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

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2012-002034
Article Type:	Research
Date Submitted by the Author:	08-Sep-2012
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Primary Subject Heading:	Smoking and tobacco
Secondary Subject Heading:	Epidemiology, Public health, Occupational and environmental medicine
Keywords:	EPIDEMIOLOGY, Child protection < PAEDIATRICS, PUBLIC HEALTH, TOXICOLOGY, PREVENTIVE MEDICINE

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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 pressure[9] 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 pregnancy[11] 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. 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 their pregnancy 2263 of the 2525 remaining women completed a questionnaire on smoking and other variables and 2290 provided urine samples for determination of UC. 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.

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

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

Other variables

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

Statistical analysis

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

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

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	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	0.74	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).

Table 4: 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.[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 seft-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 Younden´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.

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

Not exposed women to SHS compared with all smokers, daily or occasional, had a lower cut-off point of 42 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 (95% CI 55 to 136) and 106 ng/ml (95% CI 79 to 201), respectively.

DISCUSSION

Main findings in relation to the literature

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

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

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 21] 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*[22] 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 50 and 100 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/ml[18] 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 exposure do 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 (27-57) when the analysis was resticted to women who reported no SHS exposure, and increased according to the number of sources reported.. The validity of 27 ng/ml 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]

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 is not necessarily affected. There were other likely sources of misclassification in addition to maternal misreporting of smoking status, as misclassification of non-smokers as smokers because of high exposure degree to SHS. On the other hand, women who smoked occasionally but report to be non-smokers might have low UC concentrations if they had not smoked recently, and their self-report and UC levels would be in agreement

Since the optimal cut-off point for UC is determined using self-reported smoking status as the gold standard, the validity of this assumption is important. On the one hand, it is improbable that a non-smoking woman declared to be a smoker, because a battery of items should be completed detailing smoking habits in this case. On the other hand, however, it is possible that some smokers did not reveal their habit. In order to

minimize this type of bias, we excluded in the main analysis self-reported non-smokers with implausible high UC levels. In additional analysis, we excluded self-reported non-smokers who claimed to stop smoking during pregnancy, since these cases are at higher risk of misclassification as reported in table 3; the optimal cut-off point did not change after this exclusion. In general terms, the AUC shows a good overall accuracy, and we think that self-reported smoking is a reliable measure in this study. If some kind of misclassification occurs, it would lead to a shift towards the right in both distributions, and a slight overestimation of the optimal cut-off point as a result.

One of the main strengths of this study was the possibility of assessing 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. The confirmation that the cut-offs would differ according to the level of exposure to SHS emphasizes the need of taking it into account, especially in countries with elevated SHS exposure.

Implications for practice

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

CONCLUSION

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

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

Acknowledgments: The authors are grateful to all fieldworkers for their assistance in administering the questionnaires. A full listing of the INMA project researchers can be found at http://www.proyectoinma.org/presentacioninma/listado-investigadores/en_listado-investigadores.html

Declaration of interests: None.

Patient consent: Obtained.

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

Funding: INMA project is funded by grants from Instituto de Salud Carlos III (Red INMA G03/176 and CB06/02/0041) and Fundación Roger Torné. The studies in the specific regions were funded by the Spanish Ministry of Health (FIS 03/1615, 04/1436, 04/1509, 04/1112, 04/1931, 05/1079, 05/1052, 06/0867,06/1213, 07/0314, 08/1151, 09/02647, 04/2018, 09/02311), the Generalitat de Catalunya (CIRIT 1999SGR00241), the Diputación Foral de Gipuzkoa (DFG06/004), the Department of Health of the Basque Government (2005111093), the Regional Government of Andalucía (SAS 07/183), Obra social Cajastur, University of Oviedo and the Conselleria de Sanitat Generalitat Valenciana. http://www.proyectoinma.org/instituciones-participantes/en_entidades-colaboradoras/

Data Sharing Statement: There is no additional data available.

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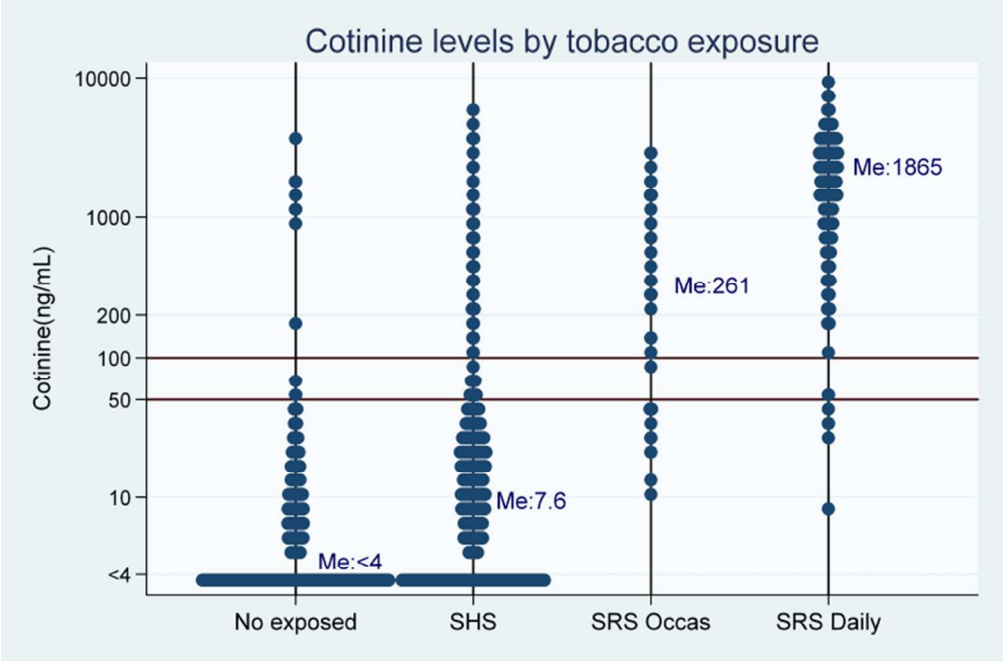
Cotinine levels by tobacco exposure
Cotinine(ng/mL)
10000
1000
200
100
50
10
<4
Me:<4
Me:7.6
Me:261
Me:1865
No exposed
SHS
SRS Occas
SRS Daily

