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RESEARCH ARTICLE

Nasal epithelium integrity, environmental stressors, and allergic sensitization: A biomarker study in adolescents

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Abstract

Changes in the airways epithelium caused by environmental insults might play a role in the development of allergic rhinitis. We measured albumin and Clara cell protein (CC16) in the nasal lavage fluid (NALF) from 474 adolescents (263 girls and 211 boys). The NALF CC16/albumin ratio, integrating the permeability and cellular integrity of the nasal epithelium, decreased mostly with time spent in chlorinated pools. In boys, a lower CC16/albumin ratio in NALF was associated with an increased risk of house dust mite sensitization. The results suggest that the CC16/albumin ratio in NALF can be used to detect nasal epithelium alterations linked to allergic sensitization.

Keywords: Nasal epithelium, environmental tobacco smoke, Clara cell protein, albumin, chlorine, allergy, permeability, biomarkers

Introduction

Allergic rhinitis and atopic asthma are chronic inflammatory diseases of the airways that develop following sensitization to air-borne allergens and progressively lead to an impairment of lung function. Patients with allergic asthma frequently also suffer from an allergic rhinitis, which frequently precedes the onset of asthma. Both diseases also share the same genetic traits and the same triggers including allergen exposure, viral infection, and air pollution, which suggest that they might be the manifestations of the same underlying allergic disease (Marple 2010, Spergel 2010, Murphy & O'Byrne 2010). Although allergic diseases are driven by the immune system, in particularly by the activation of Th2-type lymphocytes, the idea now progressively emerges that the primary cause of these diseases might not lie in the immune processes but in some functional or structural defects of the airways epithelium (Cookson 2004, Holgate et al. 2009). When occurring in certain windows of exposure or susceptibility, in particular during early life, these airways epithelium defects might create conditions promoting the IgE sensitization and later the transformation of the atopic phenotype into rhinitis and then asthma (Holgate et al. 2009).

Changes in airways epithelium, which might predispose to allergic diseases, are largely unknown. One possible type of change might be an epithelial hyperpermeability facilitating the transepithelial delivery of allergens to the antigen-presenting cells (Bernard et al. 2003). This mechanism is suggested by the fact that most potent allergens such as the house dust mite (HDM) present a proteolytic activity enabling them to open the tight junctions and thus to move more easily across epithelial barriers toward immunocompetent cells (Roelandt et al. 2008). Ambient air pollutants or lifestyle-related stressors with a membrane disrupting potential such as ozone, tobacco smoke, chlorination products, or endotoxins are also capable of opening tight junctions and thus of exerting a similar adjuvant effect in allergic sensitization (Carbonnelle et al. 2002, Bernard et al. 2003, Schelegle et al. 2003, Michel et al. 2005, Chimenti et al. 2010).

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Another possible mechanism that might be triggered by above stressors would be a damage or dysfunction of epithelial cells secreting proteins downregulating the allergic or inflammatory responses. The reduced secretion of the anti-inflammatory Clara cell protein (CC16, SCGB1A1, or secretoglobin 1A1) (Jackson et al. 2011, Miele et al. 1987) in subjects exposed to chlorination products (Lagerkvist et al. 2004, Bernard et al. 2007) and in patients with asthma (Laing et al. 2000) or allergic rhinitis (Benson et al. 2007, Johansson et al. 2005) might illustrate such a mechanism. Since cell damage in the airways is frequently accompanied by an increased epithelial permeability, the pulmonary pool of anti-inflammatory proteins might be further reduced by the leakage of lung proteins across the disrupted bronchoalveolar blood barriers (Hermans & Bernard 1999).

The purpose of this population-based study conducted among school adolescents was to test these different hypotheses by assessing the associations between nasal epithelium integrity, possible environmental insults, and the risks of allergic sensitization. We evaluated the integrity of the nasal epithelium by measuring in nasal lavage fluid (NALF) albumin as a marker of epithelial permeability and CC16 as a marker of cell damage or dysfunction.

