

In utero exposure to parental smoking, cotinine measurements, and cord blood IgE

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The relationship between parental smoking and cord blood IgE has been studied in a survey conducted in 99 unselected newborn infants with a sensitive test for IgE and cotinine as a biologic marker to validate smoking data. For both cord blood cotinine and maternal urine cotinine creatinine ratio (CCR), significantly higher levels were observed for smokers compared to nonsmokers. Furthermore, among nonsmokers, passive smokers had significantly higher cotinine levels than true nonsmokers, which demonstrates that cord blood may be used to assess active as well as passive maternal smoking. No association was observed in this study between cord blood IgE and maternal smoking assessed by questionnaire (geometric means of cord blood IgE levels were 0.11 IU/ml for newborn infants of smoking mothers and 0.12 IU/ml for newborn infants of nonsmoking mothers). The same observations were drawn from the analysis of cord blood IgE and cotinine levels, with correlation coefficients of -0.005 for cord blood CCR and 0.003 for maternal CCR. Additional studies are needed to determine whether maternal smoking is causally related to cord blood IgE and by which mechanisms. (J ALLERGY CLIN IMMUNOL 1991;87:1169-74.)

Since high cord blood IgE level is predictive of infant allergy,^{1,2} it is important to determine in which respects environmental factors increase cord blood IgE level. Association between smoking and total IgE level is established among adults,³⁻⁵ whereas discordant results have been observed among newborn infants and children. IgE level has been demonstrated to be significantly higher among 9-month-old children of smoking parents compared to children of non-smoking parents.⁶ In older children, association of maternal smoking with specific IgE and development of atopy have been reported in several studies,⁷⁻⁹ but not in other studies.¹⁰ Among newborn infants, only one study until now, conducted in Sweden,¹¹ demonstrated an association between maternal smoking and cord blood IgE, a relationship that was particularly clear among nonallergic families. Three other studies^{6, 12, 13} failed to observe a significant association between parental smoking and cord blood IgE. Two of these studies were conducted on relatively small samples

Abbreviations used

GM: Geometric mean
CI: Confidence interval
CCR: Cotinine creatinine ratio

and used low sensitive tests for IgE. No study included biologic validation of smoking data.

Infant blood cotinine, the major degradation product of nicotine, is a good marker of maternal smoking.¹⁴ Assessment of in utero exposure to maternal smoking by cotinine measurements in cord blood have already been performed in a few studies.^{15, 16} In the present study, the association of parental smoking and cord blood IgE was analyzed with a sensitive test for IgE and cotinine as a biologic marker to validate smoking data.

MATERIAL AND METHODS

An epidemiologic study, including biologic markers of smoking,¹⁷ has been conducted in a subgroup of the 799 single births that occurred between January and May 1985 in Baudelocque maternity Hospital (Paris). Still births (N = 33) and, for practical reasons, births that took place during weekends (N = 170), holidays (N = 101), or from 5 PM to 9 AM the next morning (N = 271) were excluded. Among the remaining births, those excluded were mothers who gave birth to premature babies (N = 7), had cesareans

