Prognostic value of acetylcholine challenge test: a prospective study

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ABSTRACT Eleven hundred and nine iron mine workers aged 35 to 55 with normal chest radiographs were submitted to a pulmonary examination consisting of a questionnaire, a clinical examination, and pulmonary function testing including an acetylcholine challenge test. A positive response (decrease of FEV₁ of more than 10%) was observed in 210 subjects (Ace⁺). The remaining 899 had a negative response (Ace⁻). Bronchitis, asthma, dyspnoea, and obstructive syndrome were more frequent in the Ace⁺ group. Five years later, 820 subjects were re-examined: occasional cough and sputum and chronic bronchitis appeared more frequently among subjects without symptoms at the first examination but with a positive acetylcholine challenge test. The obstructive syndrome was more often observed and regressed more rarely in the Ace⁺ group. The results confirm the use of a test of bronchial hyperreactivity as a means of identifying subjects at risk from chronic obstructive lung disease.

The concept that bronchial hyperreactivity was important in the evolution of chronic obstructive lung disease (COLD) was introduced by Dutch investigators.^{1,2} Pharmacodynamic tests using acetylcholine, metacholine, or histamine may be used to evaluate the degree of hyperreactivity.³⁻⁶ Initial work by Tiffeneau *et al*³⁻⁶ on the ventilatory effect of acetylcholine concerned asthmatic subjects during remission. It is now known, however, that bronchial hyperreactivity may occur not only in asthma but also in other conditions as a response to inhaled irritants and infections. The mechanism of this hyperreactivity is complex and not yet fully understood; nevertheless, it remains a criterion to be taken into account when evaluating the diagnosis and prognosis of COLD, the decrease in forced expiratory volume in one second (FEV₁) being correlated with the degree of methacholine reactivity as was pointed out by Barter and Campbell⁷ and Minette⁸ in their longitudinal studies of bronchitic patients.

In 1973 a recommendation was made by the working group on "functional respiratory tests in

epidemiology" of the European Society for Clinical Respiratory Physiology at a meeting held in Laanaken to include a pharmacodynamic test (the acetylcholine challenge test) in the set of respiratory function tests to be used in epidemiology.

Therefore, in order to explore the predictive value of an acetylcholine challenge test we have performed it in an epidemiological survey of iron miners and related the results to the evolution of clinical and functional pulmonary symptoms five years later.⁹

Material and methods

In 1975 (first examination) 1109 workers employed in iron mines, aged 35 to 55, with a normal chest radiograph, were submitted to a bronchial challenge test with acetylcholine that was performed at the end of all examinations planned for the epidemiological survey. Each subject inhaled a one in a thousand acetylcholine aerosol delivered by a Gauchard AMP 7 nebuliser for three minutes. The amount of acetylcholine delivered during this time was 1200 µg. More than 90% of the particles issued from the nebuliser are below 5 µ in size. (After explanation, all the subjects agreed to the test. Precautions had been taken to avoid any incident and

none occurred; some mild bronchospasm was rapidly alleviated with a salbutamol inhaler.)

Immediately after the aerosol inhalation, each subject was asked to perform three forced expiratory volumes to measure FEV₁, and the best value was compared with that obtained before the inhalation of acetylcholine. Decreases exceeding 10% were considered as a positive response, indicating a bronchial hyperreactivity.*

Other examination undertaken during the survey were as follows:

(a) Questionnaire on chronic bronchitis and emphysema of the European Community of Steel and Coal (ECSC) derived from that of the Medical Research Council, with additional questions concerning work hazards.

(b) Clinical examination (including chest and heart auscultation and measurement of systemic arterial pressure).

(c) Spirometric measurements: three vital capacity (VC) and three FEV₁ were performed using a 9 l watersealed spirometer (Boulitte N). The largest values of VC and of FEV₁ were taken.

(d) Functional residual capacity (FRC), residual volume (RV), TCO/ml/min Torr, and fractional uptake of CO (FuCO) by the steady state method were obtained using the Pneumotest Alveotest Jaeger apparatus.

The same procedures were repeated five years later (second examination) in 820 subjects by the same investigators with the same apparatus.

Table 1 Characteristics of groups at first examination

	Negative <i>Ace</i> ⁻ (<i>n</i> = 899)	Positive <i>Ace</i> ⁺ (<i>n</i> = 210)
Age (years) $\bar{x}$ SD	45.6 4.8	46.4 4.6
Smoking habits (% of each group):		
Non-smokers	16.8%	10.5%
Ex-smokers	10.1	12.9
Smokers < 20 cig/day	39.9	40.0
Smokers > 20 cig/day	29.6	32.9
Duration of work in mines (years):		
<20	12.7	10.5
20-29	73.0	78.1
>30	14.3	11.4

No significant difference is observed between the two groups.

