Eur Respir J 1999; 13: 482–488
Printed in UK – all rights reserved

European Respiratory Journal
ISSN 0903-1936

Diagnosing occupational asthma: use of induced sputum

C. Lemière, M.M.M. Pizzichini, R. Balkissoon, L. Clelland, A. Efthimiadis, D. O'Shaughnessy, J. Dolovich, F.E. Hargreave

Diagnosing occupational asthma: use of induced sputum. C. Lemière, M.M.M. Pizzichini, R. Balkissoon, L. Clelland, A. Efthimiadis, D. O'Shaughnessy, J. Dolovich, F.E. Hargreave. ©ERS Journals Ltd 1999.

ABSTRACT: The diagnosis of occupational asthma (OA) needs to be made with as much objective evidence as possible. If there is airway inflammation, measurement of this should be an asset. The objective of this study was to investigate whether there is an increase in induced sputum and blood eosinophils and eosinophil cationic protein (ECP) in OA after work exposure.

Patients were assessed after a 2–4 week period at work and away from work with cell counts and ECP assays performed blind to the clinical data. They were considered to have OA if symptoms were worse at work and there was a fall in forced expiratory volume in one second (FEV_1) ≥20% or in the provocative concentration of methacholine causing a 20% fall in FEV_1 (PC_{20}) of four-fold or more compared with away from work. Patients whose symptoms were worse at work but had a change in FEV_1 of <20% and in methacholine PC_{20} of less than four-fold were considered as controls.

Sixteen patients were studied. Ten had OA and six were controls. Patients with OA had a significant increase in median (interquartile range) sputum eosinophils and ECP when at work compared with the periods out of work, 10.0 (17.05) *versus* 0.8 (1.6)% (p=0.007) and 3,840 (6,076) *versus* 116 (180) $\mu g \cdot L^{-1}$ (p=0.01). They also had a higher blood eosinophil count, 0.3 (0.5) $\times 10^9$ *versus* 0.2 (0.1) $\times 10^9 \cdot L^{-1}$ (p=0.013), and a trend towards higher serum ECP levels, 44.0 (20.0) *versus* 32.0 (18.5) $\mu g \cdot L^{-1}$ (p=0.07).

In conclusion, the proportion of eosinophils and levels of eosinophil cationic protein in sputum are particularly high at work in patients with occupational asthma, suggesting that the measurement of these factors can supplement other physiological outcomes in establishing the diagnosis of occupational asthma.

Eur Respir J 1999; 13: 482–488.

Firestone Regional Chest and Allergy Unit, St Joseph's Hospital, Hamilton, Ontario, Canada.

Correspondence: F.E. Hargreave
Firestone Regional Chest and Allergy Unit
St Joseph's Hospital
50 Charlton Avenue East
Hamilton
Ontario
Canada L8N 4A6
Fax: 1 9055216158

Keywords: Induced sputum
occupational asthma
sputum cell counts

Received: March 9 1998
Accepted after revision June 29 1998

Supported by the Ontario Thoracic Society.

The diagnosis of occupational asthma (OA) needs to be made objectively using as many criteria as possible. These criteria include laboratory exposure tests with occupational agent(s) but these can only be performed in a few specialized centres and are time consuming; in addition, a negative result does not exclude OA [1]. Another approach, which is easier to perform, is to monitor peak expiratory flow (PEF) and/or methacholine airway responsiveness during periods at work and away from work. In patients with OA, PEF falls and methacholine airway responsiveness increases [2]. In non-OA, there may be a diurnal variation in PEF at work but airway responsiveness is not raised [3]. These measurements, however, are open to misinterpretation if they are not performed optimally, and PEF measurements are open to manipulation, fraud and poor compliance [4]. Hence, a further laboratory measurement may be useful.

OA is caused by allergens and chemical sensitizers. The reaction following exposure is often associated with an eosinophilic bronchitis [5]. The latter can now be measured directly and noninvasively by reliable and responsive methods of induced sputum examination [5, 6]. In this study, therefore, the examination of induced sputum was added to objective monitoring during periods at work and away from work. The aim was to investigate whether OA diagnosed objectively by a heightening of methacholine airway responsiveness during periods at work was associated with an increase in induced sputum eosinophilia. In addition, other sputum markers of inflammation were examined and the results compared with corresponding markers in the blood.