Materials and methods

Population studied

After approval by the ethics committee of the Faculty of Medicine of the Catholic University of Louvain, a total of 870 adolescents, aged between 15 and 18 years were recruited from three secondary schools in the southern part of Belgium (in the cities of Louvain-la-Neuve, Bastogne, and Lessines). Participation rates were very similar between the three schools as well as between girls and boys (between 70.6% and 72.1%). The study protocol was described in detail previously (Bernard et al. 2008, 2009). Briefly, the adolescents' parents were asked to complete a questionnaire about the personal or familial characteristics, the in-house and out-house environment, medical characteristics and antecedents, and self-reported symptoms. The questionnaire also included specific items to estimate the lifetime attendance of outdoor or indoor chlorinated swimming pools. The examination, which took place in schools, included the collection of a blood sample and a NALF sample from both nostrils. To avoid acute effects caused by irritants or inflammation and thus detect chronic changes in NALF biomarkers, we excluded subjects who had signs of rhinitis or who were under medication at the time of examination. The final population retained for the study included 263 girls and 211 boys.

Nasal lavage sample collection analysis

NALF samples were obtained from both nostrils using a procedure similar to that described by Tossa et al. (2009). Participants were asked to sit down, bend forward, and put their heads down. About 2.5 mL of sterile physiological saline at 37°C were instilled into each nostril by a disposable tip connected to a peristaltic pump. After 10 s, students were asked to lift their head and the NALF was collected using a small funnel. Participants were free to start the NALF collection with the left or right nostril. As majority of participants were right-handed, most of them started with the right nostril. CC16 was quantified by a semi-automated nonisotopic immunoassay based on the agglutination of latex particles coated with a polyclonal antibody (Bernard et al. 1992a,b). We used a rabbit antihuman CC16 polyclonal antibody (A0257; DAKO, Glostrup, Denmark), and as standards, the protein purified in our laboratory was used. The immunoassay was validated by comparison with a fluorescence enzyme immunoassay using monoclonal antibodies (Hermans et al. 1998). Albumin and urea were quantified by the Beckman Synchron CX5 Delta Clinical System. The reproducibility of protein determination in NALF was assessed in four healthy subjects (2 men and 2 women, aged 27–59 years), who provided twice a day for five consecutive days a NALF sample from each nostril. The mean NALF protein concentrations in these subjects ranged from 89 to 248 μg/L for CC16 and from 6.8 to 18.8 mg/L for albumin. The coefficients of variation (CVs) of CC16 and albumin determinations in the 10 samples from each nostril averaged 42.6% (range 29.0%–58.2%) and 35.2% (range 16.7%–55.1%), respectively. Adjustment for the NALF/plasma urea concentration ratio yielded rather similar values, with mean CVs of 42.8% for CC16 and of 33.2% for albumin. These overall CVs appear quite acceptable considering that they integrate the analytical variation of immunoassays (CVs between 5%–10%), the variable recovery of proteins from the nasal epithelial lining fluid (ELF) and probably also some biological variations in the ELF composition in the course of the experiment (5 days). Of note, the mean protein concentration ratio between the two nostrils, even after adjustment for the NALF/plasma urea ratio, was not necessarily equal to 1 but ranged from 0.91 to 1.55 for CC16 and from 0.88 to 1.37 for albumin. This means that the NALF has to be sampled in the two nostrils for a reliable assessment of the nasal epithelium integrity of an individual.

Total and specific IgE analysis

Total and aeroallergen-specific IgE (HDM, cat epithelium, dog dander, mould, tree pollen, grass pollen, and herbaceous pollen mixture) concentrations in serum were determined using the Immulite® IgE kit (Diagnostic Products Company, Los Angeles, CA, USA). A sensitization against aeroallergen-specific IgE was defined as a serum concentration >0.35 kIU/L. For total serum IgE, we used the same cut-off of 30 kIU/L as in our previous studies (Bernard et al. 2008, 2009). This cut-off value corresponds approximately to the 50th percentile (27.5 kIU/L) or the geometric mean (27.3 kIU/L) observed in adolescents who had no detectable aeroallergen-specific IgE in serum. This low total serum IgE threshold was used in order to identify most subjects likely to be sensitized

to aeroallergens (Kerkhof et al. 2003). Concentrations of proteins in NALF were adjusted for the variable dilution of the ELF using the NALF/plasma concentration ratio of urea (Cavaliere et al. 1986, Kaulbach et al. 1993).

