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Matrix metalloproteinases, IL-8 and glutathione in the prognosis of workers exposed to chlorine

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Abstract

Background: Workers exposed to chlorine may be at risk of deterioration in FEV1.
Methods: A prospective study of 72 workers examined over a 5.8 ± 1.9 year period. A sample of induced sputum for cells and mediators was obtained in 69 subjects at baseline (Vb) and in 36 both at Vb and at follow-up (Vf).
Results: Sixty-four workers (89%) experienced at least one accidental inhalation of chlorine in the interval. The mean decrease in FEV1 was 30 ml/year and thus was within normal limits. Among the analysed remodelling markers, the level of the MMP-9-TIMP-1 complex, but not of free MMP-9 and TIMP-1, significantly diminished from Vb to Vf. We found significant correlations between neutrophils, IL-8, MMP-9 and MMP9-TIMP-1 complex at Vb and Vf. While levels of total glutathione, IL-8, MMP9, TIMP-1 and MMP9-TIMP-1 complex were highly correlated with each other at Vb, this was inconstant at Vf. Levels of MMP9-TIMP1 complex and of TIMP1 at Vf were significantly lower in workers reporting chlorine puffs with mild acute respiratory symptoms between visits compared to those who had no, or asymptomatic inhalations ($P = 0.03$ and 0.02, respectively). The fall in FEV1 from Vb to Vf was significantly correlated with levels of glutathione at Vb. Cough between visits was associated with a decrease in FEV1 ($P = 0.06$).
Conclusion: Although no accelerated loss in FEV1 was documented in these workers exposed to chlorine, subjects with a greater fall in FEV1 were more likely to report cough and have higher levels of total glutathione at Vb.

Workers exposed to a potentially irritant material such as chlorine, either chronically or acutely at higher concentrations causing irritant-induced asthma or reactive airways dysfunction syndrome (RADS) (1), may be at risk of developing permanent airway obstruction and hyperresponsiveness that are related to airway remodelling. RADS is a type of occupational asthma (OA) without a latency period (2). Airway remodelling with intense fibrosis of the bronchial wall is a main feature that results of the inhalational accident (1). The proposed mechanism of RADS is related to an acute insult that causes important damage to the epithelium, which is a feature of this condition (3, 4), with oxidative stress reflected by a dose-dependent effect on nitrite/nitrate levels in lung lavage obtained from mice exposed to chlorine (4).

Matrix metalloproteinases (MMP), their inhibitors (TIMP), cytokines and glutathione are markers used to measure airway remodelling (5). Gueders et al. (6) as well as Demedts et al. (7) have recently reviewed the implications of MMPs and their inhibitors in asthma and chronic obstructive pulmonary disease (COPD). Neutrophils that play a role in asthma produce MMP9 that is increased in asthma (8). An imbalance between MMP9 and TIMP-1 is associated with a thickening of the airway wall (9). Because an oxidative stress is present in this condition (4), it is likely that glutathione levels might also be affected.

In a prospective study of workers exposed to chlorine over 6 years, we aimed at examining the levels of MMPs, interleukin-8 (IL-8), myeloperoxidase (MPO) and total glutathione in induced sputum, both at baseline (most often at the beginning of employment) and at follow-up visits, in relation to acute symptomatic chlorine inhalation events and changes in FEV1 over time. It was hypothesized that these workers may

be at risk of accelerated changes in FEV1 and that changes in FEV1 may be associated with changes in markers of oxidative stress and remodelling.

Subjects and methods

This prospective study was undertaken in an industry where workers were chronically exposed to chlorine as well as being at risk of acute inhalational accidents of chlorine, generated at higher concentrations. Between 1995 and 2004, 115 newly hired employees were recruited in the study; an assessment of induced sputum was proposed to all recruited workers. Of these, 69/115 produced an interpretable sputum sample. Of the initial 115 newly hired employees, 72 (63%) underwent at least one follow-up visit at the time they were still at work; a satisfactory sputum sample was obtained in 36 of these 72 participants. The first sputum induction was performed as closely as possible to the time workers who were hired by the company; the mean (SD) time was 18.6 (±1.9) months. The second induction took place 5.8 ± 1.9 years after the initial assessment. At each assessment, a standardized respiratory questionnaire was administered (10, 11). The data concerning chlorine exposure was obtained prospectively during the period between visits. Information was also obtained on the number and intensity of accidental chlorine inhalations (referred to as 'puffs') with and without accompanied nasal (acute burning) and respiratory symptoms. Information on possible visits to the first aid clinic during the study period was also obtained.

