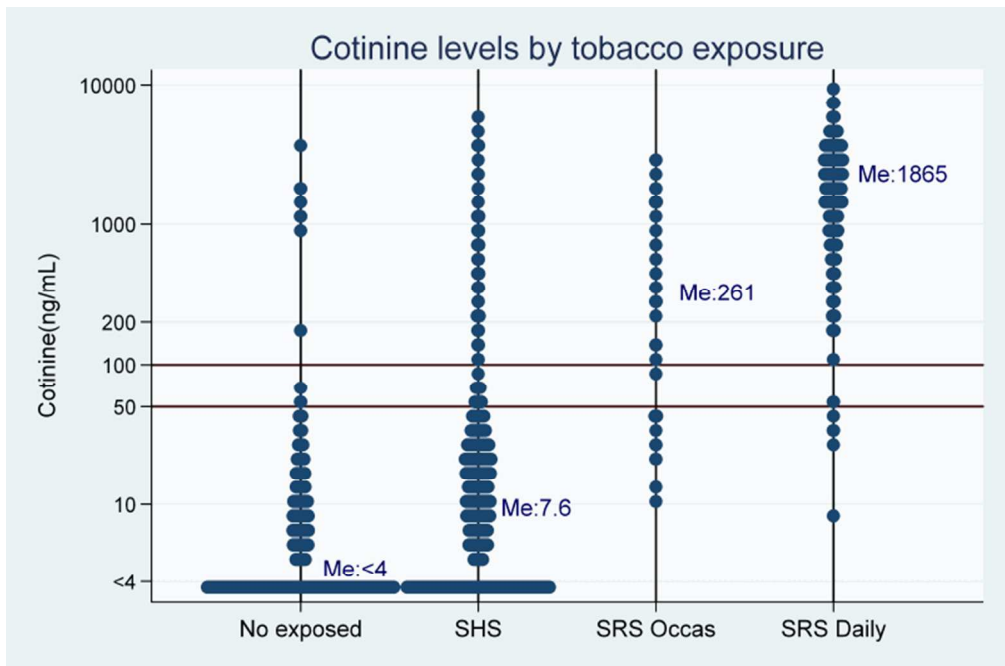
40 **Data Sharing Statement:** There is no additional data available.
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Determinants of self-reported smoking and misclassification during pregnancy, and analysis of optimal cut-off points of urinary cotinine: a cross sectional study

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5 **Determinants of self-reported smoking and misclassification during pregnancy, and analysis**
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7 **of optimal cut-off points of urinary cotinine: a cross sectional study**
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10 JJ Aurrekoetxea ^{1,2,3}, M Murcia ^{4,5}, M Rebagliato ^{4,5}, MJ López ^{4,6,7}, AM Castilla ^{1,4}, L Santa Marina ^{1,3,4}, M
11 Guxens ^{4,8,9}, A Fernández-Somoano ^{4,10}, M Espada ¹, A Lertxundi ^{2,3,4}, A Tardón ^{4,10}, F Ballester ^{4,5,11}
12
13
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16 Lertxundi ^{2,3,4}, Adonina Tardón ^{4,10}, Ferran Ballester ^{4,5,11})
17
18

19
20
21 1 *Public Health Department, Basque Government, Spain*

22 2 *University of Basque Country UPV/EHU, Spain*

23 3 *Health Research Institute (BIODONOSTIA), Spain*

24 4 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP)*

25 5 *Center for Public Health Research (CSISP), Valencia, Spain*

26 6 *Public Health Agency of Barcelona, Spain*

27 7 *Institute of Biomedical Research (IIB Sant Pau), Spain*

28 8 *Center for Research in Environmental Epidemiology (CREAL), Spain*

29 9 *Hospital del Mar Research Institute (IMIM), Barcelona, Spain*

30 10 *University of Oviedo, Spain*

31 11 *University of Valencia, Spain*

32
33
34
35
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37
38
39
40
41
42
43 **Juan J Aurrekoetxea**, *Public Health Department, Basque Government, Spain;*

44 *University of Basque Country UPV/EHU, Spain;*

45 *Health Research Institute (BIODONOSTIA), Spain.*

46 jj.aurreko@gmail.com

47
48
49 **Mario Murcia**: *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

50 *Center for Public Health Research (CSISP); Valencia, Spain.*

51 murcia_mar@gva.es

52
53
54 **Marisa Rebagliato**, *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

55 *Center for Public Health Research (CSISP); Valencia, Spain.*

56 rebagliato_mar@gva.es

57
58
59 **Maria José López**, *Public Health Agency of Barcelona, Spain;*

1 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

2 *Institute of Biomedical Research (IIB Sant Pau), Spain.*

3 mjlopez@aspb.cat

4 **Ane Miren Castilla**, *Public Health Laboratory of Bilbao, Spain.*

5 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

6 ANE_M2002@yahoo.es

7 **Loreto Santa Marina**, *Public Health Department, Basque Government, Spain;*

8 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

9 *Health Research Institute (BIODONOSTIA), Spain.*

10 ambien4ss-san@ej-gv.es

11 **Mònica Guxens**, *Center for Research in Environmental Epidemiology (CREAL), Spain;*

12 *Hospital del Mar Research Institute (IMIM), Spain;*

13 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

14 mguxens@creal.cat

15 **Ana Fernández-Somoano**, *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

16 *University of Oviedo, Spain.*

17 capua.uo@uniovi.es

18 **Mercedes Espada**, *Public Health Department, Basque Government, Spain;*

19 metabobi-san@ej-gv.es

20 **Aitana Lertxundi**, *University of Basque Country UPV/EHU, Spain;*

21 *Health Research Institute (BIODONOSTIA), Spain;*

22 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

23 aitana.lertxundi@gmail.com

24 **Adonina Tardón**, *University of Oviedo, Spain;*

25 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

26 atardon@uniovi.es

27 **Ferran Ballester**, *University of Valencia, Spain;*

28 *Center for Public Health Research (CSISP), Valencia, Spain;*

29 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

30 ballester_fer@gva.es

31 Corresponding author: Juan J Aurrekoetxea Ph.D; Departamento de Sanidad del Gobierno Vasco; C/ Sancho el Sabio 35; 20.010, San

32 Sebastián; Telephone: 34.943.023033; Fax: 34.943.023093; E-mail: jj_aurreko@gmail.com

33 **Key words:** Pregnancy; Smoking; Validation studies; Cut-off; Urinary cotinine.

ABSTRACT

Objectives: To estimate the prevalence and factors associated with smoking and misclassification in pregnant women from INMA [*Infancia y Medio Ambiente*, Environment and Childhood] project, Spain, and to assess the optimal cut-offs for urinary cotinine (UC) that best distinguishes daily and occasional smokers with varying levels of SHS exposure.

Design: We used logistic regression models to study the relationship between sociodemographic variables and self-reported smoking and misclassification (self-reported non-smokers with UC >50ng/ml). ROC curves were used to calculate the optimal cut-off point for discriminating smokers. The cut-offs were also calculated after stratification among non smokers for SHS exposure by number of sources. The cut-off points used to discriminate smoking status were the level of UC given by the Youden's Index and for 50 and 100ng/ml for daily smokers, or 25 and 50ng/ml for occasional smokers.

Participants: At third trimester of pregnancy 2263 pregnant women of the INMA Project were interviewed between 2004 and 2008 and a urine sample was collected.

Results: Prevalence of self-reported smokers at third trimester of pregnancy was 18.5%, and other 3.9% misreported their smoking status. Variables associated with self-reported smoking and misreporting were similar, including born in Europe, educational level, and exposure to SHS. The optimal cut-off was 82ng/ml (95%CI 42-133); sensitivity: 95.2% and specificity: 98.5%. The area under the ROC curve was 0.995 (95%CI 0.992-0.997). The cut-offs varied according to the SHS exposure level being 42 (95%CI 27-57), 82 (95%CI 55-136) and 106ng/ml (95%CI 79-227) for not SHS exposed, exposed to one and to two or more sources of SHS, respectively. The optimal cut-off for discriminating occasional smokers from non-smokers was 27ng/ml (95%CI 11-43).

Conclusions: Prevalence of smoking during pregnancy in Spain remains high. UC is a reliable biomarker for classifying pregnant women according to their smoking status. However, cut-offs would differ based on baseline exposure to SHS.

ARTICLE SUMMARY

Article focus

The focus of this study is on:

- There is no current consensus regarding the cut-off point for urinary cotinine in pregnant women able to discriminate regular or occasional smokers from non-smokers,
- These cut-offs would also differ according to baseline exposure to second hand smoke (SHS)
- This study assess the maternal factors influencing both self-reported and misclassification of smoking; and evaluate the optimal cut-off point for urinary cotinine that best distinguishes smokers from non-smokers according to frequency of smoking and SHS exposure.

Key messages

- The prevalence of both smoking (18.5%) and SHS exposure (45.9%) was high in a population based sample of pregnant women in Spain.
- Factors associated with self-reported smoking and misreporting were similar, including lower level of education and living in a smoking environment, which highlights the need of reinforcing the preventive interventions and policies.
- The optimal cut-off point to discriminate smokers from non-smokers varied according to the frequency of smoking (occasional or daily smokers) and to SHS exposure levels.
- This study highlights the importance of SHS exposure for selecting reference cut-offs to discriminate smoking status, especially in high SHS exposed populations.

Strengths and limitations of this study

- This study has the ability to assess the role of baseline exposures to SHS in the estimate of cut-offs, given the detailed information collected on SHS exposure and its elevated prevalence.
- This study uses population based samples of pregnant women from the INMA birth cohort, which might not be fully representative of all pregnant women in the study areas,

INTRODUCTION

Risks for mother and foetus has been widely related to smoking during pregnancy.¹ Several studies have indicated that pregnant women tend to under-report their consumption of tobacco,²⁻⁸ due to social pressure⁹ or to avoid criticism from health professionals.³ Indeed, it is known to be a higher rate of misreporting of smoking among the groups in which it is not considered as acceptable, such as pregnant women and patients with smoking-related diseases.⁹

Cotinine is the main metabolite of nicotine and the biomarker of choice for distinguishing smokers from non-smokers and for assessing exposure to second-hand smoke (SHS).¹⁰ The women's clearance of cotinine is faster during pregnancy¹¹ and its plasma half-life is a little less than 9h.¹² For this reason, urinary cotinine (UC) tests may give false negatives in pregnant women who have not recently smoked.

There is no current consensus regarding the cut-off point for UC in pregnant women. Several thresholds have been proposed being 50 ng/ml, the most widely used.¹³⁻¹⁶ On the other hand, Higgins *et al*¹⁷ proposed 25 ng/ml as the cut-off point, while Gorber *et al*⁹ underlined the need to decide on a suitable threshold for pregnant women in particular, for whom the sensitivity of the test may be different, and also suggested that a new cut-off point should be established for occasional smokers. Spierto *et al*¹⁸ found 79 ng/ml as the cut-off between non-smoker and smoker pregnant women.

The aims of our study were: 1) to assess the prevalence of self-reported smoking and the UC levels in a cohort of pregnant women; 2) to assess the prevalence of misclassification of maternal smoking status according to the most widely accepted cut-off point in the literature of 50 ng/ml, and to study maternal factors associated with both self-reported and misclassification of maternal smoking; and 3) to identify the optimal cut-off point for UC that best distinguishes smokers from non-smokers in our study sample, according to frequency of smoking (occasional or daily smokers) and SHS exposure.

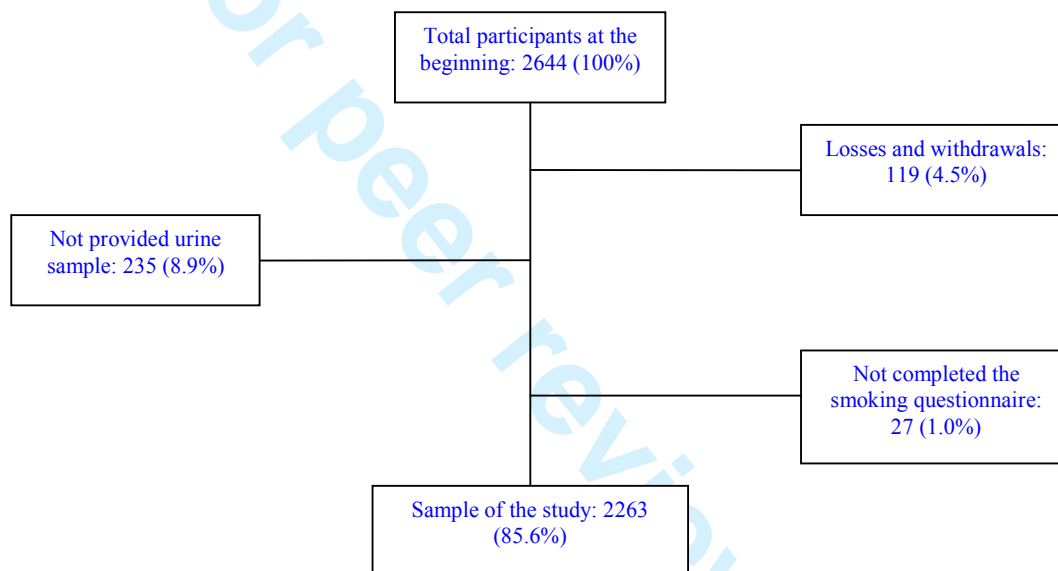
METHODS

Study population

The INMA [*Infancia y Medio Ambiente*, Environment and Childhood] project is a Spanish multi-centre prospective birth cohort study which aims to evaluate the impact of exposure to the most prevalent environmental pollutants, and the role of diet, on foetal and infant growth, health and development.¹⁹ From eligible pregnant women recruited between 2003 and 2008, a 56% agreed to participate. The inclusion criteria were at least 16 years of age, singleton pregnancy, enrolment at 10 to 13 weeks of gestation, no

assisted conception, delivery scheduled at the reference hospital, and no communication handicap. There was no upper age limit for be a member of the cohort. Of the 2644 women who agreed to participate in the study, 119 (4.5%) were lost (59 miscarriages, 8 foetal death, 47 withdrew and 5 lost to follow-up). Around week 32 of their pregnancy 2263 of the 2525 remaining women completed between 2004 and 2008 a questionnaire on smoking and other variables and provided urine samples for determination of UC (Figure 1). The hospital ethics committee of each centre approved the research protocol and all pregnant women gave written informed consent before inclusion at the first trimester of pregnancy.

Figure 1: Flow-chart of the INMA birth cohort in relation to smoking and UC quantification.



Information concerning smoking

Questionnaire on tobacco consumption included smoking history, patterns of consumption (occasional or regular) and exposure to SHS. We considered the women who, at this interview, reported smoking occasionally or daily to be smokers, regardless of their UC levels. Women who had UC levels higher than the widely used level of 50 ng/ml to distinguish smokers from non-smokers,¹³⁻¹⁶ but who did not report smoking, were classed as misclassified. It was considered that the participants were exposed to SHS when they reported exposure at least twice a week in any of the following environments: at work, at home, or in leisure time outside the home (e.g. bars/restaurants, or other homes). We analysed whether women had any passive exposure to tobacco smoke (yes or no), and also the number of exposure sources, between 0 and 3, according to the reported places of exposure: at work, at home and/or elsewhere in leisure time.

Urinary cotinine

1 The urine samples were collected in the same interview in the morning during the third trimester of
2 pregnancy. Urine was collected in 100 ml polyethylene containers and stored at -20°C. One aliquot of the
3 sample from each of the participants was sent to the Public Health Laboratory of Bilbao (Spain) to be
4 analysed. All urine samples were stored for a minimum of one year and a maximum of 5 years before
5 analysis. The analysis of the UC was performed by competitive enzyme immunoassay (EIA) using
6 commercial EIA microplate test kits (OraSure Technologies, Inc., Bio-Rad) for determining salivary cotinine
7 adapted for urine samples using urine controls (0, 2.5, 10 and 50 ng/ml, Bio-Rad). Samples with UC levels
8 above 50 ng/ml were diluted. Before testing the urine samples the method was validated; a certified reference
9 material was used (EPA/NIST Reference Material 8444) to evaluate the repeatability and reproducibility.
10 The quantification limit was 4.0 ng/ml, the coefficient of repeatability 7% and the reproducibility 10%.

11 **Other variables**

12 The women were interviewed twice during pregnancy (first and third trimester of gestation) to obtain
13 information about their sociodemographic characteristics and life-style variables. Social status of the women
14 (or her partner, if she had never worked outside the home) was defined using Spanish adaptation of British
15 classification system.²⁰

16 **Statistical analysis**

17 The chi-square test was used to test hypotheses for categorical variables, while differences in the distribution
18 of UC according to categorical covariates were evaluated using the Mann-Whitney U and the Kruskal Wallis
19 tests. In order to identify the variables independently associated with being either a smoker, a misclassified,
20 or both, logistic regression models were built including geographical area and the variables related with the
21 outcome at $p < 0.10$ in the univariate analysis, and sequentially excluding those variables not related at $p < 0.10$
22 in the adjusted model using the likelihood ratio test. For comparability purposes, variables remaining at $p <$
23 0.10 in any of the models were entered in all the models. For ordinal categorical variables, the p for a linear
24 trend was also calculated.

25 We used non-parametric receiver operating characteristic (ROC) curve to analyse the relationship between
26 the sensitivity (probability of a positive test among smokers) and false positive (probability of a positive test
27 among non-smokers, 1-specificity) cases for various different cut-off points that dichotomize UC to
28 distinguish smokers from non-smokers, using self-reported cigarette smoking status as the reference value.
29 Overall accuracy was evaluated by means of the area under the curve (AUC) (showing the ability of the
30

urinary cotinine to correctly classify smoking status with varying cut off points.²¹ The optimal cut-off point for UC to discriminate smokers from non smokers was the value (c) associated with the Youden's index (J), defined by: $J = \text{maximum}\{\text{sensitivity}(c) + \text{specificity}(c) - 1\}$.²² This value is 'optimal' in the sense that maximizes the overall rate of correct classification in the absence of a loss function (i.e., giving the same weight to errors of sensitivity and specificity). Since the shape of the distribution of the estimator of the optimal cut off point was unknown, we used the percentile bootstrap method, with 2000 resampling simulations, to establish 95% confidence intervals, with the aid of the 'boot' package of R.²³ Additionally, the data were analysed for the most widely used cut-off points, namely 50 and 100 ng/ml, or 25 and 50 ng/ml when analyses were restricted to occasional smokers. Thirty-five ~~W~~women who declared that they did not smoke but with implausible UC levels in non smokers (>500 ng/ml) were excluded from ~~the~~ these analyses in order to diminish the measurement error of self-reported cigarette smoking. The cut-off points were also calculated after stratification among non smokers for SHS exposure in three groups: 791 women that referred not exposed to SHS, 718 exposed to one source of SHS and 292 exposed to more than one source. Additional sensitivity analysis excluded 290 self-reported non-smokers who claimed to stop smoking during pregnancy, since this group is more likely to misreport their smoking status. Likewise, occasional smoking was analysed excluding non smokers exposed to SHS. Assuming $\alpha = 0.05$, 95% CI were calculated for ORs, cut-off points and area under ROC curve. All statistical tests were two-sided. Statistical analysis was carried out using SPSS (version 17.0) and R (2.11.1) statistical software.

RESULTS

Study setting and characteristics of the sample

Overall, 61.2% of women reported to have smoked at least once in their life, while 32.4% were occasional or regular smokers when they became pregnant, falling to 19.7% at first trimester and 18.5% at third trimester of their pregnancy (Table 1).

Table 1: Description of the sample and variables of interest.

	N ^a	%
Cohort		
Asturias	416	18.4
Gipuzkoa	545	24.1

	Sabadell	591	26.1
	Valencia	711	31.4
Age			
	≤ 24	154	6.8
	25-29	717	31.7
	30-34	973	43.0
	≥ 35	418	18.5
Social class			
	I-II (more affluent)	492	21.8
	III	584	25.8
	IV-V (less affluent)	1186	52.4
Level of education			
	Primary or no education	547	24.2
	Secondary	936	41.4
	University	776	34.4
BMI (pre-pregnancy)			
	<18.5	100	4.4
	18.5 – 25	1568	69.3
	25 – 30	420	18.6
	≥ 30	175	7.7
Previous parity		957	43.1
Birth In Europe		2130	94.3
Reported having smoked in their life			
	No	879	38.8
	Occasional	146	6.5
	Regular	1238	54.7
Reported smoking at the start of pregnancy			
	No	1529	67.6
	Occasional	28	1.2
	Regular	706	31.2
Reported smoking at first trimester of pregnancy			
	No	1813	80.3
	Occasional	35	1.6
	Regular	410	18.2
Reported smoking at third trimester of pregnancy			
	No	1845	81.5
	Occasional	37	1.6
	Regular	381	16.8
Year of urine sampling			

2004	321	14.2
2005	857	37.9
2006	466	20.6
2007	470	20.8
2004	149	6.6
Cigarettes/day at third trimester of pregnancy		
0	1845	81.5
Occasional	37	1.6
1-4	149	6.6
5-9	141	6.2
≥ 10	91	4.0
Exposed to SHS in non-smoking women ^b :		
At home (partner or others)	479	26.0
At work	186	10.1
Elsewhere in leisure time ^c	715	38.8
Number of sources of exposure to SHS ^d		
0	798	43.5
1	735	40.0
2	271	14.8
3	32	1.7
Cotinine (ng/ml) all the women		
< 50	1773	78.3
50-99	31	1.4
100-199	19	0.8
200-499	52	2.3
500-999	70	3.1
≥ 1000	318	14.1

a: The numbers and rates that do not match the total are due to missing data

b: Percentages calculated including non exposed women

c: Other homes or public places, e.g. pubs or restaurants

d: Work, home and elsewhere in leisure time among non smokers

Smoking and SHS exposure

The median UC level in women who did not refer to smoke and were not exposed to SHS was below the quantification level of 4.0 ng/ml while in non-smokers exposed to SHS it was 7.6 ng/ml. Among all smokers the UC median level was 1744.3 ng/ml (Table 2). Occasional smokers had a median level of 260.7 ng/ml. Among daily smokers statistically significant differences were observed between UC concentration and the number of cigarettes smoked per day ($p < 0.001$), showing a clear dose-response pattern (not statistically tested). In the same way, in non-smokers there were statistically significant differences between UC levels and the number of sources of exposure to SHS; that are, work, home and elsewhere in leisure time ($p < 0.001$), with a progressive dose-response pattern (not tested, neither). Figure 1 shows the different distribution patterns of UC among non-smokers, exposed or not to SHS, and occasional and daily smokers.