Statistical analyses

Except for age and cumulative pool attendance (CPA), all continuous variables were log-transformed before statistical analysis. Differences between boys or girls were compared by the Chi-squared test, the Mann–Whitney U-test, or the Student's *t*-test depending on the type of variable. Univariate associations were assessed using the Pearson's correlation coefficient. Multiple linear regression analysis was used to identify factors associated with biomarker concentrations in NALF. Regression models were run by testing a total of 23 independent variables, including among others, age, body mass index (BMI), breastfeeding, tobacco smoking, exposure to environmental tobacco smoke (ETS), vicinity to a busy road, aeroallergen sensitization, and doctor-diagnosed diseases of the upper airways. In these analyses, attendance of the different types of chlorinated pool (indoor, outdoor residential, outdoor during holidays) was stratified in three categories of increasing lifetime CPA (CPA < 100, CPA 100–500, and CPA > 500 h). These categories were transformed in dummy variables using as referents subjects who never swam in the considered swimming pool. We used backward logistic regression models to calculate the odds ratios of being sensitized against HDM or pollen according to the levels of CC16, albumin, and of the CC16/albumin ratio in NALF. The *p* values were two-sided, and results were considered as statistically significant at *p* values below 0.05.

Results

The characteristics of participants are presented in Table 1. With the exception of parental asthma, which was more frequent in girls than in boys, there were no significant sex differences in the major risk factors of allergic or respiratory diseases. Prevalence of doctor-diagnosed asthma, hay fever, and allergic rhinitis also did not differ between sexes. The only noticeable differences concerned the total serum IgE levels and the sensitization rates to aeroallergens and especially to HDM, which were both significantly higher in boys than in girls.

We first analyzed our data by comparing the concentrations of biomarkers in the NALF from the two nostrils. To assess the influence of the variable dilution of the ELF during nasal lavage, the comparisons were made with concentrations of CC16 and albumin, unadjusted or adjusted for the NALF/plasma urea concentration ratio. As shown in Figure 1, concentrations of CC16 and albumin expressed per liter as well as their ratio in the two nostrils were rather well correlated (CC16, $r = 0.65$; albumin, $r = 0.57$; CC16/albumin ratio, $r = 0.58$, all $p < 0.001$) with however a considerable inter-individual variations over more than four orders of magnitude. Concentrations of urea in the NALF from both nostrils were also well correlated ($r = 0.64$, $p < 0.001$). Adjustment of protein concentrations for the NALF/plasma ratio did not improve these correlations (CC16, $r = 0.36$; albumin, $r = 0.57$, $p < 0.001$) nor did this adjustment reduce the inter-individual variations. We, therefore, pursued our analyses by using the means of values measured in the NALF from the two nostrils, both with and without adjustment for the NALF/plasma urea ratio.

Table 2 compares the NALF levels of CC16 and albumin between boys and girls. The concentration of urea in the NALF of boys was slightly higher than that of girls, but there was no significant sex difference in the NALF/plasma urea ratio. Concentrations of CC16 in NALF, adjusted or not for the NALF dilution, did not differ significantly between boys and girls. This is in contrast to the albumin concentration, which on average was about 30% greater in boys than in girls.

As shown in Table 3, the determinants of NALF epithelial markers differed noticeably between the two sexes. In boys, the NALF concentration of CC16, adjusted or not for the NALF/plasma urea ratio, correlated negatively with age and positively with parental smoking. Also in boys, the NALF concentration of albumin, whether adjusted or not for the urea ratio, decreased with the number of siblings, the BMI, and the proximity to a busy road. Albumin in the NALF of boys also decreased with age, but this association disappeared after adjustment for the urea ratio. The only positive association with NALF levels of albumin, suggesting an increased epithelial permeability, was that between the urea-adjusted concentrations of albumin and the lifetime attendance at public chlorinated pools (CPA > 500 h). Quite interestingly, the CC16/albumin ratio in the NALF of boys, an index integrating changes in CC16 secretion and nasal epithelium permeability, decreased dose-dependently with the lifetime attendance of indoor chlorinated pools (CPA 100–500 and CPA > 500 h). Like for the CC16 concentration, the CC16/albumin ratio in the NALF of boys, showed a positive association with parental smoking. In girls, by contrast, the NALF concentration of CC16 decreased with both parental smoking and the vicinity of a busy road, irrespectively of the adjustment for the urea ratio. There was also a negative association between the urea-adjusted CC16 concentration in the NALF of girls and the lifetime attendance of residential outdoor chlorinated pools (CPA > 500 h). None of the other tested factors was associated with albumin levels or the CC16/albumin ratio in the NALF of girls.

When tested separately, the concentrations of CC16 and albumin in NALF, expressed per liter or adjusted for the NALF/plasma urea ratio, showed no significant associations with the risk of sensitization to HDM or pollen. However, as shown in Table 4, the CC16/albumin ratio in the NALF of boys, whether tested as a continuous variable or stratified in tertiles, was inversely related to the

Table 1. Characteristics of adolescents.