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

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2012-002034.R1
Article Type:	Research
Date Submitted by the Author:	25-Nov-2012
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Primary Subject Heading:	Smoking and tobacco
Secondary Subject Heading:	Epidemiology, Public health, Occupational and environmental medicine
Keywords:	EPIDEMIOLOGY, Child protection < PAEDIATRICS, PUBLIC HEALTH, TOXICOLOGY, PREVENTIVE MEDICINE

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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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 pressure[9] 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 pregnancy[11] 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.

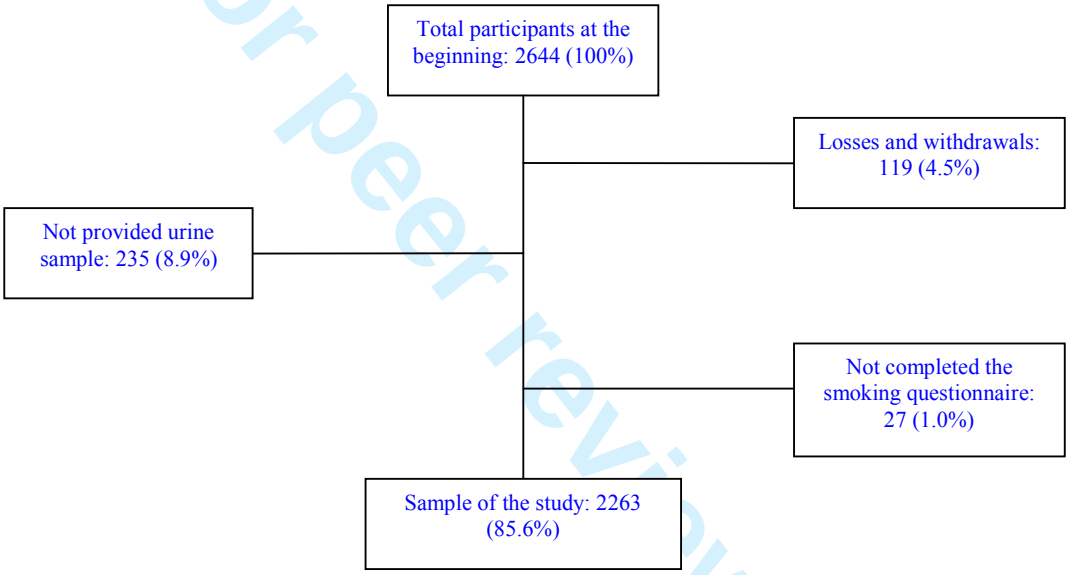
METHODS

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

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

Other variables

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

Statistical analysis

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

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

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=maximum{sensitivity(c)+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 α= 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	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 [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	-	

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 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 Younden´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 seft-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 Younden´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.

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

Not exposed women to SHS compared with all smokers, daily or occasional, had a lower cut-off point of 42 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 (95% CI 55 to 136) and 106 ng/ml (95% CI 79 to 201), respectively.

DISCUSSION

Main findings in relation to the literature

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

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

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/ml[18] 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 resticted 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)

is not necessarily affected. There were other likely sources of misclassification in addition to maternal misreporting of smoking status, as misclassification of non-smokers as smokers because of high exposure degree to SHS. On the other hand, women who smoked occasionally but report to be non-smokers might have low UC concentrations if they had not smoked recently, and their self-report and UC levels would be in agreement.

No information about last cigarette or last SHS exposure was obtained. We lost the opportunity of analysing this variable in the evolution of the UC, showing his influence in false negatives, above all, and especially relevant for occasional smokers.

Since the optimal cut-off point for UC is determined using self-reported smoking status as the gold standard, the validity of this assumption is important. On the one hand, it is unlikely that a non-smoking woman declared to be a smoker, because a battery of items should be completed detailing smoking habits in this case. On the other hand, however, it is possible that some smokers did not reveal their habit. In order to minimize this type of bias, we excluded in the main analysis self-reported non-smokers with implausible high UC levels. In additional analysis, we excluded self-reported non-smokers who claimed to stop smoking during pregnancy, since these cases are at higher risk of misclassification as reported in table 3; the optimal cut-off point did not change after this exclusion. In general terms, the AUC shows a good overall accuracy, and we think that self-reported smoking is a reliable measure in this study. If some kind of misclassification occurs, it would lead to a shift towards the right in both distributions, and a slight overestimation of the optimal cut-off point as a result.