Table 2: Active smoking and exposure to SHS in pregnant women in the INMA cohort. Median levels of urinary cotinine (ng/ml) at third trimester of pregnancy.

	N	%	Urinary cotinine ^a
Total	2263	100	7.4
Non smokers ^b	1845	81.5	4.4
No SHS exposure	798	35.3	< 4
SHS exposure	1038	45.9	7.6
1 source ^{c, d}	735	32.5	5.8
2 sources	271	12.0	11.7
3 sources	32	1.4	16.9
Smokers ^d	418	18.5	1744.3
Occasional	37	1.6	260.7
1-2 cigarettes/day	76	3.4	1036.4
3-4 cigarettes/day	73	3.2	1330.7
5-9 cigarettes/day	141	6.2	1848.5
≥ 10 cigarettes/day	91	4.0	3033.0

a: Median level of urinary cotinine ng/ml.

b: Exposed and not exposed to SHS; Mann-Whitney test: $p < 0.001$ for smoking and urinary cotinine

c: Sources of exposure to SHS at work/at home/in leisure time outside the home

d: Kruskal Wallis test $p < 0.001$

Figure 2: Distribution of urinary cotinine (ng/ml) according to active or passive tobacco exposure in pregnant women from the INMA cohort.

Me: median

SHS: Second-hand smoking

SRS Occas: Self reported smoking, occasional

SRS Daily: Self reported smoking, daily

Self-reported smoking and misclassification

Among the 2263 women studied, 1755 (77.6%) reported that they did not smoke and had UC levels below 50 ng/ml (true negative). A further 18 (0.8%) also had UC levels under 50 ng/ml despite claiming to smoke, though 13 of these claimed to be occasional smokers. On the other hand, 90 women (3.9%) reported that they did not smoke but were found to have UC levels above 50 ng/ml and were considered as misclassified and, finally, 400 women (17.7%) were true positive. Table 3 shows the ORs of the variables associated with smoking and misclassification, before and after adjusting. In the adjusted model, the risk of smoking and misclassification were associated with low educational level, country of birth, and exposure to SHS. Age was related only to misclassification risk. In regards to smoking history, only smoking at the beginning of pregnancy was associated with misclassification. The year of urine sampling and the social class were statistically associated only in the unadjusted analysis. Adding women misclassified to self-reported smokers the pattern of the association found with self-reported smoking did not vary.

Table 3: Unadjusted and adjusted odds ratios (ORs) and variables associated with smoking, self-reported and misclassification of smoking status.

	Unadjusted analysis							Adjusted analysis ^a					
	Non-smokers ^b		Self-reported smokers ^c			Misclassification ^d		Self-reported smokers ^c		Misclassification ^d		Both ^e	
	N	OR	95% CI	N	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	
Total	1755	418	-	-	90	-	-	-	-	-	-	-	
Cohort													
Asturias	326	75	1	-	15	1	-	1	-	1	-	-	
Gipuzkoa	467	65	0.60	0.42 to 0.88	13	0.60	0.27 to 1.36	0.77	0.52 to 1.15	0.63	0.28 to 1.42	0.75	0.52 to 1.09
Sabadell	466	99	0.92	0.66 to 1.29	26	1.21	0.61 to 2.44	0.68	0.47 to 0.99	0.81	0.40 to 1.64	0.72	0.51 to 1.02
Valencia	496	179	1.57	1.15 to 2.15	36	1.58	0.82 to 3.07	0.94	0.67 to 1.33	0.84	0.43 to 1.66	0.95	0.69 to 1.31
Age													
≤ 24	93	43	1	-	18	1	-	1	-	1	-	1	
25-29	537	152	0.62	0.40 to 0.94	28	0.27	0.14 to 0.53	0.87	0.55 to 1.38	0.36	0.18 to 0.73	0.73	0.49 to 1.11
30-34	792	153	0.42	0.27 to 0.64	28	0.18	0.09 to 0.36	0.75	0.48 to 1.19	0.26	0.13 to 0.54	0.61	0.41 to 0.92
≥ 35	332	70	0.46	0.29 to 0.73	16	0.25	0.12 to 0.54	0.90	0.54 to 1.49	0.46	0.21 to 1.04	0.76	0.48 to 1.19
				(p ^f < 0.001)			(p ^f = 0.001)						
Country of birth													
In Europe	1637	410	1	-	83	1	-	1	-	1	-	1	
Outside Europe	114	8	0.28	0.13 to 0.60	7	1.21	0.50 to 2.79	0.25	0.12 to 0.54	1.11	0.46 to 2.65	0.39	0.21 to 0.69
Level of education													
University	695	59	1	-	22	1	-	1	-	1	-	1	
Secondary	703	192	3.22	2.36 to 4.39	41	1.84	1.09 to 3.17	2.37	1.70 to 3.29	1.17	0.66 to 2.08	2.08	1.55 to 2.78
Primary or less	355	165	5.47	3.96 to 7.57	27	2.40	1.34 to 4.32	3.30	2.31 to 4.70	1.02	0.53 to 1.97	2.72	1.97 to 3.74
				(p ^f < 0.001)			(p ^f = 0.002)						
Social class													
I+II (highest)	438	41	1	-	13	1	-	-	-	-	-	-	

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III	469	96	2.19	1.48 to 3.22	19	1.36	0.67 to 2.80	-	-	-	-	-	-
IV+V (lowest)	847	281	3.54	2.50 to 5.02	58	2.31	1.25 to 4.26	-	-	-	-	-	-
				(p ^f < 0.001)			(p ^f = 0.002)						
Year of urine sampling													
2004	224	78	1	-	19	1	-	-	-	-	-	-	-
2005	642	179	0.80	0.59 to 1.09	36	0.66	0.37 to 1.18	-	-	-	-	-	-
2006	362	83	0.66	0.46 to 0.94	21	0.68	0.36 to 1.30	-	-	-	-	-	-
2007	399	58	0.42	0.29 to 0.61	13	0.38	0.19 to 0.79	-	-	-	-	-	-
2008	128	20	0.45	0.26 to 0.77	1	0.09	0.01 to 0.70	-	-	-	-	-	-
				(p ^f < 0.001)			(p ^f = 0.001)						
Exposure to SHS at home													
No	1328	144	1	-	34	1	-	1	-	1	-	1	-
Yes	423	274	5.97	4.75 to 7.51	56	5.17	3.33 to 8.03	4.41	3.44 to 5.64	3.26	2.03 to 5.25	4.39	3.49 to 5.51
Exposure to SHS at work													
No	1582	343	1	-	76	1	-	1	-	1	-	1	-
Yes	172	74	1.98	1.48 to 2.67	14	1.69	0.94 to 3.06	1.55	1.11 to 2.17	1.37	0.72 to 2.59	1.57	1.14 to 2.15
Exposure to SHS elsewhere in leisure time													
No	1082	175	1	-	44	1	-	1	-	1	-	1	-
Yes	669	243	2.25	1.81 to 2.79	46	1.69	1.11 to 2.59	1.88	1.44 to 2.34	1.47	0.92 to 2.34	1.80	1.44 to 2.26
Reported having smoked in their life [§]													
No	862	0	-	-	17	1	-	-	-	-	-	-	-
Yes	893	418	-	-	73	4.15	2.43 to 7.09	-	-	-	-	-	-
Reported smoking at the start of pregnancy [§]													
No	1489	1	-	-	39	1	-	-	-	1	-	-	-
Yes	266	417	-	-	51	7.32	4.73 to 11.33	-	-	6.21	3.91 to 9.86	-	-

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4 a: Only variables showed in the table were entered in the logistic equation

5 b: Non-smokers: women who reported that they did not smoke and were found to have urinary cotinine levels of less than 50 ng/ml. the reference group

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7 c: Smokers: those who reported smoking

8 d: Misclassification: those who claimed that they did not smoke but were found to have urinary cotinine levels above 50 ng/ml

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10 e: Both: c+d

11 f: p for trend

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13 g: Only analysed with regards to misclassification, given the extremely strong association with smoking at third trimester of pregnancy
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Cut-off points of UC for smoking

Optimal cut-off points for distinguishing non-smokers from smokers (daily and occasional) calculated by the Youden's index (excluding self reported non-smokers with UC values above 500 ng/ml), was 82 ng/ml, with a sensitivity of 95.2%, specificity of 98.5% and AUC 0.995 (95% CI 0.992 to 0.997) (Table 4). Sensitivity and specificity for the cut-off points of 50 and 100 ng/ml were quite close to that of 82 ng/ml. The exclusion from the analysis of 290 women who declared quitting smoking during pregnancy as possible group at risk of underreporting of their smoking status, did not improve substantially the validation parameters of the test (data not shown).

Table 4: Parameters for assessing the optimal cut-off point for urinary cotinine, ng/ml, obtained by the Youden's index, as well as the levels of 25, 50, and 100 ng/ml, for classifying pregnant women as regular or occasional smokers.^a

Youden's index ^b	Cut-off point (95% CI) ^c	Sensitivity	Specificity	Positive PV ^d	Negative PV ^d	AUC of the ROC (95% CI) ^e
Regular and occasional smokers: 1810 non-smokers, 418 smokers						
-	50	0.957	0.970	0.879	0.990	
-	100	0.950	0.985	0.936	0.988	0.995 (0.992 to 0.997)
0.937	82 (42 to 133)	0.952	0.985	0.934	0.989	
Results stratified by frequency of smoking						
Occasional smokers: 1810 non-smokers, 37 smokers						
-	25	0.892	0.887	0.139	0.998	
-	50	0.649	0.970	0.304	0.993	0.961 (0.939 to 0.984)
0.789	27 (11 to 43)	0.892	0.897	0.151	0.998	
Daily smokers: 1810 non-smokers, 381 smokers						
-	50	0.987	0.970	0.872	0.997	
-	100	0.982	0.985	0.933	0.996	0.998 (0.996 to 1)
0.968	115 (57 to 189)	0.982	0.986	0.937	0.996	
Results stratified by SHS exposure among non-smokers						
Non exposed to SHS: 791 non-smokers, 418 active smokers						
-	50	0.957	0.996	0.993	0.978	
-	100	0.950	0.999	0.997	0.974	0.998 (0.997 to 1)
0.962	42 (27 to 57)	0.971	0.991	0.983	0.985	
Exposed to SHS (1 source^f): 718 non-smokers, 418 active smokers						
-	50	0.957	0.957	0.928	0.974	
-	100	0.950	0.981	0.966	0.971	0.993 (0.990 to 0.997)
0.933	82 (55 to 136)	0.952	0.981	0.966	0.972	
Exposed to SHS (2-3 sources^f): 292 non-smokers, 418 active smokers						
-	50	0.957	0.928	0.950	0.938	
-	100	0.950	0.959	0.971	0.930	0.988 (0.982 to 0.994)
0.912	106 (79 to 227)	0.950	0.962	0.973	0.930	

a: Excluding cases with cotinine > 500 ng/ml in self-reported non-smokers (n=35).

b: Youden's index = max (Sensitivity+Specificity-1).

c: 95% bootstrap confidence interval for the cut-off point associated with the Youden's index.

d: Predictive value of a positive or negative result for the prevalence of the study: 18.5%.

e: Area under the receiver operating characteristic curve and 95% confidence interval.

f: Number of sources of exposure among: work, home and elsewhere in leisure time.

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4 The Youden's index and AUC for daily smoking were higher, with a cut-off point of 115 ng/ml. Occasional
5 smoking was analysed, by excluding from the analysis the 381 women who admitted that they smoked
6 regularly at third trimester of pregnancy. The optimal cut-off point for discriminating occasional smokers
7 from non-smokers was 27 ng/ml (95% CI 11 to 43), with a sensitivity and specificity of 89.2% and 89.7%,
8 respectively. The exclusion of women who declared to quit during pregnancy, improved the specificity to
9 91.8%, but did not almost change the Youden's Index or the sensitivity. Excluding non SHS exposed among
10 non-smokers, the optimal cut-off point was 19 ng/ml (95% CI 11 to 33), and improving the specificity to
11 93.7% and to 41.2% the positive predictive value (probability of smoking status being a positive test).
12 Nevertheless, these low positive predictive values are consequence, above all, of the low prevalence of
13 occasional smoking in this sample.

14 Not exposed women to SHS compared with all smokers, daily or occasional, had a lower cut-off point of 42
15 ng/ml (95% CI 27 to 57), while for exposed to one or to two or more sources of SHS, cut-off points were 82
16 (95% CI 55 to 136) and 106 ng/ml (95% CI 79 to 201), respectively.

30 DISCUSSION

32 Main findings in relation to the literature

34 The prevalence of smoking in pregnant women at third trimester was 18.5%. In this later stage, the
35 prevalence of active smoking increased up to 22.5% if women who did not report smoking but had UC levels
36 above 50 ng/ml were reclassified as smokers, assuming that false positives were due to maternal
37 misreporting of smoking status. Prevalence of self reported smokers and misclassified in our study is close to
38 the referred by Kendrick *et al*⁶ and Lindqvist *et al*⁷, and smoking rate and UC levels are lower than that
39 showed by Pickett *et al*¹⁵. Our study had, nevertheless, a lower rate of smoking misreporting than other
40 studies.²⁻⁸

42 There was a clear relationship between UC and smoking dose among smokers, and with the number of
43 sources of exposure to SHS among non-smokers. Specifically, those who smoked 10 or more cigarettes per
44 day had median UC levels of 3033 ng/ml, while the levels were 260 ng/ml for occasional smokers and less
45 than 17 ng/ml for non-smokers, increasing with the number of sources of exposure to SHS. This data
46 reinforces the validity of UC also as an indicator of exposure to SHS.¹⁰

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England *et al*¹³ indicated that few studies have identified differences between misclassified and self-reported smokers and the way in which this would affect epidemiological studies. Our study shows similar patterns of association and both self-reported smoking and misclassification were strongly associated with various predictive variables. In particular, we found a higher risk of smoking and misclassification among women with low education level. These results are consistent with those reported by other authors.^{6 14 24} We also found a higher risk of smoking and misclassification in women from Europe, and women exposed to SHS in different places. Women younger than 24 years had an increased risk of misreport her smoking, as indicated by Dietz *et al*.⁴ In this study, exposure to SHS was associated with smoking. In other words, there were more smoking people around pregnant women who smoked. In addition, misclassification was significantly associated with exposure to SHS at home. Jhun *et al*¹⁴ and Orr *et al*²⁵ also showed higher prevalence of smoking among pregnant women whose partners smoked at home. Having smoked previously was associated with a higher probability of misreporting the habit, as observed by England *et al*.¹³

This work showed an optimal cut-off point for discriminate pregnant women smokers from non-smokers of 82 ng/ml, with a confidence interval of 42 to 133 ng/ml. Some studies proposed a cut-off of 50 ng/ml,¹³⁻¹⁶ coherent with the women not SHS exposed in this study. Other studies proposed cut-off points of 79 ng/ml¹⁸ and 85 ng/ml,⁶ closer to the smoking dose and SHS exposure in our study sample. In our study population both prevalence of smoking and of SHS are high and this can explain in part why our optimal cut-off point is higher than those reported in other studies.¹³⁻¹⁷ This is also supported by the fact that the optimal cut-off point decreased to 42 ng/ml (27 to 57) when the analysis was restricted to women who reported no SHS exposure, and increased according to the number of sources reported.. The validity of 27 ng/ml (11 to 43) as cut-off point for differentiating occasional smokers from non-smokers was lower than that for differentiating daily smokers, and it could depend on SHS exposure and on the time spent from the last cigarette smoked given the faster elimination of cotinine in pregnant women,¹⁰⁻¹² information not collected in this study. There are not validation studies of cotinine in different biological matrices, blood (plasma or serum), saliva or urine,^{9 16} so it cannot be established which the most reliable biomarker is.

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Limitations of the study

The current study has several limitations. From the eligible population, the participation rate was 56%, and 85.6% of the women who agreed to participate completed the study. Therefore, the final study sample might not be fully representative of all pregnant women in the study areas, but its internal validity (absence of bias)

1 is not necessarily affected. There were other likely sources of misclassification in addition to maternal
2 misreporting of smoking status, as misclassification of non-smokers as smokers because of high exposure
3 degree to SHS. On the other hand, women who smoked occasionally but report to be non-smokers might
4 have low UC concentrations if they had not smoked recently, and their self-report and UC levels would be in
5 agreement.
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8 No information about last cigarette or last SHS exposure was obtained. We lost the opportunity of analysing
9 this variable in the evolution of the UC, showing his influence in false negatives, above all, and especially
10 relevant for occasional smokers.
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12 Since the optimal cut-off point for UC is determined using self-reported smoking status as the gold standard,
13 the validity of this assumption is important. On the one hand, it is unlikely that a non-smoking woman
14 declared to be a smoker, because a battery of items should be completed detailing smoking habits in this
15 case. On the other hand, however, it is possible that some smokers did not reveal their habit. In order to
16 minimize this type of bias, we excluded in the main analysis self-reported non-smokers with implausible
17 high UC levels. In additional analysis, we excluded self-reported non-smokers who claimed to stop smoking
18 during pregnancy, since these cases are at higher risk of misclassification as reported in table 3; the optimal
19 cut-off point did not change after this exclusion. In general terms, the AUC shows a good overall accuracy,
20 and we think that self-reported smoking is a reliable measure in this study. If some kind of misclassification
21 occurs, it would lead to a shift towards the right in both distributions, and a slight overestimation of the
22 optimal cut-off point as a result.
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40 One of the main strengths of this study was the possibility of assessing the role of baseline exposures to SHS
41 in the estimate of cut-offs, given the detailed information collected on SHS exposure and its elevated
42 prevalence. The confirmation that the cut-offs would differ according to the level of exposure to SHS
43 emphasizes the need of taking it into account, especially in countries with elevated SHS exposure.
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48 **Implications for practice**

49 This study shows that the efforts made to encourage women to give up smoking before or during pregnancy
50 are not sufficient or particularly effective, given that at least 18.5% of the pregnant women smoked in the
51 third trimester. The results of this study indicate that the groups to which the most effort should be directed
52 are young women, those of a European origin and those from a low social class. Further, the association
53 observed in this study between active smoking of pregnant women and the presence of smokers in their close
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1 environment supports the hypothesis that this factor makes it more difficult to stop smoking.²⁶ It is necessary
2 to undertake effective programmes for reducing smoking before and during pregnancy, reaching also
3 misclassified, and to reduce SHS exposure, in order to prevent risks for women and foetus.
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7 **CONCLUSION**

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9 Smoking is an important risk factor for health and development and should be taken into account as
10 confounder when analysing the potential effects of environmental contaminants in studies like the INMA
11 project. To have a reliable marker like UC and a valid a cut-off point able to discriminate regular or
12 occasional smokers from non-smokers is a critical issue. The cut-off point of 82 ng/ml showed a good
13 validity for discriminating smokers from non-smokers in our study sample, while 27 ng/ml is the optimal
14 point for discriminating occasional smokers from non-smokers. It should be emphasized that cut-offs would
15 differ based on baseline exposure to SHS, and this should be taken into account when selecting reference
16 cut-offs for specific populations.
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30 AL, AT and FB revised the design of the study and the results. JJA redacted the manuscript and the other
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5 **Determinants of self-reported smoking and misclassification during pregnancy, and analysis**
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7 **of optimal cut-off points of urinary cotinine: a cross sectional study**
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10 JJ Aurrekoetxea ^{1,2,3}, M Murcia ^{4,5}, M Rebagliato ^{4,5}, MJ López ^{4,6,7}, AM Castilla ^{1,4}, L Santa Marina ^{1,3,4}, M
11 Guxens ^{4,8,9}, A Fernández-Somoano ^{4,10}, M Espada ¹, A Lertxundi ^{2,3,4}, A Tardón ^{4,10}, F Ballester ^{4,5,11}
12
13
14 (Juan J Aurrekoetxea ^{1,2,3}, Mario Murcia ^{4,5}, Marisa Rebagliato ^{4,5}, María José López ^{4,6,7}, Ane Miren Castilla
15 ^{1,4}, Loreto Santa Marina ^{1,3,4}, Mónica Guxens ^{4,8,9}, Ana Fernández-Somoano ^{4,10}, Mercedes Espada ¹, Aitana
16 Lertxundi ^{2,3,4}, Adonina Tardón ^{4,10}, Ferran Ballester ^{4,5,11})
17
18

19
20
21 1 *Public Health Department, Basque Government, Spain*

22 2 *University of Basque Country UPV/EHU, Spain*

23 3 *Health Research Institute (BIODONOSTIA), Spain*

24 4 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP)*

25 5 *Center for Public Health Research (CSISP), Valencia, Spain*

26 6 *Public Health Agency of Barcelona, Spain*

27 7 *Institute of Biomedical Research (IIB Sant Pau), Spain*

28 8 *Center for Research in Environmental Epidemiology (CREAL), Spain*

29 9 *Hospital del Mar Research Institute (IMIM), Barcelona, Spain*

30 10 *University of Oviedo, Spain*

31 11 *University of Valencia, Spain*

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37
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39
40
41
42
43 **Juan J Aurrekoetxea**, *Public Health Department, Basque Government, Spain;*

44 *University of Basque Country UPV/EHU, Spain;*

45 *Health Research Institute (BIODONOSTIA), Spain.*

46 jj.aurreko@gmail.com

47
48
49 **Mario Murcia**: *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

50 *Center for Public Health Research (CSISP); Valencia, Spain.*

51 murcia_mar@gva.es

52
53
54 **Marisa Rebagliato**, *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

55 *Center for Public Health Research (CSISP); Valencia, Spain.*

56 rebagliato_mar@gva.es

57
58
59 **Maria José López**, *Public Health Agency of Barcelona, Spain;*

1 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

2 *Institute of Biomedical Research (IIB Sant Pau), Spain.*

3 mjlopez@aspb.cat

4 **Ane Miren Castilla**, *Public Health Laboratory of Bilbao, Spain.*

5 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

6 ANE_M2002@yahoo.es

7 **Loreto Santa Marina**, *Public Health Department, Basque Government, Spain;*

8 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

9 *Health Research Institute (BIODONOSTIA), Spain.*

10 ambien4ss-san@ej-gv.es

11 **Mònica Guxens**, *Center for Research in Environmental Epidemiology (CREAL), Spain;*

12 *Hospital del Mar Research Institute (IMIM), Spain;*

13 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

14 mguxens@creal.cat

15 **Ana Fernández-Somoano**, *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

16 *University of Oviedo, Spain.*

17 capua.uo@uniovi.es

18 **Mercedes Espada**, *Public Health Department, Basque Government, Spain;*

19 metabobi-san@ej-gv.es

20 **Aitana Lertxundi**, *University of Basque Country UPV/EHU, Spain;*

21 *Health Research Institute (BIODONOSTIA), Spain;*

22 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

23 aitana.lertxundi@gmail.com

24 **Adonina Tardón**, *University of Oviedo, Spain;*

25 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

26 atardon@uniovi.es

27 **Ferran Ballester**, *University of Valencia, Spain;*

28 *Center for Public Health Research (CSISP), Valencia, Spain;*

29 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

30 ballester_fer@gva.es

31 Corresponding author: Juan J Aurrekoetxea Ph.D; Departamento de Sanidad del Gobierno Vasco; C/ Sancho el Sabio 35; 20.010, San

32 Sebastián; Telephone: 34.943.023033; Fax: 34.943.023093; E-mail: jj.aurreko@gmail.com

33 **Key words:** Pregnancy; Smoking; Validation studies; Cut-off; Urinary cotinine.