For editorial comments see page 477

Patients and methods

Patients

Asthmatic adult patients seen at the Firestone Regional Chest and Allergy Clinic suspected of having OA were enrolled in the study (table 1). Asthma was defined by symptoms of variable cough, sputum production, wheeze, dyspnoea or chest tightness and if forced expiratory volume in one second (FEV_1)/vital capacity (VC) was <70%, an improvement of >15% in FEV_1 after salbutamol 200 μg or if the FEV_1/VC was ≥70% or there was methacholine hyperresponsiveness (provocative concentration of methacholine causing a 20% fall in FEV_1

Table 1. – Clinical characteristics of the subjects with occupational asthma and with non-occupational asthma

Patients	Sex	Age yrs	Smoking	Treatment	Atopy	Agent	Duration of	
							exposure yrs	symptoms yrs
Patients with occupational asthma								
1	M	45	NS	β_2 *p.r.n.* IS 800 μg·day^{-1}	-	Unknown: baker with negative skin tests to cereals	24	5
2	F	33	NS	β_2 *p.r.n.* IS 800 μg·day^{-1}	+	Methylmethacrylates/ cyanoacrylates	15	2
3	M	31	S	β_2 *p.r.n.* IS 2000 μg·day^{-1}	-	Welding stainless steel	2	2
4	F	29	Ex	β_2 *p.r.n.*	+	Methylmethacrylates/ cyanoacrylates	3	2
5	M	53	Ex	β_2 *p.r.n.*	-	Grain	2	2
6	M	34	NS	β_2 *p.r.n.*	+	Methylmethacrylates/ cyanoacrylates	2	1
7	M	38	S	β_2 *p.r.n.*	-	Aziridine	22	0.3
8	M	64	Ex	β_2 *p.r.n.*	+	MDI	10	10
9	F	48	Ex	β_2 *p.r.n.* IS 800 μg·day^{-1}	-	Unknown: working in a company testing PVC gloves	6	6
10	M	43	NS	β_2 *p.r.n.*	+	Unknown: working in a strip mill	26	26
Median (IQR)		40.5 (16.7)					8 (20.5)	2 (5.3)
Patients with non-occupational asthma								
11	F	29	S	β_2 *p.r.n.*	-	Methylmethacrylates/ cyanoacrylates	2	1
12	F	50	NS	None	+	Unknown: working in a medical laboratory	7	2
13	M	35	NS	β_2 *p.r.n.* IS 2000 μg·day^{-1}	-	Welding stainless steel and zinc	10	2
14	M	42	NS	β_2 *p.r.n.*	+	Furan	18	2
15	F	32	Ex	β_2 *p.r.n.* IS 400 μg·day^{-1}	-	Cotton	8	8
16	M	47	Ex	β_2 *p.r.n.*	+	Cutting oil	3	0.5
Median (IQR)		38.5 (15.7)					7.5 (9.3)	2.0 (2.6)

M: male; F: females; NS: nonsmokers; S: current smoker; Ex: exsmoker; β_2 *p.r.n.*: short-acting β_2-agonists as needed; IS: inhaled steroids; atopy: +: one or more weal and flare responses to skin tests with common allergen extracts; MDI: methylphenyl diisocyanate; PVC: polyvinyl chloride; IQR: interquartile range.

(PC_{20}) <8 mg·mL^{-1}). OA was diagnosed if symptoms of asthma were worse at work and there was a decrease in FEV_1 of ≥20% and/or in methacholine PC_{20} of four-fold or more during periods at work with reversal of these away from work. The reproducibility for the methacholine challenge in the authors' laboratory is high and the 95% confidence interval (CI) of PC_{20} is <1 two-fold concentration difference. Therefore, a four-fold change in the PC_{20} was considered to be of unambiguous clinical significance. Patients were considered as controls if they had asthmatic symptoms which were worse at work but were associated with a decrease in FEV_1 of <20% and/or in PC_{20} of less than four-fold. No patient had a prior history of colds or respiratory infection for 4 weeks before or during the study period. The study was approved by the hospital research committee and all subjects gave written consent.

Study design

The study was of a prospective crossover design with periods of up to 4 weeks at work and 4 weeks away from work. The patients were seen at the start and at the end of each of these periods or when there was an exacerbation of symptoms, and at a similar time of the day (in the morning). On the first occasion patient characteristics were documented and on all occasions spirometry, methacholine inhalation test, sputum induction and venesection were performed. The clinical, sputum and blood measurements were made blind to each other. If the patient was atopic, the study was not carried out during variable exposure to any relevant allergens. Concurrent treatment with inhaled steroid was maintained at the same level and inhaled β_2-agonist was used only when needed. Subjects were withdrawn from the study if an upper or lower respiratory infection occurred, which occurred for one patient. The outcomes were induced sputum, total and differential cell counts, fluid-phase eosinophil cationic protein (ECP), blood eosinophils and serum ECP.

