

Clinical evaluation of eosinophils in the sputum

V. G. VIEIRA AND J. C. PROLLA¹

*From the Department of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, and
¹Laboratory of Cytopathology, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil*

SUMMARY The sputum differential eosinophil/neutrophil count was done in 384 patients using Leishman staining. The patients were distributed in four groups: bronchial asthma (197 patients); chronic bronchitis with wheezing (45 patients); chronic bronchitis and/or emphysema without wheezing (73 patients); other pulmonary diseases (64 patients).

Eosinophils were present in patients from all groups but more frequently ($P < 0.001$) in asthma: 142 (72%) of 197 patients. In bronchial asthma and chronic bronchitis with wheezing the percentages of eosinophils were more frequently ($P < 0.001$) above 80%: 57% and 58% of the patients respectively. The other two groups had more cases with 19% or less eosinophils. There is no percentage level specific for asthma but levels above 80% of eosinophils are strongly suggestive of asthma or of chronic bronchitis with wheezing.

The presence of eosinophil leucocytes in the sputum of asthmatic patients has been recognised for a long time. Together with Charcot-Leyden crystals and Curschmann's spirals they have been associated with allergy of the respiratory tract.

More recently, reports from the British literature have shown the value of finding eosinophils in the sputum in the differential diagnosis of asthma and obstructive chronic bronchitis. Also, their presence has been associated with treatment, especially with corticosteroids (Brown, 1958; Williams, 1961).

The present study was designed to answer the following questions:

(1) Is the presence of eosinophils in the sputum a specific sign of any pulmonary disease?

(2) Is there a percentage level of eosinophils relative to neutrophils which permits a differential diagnosis of bronchial asthma from other lung affections?

(3) What is the relationship between bronchial asthma and chronic bronchitis with wheezing in regard to the proportion of eosinophils and neutrophils in the sputum?

Material and methods

PATIENT POPULATION

The differential count, as herein described, was done in 384 patients from two hospitals (Pavilhão Pereira

Filho and Hospital de Clínicas de Porto Alegre) in Porto Alegre, Brazil.

The sputum of 197 patients with bronchial asthma and of 187 patients with other pulmonary diseases was examined. Besides the usual clinical history, a special questionnaire was answered by all patients in order to identify clinical manifestations of bronchial obstruction and atopy.

The patients were divided into four groups, as follows:

Group 1 Bronchial asthma (197 patients)

Group 2 Chronic bronchitis with wheezing, with or without emphysema (45 patients)

Group 3 Chronic bronchitis and/or emphysema without wheezing (73 patients)

Group 4 Other lung diseases (69 patients)

The diagnostic criteria of bronchial asthma and chronic bronchitis were those of the British Medical Research Council (1965). The diagnosis of emphysema was made by clinical and radiological methods. Group 2 comprised the chronic bronchitics who complained of dyspnoea and who presented wheezing on physical examination. Group 4 comprised all the other lung diseases: pneumonia, tuberculosis, bronchogenic carcinoma, pulmonary fibrosis, and patients with respiratory symptoms without definite pulmonary disease.

All diagnoses were made on clinical, radiological, bacteriological, and cytopathological grounds. In some cases there was also histopathological proof.

Patients with unequivocal manifestations of atopy were excluded from groups 2, 3, and 4 in order to minimise confusion with asthma.

SPUTUM COLLECTION

Spontaneous sputum was collected in the early morning with the patients fasting. They were instructed to spit, after a deep cough, into a wide-mouthed jar, which was sent without delay to the cytology laboratory.

SPUTUM PROCESSING

In the laboratory the sputum was transferred to a Petri dish placed against a black or dark background. In this way it is easier to identify saliva or purulent sputum. Material consisting exclusively of watery saliva was rejected at this point. Whitish dense threads or streaks representing mucus with entrapped cells were chosen when possible. Small aliquots of sputum were finely distributed over two microscope slides using metal spatulas. The smears were air-dried and stained with Leishman or May-Grünwald-Giemsa methods.

CELL COUNTING

A preliminary evaluation of the smear was made using a 40 × objective to exclude material consisting predominantly of saliva.