ABSTRACT

Objectives: To estimate the prevalence and factors associated with smoking and misclassification in pregnant women from INMA [*Infancia y Medio Ambiente, Environment and Childhood*] project, Spain, and to assess the optimal cut-offs for urinary cotinine (UC) that best distinguishes **daily and occasional smokers with varying levels of SHS exposure**.

Design: We used logistic regression models to study the relationship between sociodemographic variables and self-reported smoking and misclassification (**self-reported non-smokers with UC >50ng/ml**). ROC curves were used to calculate the optimal cut-off point for discriminating smokers. The cut-offs were also calculated after stratification among non smokers for SHS exposure **by number of sources**. The cut-off points used to discriminate smoking status were the level of UC given by the Youden's Index and for 50 and 100ng/ml for daily smokers, or 25 and 50ng/ml for occasional smokers.

Participants: At third trimester of pregnancy 2263 pregnant women of the INMA Project were interviewed **between 2004 and 2008** and a urine sample was collected.

Results: Prevalence of self-reported smokers at third trimester of pregnancy was 18.5%, and other 3.9% misreported their smoking status. Variables associated with self-reported smoking and misreporting were similar, including born in Europe, educational level, and exposure to SHS. **The optimal cut-off was 82ng/ml (95%CI 42-133); sensitivity: 95.2% and specificity: 98.5%. The area under the ROC curve was 0.995 (95%CI 0.992-0.997). The cut-offs varied according to the SHS exposure level being 42 (95%CI 27-57), 82 (95%CI 55-136) and 106ng/ml (95%CI 79-227) for not SHS exposed, exposed to one and to two or more sources of SHS, respectively. The optimal cut-off for discriminating occasional smokers from non-smokers was 27ng/ml (95%CI 11-43).**

Conclusions: **Prevalence of smoking during pregnancy in Spain remains high**. UC is a reliable biomarker for classifying pregnant women according to their **smoking status**. However, cut-offs would differ based on baseline exposure to SHS.

ARTICLE SUMMARY

Article focus

The focus of this study is on:

- There is no current consensus regarding the cut-off point for urinary cotinine in pregnant women able to discriminate regular or occasional smokers from non-smokers,
- These cut-offs would also differ according to baseline exposure to second hand smoke (SHS)
- This study assess the maternal factors influencing both self-reported and misclassification of smoking; and evaluate the optimal cut-off point for urinary cotinine that best distinguishes smokers from non-smokers according to frequency of smoking and SHS exposure.

Key messages

- The prevalence of both smoking (18.5%) and SHS exposure (45.9%) was high in a population based sample of pregnant women in Spain.
- Factors associated with self-reported smoking and misreporting were similar, including lower level of education and living in a smoking environment, which highlights the need of reinforcing the preventive interventions and policies.
- The optimal cut-off point to discriminate smokers from non-smokers varied according to the frequency of smoking (occasional or daily smokers) and to SHS exposure levels.
- This study highlights the importance of SHS exposure for selecting reference cut-offs to discriminate smoking status, especially in high SHS exposed populations.

Strengths and limitations of this study

- This study has the ability to assess the role of baseline exposures to SHS in the estimate of cut-offs, given the detailed information collected on SHS exposure and its elevated prevalence.
- This study uses population based samples of pregnant women from the INMA birth cohort, which might not be fully representative of all pregnant women in the study areas,

INTRODUCTION

Risks for mother and foetus has been widely related to smoking during pregnancy.¹ Several studies have indicated that pregnant women tend to under-report their consumption of tobacco,²⁻⁸ due to social pressure⁹ or to avoid criticism from health professionals.³ Indeed, it is known to be a higher rate of misreporting of smoking among the groups in which it is not considered as acceptable, such as pregnant women and patients with smoking-related diseases.⁹

Cotinine is the main metabolite of nicotine and the biomarker of choice for distinguishing smokers from non-smokers and for assessing exposure to second-hand smoke (SHS).¹⁰ The women's clearance of cotinine is faster during pregnancy¹¹ and its plasma half-life is a little less than 9h.¹² For this reason, urinary cotinine (UC) tests may give false negatives in pregnant women who have not recently smoked.

There is no current consensus regarding the cut-off point for UC in pregnant women. Several thresholds have been proposed being 50 ng/ml, the most widely used.¹³⁻¹⁶ On the other hand, Higgins *et al*¹⁷ proposed 25 ng/ml as the cut-off point, while Gorber *et al*⁹ underlined the need to decide on a suitable threshold for pregnant women in particular, for whom the sensitivity of the test may be different, and also suggested that a new cut-off point should be established for occasional smokers. Spierto *et al*¹⁸ found 79 ng/ml as the cut-off between non-smoker and smoker pregnant women.

The aims of our study were: 1) to assess the prevalence of self-reported smoking and the UC levels in a cohort of pregnant women; 2) to assess the prevalence of misclassification of maternal smoking status according to the most widely accepted cut-off point in the literature of 50 ng/ml, and to study maternal factors associated with both self-reported and misclassification of maternal smoking; and 3) to identify the optimal cut-off point for UC that best distinguishes smokers from non-smokers in our study sample, according to frequency of smoking (occasional or daily smokers) and SHS exposure.

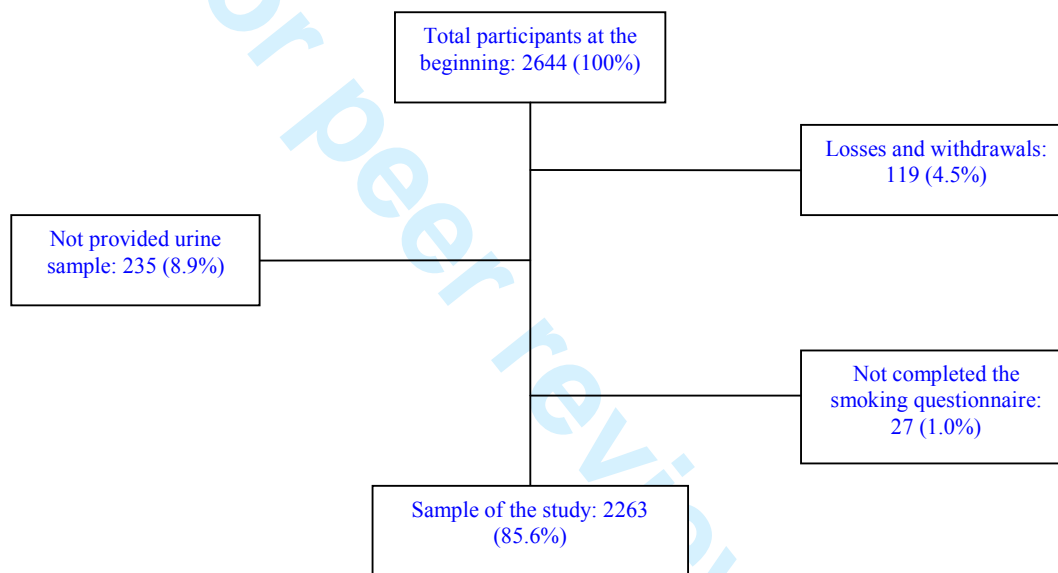
METHODS

Study population

The INMA [*Infancia y Medio Ambiente*, Environment and Childhood] project is a Spanish multi-centre prospective birth cohort study which aims to evaluate the impact of exposure to the most prevalent environmental pollutants, and the role of diet, on foetal and infant growth, health and development.¹⁹ From eligible pregnant women recruited between 2003 and 2008, a 56% agreed to participate. The inclusion criteria were at least 16 years of age, singleton pregnancy, enrolment at 10 to 13 weeks of gestation, no

assisted conception, delivery scheduled at the reference hospital, and no communication handicap. There was no upper age limit for be a member of the cohort. Of the 2644 women who agreed to participate in the study, 119 (4.5%) were lost (59 miscarriages, 8 foetal death, 47 withdrew and 5 lost to follow-up). Around week 32 of their pregnancy 2263 of the 2525 remaining women completed between 2004 and 2008 a questionnaire on smoking and other variables and provided urine samples for determination of UC (Figure 1). The hospital ethics committee of each centre approved the research protocol and all pregnant women gave written informed consent before inclusion at the first trimester of pregnancy.

Figure 1: Flow-chart of the INMA birth cohort in relation to smoking and UC quantification.



Information concerning smoking

Questionnaire on tobacco consumption included smoking history, patterns of consumption (occasional or regular) and exposure to SHS. We considered the women who, at this interview, reported smoking occasionally or daily to be smokers, regardless of their UC levels. Women who had UC levels higher than the widely used level of 50 ng/ml to distinguish smokers from non-smokers,¹³⁻¹⁶ but who did not report smoking, were classed as misclassified. It was considered that the participants were exposed to SHS when they reported exposure at least twice a week in any of the following environments: at work, at home, or in leisure time outside the home (e.g. bars/restaurants, or other homes). We analysed whether women had any passive exposure to tobacco smoke (yes or no), and also the number of exposure sources, between 0 and 3, according to the reported places of exposure: at work, at home and/or elsewhere in leisure time.

Urinary cotinine

1 The urine samples were collected in the same interview **in the morning** during the third trimester of
2 pregnancy. Urine was collected in 100 ml polyethylene containers and stored at -20°C. One aliquot of the
3 sample from each of the participants was sent to the Public Health Laboratory of Bilbao (Spain) to be
4 analysed. All urine samples were stored for a minimum of one year and a maximum of 5 years before
5 analysis. The analysis of the UC was performed by competitive enzyme immunoassay (EIA) using
6 commercial EIA microplate test kits (OraSure Technologies, Inc., Bio-Rad) for determining salivary cotinine
7 adapted for urine samples using urine controls (0, 2.5, 10 and 50 ng/ml, Bio-Rad). Samples with UC levels
8 above 50 ng/ml were diluted. Before testing the urine samples the method was validated; a certified reference
9 material was used (EPA/NIST Reference Material 8444) to evaluate the repeatability and reproducibility.
10 The quantification limit was 4.0 ng/ml, the coefficient of repeatability 7% and the reproducibility 10%.

11 **Other variables**

12 The women were interviewed twice during pregnancy (first and third trimester of gestation) to obtain
13 information about their sociodemographic characteristics and life-style variables. Social status of the women
14 (or her partner, if she had never worked outside the home) was defined using Spanish adaptation of British
15 classification system.²⁰

16 **Statistical analysis**

17 The chi-square test was used to test hypotheses for categorical variables, while differences in the distribution
18 of UC according to categorical covariates were evaluated using the Mann-Whitney U and the Kruskal Wallis
19 tests. In order to identify the variables independently associated with being either a smoker, a misclassified,
20 or both, logistic regression models were built including geographical area and the variables related with the
21 outcome at $p < 0.10$ in the univariate analysis, and sequentially excluding those variables not related at $p < 0.10$
22 in the adjusted model using the likelihood ratio test. For comparability purposes, variables remaining at $p <$
23 0.10 in any of the models were entered in all the models. For ordinal categorical variables, the p for a linear
24 trend was also calculated.

25 We used non-parametric receiver operating characteristic (ROC) curve to analyse the relationship between
26 the sensitivity (probability of a positive test among smokers) and false positive (probability of a positive test
27 among non-smokers, 1-specificity) cases for various different cut-off points that dichotomize UC to
28 distinguish smokers from non-smokers, using self-reported cigarette smoking status as the reference value.
29 Overall accuracy was evaluated by means of the area under the curve (AUC) (showing the ability of the
30

urinary cotinine to correctly classify smoking status with varying cut off points.²¹ The optimal cut-off point for UC to discriminate smokers from non smokers was the value (c) associated with the Youden's index (J), defined by: $J = \text{maximum}\{\text{sensitivity}(c) + \text{specificity}(c) - 1\}$.²² This value is 'optimal' in the sense that maximizes the overall rate of correct classification in the absence of a loss function (i.e., giving the same weight to errors of sensitivity and specificity). Since the shape of the distribution of the estimator of the optimal cut off point was unknown, we used the percentile bootstrap method, with 2000 resampling simulations, to establish 95% confidence intervals, with the aid of the 'boot' package of R.²³ Additionally, the data were analysed for the most widely used cut-off points, namely 50 and 100 ng/ml, or 25 and 50 ng/ml when analyses were restricted to occasional smokers. Thirty-five ~~W~~women who declared that they did not smoke but with implausible UC levels in non smokers (>500 ng/ml) were excluded from ~~the~~ these analyses in order to diminish the measurement error of self-reported cigarette smoking. The cut-off points were also calculated after stratification among non smokers for SHS exposure in three groups: 791 women that referred not exposed to SHS, 718 exposed to one source of SHS and 292 exposed to more than one source. Additional sensitivity analysis excluded 290 self-reported non-smokers who claimed to stop smoking during pregnancy, since this group is more likely to misreport their smoking status. Likewise, occasional smoking was analysed excluding non smokers exposed to SHS. Assuming $\alpha = 0.05$, 95% CI were calculated for ORs, cut-off points and area under ROC curve. All statistical tests were two-sided. Statistical analysis was carried out using SPSS (version 17.0) and R (2.11.1) statistical software.

RESULTS

Study setting and characteristics of the sample

Overall, 61.2% of women reported to have smoked at least once in their life, while 32.4% were occasional or regular smokers when they became pregnant, falling to 19.7% at first trimester and 18.5% at third trimester of their pregnancy (Table 1).

Table 1: Description of the sample and variables of interest.

	N ^a	%
Cohort		
Asturias	416	18.4
Gipuzkoa	545	24.1

1			
2	Sabadell	591	26.1
3	Valencia	711	31.4
4			
5	Age		
6	≤ 24	154	6.8
7	25-29	717	31.7
8			
9	30-34	973	43.0
10			
11	≥ 35	418	18.5
12	Social class		
13	I-II (more affluent)	492	21.8
14			
15	III	584	25.8
16			
17	IV-V (less affluent)	1186	52.4
18	Level of education		
19	Primary or no education	547	24.2
20			
21	Secondary	936	41.4
22			
23	University	776	34.4
24	BMI (pre-pregnancy)		
25	<18.5	100	4.4
26			
27	18.5 – 25	1568	69.3
28			
29	25 – 30	420	18.6
30			
31	≥ 30	175	7.7
32	Previous parity	957	43.1
33	Birth In Europe	2130	94.3
34	Reported having smoked in their life		
35	No	879	38.8
36			
37	Occasional	146	6.5
38			
39	Regular	1238	54.7
40	Reported smoking at the start of pregnancy		
41	No	1529	67.6
42			
43	Occasional	28	1.2
44			
45	Regular	706	31.2
46	Reported smoking at first trimester of pregnancy		
47	No	1813	80.3
48			
49	Occasional	35	1.6
50			
51	Regular	410	18.2
52	Reported smoking at third trimester of pregnancy		
53	No	1845	81.5
54			
55	Occasional	37	1.6
56			
57	Regular	381	16.8
58	Year of urine sampling		
59			
60			

2004	321	14.2
2005	857	37.9
2006	466	20.6
2007	470	20.8
2004	149	6.6
Cigarettes/day at third trimester of pregnancy		
0	1845	81.5
Occasional	37	1.6
1-4	149	6.6
5-9	141	6.2
≥ 10	91	4.0
Exposed to SHS in non-smoking women ^b :		
At home (partner or others)	479	26.0
At work	186	10.1
Elsewhere in leisure time ^c	715	38.8
Number of sources of exposure to SHS ^d		
0	798	43.5
1	735	40.0
2	271	14.8
3	32	1.7
Cotinine (ng/ml) all the women		
< 50	1773	78.3
50-99	31	1.4
100-199	19	0.8
200-499	52	2.3
500-999	70	3.1
≥ 1000	318	14.1

a: The numbers and rates that do not match the total are due to missing data

b: Percentages calculated including non exposed women

c: Other homes or public places, e.g. pubs or restaurants

d: Work, home and elsewhere in leisure time among non smokers

Smoking and SHS exposure

The median UC level in women who did not refer to smoke and were not exposed to SHS was below the quantification level of 4.0 ng/ml while in non-smokers exposed to SHS it was 7.6 ng/ml. Among all smokers the UC median level was 1744.3 ng/ml (Table 2). Occasional smokers had a median level of 260.7 ng/ml. Among daily smokers statistically significant differences were observed between UC concentration and the number of cigarettes smoked per day ($p < 0.001$), showing a clear dose-response pattern (not statistically tested). In the same way, in non-smokers there were statistically significant differences between UC levels and the number of sources of exposure to SHS; that are, work, home and elsewhere in leisure time ($p < 0.001$), with a progressive dose-response pattern (not tested, neither). Figure 1 shows the different distribution patterns of UC among non-smokers, exposed or not to SHS, and occasional and daily smokers.

Table 2: Active smoking and exposure to SHS in pregnant women in the INMA cohort. Median levels of urinary cotinine (ng/ml) at third trimester of pregnancy.

	N	%	Urinary cotinine ^a
Total	2263	100	7.4
Non smokers ^b	1845	81.5	4.4
No SHS exposure	798	35.3	< 4
SHS exposure	1038	45.9	7.6
1 source ^{c, d}	735	32.5	5.8
2 sources	271	12.0	11.7
3 sources	32	1.4	16.9
Smokers ^d	418	18.5	1744.3
Occasional	37	1.6	260.7
1-2 cigarettes/day	76	3.4	1036.4
3-4 cigarettes/day	73	3.2	1330.7
5-9 cigarettes/day	141	6.2	1848.5
≥ 10 cigarettes/day	91	4.0	3033.0

a: Median level of urinary cotinine ng/ml.

b: Exposed and not exposed to SHS; Mann-Whitney test: $p < 0.001$ for smoking and urinary cotinine

c: Sources of exposure to SHS at work/at home/in leisure time outside the home

d: Kruskal Wallis test $p < 0.001$

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Figure 2: Distribution of urinary cotinine (ng/ml) according to active or passive tobacco exposure in pregnant women from the INMA cohort.

Me: median

SHS: Second-hand smoking

SRS Occas: Self reported smoking, occasional

SRS Daily: Self reported smoking, daily

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Self-reported smoking and misclassification

Among the 2263 women studied, 1755 (77.6%) reported that they did not smoke and had UC levels below 50 ng/ml (true negative). A further 18 (0.8%) also had UC levels under 50 ng/ml despite claiming to smoke, though 13 of these claimed to be occasional smokers. On the other hand, 90 women (3.9%) reported that they did not smoke but were found to have UC levels above 50 ng/ml and were considered as misclassified and, finally, 400 women (17.7%) were true positive. Table 3 shows the ORs of the variables associated with smoking and misclassification, before and after adjusting. In the adjusted model, the risk of smoking and misclassification were associated with low educational level, country of birth, and exposure to SHS. Age was related only to misclassification risk. In regards to smoking history, only smoking at the beginning of pregnancy was associated with misclassification. The year of urine sampling and the social class were statistically associated only in the unadjusted analysis. Adding women misclassified to self-reported smokers the pattern of the association found with self-reported smoking did not vary.

Table 3: Unadjusted and adjusted odds ratios (ORs) and variables associated with smoking, self-reported and misclassification of smoking status.