	Girls ($n=263$)	Boys ($n=211$)	p
Age, mean (SD), years	15.4 (0.78)	15.6 (0.93)	0.18
BMI, mean (SD), Kg/m^2	20.7 (3.06)	20.5 (2.86)	0.5
Parental asthma, N° (%)	43 (16.4)	20 (9.5)	0.03
Parental allergy, N° (%)	105 (39.9)	69 (32.7)	0.11
Birth weight, mean (SD), g	3218 (545)	3432 (557)	<0.001
Breastfeeding, N° (%)	175 (66.5)	142 (67.3)	0.86
Day-care attendance, N° (%)	72 (27.4)	59 (28.0)	0.89
Smoking, active, N° (%)	20 (7.6)	18 (8.5)	0.71
Exposure to smoking during pregnancy, N° (%)	35 (13.3)	22 (10.4)	0.34
Parental smoking at home, N° (%)	96 (36.5)	73 (34.6)	0.67
Number of older siblings, mean (SD)	0.97 (0.94)	0.97 (1.03)	0.94
House cleaning with bleach, N° (%)	72 (27.4)	61 (28.9)	0.71
Mould on bedroom wall, N° (%)	20 (7.6)	13 (6.2)	0.54
Living at <100 m from a busy road, N° (%)	49 (18.6)	44 (20.9)	0.54
Exposure to pets since birth, N (%)	25 (9.5)	27 (12.8)	0.25
Attendance at indoor chlorinated pool			
Ever, N (%)	223 (56.0)	175 (82.9)	0.16
CPA, median (IQR), h	302 (158–549)	271 (121–526)	0.21
Attendance at outdoor residential chlorinated pool			
Ever, N (%)	47 (17.9)	41(19.4)	0.69
Median (IQR), h	192 (81–450)	192 (54–640)	0.78
Attendance at outdoor chlorinated pool during holidays			
Ever, N (%)	133 (50.6)	112 (53.1)	0.13
Median (IQR), h	126 (45–280)	186 (65–385)	0.22
Total serum IgE, median (IQR), kIU/L	43.1 (15.2–155)	34.0 (15.4–98.7)	<0.001
Sensitization to aeroallergens At least one aeroallergen, N° (%)	77 (29.3)	84 (39.8)	0.016
House dust mite, N° (%)	55 (20.9)	68 (32.2)	0.005
High sensitization, N° (%)	22 (8.4)	31(14.7)	0.03
Low sensitization, N° (%)	33 (12.5)	37 (17.5)	0.13
Cat, N° (%)	23 (8.8)	25 (11.8)	0.27
Pollen, N° (%)	33 (12.5)	40 (19.0)	0.055
Ever diagnosed respiratory diseases			
Asthma, N° (%)	15 (5.7)	17 (8.1)	0.31
Hay fever, N° (%)	25 (9.5)	17 (8.1)	0.58
Allergic rhinitis, N° (%)	28 (10.6)	19 (9.0)	0.55
Bronchitis, N° (%)	110 (41.8)	81 (38.6)	0.47
Bronchiolitis, N° (%)	39 (14.8)	28 (13.3)	0.64
Sinusitis, N° (%)	48 (18.3)	38 (18.1)	0.97
Cold, N° (%)	75 (28.5)	50 (23.8)	0.25

Abbreviations: CPA, cumulative pool attendance; IQR, interquartile range; SD, standard deviation.

Table 2. Biomarkers in the NALF of girls and boys.

Parameters	Boys ($n=211$)	Girls ($n=263$)	p
Urea (mg/L)			
Crude	43.5 (33.0–59.9)	40.0 (31.1–51.0)	0.03
NALF/plasma urea ratio	0.18 (0.13–0.23)	0.17 (0.13–0.23)	0.94
CC16 (µg/L)			
Crude	24.5 (8.80–67.7)	18.7 (7.50–70.4)	0.33
Adjusted for the NALF/plasma urea ratio	149 (59.9–327)	121.9 (46.0–370)	0.30
Albumin (mg/L)			
Crude	10.5 (6.4–19.8)	6.90 (3.61–14.4)	<0.001
Adjusted for the NALF/plasma urea ratio	57.2 (35.8–107)	41.0 (22.8–81.5)	<0.001
CC16/albumin ratio ($\times 10^4$)	25.3 (9.01–77.7)	32.9 (10.6–98.7)	0.08

Concentrations are expressed as median with interquartile range.
Abbreviations: CC16, Clara cell protein; NALF, nasal lavage fluid.