One of the main strengths of this study was the possibility of assessing 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. The confirmation that the cut-offs would differ according to the level of exposure to SHS emphasizes the need of taking it into account, especially in countries with elevated SHS exposure.

Implications for practice

This study shows that the efforts made to encourage women to give up smoking before or during pregnancy are not sufficient or particularly effective, given that at least 18.5% of the pregnant women smoked in the third trimester. The results of this study indicate that the groups to which the most effort should be directed are young women, those of a European origin and those from a low social class. Further, the association observed in this study between active smoking of pregnant women and the presence of smokers in their close

environment supports the hypothesis that this factor makes it more difficult to stop smoking.[26] It is necessary to undertake effective programmes for reducing smoking before and during pregnancy, reaching also misclassified, and to reduce SHS exposure, in order to prevent risks for women and foetus.

CONCLUSION

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

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

Acknowledgments: The authors are grateful to all fieldworkers for their assistance in administering the questionnaires. A full listing of the INMA project researchers can be found at http://www.proyectoinma.org/presentacioninma/listado-investigadores/en_listado-investigadores.html

Declaration of interests: None.

Patient consent: Obtained.

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

Funding: INMA project is funded by grants from Instituto de Salud Carlos III (Red INMA G03/176 and CB06/02/0041) and Fundación Roger Torné. The studies in the specific regions were funded by the Spanish Ministry of Health (FIS 03/1615, 04/1436, 04/1509, 04/1112, 04/1931, 05/1079, 05/1052, 06/0867,06/1213, 07/0314, 08/1151, 09/02647, 04/2018, 09/02311), the Generalitat de Catalunya (CIRIT 1999SGR00241), the Diputación Foral de Gipuzkoa (DFG06/004), the Department of Health of the Basque Government (2005111093), the Regional Government of Andalucía (SAS 07/183), Obra social Cajastur, University of

Oviedo and the Conselleria de Sanitat Generalitat Valenciana. http://www.proyectoinma.org/instituciones-participantes/en_entidades-colaboradoras/

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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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 pressure[9] 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 pregnancy[11] 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.

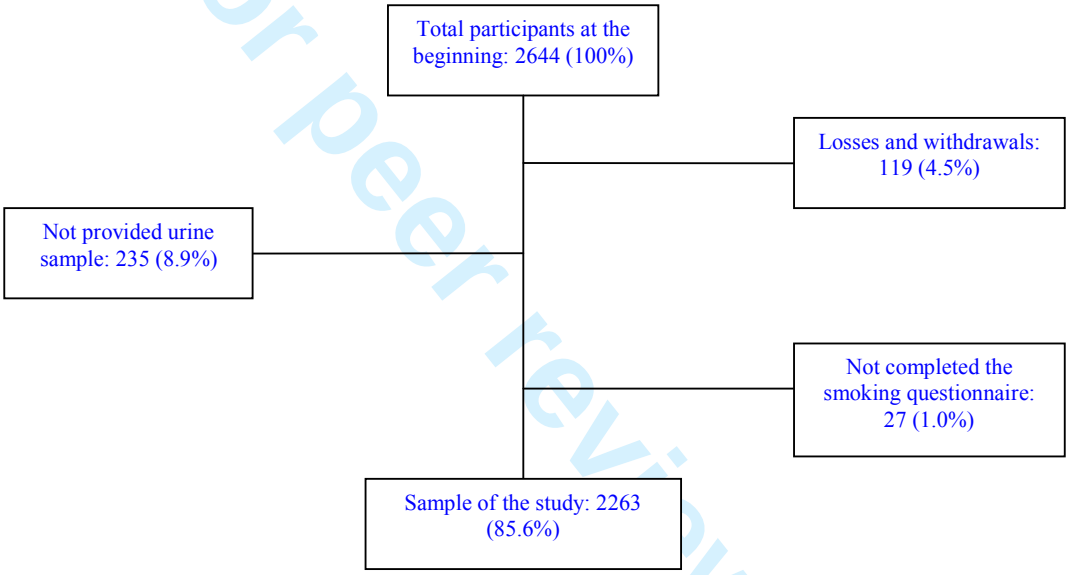
METHODS

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

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

Other variables

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

Statistical analysis

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

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

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=maximum{sensitivity(c)+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 α= 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	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 [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	-	

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 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 Younden´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 seft-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 Younden´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.

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

Not exposed women to SHS compared with all smokers, daily or occasional, had a lower cut-off point of 42 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 (95% CI 55 to 136) and 106 ng/ml (95% CI 79 to 201), respectively.

DISCUSSION

Main findings in relation to the literature

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

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

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/ml[18] 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 resticted 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)

is not necessarily affected. There were other likely sources of misclassification in addition to maternal misreporting of smoking status, as misclassification of non-smokers as smokers because of high exposure degree to SHS. On the other hand, women who smoked occasionally but report to be non-smokers might have low UC concentrations if they had not smoked recently, and their self-report and UC levels would be in agreement.

No information about last cigarette or last SHS exposure was obtained. We lost the opportunity of analysing this variable in the evolution of the UC, showing his influence in false negatives, above all, and especially relevant for occasional smokers.