	Unadjusted analysis							Adjusted analysis ^a					
	Non-smokers ^b		Self-reported smokers ^c			Misclassification ^d		Self-reported smokers ^c		Misclassification ^d		Both ^e	
	N	OR	95% CI	N	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	
Total	1755	418	-	-	90	-	-	-	-	-	-	-	
Cohort													
Asturias	326	75	1	-	15	1	-	1	-	1	-	-	
Gipuzkoa	467	65	0.60	0.42 to 0.88	13	0.60	0.27 to 1.36	0.77	0.52 to 1.15	0.63	0.28 to 1.42	0.75	0.52 to 1.09
Sabadell	466	99	0.92	0.66 to 1.29	26	1.21	0.61 to 2.44	0.68	0.47 to 0.99	0.81	0.40 to 1.64	0.72	0.51 to 1.02
Valencia	496	179	1.57	1.15 to 2.15	36	1.58	0.82 to 3.07	0.94	0.67 to 1.33	0.84	0.43 to 1.66	0.95	0.69 to 1.31
Age													
≤ 24	93	43	1	-	18	1	-	1	-	1	-	1	
25-29	537	152	0.62	0.40 to 0.94	28	0.27	0.14 to 0.53	0.87	0.55 to 1.38	0.36	0.18 to 0.73	0.73	0.49 to 1.11
30-34	792	153	0.42	0.27 to 0.64	28	0.18	0.09 to 0.36	0.75	0.48 to 1.19	0.26	0.13 to 0.54	0.61	0.41 to 0.92
≥ 35	332	70	0.46	0.29 to 0.73	16	0.25	0.12 to 0.54	0.90	0.54 to 1.49	0.46	0.21 to 1.04	0.76	0.48 to 1.19
				(p ^f < 0.001)			(p ^f = 0.001)						
Country of birth													
In Europe	1637	410	1	-	83	1	-	1	-	1	-	1	
Outside Europe	114	8	0.28	0.13 to 0.60	7	1.21	0.50 to 2.79	0.25	0.12 to 0.54	1.11	0.46 to 2.65	0.39	0.21 to 0.69
Level of education													
University	695	59	1	-	22	1	-	1	-	1	-	1	
Secondary	703	192	3.22	2.36 to 4.39	41	1.84	1.09 to 3.17	2.37	1.70 to 3.29	1.17	0.66 to 2.08	2.08	1.55 to 2.78
Primary or less	355	165	5.47	3.96 to 7.57	27	2.40	1.34 to 4.32	3.30	2.31 to 4.70	1.02	0.53 to 1.97	2.72	1.97 to 3.74
				(p ^f < 0.001)			(p ^f = 0.002)						
Social class													
I+II (highest)	438	41	1	-	13	1	-	-	-	-	-	-	

III	469	96	2.19	1.48 to 3.22	19	1.36	0.67 to 2.80	-	-	-	-	-	-
IV+V (lowest)	847	281	3.54	2.50 to 5.02	58	2.31	1.25 to 4.26	-	-	-	-	-	-
				(p ^f < 0.001)			(p ^f = 0.002)						
Year of urine sampling													
2004	224	78	1	-	19	1	-	-	-	-	-	-	-
2005	642	179	0.80	0.59 to 1.09	36	0.66	0.37 to 1.18	-	-	-	-	-	-
2006	362	83	0.66	0.46 to 0.94	21	0.68	0.36 to 1.30	-	-	-	-	-	-
2007	399	58	0.42	0.29 to 0.61	13	0.38	0.19 to 0.79	-	-	-	-	-	-
2008	128	20	0.45	0.26 to 0.77	1	0.09	0.01 to 0.70	-	-	-	-	-	-
				(p ^f < 0.001)			(p ^f = 0.001)						
Exposure to SHS at home													
No	1328	144	1	-	34	1	-	1	-	1	-	1	-
Yes	423	274	5.97	4.75 to 7.51	56	5.17	3.33 to 8.03	4.41	3.44 to 5.64	3.26	2.03 to 5.25	4.39	3.49 to 5.51
Exposure to SHS at work													
No	1582	343	1	-	76	1	-	1	-	1	-	1	-
Yes	172	74	1.98	1.48 to 2.67	14	1.69	0.94 to 3.06	1.55	1.11 to 2.17	1.37	0.72 to 2.59	1.57	1.14 to 2.15
Exposure to SHS elsewhere in leisure time													
No	1082	175	1	-	44	1	-	1	-	1	-	1	-
Yes	669	243	2.25	1.81 to 2.79	46	1.69	1.11 to 2.59	1.88	1.44 to 2.34	1.47	0.92 to 2.34	1.80	1.44 to 2.26
Reported having smoked in their life ^g													
No	862	0	-	-	17	1	-	-	-	-	-	-	-
Yes	893	418	-	-	73	4.15	2.43 to 7.09	-	-	-	-	-	-
Reported smoking at the start of pregnancy ^g													
No	1489	1	-	-	39	1	-	-	-	1	-	-	-
Yes	266	417	-	-	51	7.32	4.73 to 11.33	-	-	6.21	3.91 to 9.86	-	-

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- a: Only variables showed in the table were entered in the logistic equation
- b: Non-smokers: women who reported that they did not smoke and were found to have urinary cotinine levels of less than 50 ng/ml. the reference group
- c: Smokers: those who reported smoking
- d: Misclassification: those who claimed that they did not smoke but were found to have urinary cotinine levels above 50 ng/ml
- e: Both: c+d
- f: p for trend
- g: Only analysed with regards to misclassification, given the extremely strong association with smoking at third trimester of pregnancy

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Cut-off points of UC for smoking

Optimal cut-off points for distinguishing non-smokers from smokers (daily and occasional) calculated by the Youden's index (excluding self reported non-smokers with UC values above 500 ng/ml), was 82 ng/ml, with a sensitivity of 95.2%, specificity of 98.5% and AUC 0.995 (95% CI 0.992 to 0.997) (Table 4). Sensitivity and specificity for the cut-off points of 50 and 100 ng/ml were quite close to that of 82 ng/ml. The exclusion from the analysis of 290 women who declared quitting smoking during pregnancy as possible group at risk of underreporting of their smoking status, did not improve substantially the validation parameters of the test (data not shown).

Table 4: Parameters for assessing the optimal cut-off point for urinary cotinine, ng/ml, obtained by the Youden's index, as well as the levels of 25, 50, and 100 ng/ml, for classifying pregnant women as regular or occasional smokers.^a

Youden's index ^b	Cut-off point (95% CI) ^c	Sensitivity	Specificity	Positive PV ^d	Negative PV ^d	AUC of the ROC (95% CI) ^e
Regular and occasional smokers: 1810 non-smokers, 418 smokers						
-	50	0.957	0.970	0.879	0.990	
-	100	0.950	0.985	0.936	0.988	0.995 (0.992 to 0.997)
0.937	82 (42 to 133)	0.952	0.985	0.934	0.989	
Results stratified by frequency of smoking						
Occasional smokers: 1810 non-smokers, 37 smokers						
-	25	0.892	0.887	0.139	0.998	
-	50	0.649	0.970	0.304	0.993	0.961 (0.939 to 0.984)
0.789	27 (11 to 43)	0.892	0.897	0.151	0.998	
Daily smokers: 1810 non-smokers, 381 smokers						
-	50	0.987	0.970	0.872	0.997	
-	100	0.982	0.985	0.933	0.996	0.998 (0.996 to 1)
0.968	115 (57 to 189)	0.982	0.986	0.937	0.996	
Results stratified by SHS exposure among non-smokers						
Non exposed to SHS: 791 non-smokers, 418 active smokers						
-	50	0.957	0.996	0.993	0.978	
-	100	0.950	0.999	0.997	0.974	0.998 (0.997 to 1)
0.962	42 (27 to 57)	0.971	0.991	0.983	0.985	
Exposed to SHS (1 source^f): 718 non-smokers, 418 active smokers						
-	50	0.957	0.957	0.928	0.974	
-	100	0.950	0.981	0.966	0.971	0.993 (0.990 to 0.997)
0.933	82 (55 to 136)	0.952	0.981	0.966	0.972	
Exposed to SHS (2-3 sources^f): 292 non-smokers, 418 active smokers						
-	50	0.957	0.928	0.950	0.938	
-	100	0.950	0.959	0.971	0.930	0.988 (0.982 to 0.994)
0.912	106 (79 to 227)	0.950	0.962	0.973	0.930	

a: Excluding cases with cotinine > 500 ng/ml in self-reported non-smokers (n=35).

b: Youden's index = max (Sensitivity+Specificity-1).

c: 95% bootstrap confidence interval for the cut-off point associated with the Youden's index.

d: Predictive value of a positive or negative result for the prevalence of the study: 18.5%.

e: Area under the receiver operating characteristic curve and 95% confidence interval.

f: Number of sources of exposure among: work, home and elsewhere in leisure time.

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4 The Youden's index and AUC for daily smoking were higher, with a cut-off point of 115 ng/ml. Occasional
5 smoking was analysed, by excluding from the analysis the 381 women who admitted that they smoked
6 regularly at third trimester of pregnancy. The optimal cut-off point for discriminating occasional smokers
7 from non-smokers was 27 ng/ml (95% CI 11 to 43), with a sensitivity and specificity of 89.2% and 89.7%,
8 respectively. The exclusion of women who declared to quit during pregnancy, improved the specificity to
9 91.8%, but did not almost change the Youden's Index or the sensitivity. Excluding non SHS exposed among
10 non-smokers, the optimal cut-off point was 19 ng/ml (95% CI 11 to 33), and improving the specificity to
11 93.7% and to 41.2% the positive predictive value (probability of smoking status being a positive test).
12 Nevertheless, these low positive predictive values are consequence, above all, of the low prevalence of
13 occasional smoking in this sample.

14
15 Not exposed women to SHS compared with all smokers, daily or occasional, had a lower cut-off point of 42
16 ng/ml (95% CI 27 to 57), while for exposed to one or to two or more sources of SHS, cut-off points were 82
17 (95% CI 55 to 136) and 106 ng/ml (95% CI 79 to 201), respectively.

30 DISCUSSION

32 Main findings in relation to the literature

33
34 The prevalence of smoking in pregnant women at third trimester was 18.5%. In this later stage, the
35 prevalence of active smoking increased up to 22.5% if women who did not report smoking but had UC levels
36 above 50 ng/ml were reclassified as smokers, assuming that false positives were due to maternal
37 misreporting of smoking status. Prevalence of self reported smokers and misclassified in our study is close to
38 the referred by Kendrick *et al*⁶ and Lindqvist *et al*⁷, and smoking rate and UC levels are lower than that
39 showed by Pickett *et al*¹⁵. Our study had, nevertheless, a lower rate of smoking misreporting than other
40 studies.²⁻⁸

41
42 There was a clear relationship between UC and smoking dose among smokers, and with the number of
43 sources of exposure to SHS among non-smokers. Specifically, those who smoked 10 or more cigarettes per
44 day had median UC levels of 3033 ng/ml, while the levels were 260 ng/ml for occasional smokers and less
45 than 17 ng/ml for non-smokers, increasing with the number of sources of exposure to SHS. This data
46 reinforces the validity of UC also as an indicator of exposure to SHS.¹⁰

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England *et al*¹³ indicated that few studies have identified differences between misclassified and self-reported smokers and the way in which this would affect epidemiological studies. Our study shows similar patterns of association and both self-reported smoking and misclassification were strongly associated with various predictive variables. In particular, we found a higher risk of smoking and misclassification among women with low education level. These results are consistent with those reported by other authors.^{6 14 24} We also found a higher risk of smoking and misclassification in women from Europe, and women exposed to SHS in different places. Women younger than 24 years had an increased risk of misreport her smoking, as indicated by Dietz *et al*.⁴ In this study, exposure to SHS was associated with smoking. In other words, there were more smoking people around pregnant women who smoked. In addition, misclassification was significantly associated with exposure to SHS at home. Jhun *et al*¹⁴ and Orr *et al*²⁵ also showed higher prevalence of smoking among pregnant women whose partners smoked at home. Having smoked previously was associated with a higher probability of misreporting the habit, as observed by England *et al*.¹³

This work showed an optimal cut-off point for discriminate pregnant women smokers from non-smokers of 82 ng/ml, with a confidence interval of 42 to 133 ng/ml. Some studies proposed a cut-off of 50 ng/ml,¹³⁻¹⁶ coherent with the women not SHS exposed in this study. Other studies proposed cut-off points of 79 ng/ml¹⁸ and 85 ng/ml,⁶ closer to the smoking dose and SHS exposure in our study sample. In our study population both prevalence of smoking and of SHS are high and this can explain in part why our optimal cut-off point is higher than those reported in other studies.¹³⁻¹⁷ This is also supported by the fact that the optimal cut-off point decreased to 42 ng/ml (27 to 57) when the analysis was restricted to women who reported no SHS exposure, and increased according to the number of sources reported.. The validity of 27 ng/ml (11 to 43) as cut-off point for differentiating occasional smokers from non-smokers was lower than that for differentiating daily smokers, and it could depend on SHS exposure and on the time spent from the last cigarette smoked given the faster elimination of cotinine in pregnant women,¹⁰⁻¹² information not collected in this study. There are not validation studies of cotinine in different biological matrices, blood (plasma or serum), saliva or urine,^{9 16} so it cannot be established which the most reliable biomarker is.

Limitations of the study

The current study has several limitations. From the eligible population, the participation rate was 56%, and 85.6% of the women who agreed to participate completed the study. Therefore, the final study sample might not be fully representative of all pregnant women in the study areas, but its internal validity (absence of bias)

1 is not necessarily affected. There were other likely sources of misclassification in addition to maternal
2 misreporting of smoking status, as misclassification of non-smokers as smokers because of high exposure
3 degree to SHS. On the other hand, women who smoked occasionally but report to be non-smokers might
4 have low UC concentrations if they had not smoked recently, and their self-report and UC levels would be in
5 agreement.
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10 **No information about last cigarette or last SHS exposure was obtained. We lost the opportunity of analysing**
11 **this variable in the evolution of the UC, showing his influence in false negatives, above all, and especially**
12 **relevant for occasional smokers.**
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14 Since the optimal cut-off point for UC is determined using self-reported smoking status as the gold standard,
15 the validity of this assumption is important. On the one hand, it is unlikely that a non-smoking woman
16 declared to be a smoker, because a battery of items should be completed detailing smoking habits in this
17 case. On the other hand, however, it is possible that some smokers did not reveal their habit. In order to
18 minimize this type of bias, we excluded in the main analysis self-reported non-smokers with implausible
19 high UC levels. In additional analysis, we excluded self-reported non-smokers who claimed to stop smoking
20 during pregnancy, since these cases are at higher risk of misclassification as reported in table 3; the optimal
21 cut-off point did not change after this exclusion. In general terms, the AUC shows a good overall accuracy,
22 and we think that self-reported smoking is a reliable measure in this study. If some kind of misclassification
23 occurs, it would lead to a shift towards the right in both distributions, and a slight overestimation of the
24 optimal cut-off point as a result.
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40 One of the main strengths of this study was the possibility of assessing the role of baseline exposures to SHS
41 in the estimate of cut-offs, given the detailed information collected on SHS exposure and its elevated
42 prevalence. The confirmation that the cut-offs would differ according to the level of exposure to SHS
43 emphasizes the need of taking it into account, especially in countries with elevated SHS exposure.
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48 **Implications for practice**

49 This study shows that the efforts made to encourage women to give up smoking before or during pregnancy
50 are not sufficient or particularly effective, given that at least 18.5% of the pregnant women smoked in the
51 third trimester. The results of this study indicate that the groups to which the most effort should be directed
52 are young women, those of a European origin and those from a low social class. Further, the association
53 observed in this study between active smoking of pregnant women and the presence of smokers in their close
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1 environment supports the hypothesis that this factor makes it more difficult to stop [smoking](#).²⁶ It is necessary
2
3 to undertake effective programmes for reducing smoking before and during pregnancy, reaching also
4
5 misclassified, and to reduce SHS exposure, in order to prevent risks for women and foetus.
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8 **CONCLUSION**

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10 Smoking is an important risk factor for health and development and should be taken into account as
11
12 confounder when analysing the potential effects of environmental contaminants in studies like the INMA
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14 project. To have a reliable marker like UC and a valid a cut-off point able to discriminate regular or
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16 occasional smokers from non-smokers is a critical issue. The cut-off point of 82 ng/ml showed a good
17
18 validity for discriminating smokers from non-smokers in our study sample, while 27 ng/ml is the optimal
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20 point for discriminating occasional smokers from non-smokers. It should be emphasized that cut-offs would
21
22 differ based on baseline exposure to SHS, and this should be taken into account when selecting reference
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24 cut-offs for specific populations.
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28 **Contributors:** All authors contributed to various aspects of this paper. JJA, MM and MR designed the study
29
30 and analysed the data. AMC and ME analysed cotinine in urine samples. MJL, AMC, LSM, MG, AF-S, ME,
31
32 AL, AT and FB revised the design of the study and the results. JJA redacted the manuscript and the other
33
34 authors participated in the review of the different drafts and approved the final version.
35

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38 questionnaires. A full listing of the INMA project researchers can be found at
39
40 http://www.proyectoinma.org/presentacioninma/listado-investigadores/en_listado-investigadores.html
41

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2 Oviedo and the Conselleria de Sanitat Generalitat Valenciana. [http://www.proyectoinma.org/instituciones-](http://www.proyectoinma.org/instituciones-participantes/en_entidades-colaboradoras/)
3 [participantes/en_entidades-colaboradoras/](http://www.proyectoinma.org/instituciones-participantes/en_entidades-colaboradoras/)
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6 **Data Sharing Statement:** There is no additional data available.
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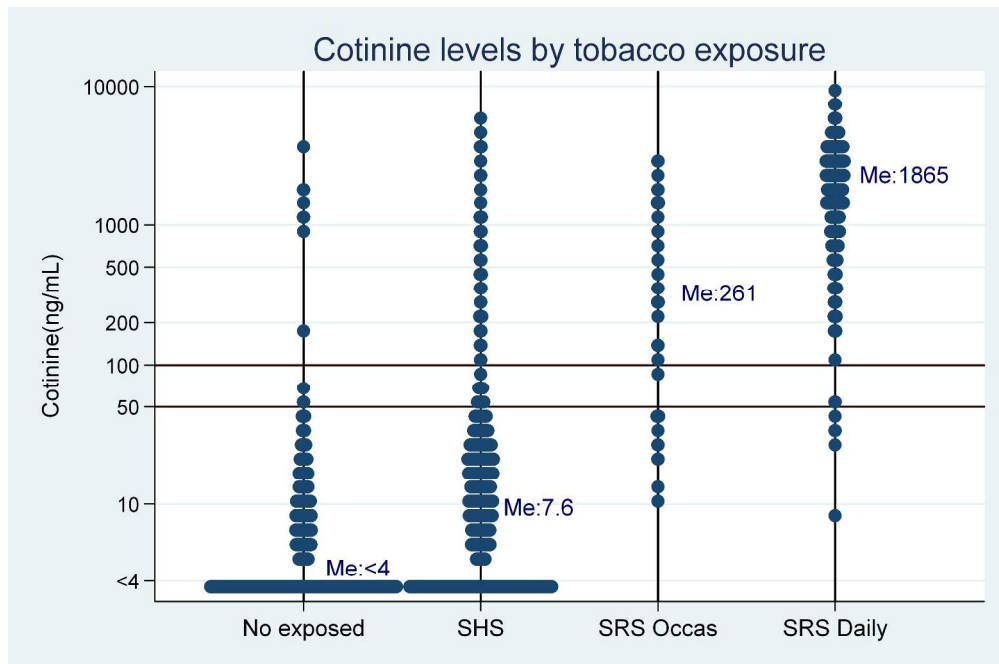
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Determinants of self-reported smoking and misclassification during pregnancy, and analysis of optimal cut-off points of urinary cotinine: a cross sectional study

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5 **Determinants of self-reported smoking and misclassification during pregnancy, and analysis**
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10 JJ Aurrekoetxea ^{1,2,3}, M Murcia ^{4,5}, M Rebagliato ^{4,5}, MJ López ^{4,6,7}, AM Castilla ^{1,4}, L Santa Marina ^{1,3,4}, M
11 Guxens ^{4,8,9}, A Fernández-Somoano ^{4,10}, M Espada ¹, A Lertxundi ^{2,3,4}, A Tardón ^{4,10}, F Ballester ^{4,5,11}
12
13
14 (Juan J Aurrekoetxea ^{1,2,3}, Mario Murcia ^{4,5}, Marisa Rebagliato ^{4,5}, María José López ^{4,6,7}, Ane Miren Castilla
15 ^{1,4}, Loreto Santa Marina ^{1,3,4}, Mónica Guxens ^{4,8,9}, Ana Fernández-Somoano ^{4,10}, Mercedes Espada ¹, Aitana
16 Lertxundi ^{2,3,4}, Adonina Tardón ^{4,10}, Ferran Ballester ^{4,5,11})
17
18

19
20
21 1 *Public Health Department, Basque Government, Spain*

22 2 *University of Basque Country UPV/EHU, Spain*

23 3 *Health Research Institute (BIODONOSTIA), Spain*

24 4 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP)*

25 5 *Center for Public Health Research (CSISP), Valencia, Spain*

26 6 *Public Health Agency of Barcelona, Spain*

27 7 *Institute of Biomedical Research (IIB Sant Pau), Spain*

28 8 *Center for Research in Environmental Epidemiology (CREAL), Spain*

29 9 *Hospital del Mar Research Institute (IMIM), Barcelona, Spain*

30 10 *University of Oviedo, Spain*

31 11 *University of Valencia, Spain*

32
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42
43 **Juan J Aurrekoetxea**, *Public Health Department, Basque Government, Spain;*

44 *University of Basque Country UPV/EHU, Spain;*

45 *Health Research Institute (BIODONOSTIA), Spain.*

46 jj.aurreko@gmail.com

47
48
49 **Mario Murcia**: *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

50 *Center for Public Health Research (CSISP); Valencia, Spain.*

51 murcia_mar@gva.es

52
53
54 **Marisa Rebagliato**, *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

55 *Center for Public Health Research (CSISP); Valencia, Spain.*

56 rebagliato_mar@gva.es

57
58
59 **Maria José López**, *Public Health Agency of Barcelona, Spain;*

1 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

2 *Institute of Biomedical Research (IIB Sant Pau), Spain.*

3 mjlopez@aspb.cat

4 **Ane Miren Castilla**, *Public Health Laboratory of Bilbao, Spain.*

5 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

6 ANE_M2002@yahoo.es

7 **Loreto Santa Marina**, *Public Health Department, Basque Government, Spain;*

8 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

9 *Health Research Institute (BIODONOSTIA), Spain.*

10 ambien4ss-san@ej-gv.es

11 **Mònica Guxens**, *Center for Research in Environmental Epidemiology (CREAL), Spain;*

12 *Hospital del Mar Research Institute (IMIM), Spain;*

13 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

14 mguxens@creal.cat

15 **Ana Fernández-Somoano**, *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

16 *University of Oviedo, Spain.*

17 capua.uo@uniovi.es

18 **Mercedes Espada**, *Public Health Department, Basque Government, Spain;*

19 metabobi-san@ej-gv.es

20 **Aitana Lertxundi**, *University of Basque Country UPV/EHU, Spain;*

21 *Health Research Institute (BIODONOSTIA), Spain;*

22 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

23 aitana.lertxundi@gmail.com

24 **Adonina Tardón**, *University of Oviedo, Spain;*

25 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

26 atardon@uniovi.es

27 **Ferran Ballester**, *University of Valencia, Spain;*

28 *Center for Public Health Research (CSISP), Valencia, Spain;*

29 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

30 ballester_fer@gva.es

31 Corresponding author: Juan J Aurrekoetxea Ph.D; Departamento de Sanidad del Gobierno Vasco; C/ Sancho el Sabio 35; 20.010, San

32 Sebastián; Telephone: 34.943.023033; Fax: 34.943.023093; E-mail: jj_aurreko@gmail.com

33 **Key words:** Pregnancy; Smoking; Validation studies; Cut-off; Urinary cotinine.

