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## A STUDY OF INFECTIVE AND OTHER FACTORS IN EXACERBATIONS OF CHRONIC BRONCHITIS

VALENTINE U. MCHARDY, J. M. INGLIS, MARGARET A. CALDER, J. W. CROFTON

*Department of Respiratory Diseases, Edinburgh University;  
Regional Virus Laboratory and Bacteriology Laboratory, City Hospital, Edinburgh*

I. GREGG, D. A. RYLAND, P. TAYLOR, MAUREEN CHADWICK,  
DOREEN COOMBS AND R. W. RIDDELL

*Department of Clinical Epidemiology in General Practice, Cardiothoracic Institute;  
Department of Pathology, Brompton Hospital, London*

### INTRODUCTION

In a study of causative factors in exacerbations of chronic bronchitis carried out in 1965–8 in Edinburgh by Fisher et al. (1969), evidence of viral or bacterial infection was found in only 44% of the exacerbations investigated. Subsequent improvements in the techniques for identifying respiratory viruses made it seem desirable to carry out a further study, using the same general methods and, in particular, the same definition of an exacerbation. In order to investigate possible geographical differences, two groups of men were followed up in the present study, one in Edinburgh and one in London. Great care was taken to standardize the methods used in each centre.

### *Materials and Methods*

Thirty-eight men were studied, 18 in Edinburgh and 20 in London. All had 'chronic or recurrent mucopurulent obstructive bronchitis', as defined by the MRC (1965). Patients with bronchiectasis, arrested pulmonary tuberculosis or other disease causing widespread lung damage were excluded from the study. No patient admitted to the study was taking long-term prophylactic antibiotics.

Patients were excluded if their forced expiratory volume in one second (FEV<sub>1</sub>) increased by more than 30% within 20 minutes of two inhalations of salbutamol aerosol. The mean FEV<sub>1</sub> of the 38 patients on recruitment was 1.40 litres (SD 0.59 litre). After salbutamol, FEV<sub>1</sub> increased by less than 10% in 29 patients and by 11–29% in eight others. In the remaining patient FEV<sub>1</sub> increased by 33% but, because the absolute increase was only 0.20 litre from a resting value of 0.60 litre and also because he was a typical bronchitic in all other respects, he was retained in the study. Of the 38 patients 20 either had previously received a trial of prednisolone (at least 20 mg a day for two weeks) or were given such a trial on the completion of the study; in none was there any improvement in daily measurements of peak expiratory flow (PEF).

At the beginning of the study 29 (76%) of the 38 patients were aged 60–69 years, the other nine being aged 44–54 years. 26 patients were currently smoking, of whom three stopped during the study. Nine (24%) of the patients' households contained children.

The study covered the period January 1975 to April 1977 and included two complete winters. Four patients were withdrawn; one because of the development of carcinoma of the bronchus and three because they proved uncooperative. One patient moved to another district and was lost to follow-up. Two patients died during the survey (one from carcinoma of the oesophagus and one

from myocardial infarction). Data obtained from these seven patients during the periods in which they were followed up have been included in the analysis.

A detailed history was taken to ensure that patients fulfilled the criteria of the MRC definition of chronic or recurrent mucopurulent obstructive bronchitis and that they had no other respiratory condition which would have excluded them from the study.

Patients attended every four weeks for routine review. Enquiry was made about any change in their symptoms or smoking habits during the previous four weeks. They were asked to notify the coordinators as soon as they began a head cold or throat infection or if they considered that their habitual symptoms of expectoration or breathlessness had increased in severity. They were seen as soon as possible after the receipt of this notification, and were questioned about the following symptoms: coryza, sore throat, hoarseness, increased cough, increased wheeze, increased sputum and a change in colour of sputum to green or yellow. An exacerbation was deemed to have occurred if a patient reported either *an increase of cough or wheeze or a change in sputum colour, plus at least one other item on the above list, or an increase in sputum volume* (whether or not it was accompanied by anything else on this list).