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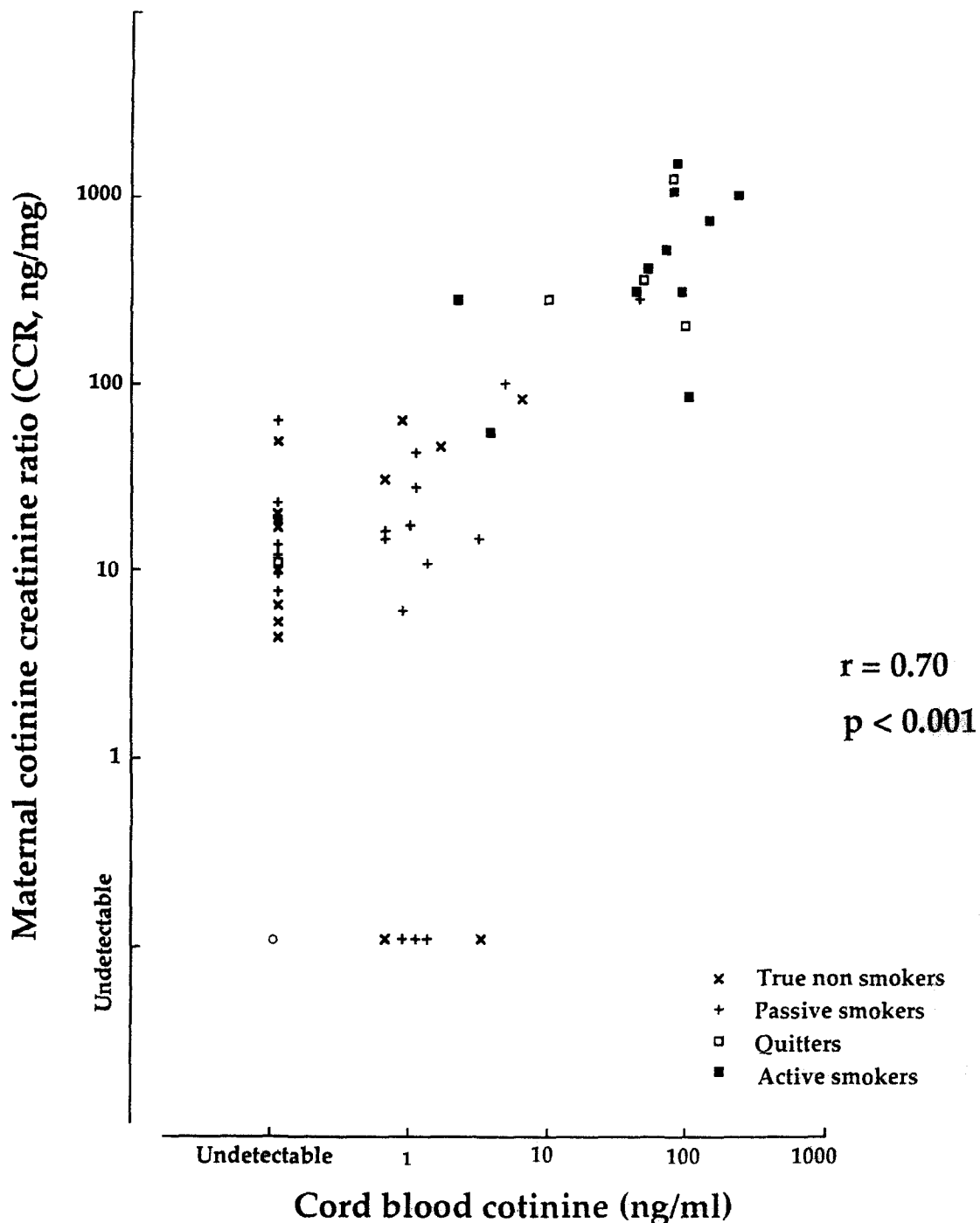


FIG. 1. Relationships between cotinine levels and smoking habits assessed by questionnaire; ○, 30 true nonsmokers, eight passive smokers, and five quitters.

($N = 36$), or did not speak French ($N = 5$). Of the 176 women solicited for the present study, eight refused, and the remaining mothers ($N = 168$) gave informed consent. For technical reasons, cord blood samples for IgE determinations were not available for 31 newborn infants whose parents had similar smoking habits like the other parents.

Tests for IgE levels were therefore performed in 137 newborn infants by Pharmacia IgE Enzymo Immuno assay

Ultra 50 (Pharmacia Diagnostic AB, Bois d'Arcy, France). Samples were run in duplicate. The 31 newborn infants with IgE levels too low to be detected by the method (<0.1 IU/ml) were ascribed a value of 0.01 IU/ml for log transformation. According to maternal ethnic origin, the highest IgE values were observed among newborn infants of non-European mothers. The difference was significant between newborn infants from Europe and infants of Asia-West In-

TABLE I. Cord blood and maternal urine cotinine levels according to active and passive smoking habits

Maternal smoking	Cord blood cotinine			Maternal urine CCR		
	No.	Median	Mean $\pm$ SD (ng/ml)	No.	Median	Mean $\pm$ SD (ng/mg)
True nonsmokers	46	0	0.27 $\pm$ 0.94	46	0	7.57 $\pm$ 16.44
Passive smokers	33	0	1.78 $\pm$ 7.37*	33	9.94	20.43 $\pm$ 45.55*
Quitters	9	0	24.36 $\pm$ 36.22*	8	96.23	237.71 $\pm$ 383.44†
Active smokers	11	75	77.04 $\pm$ 61.39‡	11	369.75	514.31 $\pm$ 409.81‡

p Values (Wilcoxon's test) refer to comparison with true nonsmokers.