Two hundred cells (neutrophil and/or eosinophil leucocytes) were counted under oil immersion objectives from randomly selected fields. The results were expressed in percentages (eg, eosinophils 80%, neutrophils 20%). The uneven distribution of cells, especially of purulent sputum, as shown by Rawlins (1955), was minimised by this technique. A comparison of counts from different aliquots of the same sputum in 11 patients did not reveal more than 10% variation between the counts.

Results

The presence or absence of eosinophils in the sputum of the 384 patients (distributed among the four groups) is shown in Table 1. In 72% of the asthmatic patients (group 1) eosinophils were present. There was a statistically significant difference between group 1 and groups 3 and 4. Group 2 did not differ statistically from group 1, nor from groups 3 and 4. Groups 3 and 4 also did not differ from each other.

Table 2 shows the distribution of patients in the four groups according to the differential counts of eosinophils in the sputum. The percentages were distributed in three classes: (a) 1-19% eosinophils; (b) 20-79%; (c) 80-100%. The chi-square test showed the following: (1) groups 1 and 2 had the same distribution; (2) groups 3 and 4 also had a similar distribution; (3) group 1 differed significantly from groups 3 and 4 ($P < 0.001$); group 2 differed significantly from groups 3 and 4 ($P < 0.001$).

Table 1 Presence of eosinophils in sputa of 384 patients distributed among the four groups

Group	Eosinophils	
	Present	Absent
	No. of patients (%)	No. of patients (%)
Group 1—Asthma (197 patients)	142 (72)*	55 (28)
Group 2—Chronic bronchitis with wheezing (45 patients)	26 (58)	19 (42)
Group 3—Chronic bronchitis and/or emphysema without wheezing (73 patients)	32 (44)*	41 (56)
Group 4—Other lung diseases (69 patients)	28 (41)*	41 (59)

*Group 1 differs from groups 3 and 4 ($P < 0.001$).

Discussion

As expected, asthmatic patients had the highest incidence of eosinophils in the sputum: 142 (72%). Also, they had the highest percentages in the differential count: 81 patients with asthma had 80-100% eosinophil counts. But in patients with chronic bronchitis with wheezing the findings were similar; the figures obtained did not show a statistically significant difference. Surprisingly, a considerable number of patients with emphysema, chronic bronchitis without wheezing, tuberculosis, pulmonary fibrosis, and other lung diseases had eosinophils in the sputum. The presence of eosinophils in the sputum is classically attributed to allergy and is mediated by eosinotactic factors (Kay and Austen, 1971; Wasserman *et al.*, 1973, 1975). Such cells were not seen in the bronchial lavage of normal individuals (Reynolds and Newball, 1974).

Some explanations or hypotheses could be advanced:

(1) Some individuals have more marked manifestations of atopy in their childhood. Later, in adulthood, such manifestations become very much attenuated but still detectable by such a test as the search for eosinophils in sputum.

(2) Sputum eosinophilia has been described in lung diseases not related to type I (Gell-Coombs) allergic reactions. For instance, in bronchogenic carcinoma the tumour probably secretes an eosinophilotactic factor responsible for the sputum eosinophilia (Wasserman *et al.*, 1974). In patients with idiopathic pulmonary fibrosis, the presence of eosinophils has been demonstrated in the bronchial lavage (Crystal *et al.*, 1976) in the absence of blood eosinophilia and elevated IgE levels in the plasma and bronchial secretions.

Table 2 Distribution of patients in the four groups according to percentage of eosinophils in the sputum

Eosinophils in sputum (%)	Group 1*	Group 2*	Group 3	Group 4
	No. (%)	No. (%)	No. (%)	No. (%)
1-19	13 (9)	5 (18)	19 (60)	24 (50)
20-79	48 (34)	6 (24)	10 (31)	11 (39)
80-100	81 (57)	15 (58)	3 (9)	3 (11)
Total	142 (100)	26 (100)	32 (100)	38 (100)

*Groups 1 and 2 differ significantly from groups 3 and 4 ($P < 0.001$).

(3) Other factors such as the eosinophil leucocyte chemotactic factor of complement (ECF-C) and the lymphocytic dependent one may be responsible for eosinophilia in bronchial secretion.

As seen in Table 2, the percentages of eosinophils in the sputum of asthmatic and bronchitic patients (with wheezing) were similar: most of them had more than 80% (high eosinophilotaxis). This was in contrast to the patients in groups 3 and 4, the majority of whom had less than 20% (slight eosinophilotaxis).