Since the optimal cut-off point for UC is determined using self-reported smoking status as the gold standard, the validity of this assumption is important. On the one hand, it is unlikely that a non-smoking woman declared to be a smoker, because a battery of items should be completed detailing smoking habits in this case. On the other hand, however, it is possible that some smokers did not reveal their habit. In order to minimize this type of bias, we excluded in the main analysis self-reported non-smokers with implausible high UC levels. In additional analysis, we excluded self-reported non-smokers who claimed to stop smoking during pregnancy, since these cases are at higher risk of misclassification as reported in table 3; the optimal cut-off point did not change after this exclusion. In general terms, the AUC shows a good overall accuracy, and we think that self-reported smoking is a reliable measure in this study. If some kind of misclassification occurs, it would lead to a shift towards the right in both distributions, and a slight overestimation of the optimal cut-off point as a result.

One of the main strengths of this study was the possibility of assessing 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. The confirmation that the cut-offs would differ according to the level of exposure to SHS emphasizes the need of taking it into account, especially in countries with elevated SHS exposure.

Implications for practice

This study shows that the efforts made to encourage women to give up smoking before or during pregnancy are not sufficient or particularly effective, given that at least 18.5% of the pregnant women smoked in the third trimester. The results of this study indicate that the groups to which the most effort should be directed are young women, those of a European origin and those from a low social class. Further, the association observed in this study between active smoking of pregnant women and the presence of smokers in their close

environment supports the hypothesis that this factor makes it more difficult to stop smoking.[26] It is necessary to undertake effective programmes for reducing smoking before and during pregnancy, reaching also misclassified, and to reduce SHS exposure, in order to prevent risks for women and foetus.

CONCLUSION

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

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

Acknowledgments: The authors are grateful to all fieldworkers for their assistance in administering the questionnaires. A full listing of the INMA project researchers can be found at http://www.proyectoinma.org/presentacioninma/listado-investigadores/en_listado-investigadores.html

Declaration of interests: None.

Patient consent: Obtained.

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

Funding: INMA project is funded by grants from Instituto de Salud Carlos III (Red INMA G03/176 and CB06/02/0041) and Fundación Roger Torné. The studies in the specific regions were funded by the Spanish Ministry of Health (FIS 03/1615, 04/1436, 04/1509, 04/1112, 04/1931, 05/1079, 05/1052, 06/0867,06/1213, 07/0314, 08/1151, 09/02647, 04/2018, 09/02311), the Generalitat de Catalunya (CIRIT 1999SGR00241), the Diputación Foral de Gipuzkoa (DFG06/004), the Department of Health of the Basque Government (2005111093), the Regional Government of Andalucía (SAS 07/183), Obra social Cajastur, University of

Oviedo and the Conselleria de Sanitat Generalitat Valenciana. http://www.proyectoinma.org/instituciones-participantes/en_entidades-colaboradoras/

Data Sharing Statement: There is no additional data available.

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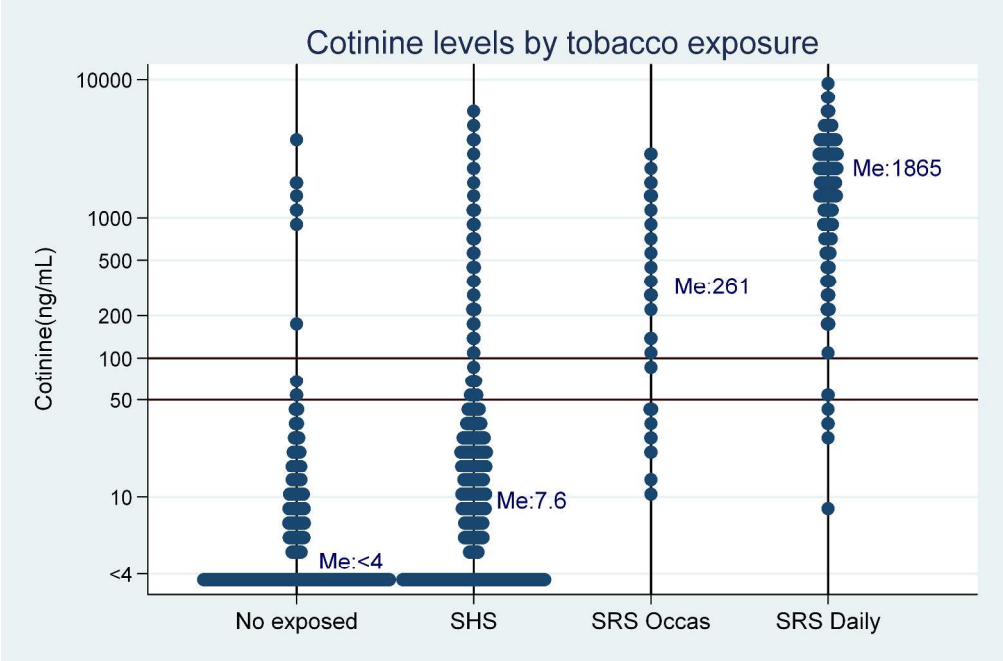