At every routine attendance the following specimens were obtained: (a) an early morning specimen of sputum; (b) nasal and pharyngeal swabs; and (c) a specimen of blood. In exacerbations the same specimens were obtained and an additional blood specimen was taken two to three weeks later. Nasal and pharyngeal swabs were placed in a bijou bottle containing virus transport medium (Hanks' BSS with 0.2% bovine albumen, ampicillin 200 µg/ml, and penicillin 150 international units/ml) and were kept at 4°C until delivered to the laboratory. Sputum specimens were collected in sterile containers and were stored at room temperature. Whenever possible, specimens were delivered to the laboratory on the same day, but occasionally they had to be stored overnight. The laboratory methods used in Edinburgh and London were identical and are described below.

#### *Infection by viruses and other non-bacterial agents*

*Isolation.* Before inoculating specimens into cell cultures, the bottles were shaken to liberate cells from the swabs into the medium. Sputum was homogenized in Hanks' BSS with glass beads. Inoculation of the suspensions was made into the following cell cultures: secondary rhesus monkey kidney, diploid human embryo lung fibroblasts (MRC5) and human embryo kidney cells, all of which were maintained on a roller drum at 33°C. Inoculation was also made into cultures of HEp2 cells which were maintained stationary at 36°C. All cultures were observed for three weeks and, if there was no evidence of the presence of virus, discarded. If, however, the cells had degenerated a second passage in similar cells was made for at least a further week. Monkey kidney cultures were tested twice weekly for haemadsorption, using guinea pig erythrocytes. The diagnostic sera used for most identification purposes were supplied by the Standards Laboratory for Serological Reagents, Central Public Health Laboratory, Colindale. Rhinoviruses were identified by their cytopathic effect in MRC5 cell cultures and by their lability to acid. A selective diphasic medium, similar to that described by Chanock et al. (1967), was used for the isolation of *Mycoplasma pneumoniae*.

*Serology.* Complement fixation (CF) tests, were carried out against the following agents: influenza viruses (types A and B), Sendai virus, adenoviruses, respiratory syncytial virus, chlamydia group B, *Coxiella burnetii* and *M. pneumoniae*. Sera were screened at a dilution of 1:16. If positive, the patient's previous specimen was titrated in parallel. A fourfold or greater rise in titre was regarded as diagnostic of recent infection. A haemagglutination inhibition (HAI) test was used to identify infection by coronavirus strain OC43, the antigen being propagated in suckling mouse brain.

#### *Bacterial infection*

*Sputum culture.* The sample of sputum was homogenized by shaking with sterile distilled water and glass beads and inoculated on (a) blood agar, (b) heated blood agar (with bacitracin 10 units/ml), and (c) MacConkey agar. All plates were incubated in 10% carbon dioxide for 18 hours. Homogenized sputum was inoculated into adult mice by the intraperitoneal route. The sensitivity of organisms to ampicillin, tetracycline, benzyl penicillin and cotrimoxazole was assessed by Stokes' method, using the Oxford *Staphylococcus* as control organism.

*Immunology.* During the early part of the study, paired specimens of sputum and serum were obtained from certain patients both at routine attendances and in exacerbations and were sent to Middlesex Hospital Department of Immunology, where they were examined for antibodies of different classes to components of *Haemophilus influenzae* by radio-immunoassay (Bull & Smith 1980).

#### *Sputum cytology*

Before homogenization of the sputum sample, one film was made from its most purulent part and stained by Gram's technique. Two films were prepared and stained by the chromotrope 2R method. Microscopic purulence was graded as follows: M=no pus cells present; Pc+ = small number of pus cells (1 to 9 cells per oil immersion ( $\times 100$ ) field); Pc++ = moderate number of pus cells (10–19 cells/field); and Pc+++ = numerous pus cells (20 or more cells/field). Eosinophil polymorphonuclear leucocytes were counted and expressed as a percentage of the total number of leucocytes per high power field.

#### *Tests of ventilatory function*

Measurements of forced expiratory volume in 1 second (FEV<sub>1</sub>), forced vital capacity (FVC) and peak expiratory flow rate (PEF) were made at every routine attendance.

#### *Meteorological data*

Data relating to temperature, wind velocity and direction, and levels of smoke and sulphur dioxide were obtained from Edinburgh Airport and Kew Observatory.