*The limit of 10% was chosen on the basis of the histogram of FEV₁ variation after acetylcholine that showed a bimodal distribution and a clear cut off at this limit. It corresponded also to an approximate doubling of the coefficient of variation of FEV₁ observed in normal subjects by the same technicians with the same apparatus.¹⁰

Results

In 1975, 899 subjects were found to have a negative response to acetylcholine challenge and 210 (19%) a positive one. For the sake of clarity, subjects with a negative test will be given the symbol *Ace*⁻ and those with a positive result *Ace*⁺.

Age, smoking habits, and duration of work did not differ significantly between the two groups (table 1). The percentage of above ground workers (having never worked underground) is higher in *Ace*⁻ (15.4%) than in *Ace*⁺ (6.7%) group (table 2).

Bronchitis, asthma, dyspnoea, and the obstructive syndrome (VC > 80% of theoretical values and FEV₁/VC < 65%) were more frequent in group *Ace*⁺, whereas no significant difference was seen for the restrictive syndrome (VC < 80% and FEV₁ < 80% of theoretical values) (tables 3 and 4).

Five years later, the percentage of subjects dead, lost, or re-examined was similar for *Ace*⁻ and *Ace*⁺ groups (3.34% v 2.86% deceased, 11.57% v 9.05% lost, 11.46% v 12.46% answered to a questionnaire but not re-examined, 73.64% v 75.24% re-examined).

Table 2 Work history of groups at first examination

	<i>Ace</i> ⁻ (<i>n</i> = 899)	<i>Ace</i> ⁺ (<i>n</i> = 210)
Percentage of time of work underground:		
0	15.4	6.7
<25	5.1	5.2
25-50	8.9	8.6
50-75	13.9	15.2
>75	56.7	64.3

χ^2 test *p* < 0.05

Table 3 Percentage occurrence of clinical symptoms in each group at first examination

	<i>Ace</i> ⁻ (<i>n</i> = 899)	<i>Ace</i> ⁺ (<i>n</i> = 210)
Cough and sputum:		
No symptoms	57.0	38.1
Occasional cough and/or sputum	24.6	25.2
Chronic bronchitis*	23.4	36.7
χ^2 test <i>p</i> < 0.001		
Asthma:		
No symptoms	89.3	75.7
Wheeze	7.2	12.9
Asthma†	3.5	10.5
χ^2 test <i>p</i> < 0.0001		
Dyspnoea:		
No dyspnoea	72.6	53.3
D grade 1	13.5	22.9
D grade ≥ 2	7.9	12.9
χ^2 test <i>p</i> < 0.001		

Chronic bronchitis,* asthma,† are defined according to definitions of American Thoracic Society 1962.

Table 4 Functional abnormalities observed at first examination. Percentage in each group

	Ace ⁻ (n = 899)	Ace ⁺ (n = 210)
Spirometry:		
Normal	97.6	85.2
Restrictive syndrome*	1.3	2.4
Obstructive syndrome†	1.1	12.4

χ^2 test $p < 0.001$

*Restrictive syndrome = VC < 80% of theoretical values.

†Obstructive syndrome = FEV₁/VC ratio < to 80% of theoretical values.

Among the 820 subjects re-examined 662 were Ace⁻ and 158 Ace⁺. The percentage of retired subjects is higher in the Ace⁺ group especially for those who worked above ground (51.9% v 33.6%). In

the second examination occasional cough and sputum and chronic bronchitis were more frequent among the subjects in the Ace⁺ group (table 5). Among subjects who were wheezing in 1975, asthma appears more often in the Ace⁺ than in the Ace⁻ subjects (table 6). Dyspnoea is more frequent in the Ace⁺ group than in the Ace⁻ (table 7) and the obstructive syndrome appears more frequently and regresses more rarely in the Ace⁺ group (table 8). No difference was observed in the evolution of the restrictive syndrome or in abnormalities of the RV or CO tests between the groups.

The reduction of tobacco consumption was similar in the two groups. Although there is a higher proportion of retired subjects in the Ace⁺ group who would not be subjected to occupational

Table 5 Evolution of symptoms: cough and sputum

	ACE ⁻				ACE ⁺		
	No symptoms (n = 342) (100%)	1st examination Occasional cough and/ or sputum (n = 170) (100%)	Chronic bronchitis (n = 141) (100%)		No symptoms (n = 61) (100%)	1st examination Symptoms ± (n = 41) (100%)	Chronic bronchitis (n = 56) (100%)
2nd examination				2nd examination			
No symptoms	75.2	46.5	24.8	0	60.7	61.0	33.9
± symptoms	17.0	31.2	27.0	±	24.6	22.0	21.5
Chronic bronchitis	7.9	22.3	48.2	Chronic bronchitis	14.7	17.0	44.6

