Thorax Online First, published on November 6, 2008 as 10.1136/thx.2008.102079

Plasma markers of inflammation and incidence of hospitalizations due to chronic obstructive pulmonary disease.

Results from a population-based cohort study

Short title: Inflammation and COPD hospitalization

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Abstract

BACKGROUND The relationship between plasma markers of inflammation and incidence of chronic obstructive pulmonary disease (COPD) is still unclear. This population-based study explored whether elevated levels of five inflammation-sensitive plasma proteins (ISPs) predicted COPD hospitalizations during 25 years of follow-up.

METHODS Spirometry and measurements of five ISPs (fibrinogen, ceruloplasmin, α1-antitrypsin, haptoglobin, orosomucoid) was performed in 5247 apparently healthy men from the city of Malmö (mean age 46 years). Incidence of hospitalizations due to COPD was studied in relation to the number of ISPs in the 4th quartile.

RESULTS During the follow-up, 258 men were hospitalized due to COPD, 211 of them were smokers at baseline. Incidence of COPD hospitalizations was significantly associated with the number of elevated ISPs. Adjusted for risk factors, the hazards ratio (95% CI) was 1.00 (reference), 1.28 (0.9-1.9), 1.29 (0.8-2.0) and 2.30 (1.6-3.2), respectively, for men with none, one, two and three or more ISPs in the top quartile (p for trend<0.001). This relationship was consistent both in men with high and low lung function at baseline. The relationship with incidence of COPD hospitalizations was largely the same for all individual ISPs.

CONCLUSION Elevated ISP levels in plasma are associated with increased incidence of COPD requiring hospitalization.

Key words: Epidemiology, inflammation, chronic obstructive pulmonary disease, FEV1

Introduction

The relationship between reduced lung function and systemic low-grade inflammation is now widely accepted (1). Increased plasma markers of various acute phase proteins have been observed in patients with COPD and in apparently healthy subjects with reduced FEV1 or FVC (2-8). However, the temporal relationship between systemic low-grade inflammation and development of COPD is still unclear. Few have explored whether increased plasma levels of acute phase proteins could predict development of COPD.

In a study from the CARDIA cohort, fibrinogen was associated with FEV1 decline over 5 years of follow-up (9). Fibrinogen (3) and CRP (8) have been associated with incidence of COPD hospitalizations in the Copenhagen Heart Study. In two recent studies of CRP in relation to FEV1 decline, no significant relationships were reported (10,11). A recent report from the Cardiovascular Health Study showed that higher levels of fibrinogen, but not CRP, independently predicted greater FEV1/FVC decline in the elderly (12).
Previous studies from the Malmö Preventive study have shown that increased plasma levels of fibrinogen, ceruloplasmin, haptoglobin, α1-antitrypsin and orosomucoid are associated with increased incidence of cardiovascular diseases (4,13), and that the risk of disease increases cumulatively with the number of elevated acute phase proteins. Cross-sectional analyses of these markers of inflammation have shown that they are inversely related to lung function (4). In the present study, we explore whether these proteins are risk factors for incidence of COPD hospitalizations over a mean follow-up of 25 years.

Methods

Between 1974 and 1984, 22444 men participated in a screening program for detection of individuals with high risk for cardiovascular diseases (14). Complete birth cohorts from the city of Malmö were invited. Participation rate was 71%. Determination of 5 plasma proteins was part of the program for 6193 men. These men were randomly selected from birth cohorts examined between 1974 and 1982. After exclusion of men with a history of myocardial infarction, stroke or cancer (according to questionnaire), 6075 men remained. FEV1 and FVC were available for 5452 men. Men with incomplete information about former smoking (n=147), men who reported that they were controlled for a pulmonary disease in a lung clinic (n=40) and men with a previous hospitalization due to COPD (n=18) were excluded. A total of 5247 men remained for the analysis. Mean age was 46.9±3.6 years (range: 28-61). The health service authority of Malmö approved and funded the screening program. All participants gave informed consent.