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Determinants of self-reported smoking and misclassification during pregnancy, and analysis of optimal cut-off points of urinary cotinine: a cross sectional study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2012-002034.R2
Article Type:	Research
Date Submitted by the Author:	18-Dec-2012
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
Primary Subject Heading:	Smoking and tobacco
Secondary Subject Heading:	Epidemiology, Public health, Occupational and environmental medicine, Obstetrics and gynaecology
Keywords:	EPIDEMIOLOGY, Child protection < PAEDIATRICS, PUBLIC HEALTH, TOXICOLOGY, PREVENTIVE MEDICINE

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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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 pressure[9] 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 pregnancy[11] 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

The urine samples were collected in the same interview in the morning during the third trimester of pregnancy. Urine was collected in 100 ml polyethylene containers and stored at -20^{o}C. One aliquot of the sample from each of the participants was sent to the Public Health Laboratory of Bilbao (Spain) to be analysed. All urine samples were stored for a minimum of one year and a maximum of 5 years before analysis. The analysis of the UC was performed by competitive enzyme immunoassay (EIA) using commercial EIA microplate test kits (OraSure Technologies, Inc., Bio-Rad) for determining salivary cotinine adapted for urine samples using urine controls (0, 2.5, 10 and 50 ng/ml, Bio-Rad). Samples with UC levels above 50 ng/ml were diluted. Before testing the urine samples the method was validated; a certified reference

material was used (EPA/NIST Reference Material 8444) to evaluate the repeatability and reproducibility. The quantification limit was 4.0 ng/ml, the coefficient of repeatability 7% and the reproducibility 10%.

Other variables

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

Statistical analysis

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

We used non-parametric receiver operating characteristic (ROC) curve to analyse the relationship between the sensitivity (probability of a positive test among smokers) and false positive (probability of a positive test among non-smokers, 1-specificity) cases for various different cut-off points that dichotomize UC to distinguish smokers from non-smokers, using self-reported cigarette smoking status as the reference value. Overall accuracy was evaluated by means of the area under the curve (AUC) (showing the ability of the 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. The cut-off points were also calculated after stratification among non smokers for SHS exposure in three groups: 798 women that referred not exposed to SHS, 735 exposed to one source of SHS and 303 exposed to more than one source. Additional sensitivity analysis was conducted (Supplementary Table) the first one excluding 1047 pregnant women non-smokers who referred SHS exposure, the second one excluding 317 self-reported non-smokers who claimed to stop smoking during pregnancy, since this group is more likely to misreport their smoking status, and the last one excluding 35 women who declared that they did not smoke but with implausible UC levels in non smokers (>500 ng/ml). Assuming α= 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 $p < 0.001$

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 [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	-	

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 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 Younden´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 Younden´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.

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

Not exposed women to SHS compared with all smokers, daily or occasional, had a lower cut-off point of 42 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 (95% CI 55 to 136) and 106 ng/ml (95% CI 79 to 201), respectively.

DISCUSSION

Main findings in relation to the literature

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

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

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 136 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/ml[18] 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)

is not necessarily affected. There were other likely sources of misclassification in addition to maternal misreporting of smoking status, as misclassification of non-smokers as smokers because of high exposure degree to SHS. On the other hand, women who smoked occasionally but report to be non-smokers might have low UC concentrations if they had not smoked recently, and their self-report and UC levels would be in agreement.

No information about last cigarette or last SHS exposure was obtained. We lost the opportunity of analysing this variable in the evolution of the UC, showing his influence in false negatives, above all, and especially relevant for occasional smokers.

Since the optimal cut-off point for UC is determined using self-reported smoking status as the gold standard, the validity of this assumption is important. On the one hand, it is unlikely that a non-smoking woman declared to be a smoker, because a battery of items should be completed detailing smoking habits in this case. On the other hand, however, it is possible that some smokers did not reveal their habit. In order to minimize this type of bias, we excluded in the additional analysis self-reported non-smokers with implausible high UC levels. In another sensitivity analysis, we excluded self-reported non-smokers who claimed to stop smoking during pregnancy, since these cases are at higher risk of misclassification as reported in table 3; the optimal cut-off point did not change after this exclusion. In general terms, the AUC shows a good overall accuracy, and we think that self-reported smoking is a reliable measure in this study. If some kind of misclassification occurs, it would lead to a shift towards the right in both distributions, and a slight overestimation of the optimal cut-off point as a result.

One of the main strengths of this study was the possibility of assessing 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. The confirmation that the cut-offs would differ according to the level of exposure to SHS emphasizes the need of taking it into account, especially in countries with elevated SHS exposure.

Implications for practice

This study shows that the efforts made to encourage women to give up smoking before or during pregnancy are not sufficient or particularly effective, given that at least 18.5% of the pregnant women smoked in the third trimester. The results of this study indicate that the groups to which the most effort should be directed are young women, those of a European origin and those from a low social class. Further, the association observed in this study between active smoking of pregnant women and the presence of smokers in their close

environment supports the hypothesis that this factor makes it more difficult to stop smoking.[26] It is necessary to undertake effective programmes for reducing smoking before and during pregnancy, reaching also misclassified, and to reduce SHS exposure, in order to prevent risks for women and foetus.

CONCLUSION

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

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

Acknowledgments: The authors are grateful to all fieldworkers for their assistance in administering the questionnaires. A full listing of the INMA project researchers can be found at http://www.proyectoinma.org/presentacioninma/listado-investigadores/en_listado-investigadores.html

Declaration of interests: None.

Patient consent: Obtained.