**p* < 0.01

†*p* < 0.05.

‡*p* < 0.0001.

dies origin. To reduce the variance in IgE values, the analysis was restricted to the 101 white mothers of European origin. Results are presented in log₁₀ IgE or in GMs. Since not enough cord blood was available to assay serum IgA levels to exclude maternal contamination of cord blood in two newborn infants with cord blood IgE level >10 IU/ml, these were excluded from the analysis (Bousquet J. Personal communication). The sample analyzed included 56 male and 43 female newborn infants.

Three days after delivery, mothers were interviewed concerning their active and passive smoking habits during the three trimesters of their pregnancy. Personal daily cigarette consumption and passive exposure at home and at work were assessed. The term "smokers" refers to mothers who smoked at least one cigarette per day during at least one of the three trimesters of their pregnancy (20.2%). Smokers were subdivided into two groups, quitters and active smokers, according to whether they stopped smoking before the last trimester of their pregnancy (11.1% and 9.1%, respectively). Passive smokers were nonsmokers environmentally exposed to at least two cigarettes per day during their pregnancy (33.3%). The remaining women were considered as true nonsmokers (46.5%). No woman started smoking during at least one of the three trimesters of her pregnancy.

Maternal urine (collected within 1 day around delivery) and cord blood cotinine levels were used to validate smoking data. Urine and cord serum were frozen at -70° C until it was analyzed. Cotinine levels were determined by competitive inhibition radioimmunoassay¹⁸ (purchased from Helen Van Vunakis, Brandeis University, Waltham, Mass.) in duplicate. The sensitivity in cord blood was 0.5 ng/ml, and in maternal urine, 6.5 ng/ml. Urinary creatinine was determined colorimetrically (diagnostics kits, Sigma Chemical Co., St. Louis, Mo.) and based on the reaction between creatinine and sodium picrate. Urinary cotinine excretion was expressed in nanograms per milligram of creatinine (CCR) because 24-hour urine samples were not available.

Since the cord blood IgE distribution was skewed, log transformation was performed. The associations of qualitative variables were evaluated with the use of χ^2 tests, and for quantitative variables with analyses of variance.¹⁹ For

cotinine levels, only nonparametric tests were used (Wilcoxon's, Kruskal-Wallis', and Spearman's rank correlation).¹⁹ For IgE levels, both parametric (on log IgE) and nonparametric tests were used and provided similar results.

RESULTS

The median value of cord serum and maternal urine CCR was, respectively, 0 ng/ml and 5.81 ng/mg. Cotinine concentrations ranged in cord serum from 0.1 to 220 ng/ml, and CCR in maternal urine ranged from 1 to 1355 ng/mg. Cotinine levels in mothers and children (Fig. 1) correlated well with a correlation coefficient (Spearman's) of 0.70 (*p* < 0.0001).

Detectable levels of cord serum cotinine were observed for 100%, 39%, and 13% for newborn infants of smoker, passive smoker, and true nonsmoker mothers, respectively. Similarly, cotinine was detectable in maternal urine of 100%, 67%, and 30% of smoker, passive smoker, and true nonsmoker mothers, respectively. As expected, significantly higher cotinine levels were observed for smokers compared to that of nonsmokers for both biologic fluids. (Means for cord serum were 53.33 ng/ml versus 0.90 ng/ml, and for maternal urine CCR, 397.85 ng/mg versus 12.94 ng/mg, respectively, with *p* < 0.0001 for both comparisons.) Furthermore, among smokers, cotinine levels were definitively higher among active smokers than among quitters (Table I). Among nonsmokers, passive smokers had significantly higher cotinine levels than true nonsmokers (Table I). The three groups of passive smokers, last trimester quitters, and active smokers had significantly higher cotinine levels than true nonsmokers. As illustrated in Fig. 1, no newborn infant whose mother was a true nonsmoker or quitter had cord serum cotinine levels >100 ng/ml; in only one newborn infant with a passive smoker mother was the level >100 ng/ml.

Cord serum IgE concentrations ranged from 0.01

TABLE II. Cord blood IgE according to maternal active and passive smoking habits

Maternal smoking	No.	GM (IU/ml)	CI
True nonsmokers	46	0.09	0.00-3.40
Passive smokers	33	0.15	0.00-8.05
Quitters	9	0.19	0.00-11.12
Active smokers	11	0.08	0.00-3.74

to 7 IU/ml (GM, 0.11 IU/ml; CI, 0.003 to 5.02 IU/ml). No difference was observed between boys and girls (0.11 IU/ml versus 0.11 IU/ml, respectively).