Spirometry was assessed (12) as well as responsiveness to inhaled methacholine up to a maximum concentration of 32 mg/ml using the Wright nebulizer at tidal volume breathing (output = 0.14 ml/min) (13, 14). Induced sputum was assessed using a standardized procedure (15). Supernatants of induced sputum samples were kept at −80°C for the assessments of inflammatory, oxidative and remodelling markers. Sputum (nonconcentrated) samples were electrophorized in nondenaturated conditions on Novex 10% Zymogram (Gelatin) Gel (Invitrogen, Mississauga, ON, Canada), and the gelatinolytic activity associated to pro-MMP-9, pro-MMP-2 and their respective active and complex forms (e.g. MMP-9-TIMP-1 complex) were revealed using a zymography kit (Invitrogen) according to the manufacturer's instructions.

For the quantification of markers, sputum samples were concentrated by centrifugation using Centricon centrifugal filters (YM-3, Millipore; FisherScientific, Montreal, PQ, Canada) with a 3000 molecular weight cut-off as previously described (Maghni, 2004). IL-8 (BD Biosciences, Mississauga, ON, Canada), MPO (Immunology Consultants Laboratory Inc., Newberg, OR, USA), MMP-9 (Raybiotech Inc., Norcross, GA, USA), TIMP-1 (EMD-Calbiochem, San Diego, CA, USA) and MMP-9-TIMP-1 complex (R&D, Minneapolis, MN, USA) levels in concentrated sputum samples were measured using commercially available ELISA kits according to the manufacturer's instructions. There is no cross-reactivity for MMP-9-TIMP-1 complex detection with free MMP-9 and free TIMP-1. The total glutathione activity (both GSH and its oxidized disulfide dimer GSSG) was determined in sputum samples using the glutathione assay kit (CaymanChemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. The optical densities were determined using the ELISA reader Elx 808iu (Bio-Tek Instruments Inc., Richmond, VA, USA), and calculations were performed using the KC4 software (Bio-Tek Instruments Inc.). Data were adjusted for the factor of concentration and expressed as pg/ml or ng/ml (16). Assays for inflammatory and remodelling mediators have also already been used and validated in our laboratory. Intra-assays coefficients of variation for the different assays used in this study were between 5.6 and 8.7%.

Analysis of results

The parameters of bronchial responsiveness were a PC20 ≤ 16 mg/ml defining significant airway hyperresponsiveness (17) and a PC20 ≤ 32 mg/ml defining measurable airway responsiveness and the slope of the dose–response curve (DRS) expressed by the percentage decline in FEV1/final cumulative dose of methacholine (13). This ratio was log transformed; higher positive values correspond to a more pronounce bronchial responsiveness. An increase in bronchial responsiveness was defined either as a 2-fold or as a 3.2-fold decrease in PC20 from the first to last assessment (18).

The results from the spirometric and bronchial challenge tests were compared between the follow-up's first and last visits using paired *t*-test or chi-squared test for continuous and categorical variables, respectively. The change in cell differentials and mediators was assessed through the Wilcoxon Signed Rank tests. The correlation between markers of inflammation levels within and between visits was assessed by the Pearson correlation analyses after the log transformation of individual results. Similarly, correlations were assessed between levels of inflammatory, oxidative and remodelling markers and changes in FEV1. The levels of markers were compared between subjects according to reported acute symptomatic exposure to chlorine by nonparametric tests (Wilcoxon Signed Rank tests).