Baseline examinations
Subjects were categorized into smokers and non-smokers. The smokers were categorised into consumers of up to 9 cigarettes per day, 10-19 cigarettes and daily consumption of 20 cigarettes or more (15).
Body mass index (BMI) was calculated as weight/height^2 (kg/m^2).
Physical inactivity in spare time was assessed using the question 'Are you mostly engaged in sedentary activities in spare time, for example, watching TV, reading, going to the movie?'
Respiratory symptoms were assessed by a self-administered questionnaire. Individuals who reported that they recently or previously had had episodes with chronic productive cough lasting more than 3 months, more than 2 consecutive years were regarded as having symptoms of chronic bronchitis (16).

Two questions were used to identify subjects with asthma or allergy: 'Have you had asthma symptoms after age of 20?' and 'Do you know or do you suspect that you have asthma, allergic rhinitis, allergic eczema, or allergic swelling of mucous membranes?' A total of 346 men answered 'yes' on any of the two questions.

FVC and FEV1 were measured using a Spirotron apparatus (Drägerwerk AG, Lübeck, Germany) with the subjects in a standing position without noseclips. Specially trained nurses performed the tests. One acceptable manoeuvre, with respect to the subject's performance and co-operation, was required. The volumes were standardised for age and height using equations derived from linear regressions of 3467 male never smokers in the present cohort (16). The following equations were used:

Predicted FEV1 (L)= 4.422*height (m)-0.038*age-2.48
Predicted FVC (L)=6.58*height (m)-0.033*age-5.54
Predicted FEV1/FVC (%) = 1.156-0.1556*height (m) -0.002025*age

Age and height were essentially unrelated to the lung volumes after adjustments. FEV1 and FVC are expressed as % of the predicted values.

Inflammation-sensitive proteins (ISPs)
Electroimmuno assay was used to assess the plasma levels of acute phase proteins (17). The analysis was performed consecutively at the time of study entry. The detection limits were 20 mg/L for ceruloplasmin, 50 mg/L for α1-antitrypsin and 350 mg/L for orosomucoid, haptoglobin and fibrinogen. The coefficient of variation was <5% (17). Median (interquartile range, g/L) for the ISPs were 3.46 (3.0-4.0) for fibrinogen, 0.80 (0.67-0.93) for orosomucoid, 1.28 (1.09-1.42) for α1-antitrypsin, 1.30 (0.89-1.75) for haptoglobin and 0.30 (0.26-0.35) for ceruloplasmin.
The relationships between the ISPs and other risk factors, eg, smoking (4,13,15) have been reported previously. We have reported that all 5 ISPs are associated with different cardiovascular diseases and that the hazard ratios (HR) are approximately the same for all ISPs (13). A composite score, ie, the number of ISPs in the 4th quartile, was therefore constructed from these proteins. Cronbach's alpha for this score is 0.65 in this sample. This score has been associated with cardiovascular diseases in many previous studies (e.g., 4,13,15).

Follow-up
The Swedish hospital discharge register was used for case retrieval. This register has been operating in the south of Sweden during the entire follow-up period and covers the whole Sweden since 1987. All men were followed from the baseline examination until hospitalization due to COPD, death, emigration from Sweden, or December 31st, 2005, whichever came first. A COPD hospitalization was defined as cases with a discharge diagnosis of COPD [490-492, 496 (ICD-9) or J40-J44 (ICD-10)] as one of the first three listed diagnoses. In addition, COPD hospitalizations with COPD as the primary diagnosis were analyzed.

Statistics
Correlations between ISPs and lung function variables were assessed using Pearsons' correlation coefficients. One-way ANOVA and logistic regression was used to compare distribution of risk factors in relation to the number of elevated ISPs. Kaplan-Meier test with

log-rank statistics and Cox proportional hazards regression was used to study incidence of COPD hospitalizations in relation to ISPs. The covariates for the Cox model were selected a priori from factors that are know to be associated with low-grade systemic inflammation and incidence of COPD. Age, BMI, FEV1 (% of predicted values) were modelled as continuous variables. Current smoking, former smoking, chronic bronchitis and the number of elevated ISPs were modelled as categorical variables. P for trend was calculated by modelling the number of elevated ISPs as an ordinal variable. The fit of the proportional hazards model was confirmed by plotting incidence of COPD over time for the different risk factors. There was no indication that the proportionality assumption was violated. SPSS software (v 11.5) was used in all statistical calculations.

Results

Baseline characteristics

The baseline characteristics of the cohort, in relation to the elevated ISPs, are presented in Table 1.