Clinical methods

Patient characteristics were documented by questionnaire. Asthmatic symptoms (chest tightness, dyspnoea, wheezing and cough) at work and away from work were graded daily on a 10-point Borg Scale [7]. The symptoms score ranged from 0 (asymptomatic) to 10 (the most severe discomfort). The use of medications was recorded. FEV_1 and slow VC were performed using a Koko spirometer (Pulmonary Data Service Instrumentation Inc., Louiseville,

Table 2. – Functional and biological characteristics of subjects with occupational asthma when at work and away from work

Patient No	Status	FEV1 L (% pred)	VC L (% pred)	PC20 mg·mL^{-1}	Sputum				Blood	
					TCC $\times 10^6$·mL^{-1}	Eo %	N %	ECP µg·L^{-1}	Eo $\times 10^9$·L^{-1}	ECP µg·L^{-1}
1	At W	2.1 (50)	4.6 (102)	< 0.03	1.7	2.3	24	208	0.2	31
	Off W	3.1 (74)	5.1 (114)	3.0	2.3	0.0	40	80	0.1	35
2	At W	2.2 (67)	3.5 (90)	ND	5.2	27.0	11	3840	0.8	50
	Off W	2.8 (85)	4.5 (116)	1.3	1.3	0.8	15	48	0.2	18
3	At W	3.8 (84)	5.5 (102)	1.6	1.6	18.0	27	856	1.0	44
	Off W	4.1 (91)	5.6 (104)	14.0	4.2	3.5	62	1016	ND	40
4	At W	4.0 (113)	4.7 (113)	3.5	3.8	3.5	5.0	4000	0.8	44
	Off W	4.2 (119)	4.6 (111)	> 64.0	2.5	0.3	9.0	56	0.2	32
5	At W	3.5 (92)	5.0 (106)	1.3	2.5	0.3	49	872	0.2	45
	Off W	4.2 (111)	5.7 (121)	28.7	3.9	1.0	86	200	0.2	51
6	At W	2.3 (58)	3.2 (79)	0.5	4.2	22.5	33	7680	0.5	44
	Off W	3.3 (84)	4.4 (92)	> 64.0	ND	ND	ND	ND	0.1	17
7	At W	3.8 (87)	4.9 (91)	0.95	2.2	11.7	38	2448	0.3	22
	Off W	3.6 (82)	4.7 (86)	32.0	3.8	0.8	68	120	0.2	16
8	At W	1.6 (55)	3.4 (106)	0.1	4.9	43.0	36	6000	0.1	49
	Off W	3.0 (105)	3.6 (101)	1.5	2.4	0.0	42	256	0.1	22
9	At W	2.5 (108)	3.2 (117)	2.9	1.0	8.0	27	672	0.1	24
	Off W	2.5 (109)	3.0 (112)	32.0	0.4	2.5	5.0	112	0.1	32
10	At W	2.5 (66)	4.0 (86)	0.4	18.4	8.3	81	1536	0.4	50
	Off W	3.1 (82)	4.6 (100)	0.9	1.9	0.3	81	ND	0.2	34

FEV1: forced expiratory volume in one second; VC: vital capacity; PC20: provocative concentration of methacholine causing a 20% fall in FEV1; TCC: total cell count; Eo: eosinophils; N: neutrophils; ECP: eosinophil cationic protein; W: work; ND: not done.

Quebec, Canada) according to the standards of the American Thoracic Society [8]. Methacholine inhalation challenge was performed using the method described by JUNIPER *et al.* [9], with any short-acting β_2-agonist withheld for at least 6 h. The results were expressed as the PC20. Allergy skin-prick tests with 12 common allergen extracts were performed using standard procedures [10].

Sputum induction

Sputum induction was performed within 48 h of the last exposure at work, after the methacholine challenge and inhaled salbutamol 200 µg, and administered using an inhalation of an aerosol of hypertonic saline at increasing concentrations (3, 4 and 5%) generated by a Fisoneb ultrasonic nebulizer (Canadian Medical Products, Markham, Ontario, Canada) with an output of 0.87 mL·min^{-1} and particle size of 5.58 µm aerodynamic mass median diameter, as described by PIN *et al.* [11]. The method was slightly modified by inhaling each of the concentrations of saline for 7 min. After each period of inhalation the FEV1 was measured to ensure the patient's safety. The patients were asked to blow their nose, rinse their mouth and swallow the water to minimize contamination with postnasal drip and saliva. They were instructed to cough into a sterile container and the expectorate was processed within 2 h.