Results

The mean age of the participants was 30.3 ± 6.4 years and all were men. Table 1 presents participants' baseline characteristics and the reported chlorine exposure because first being employed. A minority of subjects had asthma at the time of employment, though 25% had symptoms of rhinitis. The majority of subjects were nonsmokers and very few showed bronchial hyperresponsiveness. More than 50% of workers experienced inhalational accidents. Table 2 presents information pertaining to the functional characteristics of workers at Vb and Vf. The FEV1 decline was minimal, 32 subjects (44%) showing changes >40 ml per year of follow-up. Although a two-fold or greater decrease in PC20 occurred in seven subjects, the percentage of workers with a PC20 value ≤ 16 mg/ml diminished from Vb to Vf. Changes in FEV1 were not significantly different in those who experienced puffs of chlorine (−4.57 ± 9.16% and −32.4 ± 81.18 ml/year, $n = 64$)

Table 1 Baseline characteristics and reported chlorine exposure since first employed for the 72 participants in the prospective study

	Study subjects $N = 72$
Time interval between assessments, years (mean ± SD)	5.8 ± 1.9
Age, years (mean ± SD)	30.3 ± 6.4
Asthma*	4 (5.6)
Wheezing	4 (5.6)
Symptoms of rhinitis	18 (25)
Smoking, S/E × S/NS	14/14/44
Atopy	32 (45.1)
FEV1, % pred. (mean ± SD)	97.6 ± 11.8
FEV1, % pred. <80% of predicted	4 (5.6)
FEV1/FVC % (mean ± SD)	85.8 ± 6.3
FEV1/FVC % <85% of predicted	2 (2.8)
PC20 ≤ 16 mg/ml	5 (6.9)
PC20 (log) (mean ± SD)	2.25 ± 0.22
PC20 ≤ 32 mg/ml	11 (15.3)
PC20 (log) (mean ± SD)	2.69 ± 0.16
Puffs with mild symptoms	35 (48.6)
Puffs with significant symptoms	12 (16.7)

Results presented as *n* (%) unless otherwise stated.
*Reported diagnosed by physician.

when compared with the others (−1.50 ± 11.4% and −25.60 ± 150.53 ml/year, $n = 8$). There were also no significant differences in the changes in FEV1 according to the importance of respiratory symptoms categorized as mild or important. However, subjects hired before year 2000 were more likely than subjects hired after to have changes in FEV1 beyond 12% ($P = 0.002$) or changes in PC20 beyond a two-fold difference (18/24 subjects or 75% *vs* 14/48 subjects or 29%, $P < 0.001$). The mean percentage change in FEV1 was −9.8% (±0.06) and −7.5% (±1.4) for workers hired before 2000 and those hired after, respectively; this difference was not significant. However, the mean annual decline in FEV1 was −67.4 ml (±40.6) and 3.1 ml (±111.4) ($P = 0.03$) for these two subgroups, respectively.

The subjects with one or two adequate sputum specimens did not differ from the others in terms of their baseline FEV1/FVC% predicted ratio at baseline (103.5%, 105.3% and 101.8%, respectively, $P = 0.11$); FEV1% predicted at the first visit was the same in those who produced one or no adequate sputum sample (98.6% and 98.3%) while it was greater in those with sputum samples at two visits (107.4%, $P = 0.038$). In addition, the proportion of workers with significant bronchial hyperresponsiveness did not differ significantly between subjects whether or not sputum specimens were available (11.6%, 5.7% and 16.2% for one, two or no specimens, respectively). Therefore, using the available sputum specimens did not introduce a bias in favour of subjects with lower lung function parameters.

A zymograms analysis indicated that the gelatinolytic activity was mainly associated to pro-MMP-9 (gelatinase-B) and that the active form of MMP-9 was detected in around 40% of the subjects, but at markedly lower levels than pro-MMP-9 (Fig. 1). The MMP-9-TIMP-1 complex was also detected in sputum samples. Interestingly, pro-MPP-2 (gelatinase-A; 72 kDa), the second member of the gelatinase family, or its active form (expected at 66 kDa) was never detected in the sputum of workers exposed to chlorine. Because of differences in levels of pro-MMP-9, TIMP-1 and its complex MMP-9-TIMP-1 among subjects (Fig. 1), sputum supernatants were concentrated for quantification by ELISA. Table 3 illustrates the relationships between levels of markers at baseline and follow-up assessments and provides results of the correlation analyses. Levels were generally more closely related at the baseline visit. Whereas levels of glutathione were not significantly related to concentrations of IL-8 and MMP-9 at Vb, this was the case at Vf. There were generally good agreements between results for each parameter obtained at Vb and Vf (Table 4). Interestingly, there were no significant relationships between MPO and the other markers.