## RESULTS

No differences emerged between Edinburgh and London in the characteristics of the patients studied, nor in the meteorological data obtained throughout the study. Therefore the findings from the two centres have been combined.

The study lasted 28 months. The total period of observation was 695 patient-months. The duration of observation of individual patients ranged between two and 27 months: in 53% of patients it was more than 20 months, and in 79% more than 12 months.

Patients were seen for monthly follow-up on 636 occasions. Exacerbations were reported on 116 occasions, 79 of which were notified within three days of the onset of symptoms. On 37 other occasions when patients attended for routine follow-up they reported that since their last attendance they had had symptoms which fulfilled our criteria of an exacerbation. These 'missed' exacerbations were not notified for a variety of reasons, such as absence on holiday, the imminence of their next routine attendance or the wish to avoid troubling their doctors (although they were reminded at every routine attendance to report any new symptoms at once).

#### *Distribution of exacerbations*

The distribution of all the 116 notified and missed exacerbations is shown in Table I. The larger number of exacerbations in London (70) than in Edinburgh (46) is largely accounted for by four men in London who had seven or eight exacerbations. Five men had no exacerbations during the study.

The majority (75%) of exacerbations occurred between October and March. Daily climatic data, including air temperature, wind velocity and direction and levels of

Table I. The distribution of all exacerbations (79 investigated and 37 'missed') which occurred in the course of the study

Exacerbations per patient	No. of patients			Total No. of exacerbations
	Edinburgh	London	Total	
0	2	3	5	0
1	2	1	3	3
2	6	3	9	18
3	5	5	10	30
4	0	2	2	8
5	2	2	4	20
6	0	0	0	0
7	1	2	3	21
8	0	2	2	16
Total	18	20	38	116

smoke and SO<sub>2</sub> were plotted but no correlation could be discerned between changes in any of them and the incidence of exacerbations.

Examination of the serial measurements of FEV<sub>1</sub> disclosed no instance in which a persistent fall occurred in relationship to an exacerbation.

#### *Infection by viruses and other non-bacterial agents* (Table II)

Specimens were obtained on the day of notification in all of the 79 notified exacerbations. Of the 37 'missed' exacerbations, seven had begun less than eight days previously and for the purpose of analysing the virological findings it was decided to add these seven to the 79 notified exacerbations, making a total of 86. The 30 remaining 'missed' exacerbations were added to the 599 routine attendances, making a total of 629.

Infection by respiratory viruses or other non-bacterial agents was identified in 17 (20%) of exacerbations and at 36 (6%) of routine attendances (Table II), the difference between these identification rates being highly significant ( $P < 0.001$ ). Of the 30 'missed' exacerbations included with the routine attendances (because they had begun eight or more days before being investigated), there was serological evidence of recent infection in seven (23%). None of the rises in titre against influenza A and B viruses were a consequence of immunization. In some of the cases when viruses were isolated at routine attendances, patients currently had symptoms of upper respiratory illness but in the majority infection was subclinical.

Rhinoviruses were by far the most commonly isolated agents in exacerbations, accounting for more than half of all viruses identified. There was a highly significant difference ( $P < 0.001$ ) between their isolation rates in exacerbations and at routine attendances. In contrast, no significant difference was found between exacerbations and routine attendances in the identification rates of either influenza A and B viruses or parainfluenza viruses.

#### *Purulence of sputum*

There was Grade 2 or 3 purulence in over half the specimens of sputum obtained in exacerbations and routine attendances. However, a significant difference ( $P < 0.005$ ) was

Table II. Respiratory viruses and other non-bacterial agents identified at exacerbations and routine attendances

	Exacerbations		Routine attendances	
	Isolations	Serological	Isolations	Serological
Influenza A virus	2	1	2	4
Influenza B virus	0	1	0	2
Influenza A + B virus	0	0	0	1
Parainfluenza virus 1 (Sendai)	1	0	4	1
Parainfluenza virus 3*	0	—	3	—
Rhinoviruses*	11	—	5	—
Echoviruses*	0	—	2	—
Coxsackie A virus*	0	—	2	—
Respiratory syncytial virus	0	0	0	2
Adenoviruses	0	0	2	1
Coronavirus OC43†	—	1	—	2
<i>Chlamydia psittaci</i> †	—	0	—	1
<i>Coxiella burnetii</i> †	—	0	—	1
<i>Mycoplasma pneumoniae</i>	0	0	0	1
	14	3	20	16
Total identifications	17		36	
Total number of episodes investigated	86		629	
Percentage in which viral infection was identified	20%		6%	

\*Specific serological tests not undertaken. Complement fixative tests not applicable.