No difference was observed in cord serum IgE levels of newborn infants with smoker and nonsmoker mothers (GM of 0.11 and 0.12 IU/ml, respectively) as defined by smoking questionnaires. IgE levels were similar in cord serum of the newborn infants of true nonsmokers and of passive smokers (Table II). The highest value was observed for the quitters, whereas active smokers had newborn infants with lower IgE levels than did nonsmokers. The same conclusions were drawn from the analysis of cord serum IgE and biologic markers of smoking. No association was observed between cord serum IgE and cotinine levels (Fig. 2) with correlation coefficients of -0.03 for cord serum cotinine level and of 0.01 for maternal urine CCR. The values of rank correlation coefficients were -0.005 and 0.003 , respectively.

DISCUSSION

No association has been observed in this study conducted on 99 European newborn infants between cord blood IgE and maternal smoking, assessed both by questionnaire and by cotinine levels. Smoking data assessed by questionnaire correlated well with both cord blood and maternal urine cotinine levels.

IgE levels observed in the present study with a GM of 0.11 IU/ml are compatible with levels observed in the literature,²⁰ although a direct comparison is limited by differences in methods. Validation of smoking habits was searched by cotinine measurements. Cotinine levels are more relevant to consider than nicotine levels, since cotinine has a longer serum half-life (about 18 hours in adult smokers) than nicotine (about 2 hours) and is exclusively a product of *in vivo* metabolism, thus avoiding contamination problems. The reported variations between laboratories in cotinine determinations by radioimmunoassay²¹ limit comparison with other studies. Very few studies reported cotinine in cord blood.^{15, 16} Sorsa and Husgafvel-

Pursiainen¹⁶ did not observe detectable values for mothers passively exposed, but women did not have substantial passive exposure and the sample was very small. Our results demonstrate that cord blood may be used to assess active as well as passive maternal smoking. However, information from questionnaires should not be replaced by biologic markers but is complementary, since cotinine levels reflect short-term exposure only.

The first two negative studies on the relationship between cord blood IgE and parental smoking used low sensitive tests (only available at that time) for IgE levels. In the study of Michel et al.,¹² conducted in 136 newborn infants, 16 of whom had smoking mothers, 68% had undetectable IgE levels. In the study of Kjellman,⁶ there was no significant relationship at birth and at 3 months of age, when IgE levels are more easily detected than they are at birth, but only 46 children were studied. Parental smoking (without distinction between maternal and paternal) was considered. Our results do not support the association between cord blood IgE and maternal smoking reported by Magnusson.¹¹ In that study, conducted in 186 newborn infants, of whom 41 had smoking mothers, the association between maternal smoking and IgE was observed in the whole group, but the effect was more apparent in newborn infants with both non-allergic parents. This finding was interpreted by the fact that the effects of atopic heredity might overshadow a weak effect of tobacco smoke. Among adults, Barbee et al.,²² however, observed that the smoking-IgE effect was more apparent in skin test-positive than in skin test-negative subjects. Our results are consistent with the preliminary data reported by Johnson et al.¹³ on a sample of 325 newborn infants, 62 of whom had smoking mothers. A sensitive assay for IgE appears to have been used in that study in which no association between cord blood IgE and maternal smoking was observed. However, in that sample, Ownby et al.²³ did not observe the expected association between parental allergy and cord blood IgE. Self-selection against smoking by atopic adults might obscure the associations between smoking habits and IgE levels in mothers and their offspring. Biologic markers of smoking were not included in any other study on the potential association between parental smoking and cord blood IgE. In our study, cotinine determinations allowed a good assessment of recent smoking. Information obtained from questionnaire was well correlated with cord blood as well as maternal urine cotinine levels. In adults, the strength of the association of IgE with the amount of smoking is a matter of debate.^{5, 24} The lack of association in the present study between maternal smoking and cord