ABSTRACT

Objectives: To estimate the prevalence and factors associated with smoking and misclassification in pregnant women from INMA [*Infancia y Medio Ambiente*, Environment and Childhood] project, Spain, and to assess the optimal cut-offs for urinary cotinine (UC) that best distinguishes daily and occasional smokers with varying levels of SHS exposure.

Design: We used logistic regression models to study the relationship between sociodemographic variables and self-reported smoking and misclassification (self-reported non-smokers with UC >50ng/ml). ROC curves were used to calculate the optimal cut-off point for discriminating smokers. The cut-offs were also calculated after stratification among non smokers for SHS exposure by number of sources. The cut-off points used to discriminate smoking status were the level of UC given by the Youden's Index and for 50 and 100ng/ml for daily smokers, or 25 and 50ng/ml for occasional smokers.

Participants: At third trimester of pregnancy 2263 pregnant women of the INMA Project were interviewed between 2004 and 2008 and a urine sample was collected.

Results: Prevalence of self-reported smokers at third trimester of pregnancy was 18.5%, and other 3.9% misreported their smoking status. Variables associated with self-reported smoking and misreporting were similar, including born in Europe, educational level, and exposure to SHS. The optimal cut-off was 82ng/ml (95%CI 42-133); sensitivity: 95.2% and specificity: 96.6%. The area under the ROC curve was 0.986 (95%CI 0.982-0.990). The cut-offs varied according to the SHS exposure level being 42 (95%CI 27-57), 82 (95%CI 46-136) and 106ng/ml (95%CI 58-227) for not SHS exposed, exposed to one and to two or more sources of SHS, respectively. The optimal cut-off for discriminating occasional smokers from non-smokers was 27ng/ml (95%CI 11-43).

Conclusions: Prevalence of smoking during pregnancy in Spain remains high. UC is a reliable biomarker for classifying pregnant women according to their smoking status. However, cut-offs would differ based on baseline exposure to SHS.

ARTICLE SUMMARY

Article focus

The focus of this study is on:

- There is no current consensus regarding the cut-off point for urinary cotinine in pregnant women able to discriminate regular or occasional smokers from non-smokers,
- These cut-offs would also differ according to baseline exposure to second hand smoke (SHS)
- This study assess the maternal factors influencing both self-reported and misclassification of smoking; and evaluate the optimal cut-off point for urinary cotinine that best distinguishes smokers from non-smokers according to frequency of smoking and SHS exposure.

Key messages

- The prevalence of both smoking (18.5%) and SHS exposure (45.9%) was high in a population based sample of pregnant women in Spain.
- Factors associated with self-reported smoking and misreporting were similar, including lower level of education and living in a smoking environment, which highlights the need of reinforcing the preventive interventions and policies.
- The optimal cut-off point to discriminate smokers from non-smokers varied according to the frequency of smoking (occasional or daily smokers) and to SHS exposure levels.
- This study highlights the importance of SHS exposure for selecting reference cut-offs to discriminate smoking status, especially in high SHS exposed populations.

Strengths and limitations of this study

- This study has the ability to assess the role of baseline exposures to SHS in the estimate of cut-offs, given the detailed information collected on SHS exposure and its elevated prevalence.
- This study uses population based samples of pregnant women from the INMA birth cohort, which might not be fully representative of all pregnant women in the study areas,

INTRODUCTION

Risks for mother and foetus has been widely related to smoking during pregnancy.¹ Several studies have indicated that pregnant women tend to under-report their consumption of tobacco,²⁻⁸ due to social pressure⁹ or to avoid criticism from health professionals.³ Indeed, it is known to be a higher rate of misreporting of smoking among the groups in which it is not considered as acceptable, such as pregnant women and patients with smoking-related diseases.⁹

Cotinine is the main metabolite of nicotine and the biomarker of choice for distinguishing smokers from non-smokers and for assessing exposure to second-hand smoke (SHS).¹⁰ The women's clearance of cotinine is faster during pregnancy¹¹ and its plasma half-life is a little less than 9h.¹² For this reason, urinary cotinine (UC) tests may give false negatives in pregnant women who have not recently smoked.

There is no current consensus regarding the cut-off point for UC in pregnant women. Several thresholds have been proposed being 50 ng/ml, the most widely used.¹³⁻¹⁶ On the other hand, Higgins *et al*¹⁷ proposed 25 ng/ml as the cut-off point, while Gorber *et al*⁹ underlined the need to decide on a suitable threshold for pregnant women in particular, for whom the sensitivity of the test may be different, and also suggested that a new cut-off point should be established for occasional smokers. Spierto *et al*¹⁸ found 79 ng/ml as the cut-off between non-smoker and smoker pregnant women.

The aims of our study were: 1) to assess the prevalence of self-reported smoking and the UC levels in a cohort of pregnant women; 2) to assess the prevalence of misclassification of maternal smoking status according to the most widely accepted cut-off point in the literature of 50 ng/ml, and to study maternal factors associated with both self-reported and misclassification of maternal smoking; and 3) to identify the optimal cut-off point for UC that best distinguishes smokers from non-smokers in our study sample, according to frequency of smoking (occasional or daily smokers) and SHS exposure.

METHODS

Study population

The INMA [*Infancia y Medio Ambiente*, Environment and Childhood] project is a Spanish multi-centre prospective birth cohort study which aims to evaluate the impact of exposure to the most prevalent environmental pollutants, and the role of diet, on foetal and infant growth, health and development.¹⁹ From eligible pregnant women recruited between 2003 and 2008, a 56% agreed to participate. The inclusion criteria were at least 16 years of age, singleton pregnancy, enrolment at 10 to 13 weeks of gestation, no

1 assisted conception, delivery scheduled at the reference hospital, and no communication handicap. There was
2 no upper age limit for be a member of the cohort. Of the 2644 women who agreed to participate in the study,
3 119 (4.5%) were lost (59 miscarriages, 8 foetal death, 47 withdrew and 5 lost to follow-up). Around week 32
4 of their pregnancy 2263 of the 2525 remaining women completed between 2004 and 2008 a questionnaire on
5 smoking and other variables and provided urine samples for determination of UC (Figure 1). The hospital
6 ethics committee of each centre approved the research protocol and all pregnant women gave written
7 informed consent before inclusion at the first trimester of pregnancy.
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16 Figure 1: Flow-chart of the INMA birth cohort in relation to smoking and UC quantification.
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20 **Information concerning smoking**

21 Questionnaire on tobacco consumption included smoking history, patterns of consumption (occasional or
22 regular) and exposure to SHS. We considered the women who, at this interview, reported smoking
23 occasionally or daily to be smokers, regardless of their UC levels. Women who had UC levels higher than
24 the widely used level of 50 ng/ml to distinguish smokers from non-smokers,¹³⁻¹⁶ but who did not report
25 smoking, were classed as misclassified. It was considered that the participants were exposed to SHS when
26 they reported exposure at least twice a week in any of the following environments: at work, at home, or in
27 leisure time outside the home (e.g. bars/restaurants, or other homes). We analysed whether women had any
28 passive exposure to tobacco smoke (yes or no), and also the number of exposure sources, between 0 and 3,
29 according to the reported places of exposure: at work, at home and/or elsewhere in leisure time.
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40 **Urinary cotinine**

41 The urine samples were collected in the same interview in the morning during the third trimester of
42 pregnancy. Urine was collected in 100 ml polyethylene containers and stored at -20°C. One aliquot of the
43 sample from each of the participants was sent to the Public Health Laboratory of Bilbao (Spain) to be
44 analysed. All urine samples were stored for a minimum of one year and a maximum of 5 years before
45 analysis. The analysis of the UC was performed by competitive enzyme immunoassay (EIA) using
46 commercial EIA microplate test kits (OraSure Technologies, Inc., Bio-Rad) for determining salivary cotinine
47 adapted for urine samples using urine controls (0, 2.5, 10 and 50 ng/ml, Bio-Rad). Samples with UC levels
48 above 50 ng/ml were diluted. Before testing the urine samples the method was validated; a certified reference
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1 material was used (EPA/NIST Reference Material 8444) to evaluate the repeatability and reproducibility.
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3 The quantification limit was 4.0 ng/ml, the coefficient of repeatability 7% and the reproducibility 10%.
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6 **Other variables**

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8 The women were interviewed twice during pregnancy (first and third trimester of gestation) to obtain
9 information about their sociodemographic characteristics and life-style variables. Social status of the women
10 (or her partner, if she had never worked outside the home) was defined using Spanish adaptation of British
11 classification system.²⁰
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14 **Statistical analysis**

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16 The chi-square test was used to test hypotheses for categorical variables, while differences in the distribution
17 of UC according to categorical covariates were evaluated using the Mann-Whitney U and the Kruskal Wallis
18 tests. In order to identify the variables independently associated with being either a smoker, a misclassified,
19 or both, logistic regression models were built including geographical area and the variables related with the
20 outcome at $p < 0.10$ in the univariate analysis, and sequentially excluding those variables not related at $p < 0.10$
21 in the adjusted model using the likelihood ratio test. For comparability purposes, variables remaining at $p <$
22 0.10 in any of the models were entered in all the models. For ordinal categorical variables, the p for a linear
23 trend was also calculated.
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26 We used non-parametric receiver operating characteristic (ROC) curve to analyse the relationship between
27 the sensitivity (probability of a positive test among smokers) and false positive (probability of a positive test
28 among non-smokers, 1-specificity) cases for various different cut-off points that dichotomize UC to
29 distinguish smokers from non-smokers, using self-reported cigarette smoking status as the reference value.
30 Overall accuracy was evaluated by means of the area under the curve (AUC) (showing the ability of the
31 urinary cotinine to correctly classify smoking status with varying cut off points.²¹ The optimal cut-off point
32 for UC to discriminate smokers from non smokers was the value (c) associated with the Youden's index (J),
33 defined by: $J = \text{maximum}\{\text{sensitivity}(c) + \text{specificity}(c) - 1\}$.²² This value is 'optimal' in the sense that
34 maximizes the overall rate of correct classification in the absence of a loss function (i.e., giving the same
35 weight to errors of sensitivity and specificity). Since the shape of the distribution of the estimator of the
36 optimal cut off point was unknown, we used the percentile bootstrap method, with 2000 resampling
37 simulations, to establish 95% confidence intervals, with the aid of the 'boot' package of R.²³ Additionally, the
38 data were analysed for the most widely used cut-off points, namely 50 and 100 ng/ml, or 25 and 50 ng/ml
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1 when analyses were restricted to occasional smokers. The cut-off points were also calculated after
2 stratification among non smokers for SHS exposure in three groups: 798 women that referred not exposed to
3 SHS, 735 exposed to one source of SHS and 303 exposed to more than one source. Additional sensitivity
4 analysis was conducted (Supplementary Table) the first one excluding 1047 pregnant women non-smokers
5 who referred SHS exposure, the second one excluding 317 self-reported non-smokers who claimed to stop
6 smoking during pregnancy, since this group is more likely to misreport their smoking status, and the last one
7 excluding 35 women who declared that they did not smoke but with implausible UC levels in non smokers
8 (>500 ng/ml). Assuming $\alpha=0.05$, 95% CI were calculated for ORs, cut-off points and area under ROC curve.
9 All statistical tests were two-sided. Statistical analysis was carried out using SPSS (version 17.0) and R
10 (2.11.1) statistical software.
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24 RESULTS

25 Study setting and characteristics of the sample

26 Overall, 61.2% of women reported to have smoked at least once in their life, while 32.4% were occasional or
27 regular smokers when they became pregnant, falling to 19.7% at first trimester and 18.5% at third trimester
28 of their pregnancy (Table 1).
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Table 1: Description of the sample and variables of interest.

	N ^a	%
Cohort		
Asturias	416	18.4
Gipuzkoa	545	24.1
Sabadell	591	26.1
Valencia	711	31.4
Age		
≤ 24	154	6.8
25-29	717	31.7
30-34	973	43.0
≥ 35	418	18.5
Social class		
I-II (more affluent)	492	21.8
III	584	25.8
IV-V (less affluent)	1186	52.4
Level of education		
Primary or no education	547	24.2
Secondary	936	41.4
University	776	34.4
BMI (pre-pregnancy)		
<18.5	100	4.4
18.5 – 25	1568	69.3
25 – 30	420	18.6
≥ 30	175	7.7
Previous parity	957	43.1
Birth In Europe	2130	94.3
Reported having smoked in their life		
No	879	38.8
Occasional	146	6.5
Regular	1238	54.7
Reported smoking at the start of pregnancy		
No	1529	67.6
Occasional	28	1.2
Regular	706	31.2
Reported smoking at first trimester of pregnancy		
No	1813	80.3
Occasional	35	1.6

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2	Regular	410	18.2
3	Reported smoking at third trimester of pregnancy		
4	No	1845	81.5
5	Occasional	37	1.6
6	Regular	381	16.8
7	Year of urine sampling		
8	2004	321	14.2
9	2005	857	37.9
10	2006	466	20.6
11	2007	470	20.8
12	2004	149	6.6
13	Cigarettes/day at third trimester of pregnancy		
14	0	1845	81.5
15	Occasional	37	1.6
16	1-4	149	6.6
17	5-9	141	6.2
18	≥ 10	91	4.0
19	Exposed to SHS in non-smoking women ^b :		
20	At home (partner or others)	479	26.0
21	At work	186	10.1
22	Elsewhere in leisure time ^c	715	38.8
23	Number of sources of exposure to SHS ^d		
24	0	798	43.5
25	1	735	40.0
26	2	271	14.8
27	3	32	1.7
28	Cotinine (ng/ml) all the women		
29	< 50	1773	78.3
30	50-99	31	1.4
31	100-199	19	0.8
32	200-499	52	2.3
33	500-999	70	3.1
34	≥ 1000	318	14.1

a: The numbers and rates that do not match the total are due to missing data

b: Percentages calculated including non exposed women

c: Other homes or public places, e.g. pubs or restaurants

d: Work, home and elsewhere in leisure time among non smokers

Smoking and SHS exposure

The median UC level in women who did not refer to smoke and were not exposed to SHS was below the quantification level of 4.0 ng/ml while in non-smokers exposed to SHS it was 7.6 ng/ml. Among all smokers the UC median level was 1744.3 ng/ml (Table 2). Occasional smokers had a median level of 260.7 ng/ml. Among daily smokers statistically significant differences were observed between UC concentration and the number of cigarettes smoked per day ($p < 0.001$), showing a clear dose-response pattern (not statistically tested). In the same way, in non-smokers there were statistically significant differences between UC levels and the number of sources of exposure to SHS; that are, work, home and elsewhere in leisure time ($p < 0.001$), with a progressive dose-response pattern (not tested, neither). Figure 1 shows the different distribution patterns of UC among non-smokers, exposed or not to SHS, and occasional and daily smokers.

Table 2: Active smoking and exposure to SHS in pregnant women in the INMA cohort. Median levels of urinary cotinine (ng/ml) at third trimester of pregnancy.

	N	%	Urinary cotinine ^a
Total	2263	100	7.4
Non smokers ^b	1845	81.5	4.4
No SHS exposure	798	35.3	< 4
SHS exposure	1038	45.9	7.6
1 source ^{c, d}	735	32.5	5.8
2 sources	271	12.0	11.7
3 sources	32	1.4	16.9
Smokers ^d	418	18.5	1744.3
Occasional	37	1.6	260.7
1-2 cigarettes/day	76	3.4	1036.4
3-4 cigarettes/day	73	3.2	1330.7
5-9 cigarettes/day	141	6.2	1848.5
≥ 10 cigarettes/day	91	4.0	3033.0

a: Median level of urinary cotinine ng/ml.

b: Exposed and not exposed to SHS; Mann-Whitney test: $p < 0.001$ for smoking and urinary cotinine

c: Sources of exposure to SHS at work/at home/in leisure time outside the home

d: Kruskal Wallis test $p < 0.001$

Self-reported smoking and misclassification

Among the 2263 women studied, 1755 (77.6%) reported that they did not smoke and had UC levels below 50 ng/ml (true negative). A further 18 (0.8%) also had UC levels under 50 ng/ml despite claiming to smoke, though 13 of these claimed to be occasional smokers. On the other hand, 90 women (3.9%) reported that they did not smoke but were found to have UC levels above 50 ng/ml and were considered as misclassified and, finally, 400 women (17.7%) were true positive. Table 3 shows the ORs of the variables associated with smoking and misclassification, before and after adjusting. In the adjusted model, the risk of smoking and misclassification were associated with low educational level, country of birth, and exposure to SHS. Age was related only to misclassification risk. In regards to smoking history, only smoking at the beginning of pregnancy was associated with misclassification. The year of urine sampling and the social class were statistically associated only in the unadjusted analysis. Adding women misclassified to self-reported smokers the pattern of the association found with self-reported smoking did not vary.

Table 3: Unadjusted and adjusted odds ratios (ORs) and variables associated with smoking, self-reported and misclassification of smoking status.

	Unadjusted analysis							Adjusted analysis ^a					
	Non-smokers ^b		Self-reported smokers ^c			Misclassification ^d		Self-reported smokers ^c		Misclassification ^d		Both ^e	
	N	OR	95% CI	N	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	
Total	1755	418	-	-	90	-	-	-	-	-	-	-	
Cohort													
Asturias	326	75	1	-	15	1	-	1	-	1	-	-	
Gipuzkoa	467	65	0.60	0.42 to 0.88	13	0.60	0.27 to 1.36	0.77	0.52 to 1.15	0.63	0.28 to 1.42	0.75	0.52 to 1.09
Sabadell	466	99	0.92	0.66 to 1.29	26	1.21	0.61 to 2.44	0.68	0.47 to 0.99	0.81	0.40 to 1.64	0.72	0.51 to 1.02
Valencia	496	179	1.57	1.15 to 2.15	36	1.58	0.82 to 3.07	0.94	0.67 to 1.33	0.84	0.43 to 1.66	0.95	0.69 to 1.31
Age													
≤ 24	93	43	1	-	18	1	-	1	-	1	-	1	
25-29	537	152	0.62	0.40 to 0.94	28	0.27	0.14 to 0.53	0.87	0.55 to 1.38	0.36	0.18 to 0.73	0.73	0.49 to 1.11
30-34	792	153	0.42	0.27 to 0.64	28	0.18	0.09 to 0.36	0.75	0.48 to 1.19	0.26	0.13 to 0.54	0.61	0.41 to 0.92
≥ 35	332	70	0.46	0.29 to 0.73	16	0.25	0.12 to 0.54	0.90	0.54 to 1.49	0.46	0.21 to 1.04	0.76	0.48 to 1.19
				(p ^f < 0.001)			(p ^f = 0.001)						
Country of birth													
In Europe	1637	410	1	-	83	1	-	1	-	1	-	1	
Outside Europe	114	8	0.28	0.13 to 0.60	7	1.21	0.50 to 2.79	0.25	0.12 to 0.54	1.11	0.46 to 2.65	0.39	0.21 to 0.69
Level of education													
University	695	59	1	-	22	1	-	1	-	1	-	1	
Secondary	703	192	3.22	2.36 to 4.39	41	1.84	1.09 to 3.17	2.37	1.70 to 3.29	1.17	0.66 to 2.08	2.08	1.55 to 2.78
Primary or less	355	165	5.47	3.96 to 7.57	27	2.40	1.34 to 4.32	3.30	2.31 to 4.70	1.02	0.53 to 1.97	2.72	1.97 to 3.74
				(p ^f < 0.001)			(p ^f = 0.002)						
Social class													
I+II (highest)	438	41	1	-	13	1	-	-	-	-	-	-	

III	469	96	2.19	1.48 to 3.22	19	1.36	0.67 to 2.80	-	-	-	-	-	-
IV+V (lowest)	847	281	3.54	2.50 to 5.02	58	2.31	1.25 to 4.26	-	-	-	-	-	-
				(p ^f < 0.001)			(p ^f = 0.002)						
Year of urine sampling													
2004	224	78	1	-	19	1	-	-	-	-	-	-	-
2005	642	179	0.80	0.59 to 1.09	36	0.66	0.37 to 1.18	-	-	-	-	-	-
2006	362	83	0.66	0.46 to 0.94	21	0.68	0.36 to 1.30	-	-	-	-	-	-
2007	399	58	0.42	0.29 to 0.61	13	0.38	0.19 to 0.79	-	-	-	-	-	-
2008	128	20	0.45	0.26 to 0.77	1	0.09	0.01 to 0.70	-	-	-	-	-	-
				(p ^f < 0.001)			(p ^f = 0.001)						
Exposure to SHS at home													
No	1328	144	1	-	34	1	-	1	-	1	-	1	-
Yes	423	274	5.97	4.75 to 7.51	56	5.17	3.33 to 8.03	4.41	3.44 to 5.64	3.26	2.03 to 5.25	4.39	3.49 to 5.51
Exposure to SHS at work													
No	1582	343	1	-	76	1	-	1	-	1	-	1	-
Yes	172	74	1.98	1.48 to 2.67	14	1.69	0.94 to 3.06	1.55	1.11 to 2.17	1.37	0.72 to 2.59	1.57	1.14 to 2.15
Exposure to SHS elsewhere in leisure time													
No	1082	175	1	-	44	1	-	1	-	1	-	1	-
Yes	669	243	2.25	1.81 to 2.79	46	1.69	1.11 to 2.59	1.88	1.44 to 2.34	1.47	0.92 to 2.34	1.80	1.44 to 2.26
Reported having smoked in their life ^g													
No	862	0	-	-	17	1	-	-	-	-	-	-	-
Yes	893	418	-	-	73	4.15	2.43 to 7.09	-	-	-	-	-	-
Reported smoking at the start of pregnancy ^g													
No	1489	1	-	-	39	1	-	-	-	1	-	-	-
Yes	266	417	-	-	51	7.32	4.73 to 11.33	-	-	6.21	3.91 to 9.86	-	-

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- a: Only variables showed in the table were entered in the logistic equation
- b: Non-smokers: women who reported that they did not smoke and were found to have urinary cotinine levels of less than 50 ng/ml. the reference group
- c: Smokers: those who reported smoking
- d: Misclassification: those who claimed that they did not smoke but were found to have urinary cotinine levels above 50 ng/ml
- e: Both: c+d
- f: p for trend
- g: Only analysed with regards to misclassification, given the extremely strong association with smoking at third trimester of pregnancy

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Cut-off points of UC for smoking

Optimal cut-off points for distinguishing non-smokers from smokers (daily and occasional) calculated by the Youden's index (excluding self reported non-smokers with UC values above 500 ng/ml), was 82 ng/ml, with a sensitivity of 95.2%, specificity of 96.6% and AUC 0.986 (95% CI 0.982 to 0.990) (Table 4). Sensitivity and specificity for the cut-off points of 50 and 100 ng/ml were quite close to that of 82 ng/ml. The exclusion from the analysis of 317 self-reported non-smokers who claimed to stop smoking during pregnancy, since this group is more likely to misreport their smoking status, or 35 women with UC above 500 ng/ml and reporting that they did not smoke, did not improve substantially the validation parameters of the test (Supplementary table).