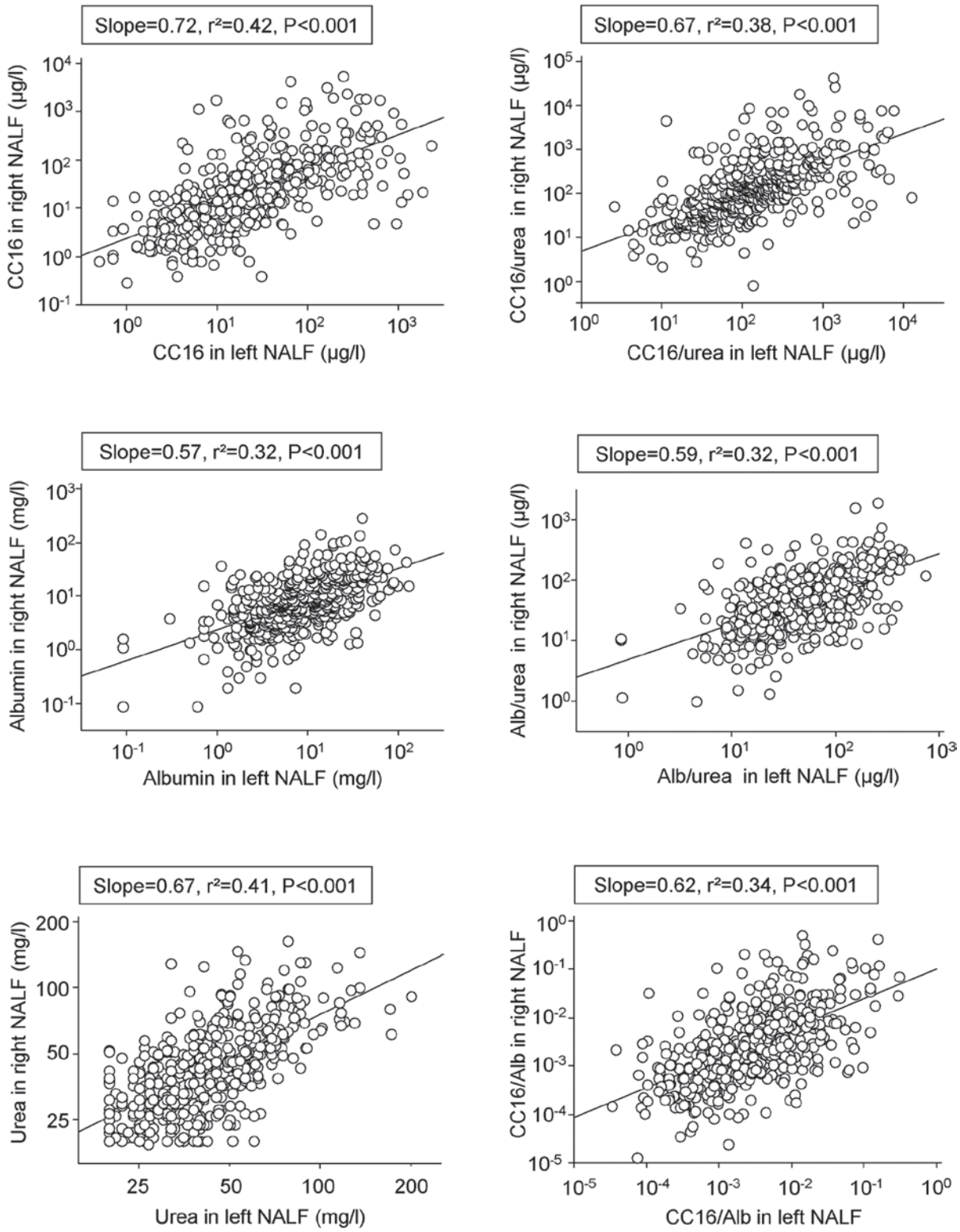

Figure 1. Correlations between biomarkers levels in the nasal lavage fluid (NALF) from the right and left nostril. CC16, Clara cell protein; Alb, albumin.

risk of sensitization to HDM, especially when total serum IgE was >30 kIU/L. Among boys with higher total serum IgE, those in the lowest tertile of the CC16/albumin ratio in NALF were three times more likely to be sensitized against HDM than those in the highest tertile. There was also a tendency for boys in the lowest tertile of the NALF CC16/albumin ratio to be more frequently sensitized to pollen, an association that again emerged only when total serum IgE > 30 kIU/L. Such associations were not seen in girls, even among those with higher serum IgE.