Our results are in accord with those of Williams (1961). In a group of 58 asthmatics, 87% had more than 10% eosinophils in the sputum, while in 56 patients with chronic bronchitis or emphysema 84% had less than 10% eosinophils. After a trial of corticosteroids in these 56 patients, clinical improvement was observed in only six. It is important to note that all these six patients had more than 10% eosinophils in the sputum. Because of this, Williams is of the opinion that they had asthma and bronchitis or emphysema. This emphasis on the eosinophil count and the corticosteroid trial in the diagnosis of asthma is shared by Crofton, personal communication (1977).

Elmes *et al.* (1953), studying the sputum of chronic bronchitics, established a correlation between purulent appearance, with abundance of neutrophil leucocytes, and the growth of *Haemophilus influenzae*. May (1954), however, calls attention to the situation in which the sputum is mucopurulent due to abundant eosinophils instead of neutrophils ('eosinophilic pus').

Helm *et al.* (1954), studying the long-term use of tetracycline in chronic respiratory infections, had 13 patients with 'infectious asthma' (diagnosis made on the basis of purulent sputum); in 11 there was no therapeutic response, and these had almost exclusively eosinophils in the sputum.

Brown (1958) notes the difficulties of the differential diagnosis between chronic bronchitis with bronchospasm and asthma. For him, eosinophilic sputum identifies the true asthmatic. However, he does not define sputum eosinophilia in terms of percentages of cells. In his experience, only patients

with groups of eosinophils are likely to have a therapeutic response to corticosteroids. In a total of 90 patients, 63 with sputum eosinophilia had partial or total relief of bronchospasm, while of 27 with few or no eosinophils only four had such relief.

Almost simultaneously, Cole *et al.* (1959) observed 30 patients with 'obstructive emphysema'; 15 patients had episodes of sputum eosinophilia not related to their clinical status, and such episodes were separated by days and even weeks of sputum free of eosinophils. These authors think that sputum eosinophilia is part of the clinical picture of 'chronic obstructive emphysema' and is not an allergic manifestation. However, the description of their patients fits well with that of chronic bronchitis with bronchospasm.

Sanerkin and Evans (1965) studied the differential diagnosis of asthmatic and chronic bronchitic patients by sputum constituents as seen in paraffin sections. In asthma, abundant eosinophils, Curschmann spirals, and 'Creola bodies' (shed epithelial fragments) were considered typical elements of sputum expectorated soon after its formation, while a laminar pattern with cellular degeneration was considered typical of retained sputum of prolonged bronchial obstruction. However, the authors do not define exactly what they consider to be an abundance of eosinophils. Curschmann spirals are a frequent finding in smokers; and the clusters of columnar cells called by Naylor and Railey (1964) 'Creola bodies' are seen in other pathological conditions (Carabelli, 1958).

Chodosh (1970) also tried to distinguish chronic bronchitis, chronic asthma, and chronic asthmatic bronchitis (as defined by the author). The main cellular differences in the sputum of asthmatic and bronchitic patients were:

(a) Chronic bronchitis: the desquamated cells of bronchial epithelium were isolated, with scanty cytoplasm, pyknotic nuclei, and few cilia. There was a preponderance of neutrophils (75 to 95% of all cells) and eosinophils were scarce (less than 2%).

(b) Asthma: large clusters of epithelial cells ('Creola bodies'), with swollen cytoplasm and many cilia. Eosinophil preponderance (20 to 90% of all cells) and few neutrophils (2 to 30%).

(c) 'Asthmatic bronchitis': same findings as in chronic bronchitis except for few epithelial cells with oedematous cytoplasm.

Turnbull *et al.* (1977) studied the mediators of immediate type hypersensitivity in the sputum of chronic bronchitics, in early-onset skin-test-positive asthmatics, in late-onset skin-test-negative asthmatics, and in bronchial carcinoma, bronchiectasis, and pneumonia. Sputum eosinophilia was found in bronchitics and asthmatics, whereas raised blood eosinophil levels were found only in early-onset skin-test-positive asthmatics. Histamine and IgE were present in considerable amounts in the sputum of bronchitics and early-onset skin-test-positive asthmatics. To the authors these findings in the sputum indicate an element of local immediate-type (type I) hypersensitivity in bronchitis.