Sputum examination

Sputum examination was performed as described by PIZZICHINI *et al.* [6].The expectorate was poured into a Petri dish and all portions that macroscopically looked more opaque and/or dense and unlike saliva (selected portion) were placed in a 15-mL polystyrene tube and weighed. This was treated with dithiothreitol (DTT) (Sputalysin 10%; Calbiochem, San Diego, CA, USA), freshly diluted 1:10 with distilled water in a volume equal to four-times the weight of the selected sputum. The mixture was agitated for 15 s on a vortex mixer, gently aspirated in and out of a pipette to ensure mixing and placed on a bench rocker (Dade Tube Rocker; Baxter Diagnostics, Miami, FL, USA) for 15 min. A further 4 volumes of Dulbecco's phosphate-buffered saline (D-PBS) was added and rocking continued for another 5 min. The suspension was filtered through 48-µm nylon mesh (BBSH Thompson, Scarborough, Ontario, Canada) to remove cell debris and remaining mucus. A total cell count was performed in a modified Neubauer haemocytometer and the cell viability was determined simultaneously by the trypan blue exclusion criteria. The total cells·mg^{-1} of processed sputum was calculated. Cytospins were prepared by placing 60 µL of the cell suspension adjusted to 1.0×10^6·mL^{-1} into a Shandon III cytocentrifuge (Shandon Southern Instruments, Sewickley, PA, USA) at $22.8 \times g$ for 6 min. One cytospin was dried and Wright-stained and a 400 nonsquamous cell differential was performed. The remaining cell suspension was spun at $790 \times g$ for 4 min and the supernatant was aspirated and stored at -70°C for later fluid phase measurements.

Fluid phase measurements

The concentration of ECP in µg·L^{-1} in the thawed supernatant was determined using radioimmunoassay (RIA; Kabi Pharmacia Diagnostics AB, Uppsala, Sweden) correcting for the dilution factor.

Blood measurements

Venous blood was collected into 5 mL 7.5% ethylenediaminetetraacetic acid (EDTA) (K3 Vacutainer; Rutherford, NJ, USA) and a differential white cell count was obtained using Coulter STKS (Coulter Corp, Hialeah, FL, USA). Serum was collected after blood coagulation for 1 h at room temperature. It was centrifuged at 20°C and 430 × *g* for 10 min and stored at -20°C until analysis. The assay used to measure serum ECP was the same as that described for sputum.

Data analysis

All data were analysed using the statistical package SPSS for Windows, release 7.5 (SPSS, Chicago, IL, USA).

Results are reported as median and interquartile range (IQR). Dependent variables with non-normal distribution were log or square root transformed before analysis. Within-group differences in the outcome measurements (FEV_1, PC_{20}, sputum and blood inflammatory indices) between periods of exposure (at work) and nonexposure (off work) were analysed using paired t-tests. A two-tailed unpaired t-test was used to compare these indices between the two groups of patients (OA and controls). Correlations were examined using the Spearman rank correlation test. All tests were two-sided. A p-value <0.05 was considered statistically significant.

Results

Eighteen patients were included in the study, of whom 10 had OA, six had non-OA (tables 1 and 2) and two were excluded (one had a cold during the study and the other completed only one visit and did not return). The patients were seen within 24 h of being at work, except for four subjects who were seen 48 h after work. There was no difference between the OA and control groups in age, sex, atopic status or smoking habits. Six patients with OA were exposed to low molecular weight agents, but the responsible agent in the other four was unknown (one was exposed to grain but had negative skin tests to cereals). There was a significant change in the FEV_1 and PC_{20} in the OA group between the periods at work and off work (2.6 (1.6) *versus* 3.2 (1.2) $mg \cdot mL^{-1}$, p=0.006 and 0.6 (1.0) *versus* 9.8 (1.4) $mg \cdot mL^{-1}$, p<0.001 respectively) (fig. 1). There was no change regarded as significant in these parameters in the control group.