†These agents cannot be isolated in cell culture under conventional laboratory conditions.

found between the purulence rate in exacerbations (73%) and that in routine attendances (53%). Although the isolation rate was higher in the most purulent sputum from exacerbations no statistically significant correlation was detected between the grades of purulence and the isolation rates either during exacerbations or at routine attendances.

#### Bacterial infection: sputum culture (Fig. 1; Table III)

Because there is general agreement that *H. influenzae* and *Streptococcus pneumoniae* are the most important pathogenic bacteria found in chronic bronchitis (May 1972) and because very few isolations were made of any other bacteria only these two bacteria have been considered in analysis.

The bacteriological findings in all 37 'missed' exacerbations have been included with those of routine attendances, since organisms identified at a subsequent routine attendance could not confidently be attributed to the preceding exacerbation.

In notified exacerbations *H. influenzae* or *S. pneumoniae* was identified in 23 (30%) of 77 exacerbations and in 135 (22%) of 628 routine attendances, there being no significant difference between these isolation rates. In only four exacerbations in which a virus was isolated was there evidence of simultaneous bacterial infection.

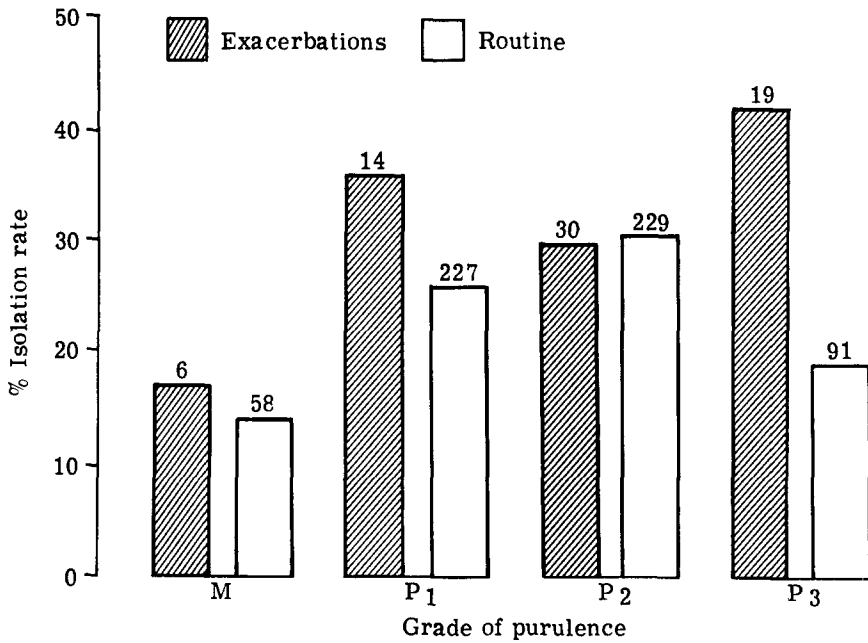


Fig. 1. Isolation rates of pathogenic bacteria in sputum, according to grade of purulence, in exacerbations and at routine attendances. The figures above each column denote the total number of specimens examined

Table III. 'Pathogenic' bacteria isolated from sputum in investigated exacerbations and at routine follow-up

Bacterial	Exacerbations (n = 77)	Routine specimens (n = 628)
<i>H. influenzae</i>	9 (12%)	69 (11%)
<i>S. pneumoniae</i>	11 (14%)	48 (8%)
<i>H. influenzae</i>	} 2 (3%)	14 (2%)
<i>S. pneumoniae</i>		
<i>Staph. pyogenes</i>	1 (1%)	4 (1%)
Total	23 (30%)	135 (22%)

The difference in isolation rates between exacerbations and routine specimens was not significant.