χ^2 test $p < 0.01$

Table 6 Evolution of symptoms: asthma

	ACE ⁻				ACE ⁺		
	No symptoms (n = 594) (100%)	1st examination Wheeze (n = 50) (100%)	Asthma (n = 17) (100%)		No symptoms (n = 119) (100%)	1st examination Wheeze (n = 22) (100%)	Asthma (n = 15) (100%)
2nd examination				2nd examination			
No symptoms	92.5	72.0	35.3	No symptoms	91.5	59.1	46.7
Wheeze	4.7	16.0	29.4	Wheeze	5.9	13.6	0
Asthma	2.8	12.0	35.3	Asthma	2.6	27.3	53.3

χ^2 test $p < 0.001$

Table 7 Evolution of symptoms: dyspnoea

	ACE ⁻				ACE ⁺		
	No dyspnoea (n = 478) (100%)	1st examination Dyspnoea grade 1 (n = 84) (100%)	Dyspnoea grade ≥ 2 (n = 51) (100%)		No dyspnoea (n = 85) (100%)	1st examination Dyspnoea grade 1 (n = 37) (100%)	Dyspnoea grade ≥ 2 (n = 20) (100%)
2nd examination				2nd examination			
No dyspnoea	79.2	59.5	39.2	No dyspnoea	76.4	37.8	25.0
Dyspnoea Grade 1	13.4	31.0	37.3	Dyspnoea Grade 1	14.1	48.6	45.0
Grade > 2	7.4	9.5	23.5	Grade ≥ 2	9.5	13.6	30.0

χ^2 test $p < 0.01$

Table 8 Evolution of lung function

	ACE ⁻			ACE ⁺	
	No obstructive syndrome (n = 635) (100%)	1st examination Obstructive syndrome (n = 7)		No obstructive syndrome (n = 135) (100%)	1st examination Obstructive syndrome (n = 15) (100%)
2nd examination			2nd examination		
No obstructive syndrome	97.6	42.8	No obstructive syndrome	90.3	20.0
Obstructive syndrome	2.4	58.2	Obstructive syndrome	9.7	80.0

 χ^2 test $p < 0.001$

hazards, the decrease of FEV₁ was significantly greater in the Ace⁺ group (fig).

Discussion

The results of the challenge test observed at first examination appear to be related to other symptomatic (bronchial) and functional (obstructive syndrome) manifestations. Similar observations were made by Minette *et al*¹¹ and Guerrin *et al*¹² in coal miners and by Navratil *et al*¹³ and by Bouhuys *et al*¹⁴ in cotton mill workers. The choice of 10% (or more) fall of FEV₁ as the limit for a positive response to the challenge test may be discussed. Though Tiffeneau and Drutel in the first work on challenge test proposed this limit,⁶ others actually preferred a limit of 20%.^{14,15} If this limit had been applied to our study only 54 subjects would have fulfilled this criterion of positive response to the challenge test. Thus in order to increase the number in the Ace⁺ group the limit of 10% fall in FEV₁ after the challenge test was chosen. This limit corresponds with the one recommended by Eiser *et al*.¹⁷

More than half the subjects (54%) who had a "positive" response at the first examination became "negative" at the second examination five years later (table 9). Despite this, the test seems to have a prognostic value. Cough and sputum as symptoms of

Mean values of FEV₁ and FEV₁/VC are lower at first and second examinations in Ace⁺ group. Decrease of mean values is significantly higher for FEV₁/VC in the Ace⁺ group. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

tructive syndrome is greater in the Ace⁺ group. The FEV₁ and FEV₁/VC decreased more between the two periods in the Ace⁺ group (but it is statistically significant only for FEV₁/VC). This finding is similar to, but not so obvious, as that of Minette *et al*¹¹ in bronchitic coal miners and of Barter and Campbell¹⁷ in patients with chronic bronchitis. Britt *et al*, during a four year survey of siblings of patient with COLD, reported that the loss of lung function was five times greater in those who had hyperreactive airways than in those without hyperreactivity.¹⁸ All these results justify the use of a challenge test in epidemiological surveys to show bronchial hyperreactivity, even if only a single dose of acetylcholine is used, as in this study. It may contribute to the identification of subjects at risk from COLD.

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Table 9 Evolution of bronchial reactivity

1st examination	2nd examination
Ace ⁻ n = 662 (100%)	Ace ⁻ 85.4%
	Ace ⁺ 14.6%
Ace ⁺ n = 158 (100%)	Ace ⁻ 54.0%
	Ace ⁺ 46.0%

chronic bronchitis developed more frequently in the subjects with a positive response to the challenge test and the stability of symptoms and of the obs-

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