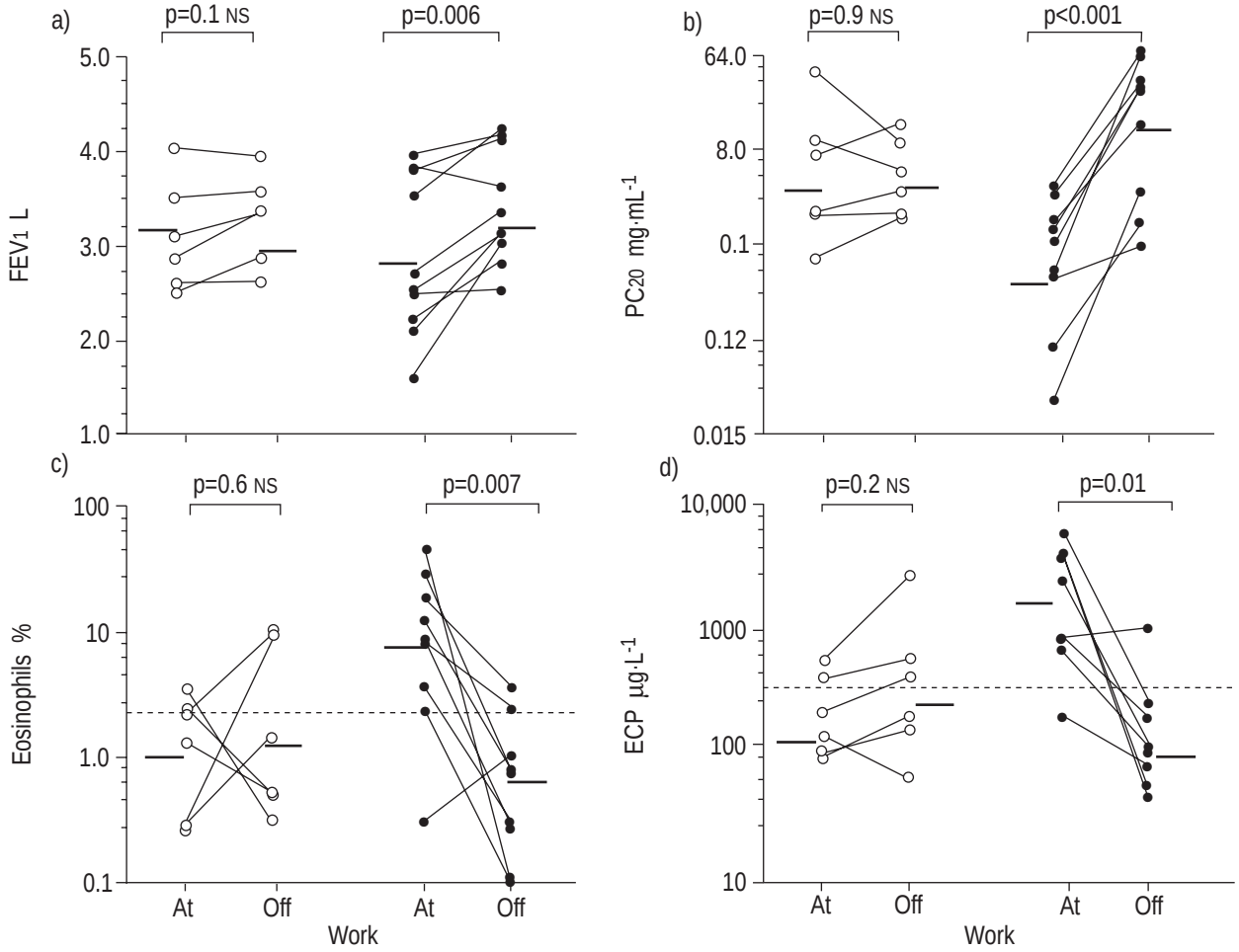

Fig. 1. – Changes in a) forced expiratory volume in one second (FEV_1); b) provocative concentration of methacholine causing a 20% fall in FEV_1 (PC_{20}); c) eosinophil count; and d) eosinophil cationic protein (ECP) levels between the period at and away from work in the occupational asthma (●) and control (○) groups. Horizontal bars represent the median, except for PC_{20} (b) where it represents the geometric mean. The area below the dashed line represents the normal range in the authors' laboratory.

Table 3. – Functional and biological characteristics of subjects without occupational asthma when at work and away from work

Patient No	Status	FEV1 L (% pred)	VC L (% pred)	PC20 mg·mL^{-1}	Sputum				Blood	
					TCC $\times 10^6$·mL^{-1}	Eo %	N %	ECP µg·L^{-1}	Eo $\times 10^9$·L^{-1}	ECP µg·L^{-1}
11	At W	3.1 (84)	4.0 (89)	2.0	1.6	1.3	23	144	0.2	ND
	Off W	3.3 (89)	4.6 (102)	3.0	0.9	0.5	61	64	0.2	21.0
12	At W	2.6 (104)	3.2 (104)	1.8	4.6	2.2	52	584	0.2	18.0
	Off W	2.3 (92)	3.4 (110)	1.9	17.1	9.8	60	2736	0.1	8.3
13	At W	3.5 (92)	4.6 (102)	10.0	1.3	0.3	35	96	0.2	6.8
	Off W	3.6 (95)	4.9 (109)	4.8	4.7	1.5	46	208	0.2	13.0
14	At W	2.9 (69)	5.1 (98)	0.7	2.5	3.5	61	400	0.4	46.0
	Off W	3.3 (80)	5.1 (98)	1.7	4.8	0.3	76	592	0.1	19.0
15	At W	2.5 (89)	3.4 (100)	6.8	1.6	2.5	39	104	ND	ND
	Off W	2.9 (104)	2.9 (85)	14.0	1.1	0.5	42	160	ND	ND
16	At W	4.0 (97)	5.4 (105)	45.0	4.8	0.3	54	224	0.5	18.0
	Off W	3.9 (95)	5.3 (104)	9.2	2.1	9.5	49	424	0.4	15.0

FEV1: forced expiratory volume in one second; VC: vital capacity; PC20: provocative concentration of methacholine causing a 20% fall in FEV1; TCC: total cell count; Eo: eosinophils; N: neutrophils; ECP: eosinophil cationic protein; W: work; ND: not done.