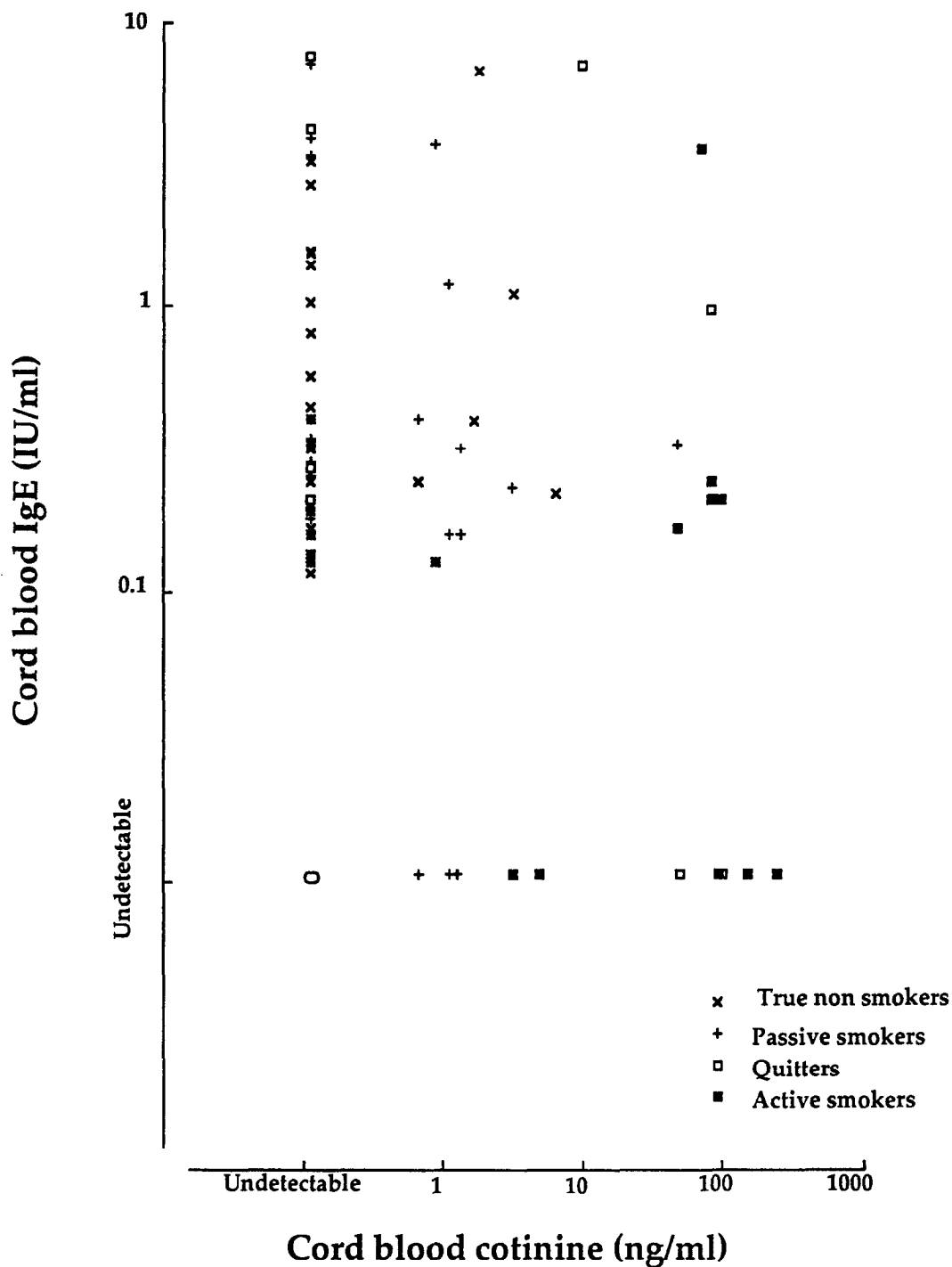


FIG. 2. Cord blood IgE according to cord blood cotinine; ○, 19 true nonsmokers and five passive smokers.

blood IgE may be explained if increased IgE occurs among newborn infants of heavy smoking mothers. (Only two women in our sample smoked >20 cigarettes per day.)

Among adults, the relationship between IgE and smoking has been observed in most studies, but the

mechanisms involved are unclear. The hypotheses suggested were infestation of low airways by microorganisms, airway inflammation, increased bronchial mucosal permeability leading to increase antigen penetration, or direct effect on the immune system.⁵ Since fetuses have never been exposed to aeroallergens,

most of these hypotheses are irrelevant to explain a potential association of maternal smoking and IgE. Additional studies are needed to determine whether maternal smoking is causally related to cord blood IgE and by which mechanisms.

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