Table 1. Baseline characteristics in relation to number of elevated ISPs.

	Number of elevated ISPs			
	None n=2023	One n=1402	Two n=794	Three or more n=1028
Age (years)	46.6±3.4	46.9±3.7	47.0±3.4	47.3±4.0
Height (cm)	177±7	176±7	176±6	176±7
BMI (kg/m^2)	24.8±3.1	25.1±3.3	25.1±3.4	25.1±3.7
Smokers (%)	29	48	61	74
>20 cigarettes/d (% of smokers)	19	23	25	31
FEV1 (% of predicted)	98±18	95±18	92±17	89±19
FVC (% of predicted)	100±17	97±16	95±16	93±17
Chronic bronchitis (%)	2.6	3.6	4.2	6.1
Asthma/allergy (%)	7.2	6.3	6.3	5.9

Presented as mean values (± standard deviation) or proportions (%).

Table 2 presents the correlation coefficients between the individual ISPs, FEV1 and FVC. FEV1 and FVC were inversely related to the ISPs, in all men and in separate analysis of non-smokers.

Table 2. Correlations between lung function and plasma proteins.

All men (n=5247)

	α1-antitrypsin	orosomucoid	ceruloplasmin	fibrinogen	haptoglobin
FEV1 (% predicted)	-0.13	-0.16	-0.14	-0.13	-0.17
FVC (% predicted)	-0.12	-0.13	-0.12	-0.13	-0.15
α1-antitrypsin	1	0.34	0.33	0.32	0.26
Orosomucoid		1	0.54	0.41	0.44
Ceruloplasmin			1	0.37	0.34
Fibrinogen				1	0.43

Non-smokers (n=2749)

	α1-antitrypsin	orosomucoid	ceruloplasmin	fibrinogen	haptoglobin
FEV1 (% predicted)	-0.08	-0.13	-0.09	-0.10	-0.10
FVC (% predicted)	-0.10	-0.12	-0.10	-0.11	-0.11
α1-antitrypsin	1	0.27	0.27	0.26	0.18
Orosomucoid		1	0.52	0.37	0.38
Ceruloplasmin			1	0.33	0.28
Fibrinogen				1	0.35

All correlations are significant at the 0.01 level (2-tailed).

Incidence of COPD hospitalizations

During the mean follow-up of 25 years, 258 men were hospitalized with a discharge diagnosis of COPD and 132 of them had COPD as the primary diagnosis. Incidence of COPD hospitalizations was strongly related to the number of elevated ISPs (Figure, Table 3). The increased incidence in men with three or more elevated ISPs persisted after adjustments for possible confounding factors (Table 3). Of the variables in the final Cox regression model, age (Hazards Ratio, HR, per 1 year:1.10, CI:1.06-1.14), BMI (HR per 1 kg/m^2:0.95, CI:0.91-0.98), chronic bronchitis (HR: 2.1, CI: 1.4-3.0), FEV1 (% pred) (HR per 1 %: 0.96, CI: 0.95-0.96) and current smoking (HR: 3.6 CI: 2.2-5.8) were significantly associated with incidence of COPD hospitalizations.

Table 3. Incidence of COPD hospitalizations during 25 years of follow-up in relation to number of elevated ISPs (i.e., in the fourth quartile).

	Number of elevated ISPs				
	None n=2023	One n=1402	Two n=794	Three or more n=1028	P, trend
COPD n (per 1000 py)	52 (1.0)	61 (1.7)	37 (2.0)	108 (4.6)	
Age-adjusted HR (95% CI)	1.0	1.76 (1.2-2.5)	2.03 (1.3-3.1)	5.08 (3.6-7.1)	<0.001
¶Risk factor-adjusted HR (95% CI)	1.0	1.28 (0.88-1.9)	1.29 (0.84-2.0)	2.30 (1.6-3.2)	<0.001

¶ Adjusted for age, current and former smoking, BMI, FEV1 (% of predicted values) and chronic bronchitis

The analysis was rerun after excluding 346 subjects who reported asthma or allergic symptoms at the baseline examination. A total of 221 incident COPD hospitalizations remained in the analysis. The relationship between the ISPs and incidence of COPD hospitalizations remained significant and was essentially unchanged.
The results were also essentially unchanged if only cases with COPD as primary diagnosis were used in the analysis.