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

Funding: INMA project is funded by grants from Instituto de Salud Carlos III (Red INMA G03/176 and CB06/02/0041) and Fundación Roger Torné. The studies in the specific regions were funded by the Spanish Ministry of Health (FIS 03/1615, 04/1436, 04/1509, 04/1112, 04/1931, 05/1079, 05/1052, 06/0867,06/1213, 07/0314, 08/1151, 09/02647, 04/2018, 09/02311), the Generalitat de Catalunya (CIRIT 1999SGR00241), the Diputación Foral de Gipuzkoa (DFG06/004), the Department of Health of the Basque Government (2005111093), the Regional Government of Andalucía (SAS 07/183), Obra social Cajastur, University of

Oviedo and the Conselleria de Sanitat Generalitat Valenciana. http://www.proyectoinma.org/instituciones-participantes/en_entidades-colaboradoras/

Data Sharing Statement: No additional data available.

Figure Legends:

Figure 1: Flow-chart of the INMA birth cohort in relation to smoking and UC quantification.
Figure 2: Distribution of urinary cotinine (ng/ml) according to active or passive tobacco exposure in pregnant women from the INMA cohort.

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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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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: 968.56%. 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 pressure[9] 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 pregnancy[11] 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.

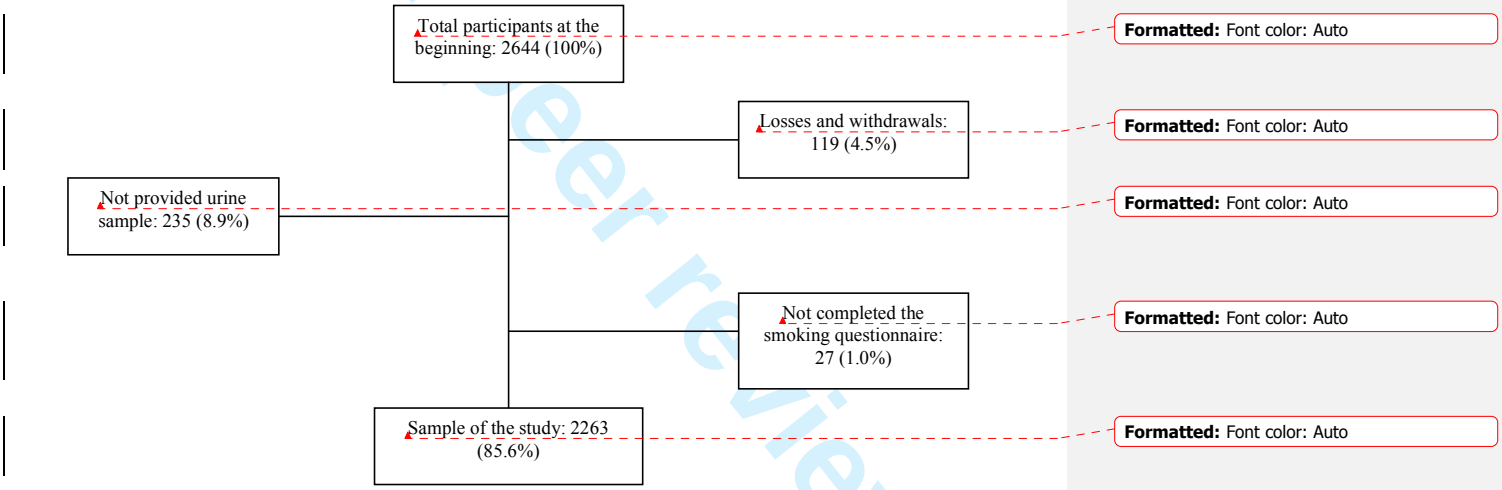
METHODS

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.



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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

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

Other variables

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

Statistical analysis

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

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

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=maximum{sensitivity(c)+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 Wwomen 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: 798~~1~~ women that referred not exposed to SHS, 735~~18~~ exposed to one source of SHS and ~~292~~ 303 exposed to more than one source. Additional sensitivity analysis was conducted (Supplementary Table) the first one excluding 1047 pregnant women non-smokers who referred SHS exposure, the second one excluding 317 self-reported non-smokers who claimed to stop smoking during pregnancy, since this group is more likely to misreport their smoking status, and the last one excluding 35 women who declared that they did not smoke but with implausible UC levels in non smokers (>500 ng/ml). ~~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 α= 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	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 [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	-	

