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The single breath nitrogen test and mortality — A 38 years follow up

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ABSTRACT

Background: Spirometry data predict mortality, but are less sensitive to detect dysfunction in small airways as compared to the slope of phase III (the N_2 slope) of the single breath nitrogen test. The association between the N_2 slope and mortality has been studied with conflicting results. In the present study the prognostic importance of the N_2 slope was tested taking spirometry variables into account.
Methods: A systematic general population sample of 595 middle-aged men had a baseline investigation with lung function tests including spirometry and the N_2 slope. Age, smoking, and anthropometry variables were registered. The cohort was followed up regarding survival for 38 years.
Results: The sample was subdivided by tertiles of the N_2 slope. A proportional hazards regression analysis was performed for each group of covariates: anthropometric, smoking variables, and spirometry variables, after accounting for age. Covariates with significant impact on mortality and the highest chi-square levels were smoking habit score and forced expired volume in 1 s corrected for height. These variables, in addition to age and the N_2 tertiles were entered into a final proportional hazards regression analysis. In this multivariate model, mortality was significantly related to age ($p < .0001$), smoking habit score ($p < .0001$) and the N_2 tertiles ($p = .0004$), but not to FEV_1 when N_2 slope was allowed for in the model.
Conclusions: Dysfunction in small airways as measured by the N_2 slope is significantly associated with overall mortality in middle-aged men, and outrivals spirometry as a predictor in multivariate analysis.

1. Introduction

Low lung function as assessed by a low vital capacity (VC or FVC) or a low forced expired volume in 1 s (FEV_1) predicts an increased mortality according to consistent studies from the seventies [1,2], and this has been confirmed by more recent studies [3–8]. Smoking is not the only explanation for this association as the increased mortality risk among subjects with low VC or FEV_1 holds true also for never smokers [3–5]. The reasons for this relationship are unclear.

VC and FEV_1 are rather insensitive measures of affected small airways [9–12]. The slope of phase III (the N_2 slope) of the single breath nitrogen test [13] may on the contrary be an indicator of small airway dysfunction and lung inhomogeneity [14,15], and has been shown to be more sensitive than spirometric variables to early injuries of tobacco smoke exposure [10,11,14]. The N_2 slope is therefore a potentially interesting variable regarding prognostication of mortality. Three previous studies have investigated the association between the N_2 slope and mortality [16–18] based on rather short follow up times. The results of these studies are not unanimous and are to some extent conflicting as will be discussed.

The present study aims at testing the hypothesis that the single breath N_2 slope is an independent predictor of all-cause mortality also when spirometry variables are taken into account.

2. Methods

2.1. Study design

The present analysis is based on a systematic sample of middle

Abbreviations: BTPS, body temperature, pressure and saturated; CV, closing volume; FEV_1, forced expired volume in one second; FEV_1/ht^2 and FEV_1/ht^3, FEV_1 divided by height to the power of 2 and 3; FEV_1res, FEV_1 residuals after allowing for height; N_2 slope, slope of the alveolar plateau of the single breath N_2 test; VC or FVC, vital capacity or forced vital capacity; TLC, total lung capacity.

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aged men with a follow up time of 38 years. Oral informed consent, which was the standard procedure at the time, was obtained from all study subjects. The study was approved on several occasions, first by the Research Ethics Committee in Gothenburg, Sweden, and later by the National Research Ethics Board.

2.2. Study population

In 1973, two systematic samples were drawn from the population register. The first sample consisted of all men born in 1913 on a date divisible by 6 of each month and living in the city of Gothenburg (n = 387) [11,17,19]. The second sample consisted of all men born in 1923 on day 3, 15 and 27 of each month, and living in the city of Gothenburg. 292 men met these criteria and 220 men (75%) participated in the study.

The lung function part of the database from 1973 [11] was not fully accessible due to outdated storage of data. Preserved original hard copy data from 1973 were, however, available and a new database was established, and carefully quality controlled. This new database contains data from 595 men, of whom 212 were 50 years old and 383 were 60 years old in 1973. Data from 12 men could not be retrieved.

Smoking habits were classified in three ways. In the first classification a smoking habit score was derived, with never-smokers (=1), ex-smokers since more than 6 months) (=2), currently smoking 1–14 cig/day (=3), smoking 15–24 cig/day (=4), or smoking ≥25 cig/day (=5). In a second classification, named smoking categories, current smokers were defined within one category, resulting in three smoking categories: never smokers (=1), ex-smokers (=2), and current smokers (=3). In a third classification tobacco consumption was calculated in terms of pack years, calculated as the product of smoking intensity (i.e. cigarettes or grams of tobacco per day) and duration (years) [20].