*Bacterial infection: immunology*

Preliminary analysis showed that antibodies in serum to the H<sub>1</sub> antigen of *H. influenzae* (Burns & May 1967) were present in high titre in the majority of patients. In a few patients sharp rises in titre accompanied exacerbations. The findings will be reported in detail elsewhere together with those from sputum.

### *Sputum and blood eosinophilia*

Of the 38 patients, 33 (87%) had a sputum eosinophilia greater than 10% on at least one occasion. Eosinophil counts were generally higher at routine attendances than in exacerbations: thus, at routine attendances 22 (58%) of the patients had a greater than 50% eosinophilia on at least one occasion, whereas in exacerbations eosinophilia of this degree was found in only six (21%) of the 29 men who had exacerbations during the study. There was no correlation between sputum eosinophilia and viral infection. Most of the patients who had the highest eosinophil counts had negative skin tests against common allergens.

There was no obvious consistency in the pattern of sputum eosinophilia. Few patients had a persistently elevated eosinophilia and in most cases high counts were found at two or three consecutive routine attendances to be followed by several months during which no, or few, eosinophils were present.

Absolute eosinophil counts in the blood did not reflect the sputum eosinophilia. In only four patients did the former exceed  $500 \times 10^9$  cells/litre and in the majority it was less than  $250 \times 10^9$  cells/litre.

### *Skin prick tests*

These were performed in 31 patients, using solutions of housedust, housedust mite, grass pollens, animal hairs and dander, *Aspergillus fumigatus* and control. One or more positive reactions were obtained in six (19%) of those tested. Three of the patients with positive reactions frequently had high eosinophil counts in the sputum whereas the other three had very low counts throughout the study.

### *Mediators of hypersensitivity reactions in sputum*

Examination of sputum from the Edinburgh patients disclosed variable levels of histamine, SRS-A and IgE: these findings have been reported elsewhere (Turnbull et al. 1977; 1978b).

## DISCUSSION

The principal aim of this study was to clarify the role of infection in exacerbations of chronic bronchitis. It was disappointing, therefore, that the identification rates of viruses and bacteria in exacerbations were lower than we had hoped. Nevertheless, the isolation rate of viruses in this study (20%) was an improvement on that obtained in the previous study (14%) carried out in Edinburgh in 1965-8 (Fisher et al. 1969).

The two most notable findings to emerge from this study were the association between rhinovirus infection and exacerbations and the frequency of high eosinophil counts in sputum during remissions in contrast to the generally low counts found in exacerbations. Comparisons of the findings of the present study with those of the earlier study in Edinburgh carried out ten years previously are also of some interest.

The severity of chronic bronchitis in the men selected for the present study was similar to and possibly greater than that of the men studied in 1965-8, but the mean age was higher with 76% older than 60 years. In the previous study only 44% of the men



were aged more than 60 years and over half of them had participated in a trial of 'early' chronic bronchitis (Medical Research Council 1966) a few years beforehand.

A striking difference between the two studies was the frequency with which purulent sputum (grades 2 and 3) was found, particularly at routine attendances (Fig. 1). In the earlier study only 17% of routine specimens were purulent, whereas 53% were purulent in the present study ( $P < 0.001$ ). In exacerbations 52% of specimens were purulent in the earlier study and 73% in the present study ( $P < 0.02$ ). These differences may have resulted from long-term antibiotic prophylaxis which was taken throughout the winter by over half of the patients in 1965-8 but by none in the present study.

Another difference between the studies may also be attributable to long-term antibiotic therapy. In 1965-8 the isolation rate of pathogenic bacteria at routine attendances was only 9%, compared with 22% in the present study. However, while antibiotic prophylaxis may have suppressed persistent infection, it would appear to have been far less effective in preventing acute bacterial infection at exacerbations, for the isolation rate was 39% in the previous study. In the present study, in which long-term chemotherapy was not used, the isolation rate in exacerbations was 30%. It is difficult to explain why this isolation rate was not higher and was inconsistent with the high rate of sputum purulence.

The identification rate of viruses and other non-bacterial agents achieved in the present study is similar to that obtained in most other similar studies. The most important finding was the relatively large number of rhinoviruses isolated and it is possible that even more might have been isolated if WI 38 cell culture, which is particularly sensitive to rhinoviruses, had not ceased to be available shortly before the study began.