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 500 ng/ml), was 82 ng/ml, with a sensitivity of 95.2%, specificity of 9~~8~~6.~~5~~6% and AUC 0.~~995~~ 986 (95% CI 0.~~992~~ 982 to 0.~~997~~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, ~~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 Younden´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,a]	Cut-off point (95% CI)[b]	Sensitivity	Specificity~~Specificity~~	Positive PV[c] ~~Positive PV[c]~~	Negative PV[d,c]	AUC of the ROC (95% CI)[e,d]
Regular and occasional smokers: ~~1810~~ 1845 non-smokers, 418 smokers						
-	50	0.957	0.951~~0.970~~	0.816~~0.879~~	0.990	0.986 (0.982 to 0.990)~~0.995 (0.992 to 0.997)~~
-	100	0.950	0.966~~0.985~~	0.865~~0.936~~	0.988	
0.918~~0.937~~	82 (42 to 136~~3~~)	0.952	0.966~~0.985~~	0.863~~0.934~~	0.989	
Results stratified by frequency of smoking						
Occasional smokers: 181~~0~~45 non-smokers, 37 smokers						
-	25	0.892	0.870~~0.887~~	0.121~~0.139~~	0.998	0.947 (0.923 to 0.970)~~0.961 (0.939 to 0.984)~~
-	50	0.649	0.951~~0.970~~	0.211~~0.304~~	0.993	
0.772~~0.789~~	27 (11 to 43)	0.892	0.880~~0.897~~	0.130~~0.151~~	0.998	
Daily smokers: ~~1810~~ 1845 non-smokers, 381 smokers						
-	50	0.987	0.951~~0.970~~	0.807~~0.872~~	0.997	0.990 (0.986 to 0.994)~~0.998 (0.996 to 1)~~
-	100	0.982	0.966~~0.985~~	0.858~~0.933~~	0.996	
0.949~~0.968~~	115 (57 to 189)	0.982	0.967~~0.986~~	0.862~~0.937~~	0.996	
Results stratified by SHS exposure among non-smokers						
Non exposed to SHS: ~~791~~ 798 non-smokers, 418 active smokers						
-	50	0.957	0.987~~0.996~~	0.976~~0.993~~	0.978	0.994 (0.990 to 0.998)~~0.998 (0.997 to 1)~~
-	100	0.950	0.990~~0.999~~	0.980~~0.997~~	0.974	
0.954~~0.962~~	42 (27 to 57)	0.971	0.982~~0.991~~	0.967~~0.983~~	0.985	
Exposed to SHS (1 source[e]): ~~718~~ 735 non-smokers, 418 active smokers						
-	50	0.957	0.935~~0.957~~	0.893~~0.928~~	0.974	0.981 (0.974 to 0.989)~~0.993 (0.990 to 0.997)~~
-	100	0.950	0.958~~0.981~~	0.928~~0.966~~	0.971	
0.910~~0.933~~	82 (~~55~~46 to 136)	0.952	0.958~~0.981~~	0.928~~0.966~~	0.972	
Exposed to SHS (2-3 sources[e]): ~~292~~ 303 non-smokers, 418 active smokers						
-	50	0.957	0.894~~0.928~~	0.926~~0.950~~	0.938	0.977 (0.966 to 0.987)~~0.988 (0.982 to 0.994)~~
-	100	0.950	0.924~~0.959~~	0.945~~0.971~~	0.930	
0.877~~0.912~~	106 (~~79~~58 to 227)	0.950	0.927~~0.962~~	0.947~~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 Younden´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.

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

Not exposed women to SHS compared with all smokers, daily or occasional, had a lower cut-off point of 42 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 (95% CI 55 to 136) and 106 ng/ml (95% CI 79 to 201), respectively.

DISCUSSION

Main findings in relation to the literature

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

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

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~~ 136 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/ml[18] 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)

is not necessarily affected. There were other likely sources of misclassification in addition to maternal misreporting of smoking status, as misclassification of non-smokers as smokers because of high exposure degree to SHS. On the other hand, women who smoked occasionally but report to be non-smokers might have low UC concentrations if they had not smoked recently, and their self-report and UC levels would be in agreement.

No information about last cigarette or last SHS exposure was obtained. We lost the opportunity of analysing this variable in the evolution of the UC, showing his influence in false negatives, above all, and especially relevant for occasional smokers.

Since the optimal cut-off point for UC is determined using self-reported smoking status as the gold standard, the validity of this assumption is important. On the one hand, it is unlikely that a non-smoking woman declared to be a smoker, because a battery of items should be completed detailing smoking habits in this case. On the other hand, however, it is possible that some smokers did not reveal their habit. In order to minimize this type of bias, we excluded in the ~~main~~ additional analysis self-reported non-smokers with implausible high UC levels. In ~~additional~~ another sensitivity analysis, we excluded self-reported non-smokers who claimed to stop smoking during pregnancy, since these cases are at higher risk of misclassification as reported in table 3; the optimal cut-off point did not change after this exclusion. In general terms, the AUC shows a good overall accuracy, and we think that self-reported smoking is a reliable measure in this study. If some kind of misclassification occurs, it would lead to a shift towards the right in both distributions, and a slight overestimation of the optimal cut-off point as a result.

One of the main strengths of this study was the possibility of assessing 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. The confirmation that the cut-offs would differ according to the level of exposure to SHS emphasizes the need of taking it into account, especially in countries with elevated SHS exposure.

Implications for practice

This study shows that the efforts made to encourage women to give up smoking before or during pregnancy are not sufficient or particularly effective, given that at least 18.5% of the pregnant women smoked in the third trimester. The results of this study indicate that the groups to which the most effort should be directed are young women, those of a European origin and those from a low social class. Further, the association observed in this study between active smoking of pregnant women and the presence of smokers in their close

environment supports the hypothesis that this factor makes it more difficult to stop smoking.[26] It is necessary to undertake effective programmes for reducing smoking before and during pregnancy, reaching also misclassified, and to reduce SHS exposure, in order to prevent risks for women and foetus.

CONCLUSION

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

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

Acknowledgments: The authors are grateful to all fieldworkers for their assistance in administering the questionnaires. A full listing of the INMA project researchers can be found at http://www.proyectoinma.org/presentacioninma/listado-investigadores/en_listado-investigadores.html

Declaration of interests: None.