Sputum inflammatory indices in the two groups of patients were examined at work and off work (tables 2 and 3). There was a significant increase in the median (IQR) sputum eosinophils (but not other cell types) in the patients with OA at work compared with the periods away from work (10.0 (17.05) *versus* 0.8 (1.6)%, p=0.007), whereas there was no difference between the two periods in the control group (fig. 1). There was an increase in sputum ECP when at work in patients with OA (3,840 (6,076) *versus* 116 (180) µg·L^{-1}, p=0.01) but not in the control group. Only one patient in the OA group did not show an increase in his sputum eosinophil count and ECP 24 h after work. There was a significant inverse correlation between the change in sputum eosinophil count and the change in airway hyperresponsiveness (Spearman′s rho= -0.68, p=0.007) as well as between the changes in sputum ECP and airway hyperresponsiveness (Spearman′s rho= -0.80, p=0.001) (fig. 2).

The blood eosinophils and the serum ECP in the two groups were also examined (tables 2 and 3). The patients with OA had a statistically significant higher median absolute blood eosinophil count when at work compared with when they were away from work, although the count remained within normal limits (0.3 (0.5) $\times 10^9$ *versus* 0.2 (0.1) $\times 10^9$·L^{-1}, p=0.013). They also showed a trend towards an increase in median serum ECP (32.0 (18.5) *versus* 44.0 (20.0) µg·L^{-1}, p=0.07). There was no increase in the median blood eosinophil count or the serum ECP for the control group.

Discussion

This study demonstrates that subjects with OA due to low molecular weight or unknown materials have an increase in induced sputum eosinophils and ECP when at work, while asthmatic subjects without OA do not. Hence, induced sputum eosinophilia in relation to work can be used to support the diagnosis of OA. This is particularly helpful in problematic cases where one must differentiate

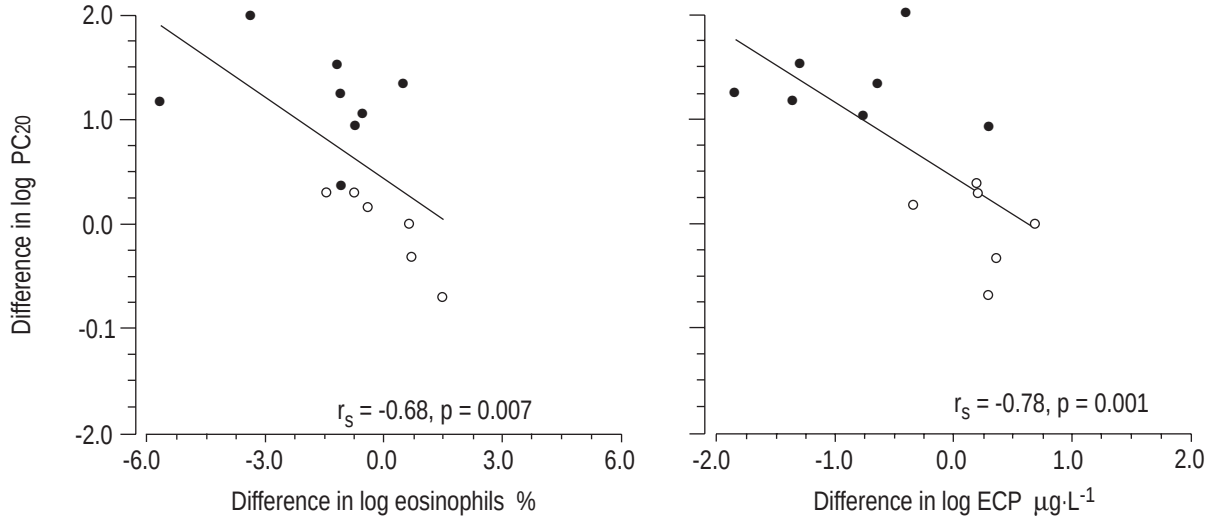

Fig. 2. – Correlation between the difference in log provocative concentration of methacholine causing a 20% fall in forced expiratory volume in one second (PC20) and difference in log eosinophils and in log eosinophil cationic protein (ECP). ●: Occupational asthma; ○: control.

between workplace aggravation of asthma and superimposed OA due to a workplace sensitizer. It also supports OA when the workplace sensitizer is unknown.