Levels of cells and mediators in induced sputum were not significantly different at baseline and follow-up visits (Table 5) except for the MMP-9-TIMP-1 complex, which diminished significantly from Vb to Vf. There were 17 subjects who changed their FEV1 by 12% (≥2 SD of the mean

Table 2 Functional characteristics of workers at baseline and follow-up assessments and their changes between visits ($n = 72$)

	Baseline	Follow-up	Changes
FEV1 (L)	4.14 ± 0.58	3.95 ± 0.60	−4.23% ± 9.40 −31.6 ml/year ± 92.3
FEV1 (%, pred)	97.57 ± 11.86	96.94 ± 13.12	−0.63 ± 9.27
FEV1/FVC	85.84 ± 6.32	83.37 ± 6.54	−2.47 ± 6.76
PC20 baseline/PC20 follow-up			7 (9.7)
≥ 2, *n* (%)			6 (8.3)
≥ 3.2, *n* (%)			
PC20 ≤ 16 mg/ml, *n* (%)	5 (6.9%)	4 (5.6%)	1 persistent at ≤16 mg/ml; 3 decreased from >16 to ≤16 mg/ml
Log dose–response slope	−0.63 ± 0.24	−0.65 ± 0.34	17.2% ± 140.8

Results expressed as means ± SD or as number (*n*) and %; dose–response slope: slope of the dose–response curve expressed as the percentage decline in FEV1/dose of last cumulative methacholine administered.

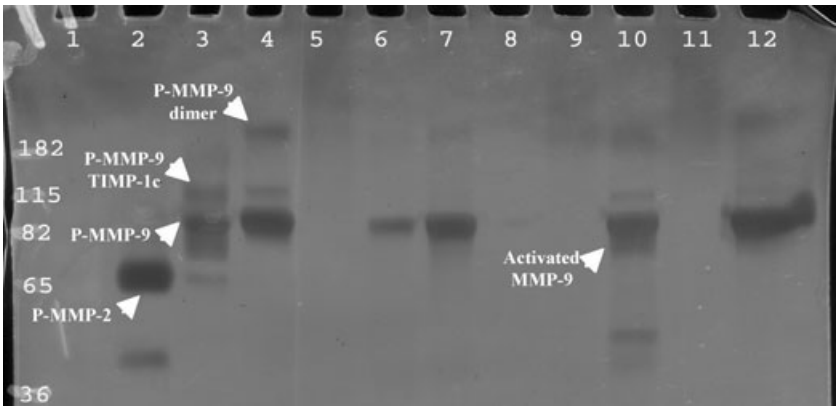

Figure 1 Gelatinolytic activities analysis. Representative illustration of gelatine zymogram of nonconcentrated sputum supernatants from nine subjects. 1: Protein molecular ladder (kDa indicated by numbers). Positive controls 2: Human purified MMP-2 and 3: Human purified MMP-9. 4 to 12: gelatinolytic activity in nonconcentrated sputum samples. P-MMP-9: pro-MMP-9, P-MMP-2: Pro-MMP-2, P-MMP-9 TIMP-1c: MMP-9-TIMP-1 complex, P-MMP-9 dimer: Pro-MMP-9 dimer.

changes) or more and seven who increased their bronchial responsiveness to methacholine by a ≥2-fold difference in PC20. In these 24 subjects by comparison with 48 without these features, asthma diagnosed by a physician at baseline as well as wheezing and rhinitis at baseline was more frequent, although of borderline significance. A lower FEV1/FVC ratio at baseline (98.2% pred *vs* 105.0% pred, $P < 0.001$) was a significant predictor of enhanced functional changes at Vf. Changes in FEV1 tended to be more pronounced in those with a higher MMP-9 at Vb ($r = -0.33$, $P = 0.08$). Interestingly, the fall in FEV1 from Vb to Vf was significantly correlated with levels of glutathione at Vb: higher values of glutathione at Vb were associated with a greater fall in FEV1 during the follow-up period (Fig. 2); the correlation reached statistical significance ($P = 0.02$).