2.3. Lung function tests

All subjects were examined with a spirometry and a single breath N_2 test. All lung function measurements were performed by well-trained technicians. Details of the single breath N_2 test, spirometric measurements, equipment and procedures were previously published [11]. In short, several variables were calculated from the N_2 tracings, e.g. the slope of phase III (the N_2 slope, $\%N_2/L$), and closing volume (CV, %VC). The N_2 slope was calculated as the increase of the nitrogen concentration from the point where 825 ml (body temperature, pressure and saturated - BTPS) had been expired from total lung capacity (TLC) until the beginning of phase IV (closing point), divided by the corresponding expired volume. The N_2 slope was also expressed in per cent of predicted normal (% pred.) according to Sixt et al. [21]. In each subject, two satisfactory tracings were attempted and the mean of these tracings were used.

All tracings were coded and examined by one well trained investigator who was unaware of the characteristics of the subjects. Two satisfactory slow vital capacity (VC) maneuvers and three satisfactory forced VC maneuvers were performed by each subject. The VC and FEV_1 were expressed in liters (BTPS) and in per cent of predicted normal (% pred.) according to Hankinson et al. [22]. The latter prediction model was chosen since the Hankinson equations have been shown superior to other equations [23]. In addition, FEV_1 was expressed as FEV_1 corrected for height (i.e. FEV_1/ht^2 and FEV_1/ht^3). Furthermore, the regression on height among non-smokers was calculated according to Vestbo et al. [18] and the residuals (FEV_1res) were calculated:

$$FEV_1res = FEV_1 - [3{,}62 - 0{,}\ 04653^*(height - 175)]$$

where 175 cm is the mean height of the present study population.

2.4. Outcome measure

Mortality data was obtained from the National Cause of Death Registry. The variable used in this study was date of death until 31 December 2011.

2.5. Statistical analysis

Data were analyzed with the SAS Software version 9.3 (URL SAS Institute, Inc., Cary, NC, USA). Simple differences between groups were tested with analysis of variance for continuous data, and with the chi-square test for categorical data. The day of examination was regarded as the individual baseline day. Follow-up time was measured as number of days until death or end of follow up. Outcome variable was all-cause mortality.

To study the effect of the N_2 slope on survival, the N_2 slope variables (i.e. N_2 slope ($\%N_2/L$) and N_2 slope (% pred.) as well as Closing volume (%VC) were entered in a Cox regression analysis with mortality (any cause) as dependent variable. The highest significant Wald's chi-square level was obtained by the N_2 slope expressed as $\%N_2/L$ (Table 2). Therefore, in further analyses only the N_2 slope ($\%N_2/L$) will be analyzed and discussed.

The N_2 slope measures were divided into tertiles. The reason for this classification was to arrive at hazard ratio measures that might be comparable between studies, since straightforward N_2 slope measures might differ between studies due to N_2 slope distributions. No similar classifications were made regarding other variables in the analyses. Admittedly, this means that the N_2 slope measure was 'handicapped' since the residual information within the tertiles would not be accounted for. On the other hand, if a disfavored N_2 slope measure would outcompete other lung function variables, it would strengthen the case that N_2 slope is a major determinant of survival.

The hazard rates were computed with SAS 'Lifetest' procedure. As shown in Fig. 1 the tertile hazards of the tertile groups were approximately proportional across time, allowing proportional hazards regression analysis.

In the next step proportional hazards regression analysis of covariables was undertaken. Since several exposure-overlapping measures were available, a preliminary Cox regression analysis was performed in each variable group (anthropometric, smoking and spirometry). The variable in each group with the best fit, measured as Wald's chi-square, was then used in the final analysis model.

Finally, in a multivariate proportional hazards regression analysis with all-cause mortality and the time when it occurred as dependent variable (outcome), N_2 slope ($\%N_2/L$, tertiles) was entered as independent variable (exposure), with best performing measure from each variable group as covariates. Forward as well as backward elimination of non-significant variables was used to avoid model overload. All analyses were two-tailed and $p < .05$ was set as the significance level.

3. Results

Characteristics of the study population are presented in Table 1, subdivided by tertiles of the N_2 slope ($\%N_2/L$). Age, anthropometry variables, smoking variables, and spirometry variables all differed significantly across the N_2 slope tertile groups, generally favoring tertile 1 and disfavoring tertile 3. The number and proportion of deceased subjects also differed significantly, being lowest in tertile 1 and highest in tertile 3.

Fig. 1 shows the hazard rate function, i.e., the proportion of