Incidence of COPD hospitalizations in relation to initial lung function
The sample was divided into three groups according to the baseline FEV1/FVC values, men who initially had low FEV1/FVC (in the low 5% of the distribution, n=259), those with low-normal FEV1/FVC (i.e., in the 5^{th} to 50^{th} percentile, n=2364), and men with high FEV1/FVC (in the top 50% of the distribution, n=2624). Among men with low FEV1/FVC, 67 (11.8 per 1000 person years) were hospitalized due to COPD during the follow-up. The HRs, adjusted for risk factors, were 1.00 (reference), 1.2 (CI:0.54-2.7), 1.2 (CI:0.51-2.9) and 2.1 (CI:1.02-4.1), respectively, in men with none, one, two, and three or more elevated ISPs. Among men with initially low-normal FEV1/FVC, 130 (2.2 per 1000) were hospitalized due to COPD. The HRs in this group were 1.00 (reference), 1.3 (CI:0.80-2.2), 1.4 (CI:0.76-2.4) and 2.0 (CI:1.2-3.3), respectively. In men with high FEV1/FVC, 61 (0.9 per 1000) were hospitalized due to COPD. The adjusted HRs were 1.00, 1.3 (CI: 0.60-2.9), 1.1 (CI: 0.42-2.8) and 3.3 (1.6-6.6), respectively, in men with none, one, two, and three or more elevated ISPs.

Incidence of COPD hospitalizations in relation to smoking
Of the cases with COPD, 211 were current smokers at the baseline examination, 27 were former smokers and 20 were never smokers (Table 4). Of the 27 former smokers, 22 had been smoking more than 10 years. The age-adjusted HR of COPD hospitalizations was significantly increased in men with three or more elevated ISPs, even in separate analyses of never smokers and former smokers,. It should be noted, however, that these analyses were based on small numbers of cases (Table 4).

Table 4. COPD hospitalizations during 25 years of follow-up in relation to number of elevated ISPs and smoking status.

	Number of elevated ISPs			
Never smokers	**None n=742**	**One n=388**	**Two n=149**	**Three or more n=136**
COPD n (per 1000 py)	5 (0.3)	10 (1.0)	1 (0.3)	4 (1.2)
Age-adjusted HR (95% CI)	1.0	3.5 (1.2-10.4)	1.0 (0.1-8.2)	4.3 (1.1-16.3)
Former smokers	**n=704**	**n=337**	**n=164**	**n=129**
COPD n (per 1000 py)	12 (0.7)	6 (0.7)	3 (0.7)	6 (2.0)
Age-adjusted HR (95% CI)	1.0	1.0 (0.39-2.8)	1.1 (0.30-3.8)	2.9 (1.1-7.8)
Current smokers	**n=577**	**n=677**	**n=481**	**n=763**
COPD n (per 1000 py)	35 (2.5)	45 (2.8)	33 (3.0)	98 (5.8)
Age-adjusted HR (95% CI)	1.0	1.1 (0.72-1.8)	1.2 (0.76-2.0)	2.5 (1.7-3.6)

Py person-years

Relationship between individual ISPs and COPD hospitalizations

All ISPs were significantly associated with incidence of COPD after adjustments for age (Table 5). The associations between the individual ISPs and COPD hospitalizations remained significant after adjustments for risk factors.

The number of men with deficiency of α1-antitrypsin was small in this cohort and only 9 men had concentrations below 50 mg/dL. The results were unchanged after exclusion of this group.

Table 5. Relationships between individual ISPs and COPD hospitalizations over 25 year of follow-up.

	Age-adjusted	Risk factor adjusted ‡
Fibrinogen (per 0.8 g/L)	1.46 (1.3-1.6)	1.25 (1.1-1.4)
Haptoglobin (per 0.68 g/L)	1.69 (1.5-1.9)	1.34 (1.2-1.5)
Ceruloplasmin (per 0.067 g/L)	1.55 (1.4-1.7)	1.25 (1.1-1.4)
α1-antitrypsin (per 0.27 g/L)	1.50 (1.3-1.7)	1.16 (1.02-1.3)
Orosomucoid (per 0.20 g/L)	1.49 (1.3-1.7)	1.26 (1.12-1.4)