Table 4: Parameters for assessing the optimal cut-off point for urinary cotinine. ng/ml. obtained by the Youden's index. as well as the levels of 25, 50, and 100 ng/ml. for classifying pregnant women as regular or occasional smokers.

Youden's index ^a	Cut-off point (95% CI) ^b	Sensitivity	Specificity	Positive PV ^c	Negative PV ^c	AUC of the ROC (95% CI) ^d
Regular and occasional smokers: 1845 non-smokers, 418 smokers						
-	50	0.957	0.951	0.816	0.990	
-	100	0.950	0.966	0.865	0.988	0.986 (0.982 to 0.990)
0.918	82 (42 to 136)	0.952	0.966	0.863	0.989	
Results stratified by frequency of smoking						
Occasional smokers: 1845 non-smokers, 37 smokers						
-	25	0.892	0.870	0.121	0.998	
-	50	0.649	0.951	0.211	0.993	0.947 (0.923 to 0.970)
0.772	27 (11 to 43)	0.892	0.880	0.130	0.998	
Daily smokers: 1845 non-smokers, 381 smokers						
-	50	0.987	0.951	0.807	0.997	
-	100	0.982	0.966	0.858	0.996	0.990 (0.986 to 0.994)
0.949	115 (57 to 189)	0.982	0.967	0.862	0.996	
Results stratified by SHS exposure among non-smokers						
Non exposed to SHS: 798 non-smokers, 418 active smokers						
-	50	0.957	0.987	0.976	0.978	
-	100	0.950	0.990	0.980	0.974	0.994 (0.990 to 0.998)
0.954	42 (27 to 57)	0.971	0.982	0.967	0.985	
Exposed to SHS (1 source^e): 735 non-smokers, 418 active smokers						
-	50	0.957	0.935	0.893	0.974	
-	100	0.950	0.958	0.928	0.971	0.981 (0.974 to 0.989)
0.910	82 (46 to 136)	0.952	0.958	0.928	0.972	
Exposed to SHS (2-3 sources^e): 303 non-smokers, 418 active smokers						
-	50	0.957	0.894	0.926	0.938	
-	100	0.950	0.924	0.945	0.930	0.977 (0.966 to 0.987)
0.877	106 (58 to 227)	0.950	0.927	0.947	0.930	

a: Youden's index = max (Sensitivity+Specificity-1).

b: 95% bootstrap confidence interval for the cut-off point associated with the Youden's index.

c: Predictive value of a positive or negative result for the prevalence of smoking in the study group.

d: Area under the receiver operating characteristic curve and 95% confidence interval.

e: Number of sources of exposure among: work, home, and elsewhere in leisure time.

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4 The Youden's index and AUC for daily smoking were higher, with a cut-off point of 115 ng/ml. Occasional
5 smoking was analysed, by excluding from the analysis the 381 women who admitted that they smoked
6 regularly at third trimester of pregnancy. The optimal cut-off point for discriminating occasional smokers
7 from non-smokers was 27 ng/ml (95% CI 11 to 43), with a sensitivity and specificity of 89.2% and 89.7%,
8 respectively. The exclusion of women who declared to quit during pregnancy, improved the specificity to
9 91.8%, but did not almost change the Youden's Index or the sensitivity. Excluding non SHS exposed among
10 non-smokers, the optimal cut-off point was 19 ng/ml (95% CI 11 to 33), and improving the specificity to
11 93.7% and to 41.2% the positive predictive value (probability of smoking status being a positive test).
12 Nevertheless, these low positive predictive values are consequence, above all, of the low prevalence of
13 occasional smoking in this sample.

14 Not exposed women to SHS compared with all smokers, daily or occasional, had a lower cut-off point of 42
15 ng/ml (95% CI 27 to 57), while for exposed to one or to two or more sources of SHS, cut-off points were 82
16 (95% CI 55 to 136) and 106 ng/ml (95% CI 79 to 201), respectively.

30 DISCUSSION

32 Main findings in relation to the literature

34 The prevalence of smoking in pregnant women at third trimester was 18.5%. In this later stage, the
35 prevalence of active smoking increased up to 22.5% if women who did not report smoking but had UC levels
36 above 50 ng/ml were reclassified as smokers, assuming that false positives were due to maternal
37 misreporting of smoking status. Prevalence of self reported smokers and misclassified in our study is close to
38 the referred by Kendrick *et al*⁶ and Lindqvist *et al*⁷, and smoking rate and UC levels are lower than that
39 showed by Pickett *et al*¹⁵. Our study had, nevertheless, a lower rate of smoking misreporting than other
40 studies.²⁻⁸

42 There was a clear relationship between UC and smoking dose among smokers, and with the number of
43 sources of exposure to SHS among non-smokers. Specifically, those who smoked 10 or more cigarettes per
44 day had median UC levels of 3033 ng/ml, while the levels were 260 ng/ml for occasional smokers and less
45 than 17 ng/ml for non-smokers, increasing with the number of sources of exposure to SHS. This data
46 reinforces the validity of UC also as an indicator of exposure to SHS.¹⁰

1 England *et al*¹³ indicated that few studies have identified differences between misclassified and self-reported
2 smokers and the way in which this would affect epidemiological studies. Our study shows similar patterns of
3 association and both self-reported smoking and misclassification were strongly associated with various
4 predictive variables. In particular, we found a higher risk of smoking and misclassification among women
5 with low education level. These results are consistent with those reported by other authors.^{6 14 24} We also
6 found a higher risk of smoking and misclassification in women from Europe, and women exposed to SHS in
7 different places. Women younger than 24 years had an increased risk of misreport her smoking, as indicated
8 by Dietz *et al*.⁴ In this study, exposure to SHS was associated with smoking. In other words, there were more
9 smoking people around pregnant women who smoked. In addition, misclassification was significantly
10 associated with exposure to SHS at home. Jhun *et al*¹⁴ and Orr *et al*²⁵ also showed higher prevalence of
11 smoking among pregnant women whose partners smoked at home. Having smoked previously was
12 associated with a higher probability of misreporting the habit, as observed by England *et al*.¹³

13 This work showed an optimal cut-off point for discriminate pregnant women smokers from non-smokers of
14 82 ng/ml, with a confidence interval of 42 to 136 ng/ml. Some studies proposed a cut-off of 50 ng/ml,¹³⁻¹⁶
15 coherent with the women not SHS exposed in this study. Other studies proposed cut-off points of 79 ng/ml¹⁸
16 and 85 ng/ml,⁶ closer to the smoking dose and SHS exposure in our study sample. In our study population
17 both prevalence of smoking and of SHS are high and this can explain in part why our optimal cut-off point is
18 higher than those reported in other studies.¹³⁻¹⁷ This is also supported by the fact that the optimal cut-off
19 point decreased to 42 ng/ml (27 to 57) when the analysis was restricted to women who reported no SHS
20 exposure, and increased according to the number of sources reported.. The validity of 27 ng/ml (11 to 43) as
21 cut-off point for differentiating occasional smokers from non-smokers was lower than that for differentiating
22 daily smokers, and it could depend on SHS exposure and on the time spent from the last cigarette smoked
23 given the faster elimination of cotinine in pregnant women,¹⁰⁻¹² information not collected in this study. There
24 are not validation studies of cotinine in different biological matrices, blood (plasma or serum), saliva or
25 urine,^{9 16} so it cannot be established which the most reliable biomarker is.

26 **Limitations of the study**

27 The current study has several limitations. From the eligible population, the participation rate was 56%, and
28 85.6% of the women who agreed to participate completed the study. Therefore, the final study sample might
29 not be fully representative of all pregnant women in the study areas, but its internal validity (absence of bias)
30

1 is not necessarily affected. There were other likely sources of misclassification in addition to maternal
2 misreporting of smoking status, as misclassification of non-smokers as smokers because of high exposure
3 degree to SHS. On the other hand, women who smoked occasionally but report to be non-smokers might
4 have low UC concentrations if they had not smoked recently, and their self-report and UC levels would be in
5 agreement.
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10 No information about last cigarette or last SHS exposure was obtained. We lost the opportunity of analysing
11 this variable in the evolution of the UC, showing his influence in false negatives, above all, and especially
12 relevant for occasional smokers.
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14 Since the optimal cut-off point for UC is determined using self-reported smoking status as the gold standard,
15 the validity of this assumption is important. On the one hand, it is unlikely that a non-smoking woman
16 declared to be a smoker, because a battery of items should be completed detailing smoking habits in this
17 case. On the other hand, however, it is possible that some smokers did not reveal their habit. In order to
18 minimize this type of bias, we excluded in the additional analysis self-reported non-smokers with
19 implausible high UC levels. In another sensitivity analysis, we excluded self-reported non-smokers who
20 claimed to stop smoking during pregnancy, since these cases are at higher risk of misclassification as
21 reported in table 3; the optimal cut-off point did not change after this exclusion. In general terms, the AUC
22 shows a good overall accuracy, and we think that self-reported smoking is a reliable measure in this study. If
23 some kind of misclassification occurs, it would lead to a shift towards the right in both distributions, and a
24 slight overestimation of the optimal cut-off point as a result.
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40 One of the main strengths of this study was the possibility of assessing the role of baseline exposures to SHS
41 in the estimate of cut-offs, given the detailed information collected on SHS exposure and its elevated
42 prevalence. The confirmation that the cut-offs would differ according to the level of exposure to SHS
43 emphasizes the need of taking it into account, especially in countries with elevated SHS exposure.
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48 **Implications for practice**

49 This study shows that the efforts made to encourage women to give up smoking before or during pregnancy
50 are not sufficient or particularly effective, given that at least 18.5% of the pregnant women smoked in the
51 third trimester. The results of this study indicate that the groups to which the most effort should be directed
52 are young women, those of a European origin and those from a low social class. Further, the association
53 observed in this study between active smoking of pregnant women and the presence of smokers in their close
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1 environment supports the hypothesis that this factor makes it more difficult to stop smoking.²⁶ It is necessary
2 to undertake effective programmes for reducing smoking before and during pregnancy, reaching also
3 misclassified, and to reduce SHS exposure, in order to prevent risks for women and foetus.
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7 **CONCLUSION**

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9 Smoking is an important risk factor for health and development and should be taken into account as
10 confounder when analysing the potential effects of environmental contaminants in studies like the INMA
11 project. To have a reliable marker like UC and a valid a cut-off point able to discriminate regular or
12 occasional smokers from non-smokers is a critical issue. The cut-off point of 82 ng/ml showed a good
13 validity for discriminating smokers from non-smokers in our study sample, while 27 ng/ml is the optimal
14 point for discriminating occasional smokers from non-smokers. It should be emphasized that cut-offs would
15 differ based on baseline exposure to SHS, and this should be taken into account when selecting reference
16 cut-offs for specific populations.
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28 **Contributors:** All authors contributed to various aspects of this paper. JJA, MM and MR designed the study
29 and analysed the data. AMC and ME analysed cotinine in urine samples. MJL, AMC, LSM, MG, AF-S, ME,
30 AL, AT and FB revised the design of the study and the results. JJA redacted the manuscript and the other
31 authors participated in the review of the different drafts and approved the final version.
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38 http://www.proyectoinma.org/presentacioninma/listado-investigadores/en_listado-investigadores.html
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42 **Declaration of interests:** None.

43 **Patient consent:** Obtained.

44 **Ethics approval:** This study was conducted with the approval for each of the four cohorts.

45
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2 Oviedo and the Conselleria de Sanitat Generalitat Valenciana. [http://www.proyectoinma.org/instituciones-](http://www.proyectoinma.org/instituciones-participantes/en_entidades-colaboradoras/)
3 [participantes/en_entidades-colaboradoras/](http://www.proyectoinma.org/instituciones-participantes/en_entidades-colaboradoras/)
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6 **Data Sharing Statement:** No additional data available.
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8 Figure Legends:

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10 Figure 1: Flow-chart of the INMA birth cohort in relation to smoking and UC quantification.

11 Figure 2: Distribution of urinary cotinine (ng/ml) according to active or passive tobacco exposure in pregnant
12 women from the INMA cohort.
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9 **Determinants of self-reported smoking and misclassification during pregnancy, and analysis**
10 **of optimal cut-off points of urinary cotinine: a cross sectional study**
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13 JJ Aurrekoetxea^{1,2,3}, M Murcia^{4,5}, M Rebagliato^{4,5}, MJ López^{4,6,7}, AM Castilla^{1,4}, L Santa Marina^{1,3,4}, M
14 Guxens^{4,8,9}, A Fernández-Somoano^{4,10}, M Espada¹, A Lertxundi^{2,3,4}, A Tardón^{4,10}, F Ballester^{4,5,11}
15
16 (Juan J Aurrekoetxea^{1,2,3}, Mario Murcia^{4,5}, Marisa Rebagliato^{4,5}, María José López^{4,6,7}, Ane Miren Castilla
17^{1,4}, Loreto Santa Marina^{1,3,4}, Mónica Guxens^{4,8,9}, Ana Fernández-Somoano^{4,10}, Mercedes Espada¹, Aitana
18 Lertxundi^{2,3,4}, Adonina Tardón^{4,10}, Ferran Ballester^{4,5,11})
19

20
21 1 Public Health Department, Basque Government, Spain

22 2 University of Basque Country UPV/EHU, Spain

23 3 Health Research Institute (BIODONOSTIA), Spain

24 4 Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP)

25 5 Center for Public Health Research (CSISP), Valencia, Spain

26 6 Public Health Agency of Barcelona, Spain

27 7 Institute of Biomedical Research (IIB Sant Pau), Spain

28 8 Center for Research in Environmental Epidemiology (CREAL), Spain

29 9 Hospital del Mar Research Institute (IMIM), Barcelona, Spain

30 10 University of Oviedo, Spain

31 11 University of Valencia, Spain

32
33 **Juan J Aurrekoetxea**, Public Health Department, Basque Government, Spain;

34 University of Basque Country UPV/EHU, Spain;

35 Health Research Institute (BIODONOSTIA), Spain.

36 jj.aurreko@gmail.com

37 **Mario Murcia**: Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;

38 Center for Public Health Research (CSISP); Valencia, Spain.

39 murcia_mar@gva.es

40 **Marisa Rebagliato**, Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;

41 Center for Public Health Research (CSISP); Valencia, Spain.

42 rebagliato_mar@gva.es

43 **María José López**, Public Health Agency of Barcelona, Spain;

1
2
3
4
5
6 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*
7 *Institute of Biomedical Research (IIB Sant Pau), Spain.*

8
9 mjlopez@aspb.cat

10 **Ane Miren Castilla**, *Public Health Laboratory of Bilbao, Spain.*

11 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

12 ANE_M2002@yahoo.es

13
14 **Loreto Santa Marina**, *Public Health Department, Basque Government, Spain;*

15 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

16 *Health Research Institute (BIODONOSTIA), Spain.*

17
18 ambien4ss-san@ej-gv.es

19
20 **Mònica Guxens**, *Center for Research in Environmental Epidemiology (CREAL), Spain;*

21 *Hospital del Mar Research Institute (IMIM), Spain;*

22 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

23
24 mguxens@creal.cat

25
26 **Ana Fernández-Somoano**, *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain;*

27 *University of Oviedo, Spain.*

28
29 capua.uo@uniovi.es

30 **Mercedes Espada**, *Public Health Department, Basque Government, Spain;*

31
32 metabobi-san@ej-gv.es

33 **Aitana Lertxundi**, *University of Basque Country UPV/EHU, Spain;*

34 *Health Research Institute (BIODONOSTIA), Spain;.*

35 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

36
37 aitana.lertxundi@gmail.com

38 **Adonina Tardón**, *University of Oviedo, Spain;*

39 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

40
41 atardon@uniovi.es

42 **Ferran Ballester**, *University of Valencia, Spain;*

43 *Center for Public Health Research (CSISP), Valencia, Spain;*

44 *Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Spain.*

45
46 ballester_fer@gva.es

47
48
49
50 Corresponding author: Juan J Aurrekoetxea Ph.D; Departamento de Sanidad del Gobierno Vasco; C/ Sancho el Sabio 35; 20.010, San

51 Sebastián; Telephone: 34.943.023033; Fax: 34.943.023093; E-mail: jj.aurreko@gmail.com

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54 **Key words:** Pregnancy; Smoking; Validation studies; Cut-off; Urinary cotinine.

ABSTRACT

Objectives: To estimate the prevalence and factors associated with smoking and misclassification in pregnant women from INMA [*Infancia y Medio Ambiente*, Environment and Childhood] project, Spain, and to assess the optimal cut-offs for urinary cotinine (UC) that best distinguishes daily and occasional smokers with varying levels of SHS exposure.

Design: We used logistic regression models to study the relationship between sociodemographic variables and self-reported smoking and misclassification (self-reported non-smokers with UC >50ng/ml). ROC curves were used to calculate the optimal cut-off point for discriminating smokers. The cut-offs were also calculated after stratification among non smokers for SHS exposure by number of sources. The cut-off points used to discriminate smoking status were the level of UC given by the Youden's Index and for 50 and 100ng/ml for daily smokers, or 25 and 50ng/ml for occasional smokers.

Participants: At third trimester of pregnancy 2263 pregnant women of the INMA Project were interviewed between 2004 and 2008 and a urine sample was collected.

Results: Prevalence of self-reported smokers at third trimester of pregnancy was 18.5%, and other 3.9% misreported their smoking status. Variables associated with self-reported smoking and misreporting were similar, including born in Europe, educational level, and exposure to SHS. The optimal cut-off was 82ng/ml (95%CI 42-133); sensitivity: 95.2% and specificity: 96.56%. The area under the ROC curve was 0.995-986 (95%CI 0.992982-0.9907). The cut-offs varied according to the SHS exposure level being 42 (95%CI 27-57), 82 (95%CI 5546-136) and 106ng/ml (95%CI 7958-227) for not SHS exposed, exposed to one and to two or more sources of SHS, respectively. The optimal cut-off for discriminating occasional smokers from non-smokers was 27ng/ml (95%CI 11-43).

Conclusions: Prevalence of smoking during pregnancy in Spain remains high. UC is a reliable biomarker for classifying pregnant women according to their smoking status. However, cut-offs would differ based on baseline exposure to SHS.

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ARTICLE SUMMARY

Article focus

The focus of this study is on:

- There is no current consensus regarding the cut-off point for urinary cotinine in pregnant women able to discriminate regular or occasional smokers from non-smokers,
- These cut-offs would also differ according to baseline exposure to second hand smoke (SHS)
- This study assess the maternal factors influencing both self-reported and misclassification of smoking; and evaluate the optimal cut-off point for urinary cotinine that best distinguishes smokers from non-smokers according to frequency of smoking and SHS exposure.

Key messages

- The prevalence of both smoking (18.5%) and SHS exposure (45.9%) was high in a population based sample of pregnant women in Spain.
- Factors associated with self-reported smoking and misreporting were similar, including lower level of education and living in a smoking environment, which highlights the need of reinforcing the preventive interventions and policies.
- The optimal cut-off point to discriminate smokers from non-smokers varied according to the frequency of smoking (occasional or daily smokers) and to SHS exposure levels.
- This study highlights the importance of SHS exposure for selecting reference cut-offs to discriminate smoking status, especially in high SHS exposed populations.

Strengths and limitations of this study

- This study has the ability to assess the role of baseline exposures to SHS in the estimate of cut-offs, given the detailed information collected on SHS exposure and its elevated prevalence.
- This study uses population based samples of pregnant women from the INMA birth cohort, which might not be fully representative of all pregnant women in the study areas,

INTRODUCTION

Risks for mother and foetus has been widely related to smoking during pregnancy.¹ Several studies have indicated that pregnant women tend to under-report their consumption of tobacco,²⁻⁸ due to social pressure⁹ or to avoid criticism from health professionals.³ Indeed, it is known to be a higher rate of misreporting of smoking among the groups in which it is not considered as acceptable, such as pregnant women and patients with smoking-related diseases.⁹

Cotinine is the main metabolite of nicotine and the biomarker of choice for distinguishing smokers from non-smokers and for assessing exposure to second-hand smoke (SHS).¹⁰ The women's clearance of cotinine is faster during pregnancy¹¹ and its plasma half-life is a little less than 9h.¹² For this reason, urinary cotinine (UC) tests may give false negatives in pregnant women who have not recently smoked.

There is no current consensus regarding the cut-off point for UC in pregnant women. Several thresholds have been proposed being 50 ng/ml, the most widely used.¹³⁻¹⁶ On the other hand, Higgins *et al*¹⁷ proposed 25 ng/ml as the cut-off point, while Gorber *et al*⁹ underlined the need to decide on a suitable threshold for pregnant women in particular, for whom the sensitivity of the test may be different, and also suggested that a new cut-off point should be established for occasional smokers. Spierto *et al*¹⁸ found 79 ng/ml as the cut-off between non-smoker and smoker pregnant women.

The aims of our study were: 1) to assess the prevalence of self-reported smoking and the UC levels in a cohort of pregnant women; 2) to assess the prevalence of misclassification of maternal smoking status according to the most widely accepted cut-off point in the literature of 50 ng/ml, and to study maternal factors associated with both self-reported and misclassification of maternal smoking; and 3) to identify the optimal cut-off point for UC that best distinguishes smokers from non-smokers in our study sample, according to frequency of smoking (occasional or daily smokers) and SHS exposure.