Table 3. Factors associated with the concentrations of biomarkers in NALF of boys and girls.

	Boys			Girls		
Dependent variable	Independent variable	Regression coefficient (95% CI)	*p*	Independent variable	Regression coefficient (95% CI)	*p*
Urea (mg/L)	Allergic rhinitis	−0.10 (−0.18 to −0.02)	0.02			
	Birth weight	−0.41 (−0.72 to −0.09)	0.01			
NALF/plasma urea ratio	Number of older siblings	−0.02 (−0.05 to −0.002)	0.048	Residential outdoor pool (CPA > 500 h)	0.13 (0.02–0.24)	0.02
CC16 (µg/L)	Age	−0.16 (−0.25 to −0.06)	0.002	Parental smoking	−0.22 (−0.40 to −0.04)	0.01
	Parental smoking	0.19 (0.004 to 0.38)	0.049	Living <100 m from a busy road	−0.22 (−0.44 to −0.005)	0.045
CC16 adjusted for the NALF/plasma urea ratio	Parental smoking	0.18 (0.01 to 0.35)	0.042	Living <100 m from a busy road	−0.21 (−0.41 to −0.01)	0.04
	Age	−0.16 (−0.25 to −0.07)	0.001	Parental smoking	−0.25 (−0.40 to −0.07)	0.004
				Residential outdoor pool (CPA > 500 h)	−0.43 (−0.83 to −0.02)	0.04
Albumin (mg/L)	Number of older siblings	−0.07 (−0.12 to −0.03)	0.004			
	BMI	−0.91 (−1.80 to −0.02)	0.046			
	Living <100 m from a busy road	−0.15 (−0.27 to −0.02)	0.03			
	Age	−0.06 (−0.12 to −0.01)	0.03			
	Mold on bedroom walls	−0.23 (−0.44 to −0.02)	0.03			
Albumin adjusted for the NALF/plasma urea ratio	Number of older siblings	−0.07 (−0.11 to −0.02)	0.008			
	BMI	−1.5 (−0.28 to −0.03)	0.02			
	Living <100 m from a busy road	−0.13 (−2.35 to −0.65)	0.001			
	Public indoor pool (CPA > 500 h)	0.13 (0.01–0.25)	0.04			
CC16/albumin ratio	Public indoor pool (CPA > 500 h)	−0.42 (−0.68 to −0.17)	0.001			
	Public indoor pool (CPA 100–500 h)	−0.24 (−0.45 to −0.03)	0.02			
	Parental smoking	0.26 (0.06 to 0.45)	0.01			

All biological parameters were normalized by logarithmic transformation.
Abbreviations: BMI, body mass index; CC16, Clara cell protein; CI, confidence interval; CPA, cumulative pool attendance; NALF, nasal lavage fluid.

Table 4. Risks of sensitization to house dust mite or pollen with decreasing CC16/albumin ratio in the NALF of girls and boys with low or high total serum IgE.

			Boys			Girls		
Sensitization	CC16/albumin ratio	All ($n=474$) OR (95% CI)[a]	All ($n=211$) OR (95% CI)[e]	<30 kIU/L ($n=63$) OR (95% CI)	>30 kIU/L ($n=148$) OR (95% CI)[e]	All ($n=263$) OR (95% CI)[b]	<30 kIU/L ($n=129$) OR (95% CI)[c]	>30 kIU/L ($n=134$) OR (95% CI)[d]
Dermatophagoïdes	Continuous	1.34 (0.97–1.87)	1.86 (1.14–3.03)		1.84 (1.12–3.01)	1.10 (0.69–1.76)	0.88 (0.29–2.63)	1.16 (0.67–2.02)
	Tertile 1	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)
	Tertile 2	1.17 (0.66)	1.82 (0.77)		1.64 (0.65)	1.34 (0.62)	1.26 (0.21)	1.15 (0.47)
	Tertile 3	1.67 (0.96)	2.54 (1.11)		2.83 (1.14)	1.12 (0.50)	0.76 (0.13)	1.48 (0.54)
p for trend		0.03	0.02	0.58	0.02	0.51	1.00	0.17
Pollen	Continuous	1.29 (0.87–1.92)	1.46 (0.85–2.54)	1.01 (0.12–8.42)	1.54 (0.85–2.78)	1.20 (0.67–2.15)	0.75 (0.16–3.64)	1.40 (0.73–2.69)
	Tertile 1	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)
	Tertile 2	1.48 (0.75)	1.16 (0.45)		0.81 (0.27)	1.49 (0.59)	0.67 (0.05)	1.55 (0.56)
	Tertile 3	1.37 (0.69)	2.00 (0.84)		2.00 (0.75)	0.97 (0.35)	0.50 (0.03)	1.28 (0.40)
p for trend		0.21	0.095	0.89	0.07	0.79	0.54	0.32