Twenty-one workers reported cough in the interval between the two visits; one worker reported breathlessness. The percentage decline in FEV1 (percent predicted) was greater among workers reporting cough between visits ($n = 21/72$) (mean ± SD) i.e., −11,7% ±7.4 compared to −7.8% ±11.8 in those not reporting cough ($P = 0.06$).

We also investigated the association between acute symptomatic chlorine inhalation events and levels of markers of inflammation in induced sputum. We found associations between symptomatic inhalation of chlorine puffs and markers of inflammation at Vf among workers hired after 2000, but not among those hired earlier. The level of the MMP-9-TIMP-1 complex was lower in those reporting chlorine puffs with mild acute symptoms between the two visits ($n = 9$) compared to those reporting asymptomatic inhalations or no inhalation ($n = 25$) (median (inter quartile range)): 0.24 ng/ml (0.33) and 0.66 ng/ml (1.26), $P = 0.03$, respectively; similarly, levels of TIMP-1 were lower in workers reporting symptomatic inhalations (1.5 ng/ml (2.4) *vs* 6.24 ng/ml (6.56), $P = 0.02$). Levels of the MMP-9-TIMP-1 complex were also significantly lower ($P = 0.006$) in workers reporting important acute symptomatic inhalations between Vf and Vb ($n = 5$) with respect to the same comparison group.

The levels of neutrophils, Il-8, MMP-9 and of the complex MMP-9-TIMP1 at the first visit did not differ according to the smoking status at baseline. The number of chlorine puffs reported, with or without symptoms, did not differ according to the smoking status. The decline in lung function was not significantly greater in smokers compared to nonsmokers.

Discussion

In this prospective study of workers exposed to chlorine, we examined the biological markers of airway remodelling in induced sputum. We found (i) satisfactory correlations between several markers of airway inflammation and remodelling at baseline and follow-up visits and from baseline to follow-up; (ii) a significant diminution in the MMP-9-TIMP-1 complex whereas there were no significant changes in the other markers; and (iii) a significant correlation between changes in FEV1 and baseline levels of glutathione. To the best of our knowledge, our study is the first to assess markers of airway inflammation and remodelling in subjects exposed to an irritant product, chlorine. A recent publication has found that cytokine production profile was not associated with the development of allergic sensitization to laboratory animals whereas atopy and total IgE levels were (19).

Workers included in our study were both exposed chronically to chlorine and at risk of acute inhalation accidents. In two previous studies completed in workers of the same plant, we found that changes in airway calibre and responsiveness were related to the number and severity of inhalational accidents to chlorine (19, 20). Such associations were not found in the current study; however, we found an association of borderline statistical significance ($P = 0.06$) between decline in FEV1 and reporting cough between visits. Mean changes in FEV1 with time correspond to the usual decline of spirometric values with time; for example, ∼40 ml/year. Moreover, only six subjects increased their airway responsiveness by a value of ≥3.2-fold PC20, and there was even a reduction in the number of workers with a PC20 level ≤16 mg/ml.

Table 3 Correlations between markers of inflammation and remodelling at each of the two visits*