Expressed as hazards ratios (95% confidence interval) per 1 standard deviation.
‡ adjusted for age, current and former smoking, BMI, FEV1 (% of predicted values) and chronic bronchitis

Discussion

Several cross-sectional studies have reported inverse relationships between markers of inflammation and lung function (1-8). However, few have explored whether a low-grade inflammation in apparently healthy men, as measured by raised acute phase proteins in plasma, could predict future COPD hospitalizations. This study shows that incidence of COPD hospitalizations was strongly related to the number of raised acute phase proteins. The ISPs were measured at a mean age of 46 years and they predicted COPD hospitalizations over a very long time period. This relationship remained significant after adjustments for initial lung function and other potential confounding factors.

Smoking is the most important risk factor for COPD in the general population, and smoking is a major cause of systemic low-grade inflammation (15). In this study 211 out of 258 COPD cases were current smokers at the baseline examination. However, relationships between three or more elevated ISPs and incidence of COPD hospitalizations were also observed in never smokers and former smokers. Due to the small number, we cannot make any conclusion about the association between ISPs and COPD hospitalizations in never smokers. The results suggest, however, that three or more elevated ISPs may be a marker of increased vulnerability regardless of smoking status.

The reason for the association between ISPs and COPD is unclear. The inflammatory proteins were elevated many years before the patients were hospitalized due to COPD and high ISP levels were associated with incidence of COPD hospitalizations even after adjustments for initial lung function. Significant relationships were also observed in men who initially had high FEV1/FVC, in the top 50% of the distribution. Reverse confounding, ie, that impaired lung function caused elevated ISPs, is therefore an unlikely explanation.

These plasma proteins are widely used as down-steam markers of inflammation, and their synthesis is regulated by different proinflammatory cytokines (18,19). It is also widely recognized that COPD is an inflammatory disease (1,20-23). Raised ISPs could thus be a marker of a predisposition to exaggerated inflammatory responses, which could cause increased ISPs, reduced lung function and incidence of COPD. This predisposition could be genetically determined (23,24). The fact that high ISPs were risk factors even in men who initially had FEV1/FVC in the high range supports the view that these proteins are associated with development of COPD. However, because COPD hospitalization was the endpoint in this study, an alternative explanation could be that these inflammatory markers indicate an increased risk of COPD exacerbations in vulnerable individuals, rather than development of COPD.

The health examination program was performed several years before the now commonly used guidelines for standardization of spirometry were published. The equipment and procedure of the lung function test did not meet the standards of these guidelines. For example, only 1 acceptable test was required and no nose-clips were used. Despite this limitation, previous studies from this cohort have shown that the validity of the lung function tests is acceptable (4,16,25).

The end-points in this study were retrieved from the national Swedish hospital discharge register. There is a considerable overlap between COPD and, e.g., heart failure (26) and one question is whether the diagnoses are valid. However, the results were essentially the same if the study was limited to cases with COPD as primary diagnosis, and these cases are most likely to be accurate. Furthermore, the diagnosis was settled during the hospital stay. The strong relationships between initial lung function, smoking and COPD hospitalizations also support the view that the diagnoses are valid. It is obvious though, that many individuals with less severe COPD are treated as out patients and therefore not included in this study. This study only explored the relationship with incidence of COPD requiring hospitalization, which generally is the most severe cases.

This population-based study of initially healthy middle-aged men showed that incidence of COPD hospitalizations was increased in those with elevated levels of acute phase proteins in plasma. These proteins may be markers of increased vulnerability for development of COPD.

Acknowledgements
The study was supported by grants from the Swedish Heart and Lung foundation, the Swedish Research council, Malmö University hospital and grants from LU-ALF.

Competing interests:
Prof Gunnar Engström is employed as senior epidemiologist by AstraZeneca R&D.
Prof Claes-Göran Löfdahl has been paid for lectures by AstraZeneca, Boehringer-Ingelheim, GlaxoSmithKline and Pfizer; taken part and been paid for ad hoc advisory boards for the same companies; and has had institutional support as unrestricted grants from AstraZeneca, Boehringer-Ingelheim and GlaxoSmithKline.

Legend to figure:
Figure. Incidence of COPD hospitalizations in men with none, one, two and three or more ISPs in the fourth quartile (log rank test $p<0.001$).

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