An association between infection by rhinoviruses and acute episodes of bronchitis and asthma has been reported by other workers, both in adults (Eadie et al. 1966; Stenhouse 1967; Lambert & Stern 1972; Gregg 1975; Minor et al. 1976) and in children (Gregg 1970; Minor et al. 1974*a, c*; Mitchell et al. 1978; Horn et al. 1979).

The mechanisms whereby respiratory virus infection provokes bronchitis and/or wheeze have been discussed by Gregg (1975) and Horn et al. (1979), who postulated that one essential factor must be some defect in the defences of the lower respiratory tract against viral infection. The highly significant difference between the isolation rates of rhinoviruses in exacerbations and routine attendances suggests that in patients with chronic bronchitis infection by rhinoviruses is seldom confined to the upper respiratory tract and that these agents have a particular tendency to provoke an exacerbation. It seems probable that their importance is due to their large number of antigenically distinct serotypes (Roebuck 1976) and the absence of cross-immunity between them (Minor et al. 1974*b*; Horn et al. 1979). Thus, if a bronchitic is infected by a rhinovirus he would be unlikely to escape an exacerbation unless he was immune as a result of a recent infection with that particular serotype.

Lambert and Stern (1972) drew attention to the importance of family structure as a determinant of the risk of infection in patients with chronic chest disease. In both the previous and the present study a quarter of the patients had children in their households. In the study by Eadie et al. (1966), in which they demonstrated rhinovirus infection in 23% of exacerbations in chronic bronchitics, a higher proportion (39%) of households contained children.

Influenza is well recognized to be a danger to chronic bronchitics and it was surprising

that infection by influenza A and B viruses seldom occurred in either the Edinburgh or London patients, despite epidemics during two of the three winters of the present study. Whereas in the previous study these viruses accounted for almost half of all those identified in exacerbations, they were seldom associated with exacerbations in the present study, despite the fact that only four patients received influenza vaccine during the study.

Although sputum eosinophilia is commonly believed to be a distinguishing feature between chronic obstructive bronchitis and asthma, other workers have also found sputum eosinophilia in patients with chronic respiratory disease who did not apparently have asthma. In 30 patients, described as having emphysema, Cole et al. (1959) examined sputum daily for 28 consecutive weeks. In half the patients they observed periodic 'showers' of eosinophils with counts between 40% and 100% which lasted a few days and occurred every few weeks. Their observations were similar to our own, as was their failure to find any correlation between eosinophilia and changes in symptoms or meteorological conditions. Miller (1963) studied 1174 working men and found that 39% of them had sputum eosinophilia at some time. O'Connell et al. (1978) studied 115 men with chronic bronchitis, asthma or both to determine the diagnostic, therapeutic and prognostic implications of sputum eosinophilia. They, too, found no correlation with positive skin tests. They concluded that sputum eosinophilia was of little value for distinguishing between chronic bronchitis and asthma and they postulated the existence of local allergic and non-allergic factors which might be responsible for eosinophil release in the bronchi.

In the present study patients were carefully screened to exclude any with asthma and were given a trial of steroids which showed that none had latent reversibility of airflow obstruction. Our finding of the high frequency of sputum eosinophilia led to subsidiary studies by Turnbull et al. (1977, 1978*a, b*). They demonstrated that IgE and mediators of type 1 hypersensitivity were frequently present in the sputum of chronic bronchitics and they also showed a significant inverse relationship between the concentration of these mediators and changes in airflow obstruction, measured by serial PEF. Our findings and theirs suggest that hypersensitivity reactions may play a part in at least some exacerbations of chronic bronchitis. This possibility needs to be explored further.

The rate of exacerbations per patient-month of observation in the recent study was not significantly different from that in 1965-8. In both there was a clear tendency for exacerbations to occur during the winter months, but it was impossible to discern any relationship between their incidence and changes in various meteorological indices including atmospheric pollution.

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*Requests for reprints to Dr Ian Gregg, Cardiothoracic Institute, Fulham Road, London SW3 6HP.*

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