	Neutrophils	IL-8	MPO	MMP-9	TIMP-1	MMP-9-TIMP-1 Complex	Glutathione μM
Baseline visit							
Neutrophils							
r-value		0.47*	−0.12	0.51***	0.35*	0.40**	−0.36*
P		0.001	0.43	0.000	0.015	0.004	0.01
n		48	45	51	46	51	50
IL-8							
r-value			0.40**	0.79***	0.56***	0.46**	−0.18
P			0.006	0.000	0.000	0.001	0.22
n			45	52	46	52	51
MPO							
r-value				0.40**	0.47**	0.30*	−0.22
P				0.005	0.002	0.04	0.14
n				48	43	48	47
MMP-9							
r-value					0.58***	0.54***	−0.21
P					0.000	0.000	0.14
n					49	55	54
TIMP-1							
r-value						0.61***	−0.55***
P						0.000	0.000
n						49	34
MMP-9- TIMP-1 complex							
							−0.39**
							0.003
							54
Follow-up visit							
Neutrophils							
r-value		0.54**	0.12	0.58***	0.32	0.05	−0.62
P		0.001	0.55	0.000	0.08	0.78	0.000
n		35	26	36	31	36	36
IL-8							
r-value			0.08	0.64***	0.58**	0.28	−0.37*
P			0.69	0.000	0.001	0.103	0.03
n			26	36	31	36	36
MPO							
r-value				0.302	−0.13	0.10	0.15
P				0.13	0.54	0.62	0.45
n				27	25	27	27
MMP-9							
r-value					0.39*	0.15	−0.31•
P					0.03	0.37	0.07
n					32	36	36
TIMP-1							
r-value						0.23	−0.29
P						0.21	0.11
n						32	32
MMP-9- TIMP-1 complex							
							−0.02
							0.93
							36

Values of markers were ln transformed. At baseline, the assessment of cell differentials was performed for 69 subjects and quantification of mediators performed for up to 55 subjects when sufficient supernatant volume was available. At follow-up, the assessment of cell differentials was performed for 36 subjects and quantification of mediators performed for up to 36 subjects if sufficient supernatant volume was available $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$.

Table 4 Correlation between levels of markers of inflammation and remodelling at baseline and follow-up

		Follow-up						
Baseline		Neutrophils	IL-8	MPO	MMP-9	TIMP-1	MMP-9/TIMP-1 complex	Glutathione
Neutrophils	*r*-value	0.51**	0.54*	0.26	0.34*	0.05	−0.01	−0.26
	P	0.002	0.001	0.24	0.05	0.81	0.96	0.15
	n	33	32	23	33	28	33	33
IL-8	*r*-value	0.34*	0.49*	−0.046	0.43*	0.47**	0.04	−0.05
	P	0.05	0.003	0.82	0.01	0.008	0.81	0.7836
	n	35	35	26	36	31	36	36
MPO	*r*-value	−0.17	−0.03	0.17	−0.02	0.21	0.14	0.03
	P	0.37	0.86	0.42	0.90	0.31	0.46	0.86
	n	29	29	26	30	26	30	30
MMP-9	*r*-value	0.35*	.0.42*	−0.20	0.39*	0.34•	−0.02	0.13
	P	0.04	0.01	0.33	0.02	0.06	0.91	0.44
	n	35	35	26	36	31	36	36
TIMP-1	*r*-value	0.40*	0.40*	−0.25	0.18	0.53*	0.36*	−0.16
	P	0.02	0.02	0.22	0.30	0.002	0.03	0.35
	n	35	35	26	36	31	36	36
MMP-9/TIMP-1 Complex	*r*-value	0.45**	0.29•	−0.13	0.33*	0.42*	0.38*	−0.16
	P	0.006	0.09	0.54	0.05	0.02	0.02	0.35
	n	35	35	26	36	31	36	36
Glutathione	*r*-value	−0.63**	−0.19	−0.13	−0.15	−0.16	−0.31•	0.27
	P	<0.001	0.27	0.53	0.39	0.39	0.07	0.11
	n	34	34	26	35	30	35	35

Values of markers were ln transformed.
At follow-up, the assessment of cell differentials was performed for 36 subjects and quantification of mediators performed for up to 36 subjects if sufficient supernatant volume was available $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$.

A follow-up of 6 years may be insufficient to identify significant functional changes in a rather young population (30 years old at baseline). Workers included in the previous studies were those hired when the industry started its activities, at which time exposure to puffs of chlorine were both more frequent and more important. The working conditions improved with time and workers were less at risk of inhalational accidents. However, they were still exposed to chlorine, but in a more stable way and at lower levels. This can explain why subjects hired before year 2000 were more likely than subjects hired after to have changes in FEV1 beyond 12% or changes in PC20 beyond two-fold differences.