The continuous models are based on log transformed data.
[a]Adjusted for BMI, pets, parental asthma, and gender.
[b]Adjusted for parental asthma, breastfeeding, pets, and day-care attendance.
[c]Adjusted for house cleaning with bleach and mould on bedroom walls.
[d]Adjusted for pets and breastfeeding.
[e]Adjusted for BMI, pets, and living <100 m from a busy road.
[f]Adjusted for pets and gender.
[g]Adjusted for breastfeeding, pets, day-care attendance, and living <100 m from a busy road.
[h]Adjusted for parental asthma, breastfeeding, and day-care attendance.
[i]Adjusted for pets.
[j]Adjusted for pets and day-care attendance.

Discussion

Our study shows that concentrations of CC16 and albumin in the NALF greatly vary despite adjustment for the variable dilution of the recovered ELF on the basis of the NALF/plasma urea ratio. Variations were just important as for the CC16/albumin ratio, which theoretically should be independent of the dilution of ELF. There were also some variations between the two nostrils, the protein concentrations in the right nostril being on average about 30% lower than in the left one. We think that this systematic difference stems from the fact that the left nostril was in most cases the second one sampled with presumably a better ELF recovery because of some training effect or of a carry-over of proteins from the first nostril. The adjustment made for the NALF/plasma urea ratio ensured a complete elimination of the variations due to the variable recovery of ELF proteins. Variations in the levels of albumin and CC16 in NALF observed under these conditions can thus be assumed to reflect true variations in the permeability (albumin) or the cellular integrity (CC16) of the nasal epithelium.

One of the most prominent observations in this study is the inverse association found between the CC16/albumin ratio in the NALF and the risk of having aeroallergen-specific serum IgE, in particular, IgE specific of HMD. The fact that this association emerges only in boys is interesting from a mechanistic point of view. This suggests indeed that the association between the NALF CC16/albumin ratio and the risk of allergic sensitization is driven by interactions with some gender-related risk factors. Clearly, one interacting factor appears to be the atopic status as assessed on the basis of the total serum IgE level, which was significantly higher in boys than in girls (by 25% on average). Total serum IgE strongly influences the association between the CC16/albumin ratio in NALF and the risk of allergic sensitization, which was increased only among boys with serum IgE > 30 kIU/L. Another source of interaction appears to be related to the response of nasal epithelium to some environmental stressors. The CC16/albumin ratio in NALF of boys was indeed significantly lower than that of girls, a difference that might be linked to the exposure to chlorinated pools. While no predictor could be identified for the CC16/albumin ratio in the NALF of girls, the attendance of public chlorinated pools emerged as a very significant predictor associated with a dose-dependent decrease of the CC16/albumin ratio in the NALF of boys.

Predictors of CC16 and albumin levels in NALF were mostly factors that have been associated with the risk of respiratory diseases such as age, gender, ETS, BMI, traffic-related air pollution, or attendance at chlorinated swimming pools (Bernard et al. 2008, 2009). Associations found between these predictors and the NALF biomarkers were, however, not necessarily pointing to the same direction as those reported with respiratory diseases, and in some cases, they were totally inconsistent between sexes. While in girls parental smoking was associated with a decrease of NALF CC16, suggesting some epithelial damage, opposite associations were seen in boys exposed to ETS who had higher NALF levels of CC16 and of the CC16/albumin ratio. The same inconsistency was observed with the vicinity of a busy road. In girls, this factor was associated with a decrease of NALF CC16, suggesting an impaired CC16 secretion, while in boys, it was associated with a decrease of NALF albumin, which on the contrary points to a more efficient barrier function. These inconsistencies might reflect gender-related differences in the response of the nasal epithelium to ETS or to the ambient air pollution. Women for instance are known to be more vulnerable to the harmful effects of tobacco smoke than men when being actively or passively exposed to tobacco smoke (Dransfield et al. 2006, Li et al. 2000, Chen et al. 2005). These opposite associations might also merely reflect the influence of some unidentified confounders or a distortion of our analyses by the lack of quantitative exposure data. The apparently protective effects of BMI and number of siblings on the permeability of nasal epithelium to albumin observed in boys might also arise from confounding.