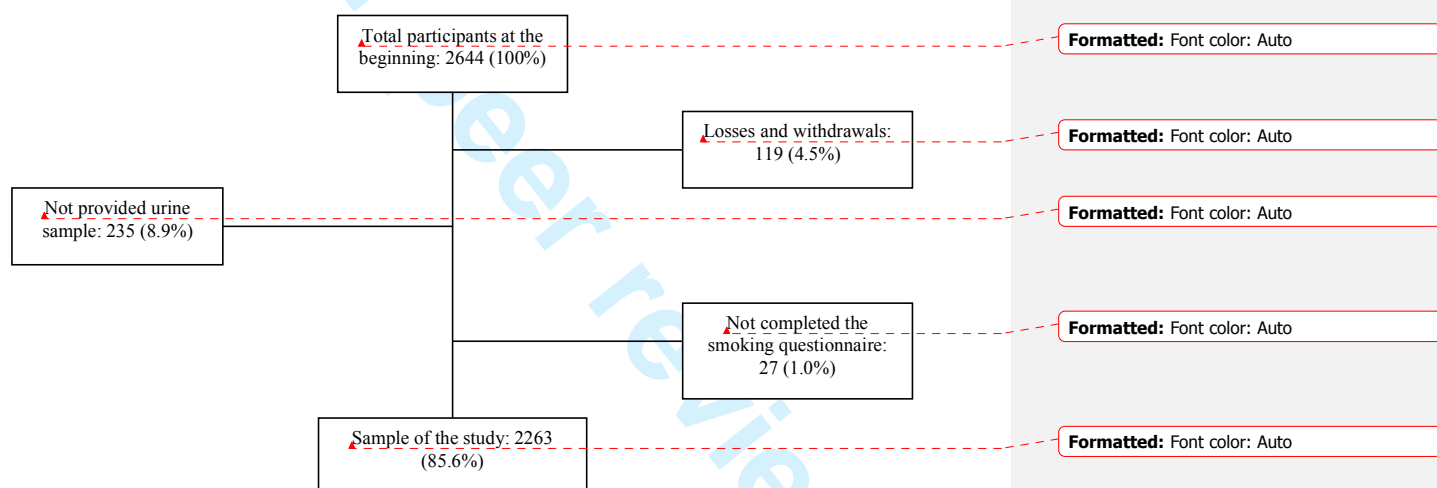
METHODS

Study population

The INMA [*Infancia y Medio Ambiente*, Environment and Childhood] project is a Spanish multi-centre prospective birth cohort study which aims to evaluate the impact of exposure to the most prevalent environmental pollutants, and the role of diet, on foetal and infant growth, health and development.¹⁹ From eligible pregnant women recruited between 2003 and 2008, a 56% agreed to participate. The inclusion criteria were at least 16 years of age, singleton pregnancy, enrolment at 10 to 13 weeks of gestation, no

assisted conception, delivery scheduled at the reference hospital, and no communication handicap. There was no upper age limit for be a member of the cohort. Of the 2644 women who agreed to participate in the study, 119 (4.5%) were lost (59 miscarriages, 8 foetal death, 47 withdrew and 5 lost to follow-up). Around week 32 of their pregnancy 2263 of the 2525 remaining women completed between 2004 and 2008 a questionnaire on smoking and other variables and provided urine samples for determination of UC (Figure 1). The hospital ethics committee of each centre approved the research protocol and all pregnant women gave written informed consent before inclusion at the first trimester of pregnancy.

Figure 1: Flow-chart of the INMA birth cohort in relation to smoking and UC quantification.



Information concerning smoking

Questionnaire on tobacco consumption included smoking history, patterns of consumption (occasional or regular) and exposure to SHS. We considered the women who, at this interview, reported smoking occasionally or daily to be smokers, regardless of their UC levels. Women who had UC levels higher than the widely used level of 50 ng/ml to distinguish smokers from non-smokers,¹³⁻¹⁶ but who did not report smoking, were classed as misclassified. It was considered that the participants were exposed to SHS when they reported exposure at least twice a week in any of the following environments: at work, at home, or in leisure time outside the home (e.g. bars/restaurants, or other homes). We analysed whether women had any passive exposure to tobacco smoke (yes or no), and also the number of exposure sources, between 0 and 3, according to the reported places of exposure: at work, at home and/or elsewhere in leisure time.

Urinary cotinine

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6 The urine samples were collected in the same interview in the morning during the third trimester of
7 pregnancy. Urine was collected in 100 ml polyethylene containers and stored at -20°C. One aliquot of the
8 sample from each of the participants was sent to the Public Health Laboratory of Bilbao (Spain) to be
9 analysed. All urine samples were stored for a minimum of one year and a maximum of 5 years before
10 analysis. The analysis of the UC was performed by competitive enzyme immunoassay (EIA) using
11 commercial EIA microplate test kits (OraSure Technologies, Inc., Bio-Rad) for determining salivary cotinine
12 adapted for urine samples using urine controls (0, 2.5, 10 and 50 ng/ml, Bio-Rad). Samples with UC levels
13 above 50 ng/ml were diluted. Before testing the urine samples the method was validated; a certified reference
14 material was used (EPA/NIST Reference Material 8444) to evaluate the repeatability and reproducibility.
15 The quantification limit was 4.0 ng/ml, the coefficient of repeatability 7% and the reproducibility 10%.

23 **Other variables**

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25 The women were interviewed twice during pregnancy (first and third trimester of gestation) to obtain
26 information about their sociodemographic characteristics and life-style variables. Social status of the women
27 (or her partner, if she had never worked outside the home) was defined using Spanish adaptation of British
28 classification system.²⁰

32 **Statistical analysis**

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34 The chi-square test was used to test hypotheses for categorical variables, while differences in the distribution
35 of UC according to categorical covariates were evaluated using the Mann-Whitney U and the Kruskal Wallis
36 tests. In order to identify the variables independently associated with being either a smoker, a misclassified,
37 or both, logistic regression models were built including geographical area and the variables related with the
38 outcome at $p < 0.10$ in the univariate analysis, and sequentially excluding those variables not related at $p < 0.10$
39 in the adjusted model using the likelihood ratio test. For comparability purposes, variables remaining at $p < 0.10$
40 in any of the models were entered in all the models. For ordinal categorical variables, the p for a linear
41 trend was also calculated.

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43 We used non-parametric receiver operating characteristic (ROC) curve to analyse the relationship between
44 the sensitivity (probability of a positive test among smokers) and false positive (probability of a positive test
45 among non-smokers, 1-specificity) cases for various different cut-off points that dichotomize UC to
46 distinguish smokers from non-smokers, using self-reported cigarette smoking status as the reference value.
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48 Overall accuracy was evaluated by means of the area under the curve (AUC) (showing the ability of the

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6 urinary cotinine to correctly classify smoking status with varying cut off points.²¹ The optimal cut-off point
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8 for UC to discriminate smokers from non smokers was the value (c) associated with the Youden's index (J),
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10 defined by: $J = \text{maximum}\{\text{sensitivity}(c) + \text{specificity}(c) - 1\}$.²² This value is 'optimal' in the sense that
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12 maximizes the overall rate of correct classification in the absence of a loss function (i.e., giving the same
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14 weight to errors of sensitivity and specificity). Since the shape of the distribution of the estimator of the
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16 optimal cut off point was unknown, we used the percentile bootstrap method, with 2000 resampling
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18 simulations, to establish 95% confidence intervals, with the aid of the 'boot' package of R.²³ Additionally, the
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20 data were analysed for the most widely used cut-off points, namely 50 and 100 ng/ml, or 25 and 50 ng/ml
21
22 when analyses were restricted to occasional smokers. ~~Thirty five Wwomen who declared that they did not
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24 smoke but with implausible UC levels in non smokers (>500 ng/ml) were excluded from the these analyses
25
26 in order to diminish the measurement error of self reported cigarette smoking.~~ The cut-off points were also
27
28 calculated after stratification among non smokers for SHS exposure in three groups: 79~~81~~ women that
29
30 referred not exposed to SHS, 7~~35~~~~48~~ exposed to one source of SHS and ~~292~~~~303~~ exposed to more than one
31
32 source. ~~Additional sensitivity analysis was conducted (Supplementary Table) the first one excluding 1047
33
34 pregnant women non-smokers who referred SHS exposure, the second one excluding 317 self-reported non-
35
36 smokers who claimed to stop smoking during pregnancy, since this group is more likely to misreport their
37
38 smoking status, and the last one excluding 35 women who declared that they did not smoke but with
39
40 implausible UC levels in non smokers (>500 ng/ml). Additional sensitivity analysis excluded 290 self-
41
42 reported non-smokers who claimed to stop smoking during pregnancy, since this group is more likely to
43
44 misreport their smoking status. Likewise, occasional smoking was analysed excluding non smokers exposed
45
46 to SHS.~~ Assuming $\alpha = 0.05$, 95% CI were calculated for ORs, cut-off points and area under ROC curve. All
47
48 statistical tests were two-sided. Statistical analysis was carried out using SPSS (version 17.0) and R (2.11.1)
49
50 statistical software.

51 RESULTS

52 Study setting and characteristics of the sample

53 Overall, 61.2% of women reported to have smoked at least once in their life, while 32.4% were occasional or
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55 regular smokers when they became pregnant, falling to 19.7% at first trimester and 18.5% at third trimester
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57 of their pregnancy (Table 1).
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Table 1: Description of the sample and variables of interest.

	N ^a	%
Cohort		
Asturias	416	18.4
Gipuzkoa	545	24.1
Sabadell	591	26.1
Valencia	711	31.4
Age		
≤ 24	154	6.8
25-29	717	31.7
30-34	973	43.0
≥ 35	418	18.5
Social class		
I-II (more affluent)	492	21.8
III	584	25.8
IV-V (less affluent)	1186	52.4
Level of education		
Primary or no education	547	24.2
Secondary	936	41.4
University	776	34.4
BMI (pre-pregnancy)		
<18.5	100	4.4
18.5 – 25	1568	69.3
25 – 30	420	18.6
≥ 30	175	7.7
Previous parity	957	43.1
Birth In Europe	2130	94.3
Reported having smoked in their life		
No	879	38.8
Occasional	146	6.5
Regular	1238	54.7
Reported smoking at the start of pregnancy		
No	1529	67.6
Occasional	28	1.2
Regular	706	31.2
Reported smoking at first trimester of pregnancy		
No	1813	80.3
Occasional	35	1.6

Regular	410	18.2
Reported smoking at third trimester of pregnancy		
No	1845	81.5
Occasional	37	1.6
Regular	381	16.8
Year of urine sampling		
2004	321	14.2
2005	857	37.9
2006	466	20.6
2007	470	20.8
2004	149	6.6
Cigarettes/day at third trimester of pregnancy		
0	1845	81.5
Occasional	37	1.6
1-4	149	6.6
5-9	141	6.2
≥ 10	91	4.0
Exposed to SHS in non-smoking women ^b :		
At home (partner or others)	479	26.0
At work	186	10.1
Elsewhere in leisure time ^c	715	38.8
Number of sources of exposure to SHS ^d		
0	798	43.5
1	735	40.0
2	271	14.8
3	32	1.7
Cotinine (ng/ml) all the women		
< 50	1773	78.3
50-99	31	1.4
100-199	19	0.8
200-499	52	2.3
500-999	70	3.1
≥ 1000	318	14.1

a: The numbers and rates that do not match the total are due to missing data

b: Percentages calculated including non exposed women

c: Other homes or public places, e.g. pubs or restaurants

d: Work, home and elsewhere in leisure time among non smokers

Smoking and SHS exposure

The median UC level in women who did not refer to smoke and were not exposed to SHS was below the quantification level of 4.0 ng/ml while in non-smokers exposed to SHS it was 7.6 ng/ml. Among all smokers the UC median level was 1744.3 ng/ml (Table 2). Occasional smokers had a median level of 260.7 ng/ml. Among daily smokers statistically significant differences were observed between UC concentration and the number of cigarettes smoked per day ($p < 0.001$), showing a clear dose-response pattern (not statistically tested). In the same way, in non-smokers there were statistically significant differences between UC levels and the number of sources of exposure to SHS; that are, work, home and elsewhere in leisure time ($p < 0.001$), with a progressive dose-response pattern (not tested, neither). Figure 1 shows the different distribution patterns of UC among non-smokers, exposed or not to SHS, and occasional and daily smokers.

Table 2: Active smoking and exposure to SHS in pregnant women in the INMA cohort. Median levels of urinary cotinine (ng/ml) at third trimester of pregnancy.

	N	%	Urinary cotinine ^a
Total	2263	100	7.4
Non smokers ^b	1845	81.5	4.4
No SHS exposure	798	35.3	< 4
SHS exposure	1038	45.9	7.6
1 source ^{c,d}	735	32.5	5.8
2 sources	271	12.0	11.7
3 sources	32	1.4	16.9
Smokers ^d	418	18.5	1744.3
Occasional	37	1.6	260.7
1-2 cigarettes/day	76	3.4	1036.4
3-4 cigarettes/day	73	3.2	1330.7
5-9 cigarettes/day	141	6.2	1848.5
≥ 10 cigarettes/day	91	4.0	3033.0

a: Median level of urinary cotinine ng/ml.

b: Exposed and not exposed to SHS; Mann-Whitney test: $p < 0.001$ for smoking and urinary cotinine

c: Sources of exposure to SHS at work/at home/in leisure time outside the home

d: Kruskal Wallis test $p < 0.001$

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Figure 2: Distribution of urinary cotinine (ng/ml) according to active or passive tobacco exposure in pregnant women from the INMA cohort.

Me: median
SHS: Second-hand smoking
SRS Occas: Self reported smoking, occasional
SRS Daily: Self reported smoking, daily

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Self-reported smoking and misclassification

Among the 2263 women studied, 1755 (77.6%) reported that they did not smoke and had UC levels below 50 ng/ml (true negative). A further 18 (0.8%) also had UC levels under 50 ng/ml despite claiming to smoke, though 13 of these claimed to be occasional smokers. On the other hand, 90 women (3.9%) reported that they did not smoke but were found to have UC levels above 50 ng/ml and were considered as misclassified and, finally, 400 women (17.7%) were true positive. Table 3 shows the ORs of the variables associated with smoking and misclassification, before and after adjusting. In the adjusted model, the risk of smoking and misclassification were associated with low educational level, country of birth, and exposure to SHS. Age was related only to misclassification risk. In regards to smoking history, only smoking at the beginning of pregnancy was associated with misclassification. The year of urine sampling and the social class were statistically associated only in the unadjusted analysis. Adding women misclassified to self-reported smokers the pattern of the association found with self-reported smoking did not vary.

Table 3: Unadjusted and adjusted odds ratios (ORs) and variables associated with smoking, self-reported and misclassification of smoking status.

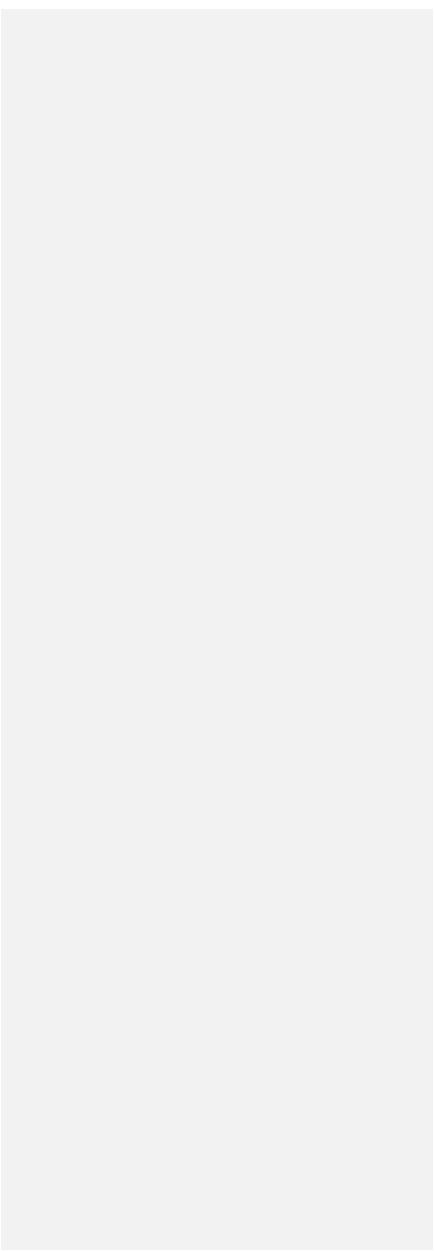
	Unadjusted analysis							Adjusted analysis ^a					
	Non-smokers ^b		Self-reported smokers ^c			Misclassification ^d		Self-reported smokers ^c		Misclassification ^d		Both ^e	
	N	N	OR	95% CI	N	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Total	1755	418	-	-	90	-	-	-	-	-	-	-	-
Cohort													
Asturias	326	75	1	-	15	1	-	1	-	1	-	1	-
Gipuzkoa	467	65	0.60	0.42 to 0.88	13	0.60	0.27 to 1.36	0.77	0.52 to 1.15	0.63	0.28 to 1.42	0.75	0.52 to 1.09
Sabadell	466	99	0.92	0.66 to 1.29	26	1.21	0.61 to 2.44	0.68	0.47 to 0.99	0.81	0.40 to 1.64	0.72	0.51 to 1.02
Valencia	496	179	1.57	1.15 to 2.15	36	1.58	0.82 to 3.07	0.94	0.67 to 1.33	0.84	0.43 to 1.66	0.95	0.69 to 1.31
Age													
≤ 24	93	43	1	-	18	1	-	1	-	1	-	1	-
25-29	537	152	0.62	0.40 to 0.94	28	0.27	0.14 to 0.53	0.87	0.55 to 1.38	0.36	0.18 to 0.73	0.73	0.49 to 1.11
30-34	792	153	0.42	0.27 to 0.64	28	0.18	0.09 to 0.36	0.75	0.48 to 1.19	0.26	0.13 to 0.54	0.61	0.41 to 0.92
≥ 35	332	70	0.46	0.29 to 0.73	16	0.25	0.12 to 0.54	0.90	0.54 to 1.49	0.46	0.21 to 1.04	0.76	0.48 to 1.19
				(p ^f < 0.001)			(p ^f = 0.001)						
Country of birth													
In Europe	1637	410	1	-	83	1	-	1	-	1	-	1	-
Outside Europe	114	8	0.28	0.13 to 0.60	7	1.21	0.50 to 2.79	0.25	0.12 to 0.54	1.11	0.46 to 2.65	0.39	0.21 to 0.69
Level of education													
University	695	59	1	-	22	1	-	1	-	1	-	1	-
Secondary	703	192	3.22	2.36 to 4.39	41	1.84	1.09 to 3.17	2.37	1.70 to 3.29	1.17	0.66 to 2.08	2.08	1.55 to 2.78
Primary or less	355	165	5.47	3.96 to 7.57	27	2.40	1.34 to 4.32	3.30	2.31 to 4.70	1.02	0.53 to 1.97	2.72	1.97 to 3.74
				(p ^f < 0.001)			(p ^f = 0.002)						
Social class													
I+II (highest)	438	41	1	-	13	1	-	-	-	-	-	-	-

III	469	96	2.19	1.48 to 3.22	19	1.36	0.67 to 2.80	-	-	-	-	-	-
IV+V (lowest)	847	281	3.54	2.50 to 5.02	58	2.31	1.25 to 4.26	-	-	-	-	-	-
				(p ^f < 0.001)			(p ^f = 0.002)						
Year of urine sampling													
2004	224	78	1	-	19	1	-	-	-	-	-	-	-
2005	642	179	0.80	0.59 to 1.09	36	0.66	0.37 to 1.18	-	-	-	-	-	-
2006	362	83	0.66	0.46 to 0.94	21	0.68	0.36 to 1.30	-	-	-	-	-	-
2007	399	58	0.42	0.29 to 0.61	13	0.38	0.19 to 0.79	-	-	-	-	-	-
2008	128	20	0.45	0.26 to 0.77	1	0.09	0.01 to 0.70	-	-	-	-	-	-
				(p ^f < 0.001)			(p ^f = 0.001)						
Exposure to SHS at home													
No	1328	144	1	-	34	1	-	1	-	1	-	1	-
Yes	423	274	5.97	4.75 to 7.51	56	5.17	3.33 to 8.03	4.41	3.44 to 5.64	3.26	2.03 to 5.25	4.39	3.49 to 5.51
Exposure to SHS at work													
No	1582	343	1	-	76	1	-	1	-	1	-	1	-
Yes	172	74	1.98	1.48 to 2.67	14	1.69	0.94 to 3.06	1.55	1.11 to 2.17	1.37	0.72 to 2.59	1.57	1.14 to 2.15
Exposure to SHS elsewhere in leisure time													
No	1082	175	1	-	44	1	-	1	-	1	-	1	-
Yes	669	243	2.25	1.81 to 2.79	46	1.69	1.11 to 2.59	1.88	1.44 to 2.34	1.47	0.92 to 2.34	1.80	1.44 to 2.26
Reported having smoked in their life [§]													
No	862	0	-	-	17	1	-	-	-	-	-	-	-
Yes	893	418	-	-	73	4.15	2.43 to 7.09	-	-	-	-	-	-
Reported smoking at the start of pregnancy [§]													
No	1489	1	-	-	39	1	-	-	-	1	-	-	-
Yes	266	417	-	-	51	7.32	4.73 to 11.33	-	-	6.21	3.91 to 9.86	-	-

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- a: Only variables showed in the table were entered in the logistic equation
- b: Non-smokers: women who reported that they did not smoke and were found to have urinary cotinine levels of less than 50 ng/ml. the reference group
- c: Smokers: those who reported smoking
- d: Misclassification: those who claimed that they did not smoke but were found to have urinary cotinine levels above 50 ng/ml
- e: Both: c+d
- f: p for trend
- g: Only analysed with regards to misclassification, given the extremely strong association with smoking at third trimester of pregnancy

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Cut-off points of UC for smoking

Optimal cut-off points for distinguishing non-smokers from smokers (daily and occasional) calculated by the Youden's index (excluding self reported non-smokers with UC values above 500 ng/ml), was 82 ng/ml, with a sensitivity of 95.2%, specificity of 98.56% and AUC 0.995-986 (95% CI 0.992-982 to 0.997990) (Table 4). Sensitivity and specificity for the cut-off points of 50 and 100 ng/ml were quite close to that of 82 ng/ml.