Sputum analysis revealed that the cellular inflammation is mainly neutrophilic and that inactive pro-MMP-9 is the major gelatinase expressed. These findings are similar to previous reports on neutrophilic asthma, which differs from eosinophilic asthma wherein sputum levels of active MMP-9 are higher than pro-MMP-9 (21). However, in contrast to allergic asthma and COPD (22), pro-MMP-2 or its active form was not detected in sputum of workers exposed to chlorine. Our findings suggest that the profile of gelatinases expression is specific to the obstructive pulmonary disease and that the detection of MMP-2 in sputum may be a helpful tool in the diagnosis of irritant-induced asthma over other obstructive pulmonary diseases; for example, detection of MMP-9 in the absence of MMP-2.

Interestingly, whereas the number of neutrophils and the levels of MMP-9 and TIMP-1 did not change from Vb to Vf, the MMP-9-TIMP-1 complex significantly diminished. The imbalance between MMP-9 and TIMP-1 has been found and previously described in asthma, but in an inconsistent way (6, 23). In our study, the formation of the MMP-9-TIMP-1 complex was determined instead of the calculation of the ratio MMP-9/TIMP-1 and revealed that most of the MMP-9 and TIMP-1 molecules present in sputum samples remain unbound to each other. The diminution in the formation of the MMP-9-TIMP-1 complex found in our study would favour the deposition of an extracellular matrix as suggested by Vignola et al. (24) and Mautino et al. (25). Airway wall thickening has been found to be related to an imbalance between MMP-9 and TIMP-1, an excess of TIMP-1 promoting airway wall thickening (9).

Whereas levels of neutrophils, MPO, IL-8, MMP-9, TIMP-1 and the MMP-9-TIMP-1 complex were all generally significantly correlated both at Vb and at VF, the most closely related indices in our study being IL8-MMP9 and IL8-TIMP-1. IL-8 being a chemotatic factor for neutrophils, a significant correlation between IL-8 and sputum neutrophilia was expected. Furthermore, correlations between neutrophils, IL-8 and MMP-9 may be explained because IL-8 has been shown to induce the release of MMP-9 by human neutrophils (26). Correlations of these factors with TIMP-1

Table 5 Levels of markers of airway inflammation and remodelling at baseline and follow-up and their changes between visits Complex

Median (IQR)	Baseline	Follow-up	Changes
Eosinophils, %			
N = 36	0 (0.7)	0.3 (0.5)	−0.69 ± 4.8
N = 69	0 (0.5)		ns
Neutrophils, %			
N = 36	19.8 (30.9)	20.8 ± (29.7)	5.0 ± 19.59
N = 69	19.8 (21.4)		0.15
IL-8, ng/ml			
N = 35	1008.1 (1706.2)	618.0 (1787.0)	−314.69 ± 2144.26
N = 52	679.0 (1399.2)		ns
MPO, ng/ml			
N = 26	1.26 (4.87)	2.35 (4.75)	5.48 ± 27.88
N = 48	0.29 (3.29)		ns
MMP-9, ng/ml			
N = 36	97.40 (340.93)	98.31 (201.53)	−22.83 ± 378.24
N = 55	83.7 (298.90)		ns
TIMP-1, ng/ml			
N = 31	5.74 (10.12)	3.96 (7.84)	−7.09 ± 24.40
N = 49	2.58 (9.04))		0.12
MMP-9/TIMP-1 Complex, ng/ml			
N = 36	1.06 (2.09)	0.45 (1.02)	−1.08 ± 2.45
N = 49	0.5 (1.59)		0.007
Glutathione, uM			
N = 35	15.99 (15.87)	14.75 (13.72)	−3.2 (19.48)
N = 54	15.96 (10.59)		ns

At baseline, the assessment of cell differentials was performed for 69 subjects and quantification of mediators performed for up to 55 subjects when sufficient supernatant volume was available. At follow-up, the assessment of cell differentials was performed for 36 subjects and quantification of mediators performed for up to 36 subjects if sufficient supernatant volume was available.
Median with inter quartile range (IQR) values in parenthesis.
ns: no significant differences as assessed by Wilcoxon Signed Rank test.