Patient consent: Obtained.

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

Funding: INMA project is funded by grants from Instituto de Salud Carlos III (Red INMA G03/176 and CB06/02/0041) and Fundación Roger Torné. The studies in the specific regions were funded by the Spanish Ministry of Health (FIS 03/1615, 04/1436, 04/1509, 04/1112, 04/1931, 05/1079, 05/1052, 06/0867,06/1213, 07/0314, 08/1151, 09/02647, 04/2018, 09/02311), the Generalitat de Catalunya (CIRIT 1999SGR00241), the Diputación Foral de Gipuzkoa (DFG06/004), the Department of Health of the Basque Government (2005111093), the Regional Government of Andalucía (SAS 07/183), Obra social Cajastur, University of

Oviedo and the Conselleria de Sanitat Generalitat Valenciana. http://www.proyectoinma.org/instituciones-participantes/en_entidades-colaboradoras/

Data Sharing Statement: There is no additional data available.

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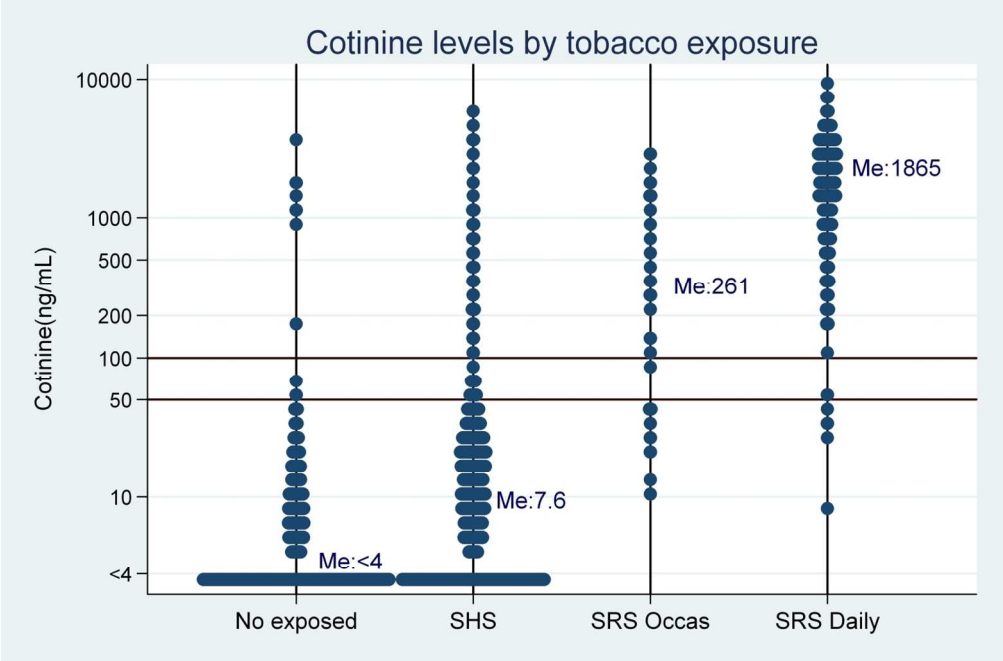

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Supplementary Table: Parameters for assessing the optimal cut-off point for urinary cotinine, ng/ml, obtained by the Younden´s index, as well as the levels of 25, 50, and 100 ng/ml, for classifying pregnant women as regular or occasional smokers. Sensitivity analysis: 1) Excluding SHS exposed non-smokers, 2) Excluding non-smokers who claimed to stop smoking during pregnancy, and 3) Excluding seft-reported non-smokers with cotinine above 500 ng/ml.

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]
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 seft-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).

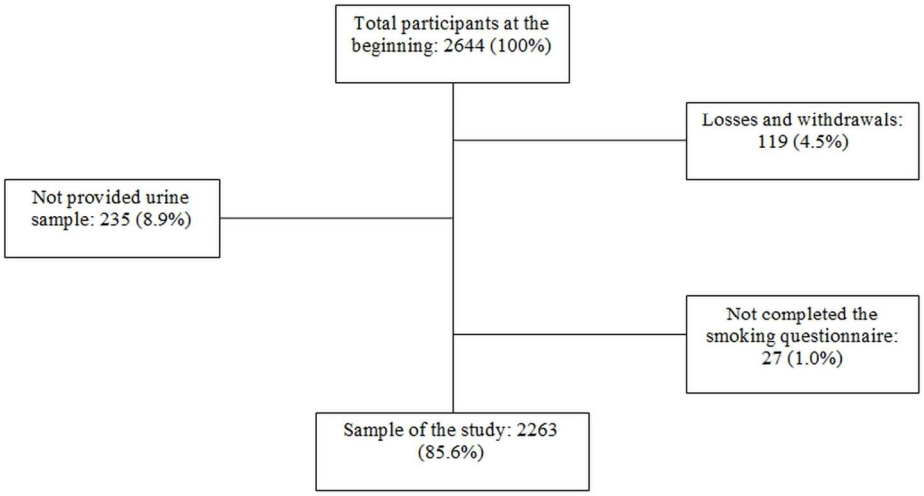
b: 95% bootstrap confidence interval for the cut-off point associated with the Younden´s index.

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.

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



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