The diagnosis of OA was made by airway physiological changes (fall in FEV_1 or PC_{20}) within 48 h of the last day at work, independent of the sputum measurements. The sputum examination was also performed without knowledge of the physiological measurements. The sputum measurements were made on sputum selected from saliva and processed by new reliable and responsive methods [6, 12]. The results show that when the FEV_1 or PC_{20} methacholine was reduced after periods at work, sputum eosinophils and ECP were increased in every subject except for one. The investigation was repeated in this one patient in the same way and the same results were found. It is, therefore, unlikely that the results were due to inaccurate measurements on both occasions. Hence, in some instances some patients with OA do not have an increase in their eosinophil count despite an increase in asthma symptoms and airway responsiveness when at work. The cause of this is not known. However, it is possible that some occupational agents cause OA by noneosinophilic mechanisms, as can occur in non-OA [13–15].

The control patients did not show significant changes in their physiological measurements between the periods at and away from work. Therefore, they were considered to have OA according to the criteria chosen for this study. However, in some instances the reduced exposure at work at the time of the investigation may have been low enough to have had no clinical effect.

The increase in sputum eosinophils in patients with OA is to be expected. Laboratory exposure tests with toluene diisocyanate produce an increase in induced sputum eosinophils, as do allergen inhalation tests [5, 16]. However, sputum examination for inflammatory indices with reliable methods has not been performed previously during periods at work and periods away from work. This approach to investigate the diagnosis is particularly relevant to most centres of investigation where laboratory exposure tests cannot be performed or where the specific agent is unknown. Such laboratories usually investigate OA by using serial monitoring of PEF and/or PC_{20}. This is also the best first line of investigation when the agent is unknown. Induced sputum is helpful in confirming the presence of sensitization in these cases. For specialists in OA, further investigation using specific inhalation challenges might be considered to identify the causal agent.

Induced sputum is an objective measurement which, unlike PEF and PC_{20}, is not open to interference. However, it needs to be processed carefully to obtain accurate and reliable results. The results of the present study show that induced sputum examination can be used as an additional, relatively noninvasive investigation to validate the diagnosis of OA. The sensitivity and specificity of sputum eosinophilia need to be investigated in patients in whom the sensitizer is known and sensitization can be confirmed by exposure tests in the laboratory. Using this approach, the sensitivity and the specificity of the combination of PEF (visual analysis) and changes in methacholine PC_{20} at and off work have been found to be 84% and 61%, respectively [17]. The addition of induced sputum examination might improve this score and needs to be assessed.

Some studies have reported an increase in neutrophils in bronchoalveolar lavage after allergen [18] and TDI laboratory exposure [19]. In the patients with OA, no increase in the percentage of neutrophils when at work was found in the present study. This discrepancy could be due to the different conditions of exposure or timing of measurements. In a laboratory exposure test, the dose of agent is administered over a short period, while at work the exposure may be at lower concentrations over a longer period. The airway inflammation might show different characteristics in these circumstances. This is consistent with the recent finding that after repeated exposure to low doses of allergen there is an increase in sputum eosinophils but not in sputum neutrophils [20]. Another possible explanation is that the increase in neutrophils is an early feature of the inflammatory reaction and is only found in the first hours after exposure [18, 19]. If patients were seen later, as in this study, sputum neutrophilia may have cleared.

Peripheral blood eosinophils and serum ECP increased in some patients with OA after the period at work. However, while the median percentage increase in sputum eosinophils was 92%, the increase in blood eosinophils was 43% and in sputum ECP was 96%, whereas the increase in serum ECP was only 27%. In addition, the magnitude of the fall in serum ECP in patients with OA, after a period away from work, was less consistent. It is, therefore, difficult to interpret changes in blood eosinophils or serum ECP in a patient, unless they are major. In contrast, the magnitude of the increase in sputum eosinophils was consistently high and, except in one patient, the proportion of sputum eosinophils returned to normal after a period away from work. These findings support a previous report showing that sputum eosinophils are a more sensitive and specific marker of airway eosinophilic inflammation than ECP [21].

In conclusion, sputum eosinophils and eosinophil cationic protein increase during periods at work in most patients with occupational asthma and can be used as objective evidence to support the diagnosis. Proper timing, collection and processing of samples are critical. Sputum analysis may be particularly useful in problematic cases where the diagnosis is in doubt owing to questionable patient cooperation, spirometric and peak expiratory flow techniques or concomitant nonoccupational asthma and where there is no recognized causative agent.

Acknowledgements. The authors thank the patients who participated in this study, S. Weston and S. Carruthers-Elliot for helping with cell counts and S. Evans for performing the fluid-phase measurements.