Our findings are generally in accord with these reports in the literature, but we would emphasise our findings in the patients with chronic bronchitis with wheezing, who represent a transition between asthma and bronchitis. These patients are chronic bronchitics with a variable degree of dyspnoea and wheezing. Clinically, they do not have typical bronchospastic attacks, and bronchospasm is not a preponderant clinical feature. The cytological analysis was in accord with the clinical picture in that these patients had more eosinophils than any of the other patients except asthmatics.

References

- Brown, H. M. (1958). Treatment of chronic asthma with prednisolone: significance of eosinophils in the sputum. *Lancet*, **2**, 1245-1247.
- Carabelli, A. A. (1958). Cytologic patterns in bronchopulmonary disease. *American Review of Respiratory Diseases*, **77**, 22-31.
- Chodosh, S. (1970). Examination of sputum cells. *New England Journal of Medicine*, **282**, 854-857.
- Cole, M. B., Hammel, J. V., Lawton, A. H., Meindersma, M. S., and Macintire, S. (1959). Longitudinal studies in emphysema; III sputum eosinophilia. *American Review of Respiratory Diseases*, **80**, 915-918.
- Crystal, R. G., Fulmer, J. D., Roberts, W. C., Moss, M. L., Line, B. R., and Reynolds, H. Y. (1976). Idiopathic pulmonary fibrosis. *Annals of Internal Medicine*, **85**, 769-788.
- Elmes, P. C., Knox, K., and Fletcher, C. M. (1953). Sputum in chronic bronchitis: effects of antibiotics. *Lancet*, **2**, 903-906.
- Helm, W. H., May, J. R., and Livingstone, J. L. (1954). Long-term oxytetracycline (Terramycin) therapy in advanced chronic respiratory infections. *Lancet*, **2**, 630-633.
- Kay, A. B., and Austen, K. F. (1971). The IgE-mediated release of an eosinophil leukocyte chemotactic factor from human lung. *Journal of Immunology*, **107**, 899-902.
- May, J. R. (1954). Pathogenic bacteria in chronic bronchitis. *Lancet*, **2**, 839-842.
- Medical Research Council (1965). Definition and classification of chronic bronchitis for clinical and epidemiological purposes. A report to the Medical Research Council by their committee on the aetiology of chronic bronchitis. *Lancet*, **1**, 775-779.
- Naylor, B., and Railey, C. (1964). A pitfall in the cyto-diagnosis of sputum of asthmatics. *Journal of Clinical Pathology*, **17**, 84-89.
- Rawlins, G. A. (1955). Cytological examination of sputum in relation to its macroscopic purulence. *Journal of Clinical Pathology*, **8**, 114-116.
- Reynolds, H. Y., and Newball, H. H. (1974). Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. *Journal of Laboratory and Clinical Medicine*, **84**, 559-573.
- Sanerkin, N. G., and Evans, D. M. D. (1965). The sputum in bronchial asthma: pathognomonic patterns. *Journal of Pathology and Bacteriology*, **89**, 535-541.
- Turnbull, L. S., Turnbull, L. W., Leitch, A. G., Crofton, J. W., and Kay, A. B. (1977). Mediators of immediate-type hypersensitivity in sputum from patients with chronic bronchitis and asthma. *Lancet*, **10**, 526-529.
- Wasserman, S. I., Goetzel, E. J., and Austen, K. F. (1973). Immunologic release of preformed eosinophil chemotactic factor of anaphylaxis (ECF-A) from isolated mast cells (Abstract). *Federation Proceedings*, **32**, 819.
- Wasserman, S. I., Goetzel, E. J., and Austen, K. F. (1975). Inactivation of slow reacting substance of anaphylaxis by human eosinophil arylsulfatase. *Journal of Immunology*, **114**, 645-649.
- Wasserman, S. I., Goetzel, E. J., Ellman, L., and Austen, K. F. (1974). Tumor-associated eosinophilotactic factor. *New England Journal of Medicine*, **290**, 420-424.
- Williams, D. A. (1961). Asthma and bronchitis with emphysema. *Acta Allergologica*, **16**, 400-406.

Requests for reprints to: Dr V. G. Vieira, Department of Medicine, Federal University of Rio Grande do Sul, Porto Alegre Brazil.