The exclusion from the analysis of 317 self-reported non-smokers who claimed to stop smoking during pregnancy, since this group is more likely to misreport their smoking status, or 35 women with UC above 500 ng/ml and reporting that they did not smoke, 290 women who declared quitting smoking during pregnancy as possible group at risk of underreporting of their smoking status, did not improve substantially the validation parameters of the test (Supplementary table data not shown).

Table 4: Parameters for assessing the optimal cut-off point for urinary cotinine. ng/ml. obtained by the Youden's index. as well as the levels of 25%, 50%, and 100 ng/ml. for classifying pregnant women as regular or occasional smokers.*

Youden's index ^a	Cut-off point (95% CI) ^b	Sensitivity	Specificity	Positive PV ^c	Negative PV ^c	AUC of the ROC (95% CI) ^d
Regular and occasional smokers: 4810-1845 non-smokers, 418 smokers						
-	50	0.957	0.9510-970	0.8160-879	0.990	0.986 (0.982 to 0.990)
-	100	0.950	0.9660-985	0.8650-936	0.988	0.995 (0.992 to 0.997)
0.9180-937	82 (42 to 136)	0.952	0.9660-985	0.8630-934	0.989	
Results stratified by frequency of smoking						
Occasional smokers: 1810-45 non-smokers, 37 smokers						
-	25	0.892	0.8700-887	0.1210-139	0.998	0.947 (0.923 to 0.970)
-	50	0.649	0.9510-970	0.2110-304	0.993	0.961 (0.939 to 0.984)
0.7720-789	27 (11 to 43)	0.892	0.8800-897	0.1300-151	0.998	
Daily smokers: 4810-1845 non-smokers, 381 smokers						
-	50	0.987	0.9510-970	0.8070-872	0.997	0.990 (0.986 to 0.994)
-	100	0.982	0.9660-985	0.8580-933	0.996	0.998 (0.996 to +)
0.9490-968	115 (57 to 189)	0.982	0.9670-986	0.8620-937	0.996	
Results stratified by SHS exposure among non-smokers						
Non exposed to SHS: 791-798 non-smokers, 418 active smokers						
-	50	0.957	0.9870-996	0.9760-993	0.978	0.994 (0.990 to 0.998)
-	100	0.950	0.9900-999	0.9800-997	0.974	+
0.9540-962	42 (27 to 57)	0.971	0.9820-991	0.9670-983	0.985	
Exposed to SHS (1 source^e): 718-735 non-smokers, 418 active smokers						
-	50	0.957	0.9350-957	0.8930-928	0.974	0.981 (0.974 to 0.989)
-	100	0.950	0.9580-981	0.9280-966	0.971	0.993 (0.990 to 0.997)
0.9100-933	82 (55-46 to 136)	0.952	0.9580-981	0.9280-966	0.972	
Exposed to SHS (2-3 sources^e): 292-303 non-smokers, 418 active smokers						
-	50	0.957	0.8940-928	0.9260-950	0.938	0.977 (0.966 to 0.987)
-	100	0.950	0.9240-959	0.9450-971	0.930	0.988 (0.982 to 0.994)
0.8770-912	106 (79-58 to 227)	0.950	0.9270-962	0.9470-973	0.930	

a: Youden's index = max (Sensitivity+Specificity-1).
 b: 95% bootstrap confidence interval for the cut-off point associated with the Youden's index.
 c: Predictive value of a positive or negative result for the prevalence of smoking in the study group.
 d: Area under the receiver operating characteristic curve and 95% confidence interval.
 e: Number of sources of exposure among: work, home, and elsewhere in leisure time.
 f: Excluding cases with cotinine > 500 ng/ml in self-reported non-smokers (n=35).
 b: Youden's index = max (Sensitivity+Specificity-1).
 e: 95% bootstrap confidence interval for the cut-off point associated with the Youden's index.
 d: Predictive value of a positive or negative result for the prevalence of the study: 18.5%.
 e: Area under the receiver operating characteristic curve and 95% confidence interval.
 f: Number of sources of exposure among: work, home and elsewhere in leisure time.

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8 The Youden's index and AUC for daily smoking were higher, with a cut-off point of 115 ng/ml. Occasional
9 smoking was analysed, by excluding from the analysis the 381 women who admitted that they smoked
10 regularly at third trimester of pregnancy. The optimal cut-off point for discriminating occasional smokers
11 from non-smokers was 27 ng/ml (95% CI 11 to 43), with a sensitivity and specificity of 89.2% and 89.7%,
12 respectively. The exclusion of women who declared to quit during pregnancy, improved the specificity to
13 91.8%, but did not almost change the Youden's Index or the sensitivity. Excluding non SHS exposed among
14 non-smokers, the optimal cut-off point was 19 ng/ml (95% CI 11 to 33), and improving the specificity to
15 93.7% and to 41.2% the positive predictive value (probability of smoking status being a positive test).
16 Nevertheless, these low positive predictive values are consequence, above all, of the low prevalence of
17 occasional smoking in this sample.
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20 Not exposed women to SHS compared with all smokers, daily or occasional, had a lower cut-off point of 42
21 ng/ml (95% CI 27 to 57), while for exposed to one or to two or more sources of SHS, cut-off points were 82
22 (95% CI 55 to 136) and 106 ng/ml (95% CI 79 to 201), respectively.
23

24 25 **DISCUSSION**

26 27 **Main findings in relation to the literature**

28
29 The prevalence of smoking in pregnant women at third trimester was 18.5%. In this later stage, the
30 prevalence of active smoking increased up to 22.5% if women who did not report smoking but had UC levels
31 above 50 ng/ml were reclassified as smokers, assuming that false positives were due to maternal
32 misreporting of smoking status. Prevalence of self reported smokers and misclassified in our study is close to
33 the referred by Kendrick *et al*⁶ and Lindqvist *et al*⁷, and smoking rate and UC levels are lower than that
34 showed by Pickett *et al*¹⁵. Our study had, nevertheless, a lower rate of smoking misreporting than other
35 studies.²⁻⁸
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38 There was a clear relationship between UC and smoking dose among smokers, and with the number of
39 sources of exposure to SHS among non-smokers. Specifically, those who smoked 10 or more cigarettes per
40 day had median UC levels of 3033 ng/ml, while the levels were 260 ng/ml for occasional smokers and less
41 than 17 ng/ml for non-smokers, increasing with the number of sources of exposure to SHS. This data
42 reinforces the validity of UC also as an indicator of exposure to SHS.¹⁰
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6 England *et al*¹³ indicated that few studies have identified differences between misclassified and self-reported
7 smokers and the way in which this would affect epidemiological studies. Our study shows similar patterns of
8 association and both self-reported smoking and misclassification were strongly associated with various
9 predictive variables. In particular, we found a higher risk of smoking and misclassification among women
10 with low education level. These results are consistent with those reported by other authors.^{6 14 24} We also
11 found a higher risk of smoking and misclassification in women from Europe, and women exposed to SHS in
12 different places. Women younger than 24 years had an increased risk of misreport her smoking, as indicated
13 by Dietz *et al*.⁴ In this study, exposure to SHS was associated with smoking. In other words, there were more
14 smoking people around pregnant women who smoked. In addition, misclassification was significantly
15 associated with exposure to SHS at home. Jhun *et al*¹⁴ and Orr *et al*²⁵ also showed higher prevalence of
16 smoking among pregnant women whose partners smoked at home. Having smoked previously was
17 associated with a higher probability of misreporting the habit, as observed by England *et al*.¹³

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20 This work showed an optimal cut-off point for discriminate pregnant women smokers from non-smokers of
21 82 ng/ml, with a confidence interval of 42 to ~~433-136~~ ng/ml. Some studies proposed a cut-off of 50 ng/ml,¹³⁻
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16 coherent with the women not SHS exposed in this study. Other studies proposed cut-off points of 79
ng/ml¹⁸ and 85 ng/ml,⁶ closer to the smoking dose and SHS exposure in our study sample. In our study
population both prevalence of smoking and of SHS are high and this can explain in part why our optimal cut-
off point is higher than those reported in other studies.¹³⁻¹⁷ This is also supported by the fact that the optimal
cut-off point decreased to 42 ng/ml (27 to 57) when the analysis was restricted to women who reported no
SHS exposure, and increased according to the number of sources reported.. The validity of 27 ng/ml (11 to
43) as cut-off point for differentiating occasional smokers from non-smokers was lower than that for
differentiating daily smokers, and it could depend on SHS exposure and on the time spent from the last
cigarette smoked given the faster elimination of cotinine in pregnant women,¹⁰⁻¹² information not collected in
this study. There are not validation studies of cotinine in different biological matrices, blood (plasma or
serum), saliva or urine,^{9 16} so it cannot be established which the most reliable biomarker is.

59 Limitations of the study

60 The current study has several limitations. From the eligible population, the participation rate was 56%, and
85.6% of the women who agreed to participate completed the study. Therefore, the final study sample might
not be fully representative of all pregnant women in the study areas, but its internal validity (absence of bias)

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6 is not necessarily affected. There were other likely sources of misclassification in addition to maternal
7 misreporting of smoking status, as misclassification of non-smokers as smokers because of high exposure
8 degree to SHS. On the other hand, women who smoked occasionally but report to be non-smokers might
9 have low UC concentrations if they had not smoked recently, and their self-report and UC levels would be in
10 agreement.
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14 No information about last cigarette or last SHS exposure was obtained. We lost the opportunity of analysing
15 this variable in the evolution of the UC, showing his influence in false negatives, above all, and especially
16 relevant for occasional smokers.
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19 Since the optimal cut-off point for UC is determined using self-reported smoking status as the gold standard,
20 the validity of this assumption is important. On the one hand, it is unlikely that a non-smoking woman
21 declared to be a smoker, because a battery of items should be completed detailing smoking habits in this
22 case. On the other hand, however, it is possible that some smokers did not reveal their habit. In order to
23 minimize this type of bias, we excluded in the ~~main~~-~~additional~~ analysis self-reported non-smokers with
24 implausible high UC levels. In ~~additional~~-~~another sensitivity~~ analysis, we excluded self-reported non-
25 smokers who claimed to stop smoking during pregnancy, since these cases are at higher risk of
26 misclassification as reported in table 3; the optimal cut-off point did not change after this exclusion. In
27 general terms, the AUC shows a good overall accuracy, and we think that self-reported smoking is a reliable
28 measure in this study. If some kind of misclassification occurs, it would lead to a shift towards the right in
29 both distributions, and a slight overestimation of the optimal cut-off point as a result.
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33 One of the main strengths of this study was the possibility of assessing the role of baseline exposures to SHS
34 in the estimate of cut-offs, given the detailed information collected on SHS exposure and its elevated
35 prevalence. The confirmation that the cut-offs would differ according to the level of exposure to SHS
36 emphasizes the need of taking it into account, especially in countries with elevated SHS exposure.
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38 39 **Implications for practice**

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41 This study shows that the efforts made to encourage women to give up smoking before or during pregnancy
42 are not sufficient or particularly effective, given that at least 18.5% of the pregnant women smoked in the
43 third trimester. The results of this study indicate that the groups to which the most effort should be directed
44 are young women, those of a European origin and those from a low social class. Further, the association
45 observed in this study between active smoking of pregnant women and the presence of smokers in their close
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6 environment supports the hypothesis that this factor makes it more difficult to stop smoking.²⁶ It is necessary
7 to undertake effective programmes for reducing smoking before and during pregnancy, reaching also
8 misclassified, and to reduce SHS exposure, in order to prevent risks for women and foetus.
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11 CONCLUSION

12 Smoking is an important risk factor for health and development and should be taken into account as
13 confounder when analysing the potential effects of environmental contaminants in studies like the INMA
14 project. To have a reliable marker like UC and a valid a cut-off point able to discriminate regular or
15 occasional smokers from non-smokers is a critical issue. The cut-off point of 82 ng/ml showed a good
16 validity for discriminating smokers from non-smokers in our study sample, while 27 ng/ml is the optimal
17 point for discriminating occasional smokers from non-smokers. It should be emphasized that cut-offs would
18 differ based on baseline exposure to SHS, and this should be taken into account when selecting reference
19 cut-offs for specific populations.
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28 **Contributors:** All authors contributed to various aspects of this paper. JJA, MM and MR designed the study
29 and analysed the data. AMC and ME analysed cotinine in urine samples. MJL, AMC, LSM, MG, AF-S, ME,
30 AL, AT and FB revised the design of the study and the results. JJA redacted the manuscript and the other
31 authors participated in the review of the different drafts and approved the final version.
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36 questionnaires. A full listing of the INMA project researchers can be found at
37 http://www.proyectoinma.org/presentacioninma/listado-investigadores/en_listado-investigadores.html
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40 **Declaration of interests:** None.

41 **Patient consent:** Obtained.

42 **Ethics approval:** This study was conducted with the approval for each of the four cohorts.

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7 [participantes/en_entidades-colaboradoras/](http://www.proyectoinma.org/instituciones-participantes/en_entidades-colaboradoras/)
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9 **Data Sharing Statement:** There is no additional data available.
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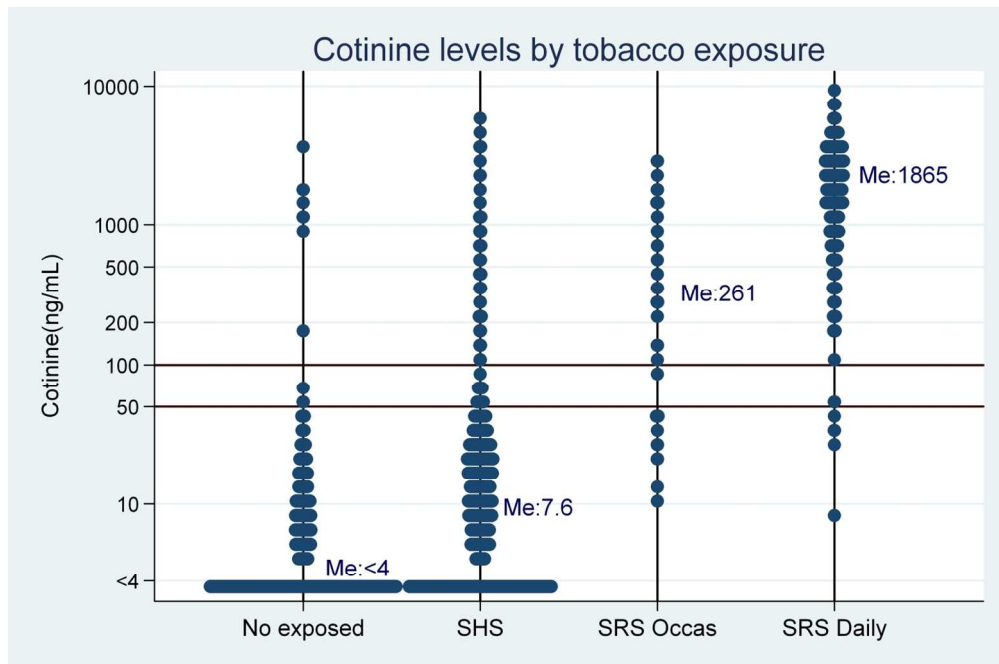
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Supplementary Table: Parameters for assessing the optimal cut-off point for urinary cotinine, ng/ml, obtained by the Younden's index, as well as the levels of 25, 50, and 100 ng/ml, for classifying pregnant women as regular or occasional smokers. Sensitivity analysis: 1) Excluding SHS exposed non-smokers, 2) Excluding non-smokers who claimed to stop smoking during pregnancy, and 3) Excluding self-reported non-smokers with cotinine above 500 ng/ml.

Younden's index ^a	Cut-off point (95% CI) ^b	Sensitivity	Specificity	Positive PV ^c	Negative PV ^c	AUC of the ROC (95% CI) ^d
Sensitivity analysis 1: Frequency of smoking, compared with non-smokers non-exposed to SHS						
Occasional smokers: 798 non-smokers and non-exposed to SHS, 37 smokers						
-	25	0,892	0,956	0,485	0,995	
-	50	0,649	0,987	0,706	0,984	0,979 (0,968 to 0,991)
0,875	19 (11 to 33)	0,946	0,929	0,38	0,997	
Daily smokers: 798 non-smokers and non-exposed to SHS, 381 smokers						
-	50	0,987	0,987	0,974	0,994	
-	100	0,982	0,999	0,979	0,991	0,995 (0,992 to 0,999)
0,976	57 (32 to 121)	0,987	0,989	0,977	0,994	
Sensitivity analysis 2: Excluding 317 self-reported non-smokers who claimed to stop smoking during pregnancy (more likely to misreport their smoking status):						
Regular and occasional smokers: 1528 non-smokers, 418 smokers						
-	50	0,957	0,974	0,911	0,988	
-	100	0,95	0,988	0,957	0,986	0,993 (0,989 to 0,996)
0,94	82 (42 to 129)	0,952	0,988	0,957	0,987	
Results stratified by frequency of smoking						
Occasional smokers: 1528 non-smokers, 37 smokers						
-	25	0,892	0,904	0,184	0,997	
-	50	0,649	0,974	0,381	0,991	0,965 (0,946 to 0,985)
0,805	27 (11 to 42)	0,892	0,913	0,199	0,997	
Daily smokers: 1528 non-smokers, 381 smokers						
-	50	0,987	0,974	0,906	0,997	
-	100	0,982	0,988	0,954	0,995	0,995 (0,993 to 0,998)
0,971	115 (56 to 169)	0,982	0,99	0,959	0,995	
Results stratified by SHS exposure among non-smokers						
Non exposed to SHS: 710 non-smokers, 418 active smokers						
-	50	0,957	0,993	0,988	0,975	
-	100	0,95	0,996	0,993	0,971	0,997 (0,996 to 0,999)
0,961	31 (26 to 57)	0,981	0,98	0,967	0,989	
Exposed to SHS (1 source ^e): 593 non-smokers, 418 active smokers						
-	50	0,957	0,956	0,939	0,969	
-	100	0,95	0,981	0,973	0,965	0,988 (0,981 to 0,994)
0,934	82 (43 to 120)	0,952	0,981	0,973	0,967	
Exposed to SHS (2-3 sources ^e): 216 non-smokers, 418 active smokers						
-	50	0,957	0,963	0,98	0,92	
-	100	0,95	0,981	0,99	0,91	0,991 (0,986 to 0,996)
0,936	106 (42 to 169)	0,95	0,986	0,993	0,91	
Sensitivity analysis 3: Excluding 35 self-reported non-smokers with cotinine > 500 ng/ml (implausible UC levels in non smokers)						
Regular and occasional smokers: 1810 non-smokers, 418 smokers						
-	50	0,957	0,970	0,879	0,990	
-	100	0,950	0,985	0,936	0,988	0,995 (0,992 to 0,997)
0,937	82 (42 to 133)	0,952	0,985	0,934	0,989	
Results stratified by frequency of smoking						
Occasional smokers: 1810 non-smokers, 37 smokers						
-	25	0,892	0,887	0,139	0,998	
-	50	0,649	0,970	0,304	0,993	0,961 (0,939 to 0,984)
0,789	27 (11 to 43)	0,892	0,897	0,151	0,998	
Daily smokers: 1810 non-smokers, 381 smokers						
-	50	0,987	0,970	0,872	0,997	
-	100	0,982	0,985	0,933	0,996	0,998 (0,996 to 1)

	0.968	115 (57 to 189)	0.982	0.986	0.937	0.996
Results stratified by SHS exposure among non-smokers						
Non exposed to SHS: 791 non-smokers, 418 active smokers						
-	50	0.957	0.996	0.993	0.978	
-	100	0.950	0.999	0.997	0.974	0.998 (0.997 to 1)
0.962	42 (27 to 57)	0.971	0.991	0.983	0.985	
Exposed to SHS (1 source ^e): 718 non-smokers, 418 active smokers						
-	50	0.957	0.957	0.928	0.974	
-	100	0.950	0.981	0.966	0.971	0.993 (0.990 to 0.997)
0.933	82 (55 to 136)	0.952	0.981	0.966	0.972	
Exposed to SHS (2-3 sources ^e): 292 non-smokers, 418 active smokers						
-	50	0.957	0.928	0.950	0.938	
-	100	0.950	0.959	0.971	0.930	0.988 (0.982 to 0.994)
0.912	106 (79 to 227)	0.950	0.962	0.973	0.930	

a: Youden’s index = max (Sensitivity+Specificity-1).

b: 95% bootstrap confidence interval for the cut-off point associated with the Youden’s index.

c: Predictive value of a positive or negative result for the prevalence of smoking in the study group.

d: Area under the receiver operating characteristic curve and 95% confidence interval.

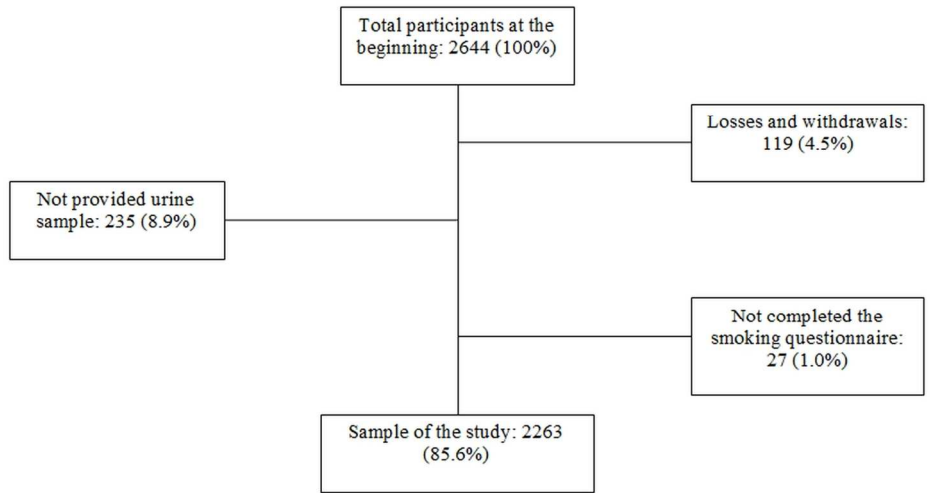
e: Number of sources of exposure among: work, home, and elsewhere in leisure time.

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Figure 1: Flow-chart of the INMA birth cohort in relation to smoking and UC quantification.



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