References

1. Lemière C, Cartier A, Dolovich J, *et al*. Outcome of specific bronchial responsiveness to occupational agents after removal from exposure. *Am J Respir Crit Care Med* 1996; 154: 329–333.
2. Chan-Yeung M. Nonspecific Bronchial Hyperresponsiveness. Asthma in the Workplace. New York, Marcel Dekker, 1993; pp. 189–214.
3. DeLuca S, Cair N, Cloutier Y, Cartier A, Ghezzo H, Malo JL. Acute exposure to sawdust does not alter airway calibre and responsiveness to histamine in asthmatic subjects. *Eur Respir J* 1988; 1: 540–546.

4. Malo JL, Trudeau C, Ghezzo H, L′Archevèque J, Cartier A. Do subjects investigated for occupational asthma through serial peak expiratory flow measurements falsify their results? *J Allergy Clin Immunol* 1995; 96: 601–607.
5. Maestrelli P, Calcagni PG, Saetta M, *et al*. Sputum eosinophilia after asthmatic responses induced by isocyanates in sensitized subjects. *Clin Exp Allergy* 1994; 24: 29–34.
6. Pizzichini E, Pizzichini MMM, Efthimiadis A, *et al*. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. *Am J Respir Crit Care Med* 1996; 154: 308–317.
7. Borg GAV. Psychophysiological bases of perceived exertion. *Med Sci Sports Exercise* 1992; 14: 377–381.
8. American Thoracic Society. Standardization of spirometry. 1987 update. *Am Rev Respir Dis* 1987; 136: 1285–1307.
9. Juniper EF, Cockcroft DW, Hargreave FE. Histamine and Methacholine Inhalation Test: a Laboratory Tidal Breathing Protocol. 2nd Edn. Lund, Astra Draco AB, 1994.
10. Pepys J. Skin tests in diagnosis. *In:* Gell PGH, Coombs RRA, Lachmann PJ, eds. Clinical Aspects of Immunology. Oxford, Blackwell Scientific Publications, 1975; pp. 55–80.
11. Pin I, Gibson PG, Kolendowicz R, Denburg JA, Hargreave FE, Dolovich J. Use of induced sputum cell counts to investigate airway inflammation in asthma. *Thorax* 1992; 47: 25–29.
12. Pizzichini E, Pizzichini MMM, Efthimiadis A, Hargreave FE, Dolovich J. Measurement of inflammatory indices in induced sputum: effect of selection of sputum to minimize salivary contamination. *Eur Respir J* 1996; 9: 1174–1180.
13. Fahy JV, Kim KW, Liu J, Boushey HA. Prominent neutrophilic inflammation in sputum from subjects with asthma exacerbation. *J Allergy Clin Immunol* 1995; 95: 843–852.
14. Turner MO, Hussak P, Sears NM, Dolovich J, Hargreave FE. Exacerbations of asthma without sputum eosinophilia. *Thorax* 1995; 50: 1057–1061.
15. Pizzichini MM, Pizzichini E, Clelland L, *et al*. Sputum in severe exacerbations of asthma: kinetics of inflammatory indices after prednisone treatment. *Am J Respir Crit Care Med* 1997; 155: 1501–1508.
16. Pin I, Freitag AP, O′Byrne P, *et al*. Changes in the cellular profile of induced sputum after allergen-induced asthmatic response. *Am Rev Respir Dis* 1992; 145: 1265–1269.
17. Perrin B, Lagier F, L′Archeveque J, *et al*. Occupational asthma: validity of monitoring of peak expiratory flow rates and non-allergic bronchial responsiveness as compared to specific inhalation challenge. *Eur Respir J* 1992; 5: 40–48.
18. Metzger WJ, Richerson HB, Worden K, Monick M, Hunninghake GW. Bronchoalveolar lavage of allergic asthmatic patients following allergen bronchoprovocation. *Chest* 1986; 89: 477–483.
19. Fabbri LM, Boschetto P, Zocca E, *et al*. Bronchoalveolar neutrophilia during late asthmatic reactions induced by toluene diisocyanate. *Am Rev Respir Dis* 1987; 136: 36–42.
20. Sulakvelidze I, Watson R, Killian K, O′Byrne P. Airway hyperresponsiveness and inflammation after repeated low dose allergen challenge. *Eur Respir J* 1997; 10: Suppl. 25, 474s.
21. Pizzichini E, Pizzichini MMM, Efthimiadis A, Dolovich J, Hargreave FE. Measuring airway inflammation in asthma: eosinophils and eosinophilic cationic protein in induced sputum compared with peripheral blood. *J Allergy Clin Immunol* 1997; 99: 539–544.