Actually, the predictor that was most consistently associated with nasal epithelium alterations in both sexes was the attendance of chlorinated swimming pools. Changes observed with CC16 (decrease) or albumin (increase) observed in boys and girls were all suggestive of functional or structural alterations of the nasal epithelium. Attendance at chlorinated pools was also the only predictor to display a significant dose-response relationship, in particular with the CC16/albumin ratio. There were yet still some inconsistencies between sexes as NALF biomarkers were associated in boys with indoor public pools and in girls with outdoor chlorinated pools. We think that these inconsistencies might reflect gender-related differences in the exposure patterns or in the deposition of chlorination products in the nasal cavity. Because of their higher activity, boys might behave differently from girls when attending indoor or outdoor pools. For instance, it is possible that boys remain less time in the water of residential outdoor pools than girls and consequently are less exposed to the chlorine-based irritants building up at the surface of the pool. By contrast, in indoor public pools, where it is really possible to develop own swimming capacity, boys might train more intensively than girls and thus be more exposed to chlorination products. These gender-related differences in the response of the nasal epithelium to chlorinated pools might also be linked to differences in the anatomic features of the nasal cavity. Women are indeed known to have smaller nasal dimensions than men, which may favor the deposition of aerosolized and gaseous chlorination products in the nasal cavity. The existence of gender difference in response to chlorinated pools is supported by a recent study among teenagers, showing that attendance at outdoor

pools increases the risk of asthma in girls but not in boys (Siegel et al. 2010).

In agreement with previous studies based on serum CC16 and surfactant-associated proteins (Carbonnelle et al. 2002, Bernard et al. 2003, Lagerkvist et al. 2004, Bernard et al. 2007), our data provide further evidence that chlorination products can affect the cellular integrity and permeability of airways epithelium. A decrease of CC16 as found in girls having attended outdoor pools can be interpreted only as the consequence of a loss of CC16-secreting cells in the nasal cavity since the urea corrected concentrations of CC16 in NALF are approximately 10 times higher than the serum concentrations of CC16. The concentration of plasma-derived albumin in NALF is commonly used as an indicator of the nasal epithelium permeability (Douwes et al. 2000, Mochca-Morales 2000, Proud et al. 2010, Pupek et al. 2003). The higher concentrations of albumin in the NALF of boys who regularly visited indoor chlorinated pools might thus be the reflection of an increased epithelial permeability due to the disruption of tight junction by hypochlorous acid and chloramines.

While no association was found with the concentrations of CC16 and albumin in NALF when tested separately, in the NALF of boys, the ratio between the proteins correlated with an increased risk of sensitization to aeroallergens. This finding suggests that changes of the nasal epithelium facilitating atopic sensitization might consist in an increased epithelial permeability associated to a decreased secretion of proteins with anti-inflammatory properties such as CC16. In other terms, this suggests that only stressors or combinations of stressors capable of causing such epithelial defects are implicated in the development of allergic sensitization. Our study points to chlorination products as important drivers of this type of epithelial defect, but of course, other stressors such as dry air, ambient air pollutants, or infectious diseases might also contribute to these epithelial changes (Simoni et al. 2010, Walinder et al. 2000).

This study has some limitations. The first limitation is the relatively small size of the subpopulations obtained after stratification for sex, which has reduced the statistical power of some analyses. The initial cohort included more than 800 subjects, but we lost about half of them after excluding subjects with signs of rhinitis or who were under medication for allergic diseases at the time of examination. The second limitation is the lack of exposure levels data, in particular for environmental risk factors. It was of course impossible to perform air measurements for assessing the individual exposure to ETS or to chlorination products and *a fortiori* to obtain such data for the past exposure when adolescents were infants or children.

Conclusion

In conclusion, this study shows that the concentrations CC16 and albumin in NALF might serve as noninvasive biomarkers to detect nasal epithelium damage caused by air pollutants and other stressors. In our adolescents, defects in nasal epithelium detected by the CC16/albumin ratio appear to be mostly caused by the attendance of chlorinated pools. In boys, these effects were associated with an increased risk of IgE sensitization to HDM.

Conception and design

AB, AS, IAM: Examination of adolescents and biological analyses; AS, MN, CV, MN, XD, AB: Data analysis and interpretation; AS, AB: Drafting the manuscript; AS, AB. AB is the guarantor of the paper and takes responsibility for the integrity of the work as a whole, from inception to published article.

Declaration of interest

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