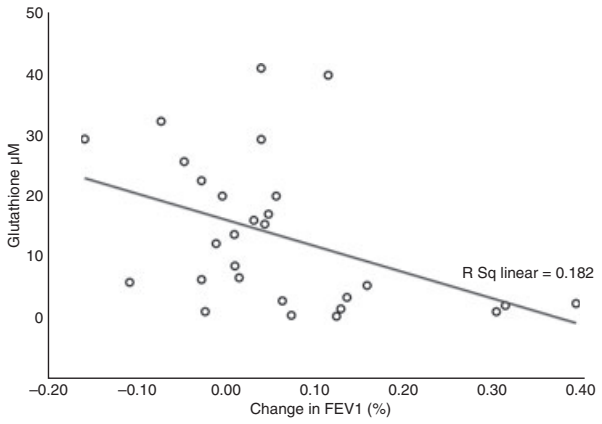

Figure 2 Relationship between changes in FEV1 between Vb and Vf on the abscissa and levels of glutathione at baseline visit.

may be attributed to the increase in TIMP-1 synthesis as a feedback regulating mechanism in response to MMP-9 production.

It was also relevant to examine the correlations of biological markers at Vb and Vf. Except for MPO, all indices were significantly related. Therefore, whereas the environmental exposure may affect some aspects of remodelling, the baseline status, most likely conditioned by a genetic predisposition, is also a significant conditioner of the persistence of airway inflammation and remodelling. We are not aware of prospective studies where the correlations between these indices have been examined at different time intervals, the relative stability of these indices with time suggesting the reliability and relevance of serial assessments. The absence of significant correlations among MPO and the other factors analysed, particularly with neutrophils and IL-8, is not presently understood because IL-8 has been reported to induce the release of MPO (and MMP-9) from human neutrophils (27). Moreover, we had previously reported sputum neutrophilia, IL-8 and MPO correlations in a group of subjects after cessation of exposure to an agent causing occupational asthma (16). The levels of the complex MMP9-TIMP1 and of TIMP1et Vb were significantly lower in workers experiencing acute symptomatic inhalation to chlorine compared to those reporting no or asymptomatic inhalations between Vf and Vb. Although we have not currently an explanation for the decrease in MMP-9-TIMP-1 complex and TIMP-1 synthesis, it is plausible that acute inhalation of chlorine in workers with symptoms results in changes in the epithelium lining fluid composition leading to conditions disfavouring the for-

mation of this complex concomitantly to the reduction in TIMP-1 production.

The main objective of this prospective study was to find a biological marker that can predict changes in airway calibre. We found that baseline levels of MMP-9 were higher in subjects who experienced a more important diminution in FEV1 from Vb to Vf. Workers who experienced more important functional changes were more likely to have asthma diagnosed by a physician at baseline, which can explain higher levels of MMP-9. This hypothesis could not be verified in this study, because among subjects with valid measures of MMP-9 levels, only two workers had reported asthma at baseline. Although a decrease in airway glutathione levels is acknowledged to result in the development and progression of a lung disease (28), contrary to our expectations, we found that baseline levels of total glutathione (GSH + GSSG) were higher at baseline in subjects who experienced a more important diminution in FEV1 from Vb to Vf. We postulate that in these subjects the increased levels of total glutathione at baseline may be the result of a compensatory mechanism in response to a genetic susceptibility to oxidative stress; for example, in response to genetic alterations of other anti-oxidative defence mechanisms. However, the increased levels of total glutathione seem to be inadequate to protect these subjects from developing airway dysfunction in response to chlorine exposure. Interestingly, in cystic fibrosis, subjects with a more severe respiratory dysfunction were reported having an increase erythrocytic glutathione levels although this potential compensatory mechanism appears to be inadequate to control airway oxidative stress (29).

Further studies are required in the general population and in samples exposed to general environmental and occupational pollutants to identify biomarkers of possible functional changes.

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