Development and use of biomarkers in clinical development of new therapies for chronic airway disease

Zuiker, Robertus Gerardus Johannes Antonius

CHAPTER 4

SPUTUM INDUCTION WITH HYPERTONIC SALINE REDUCES FRACTIONAL EXHALED NITRIC OXIDE IN CHRONIC SMOKERS AND NON-SMOKERS

Respiratory Medicine 2010 Jun; 104(6):917-20

Rob GJA Zuiker [1*], Catherine Tribouley [2,3*], Zuzana Diamant [1,4], J. Diderik Boot [1,5], Adam F Cohen [1], K. Van Dyck [2], I. De Lepeleire [2], Veronica M Rivas [2], Vlad Malkov [2] , Jacobus Burggraaf [1], and Marcella K Ruddy [2,6].

**both authors contributed equally*

1. Centre for Human Drug Research, Leiden,
2. Merck Research Laboratories, New Jersey, USA and Brussels Belgium
3. Present affiliation: Novartis, New York, USA
4. Dept. of Respiratory Medicine and Allergology, Lund, Sweden and University Medical Center Groningen, Dept. of Clinical Pharmacy & Pharmacology and Dept. of General Practice and QPS, Groningen
5. Present affiliation: Janssen Biologics B.V., Leiden,
6. Present affiliations: EMD Serono, Rockland, Massachusetts, USA

ABSTRACT

BACKGROUND Nitric oxide (NO) measurements in exhaled air and hypertonic saline-induced sputum are commonly used biomarker sampling methods of the lower airways. Both sampling methods have been validated in asthmatic patients and healthy controls, however, data from chronic smokers are scarce.

OBJECTIVES To evaluate the reproducibility and differences in fractional exhaled NO (FENO) values in asymptomatic chronic smokers and healthy, non-smoking controls. Furthermore, to test the effect of hypertonic saline sputum induction (SI) on FENO levels in both study groups.

METHODS 16 asymptomatic chronic smokers and 16 non-smokers participated in this study. Baseline FENO and forced expiratory volume in 1 s (FEV1) were recorded pre- and 30 MIN post-NaCL 4.5% SI (3 x 5 MIN) on 2 study days (± 2 H; 4-10 days apart). Mixed ANOVA was used to estimate the intra-subject Coefficient of Variation (CV) % over days; changes in FENO and FEV1 values before and after SI, were analyzed by a Student's paired t-test. The difference between smokers and non-smokers was estimated by a Student's t-test.

RESULTS On day 1, FENO values in smokers were significantly lower than in non-smokers, 10.6 PPB, and 18.4 PPB, respectively, (42% difference, p = 0.0028, 95% CI: -59%, -19%). In both study groups, FENO measurements were reproducible, with an intra-subject CV of 27.2% and 19.2%, for smokers and non-smokers, respectively. SI significantly decreased FENO levels in both study groups on day 1. In smokers, there was a mean reduction in FENO of almost 37% (p<0.01, 95% CI (-53.2%, -14.2%), and in non-smokers a mean decrease of almost 35% (p=0.047, 95% CI -57%, -0.6%). In both study groups SI did not affect FEV1 (p>0.94).

CONCLUSIONS Our data extend previous findings in asthmatics and healthy controls to asymptomatic chronic smokers: 1. FENO measurements are reproducible in both smokers and non-smokers; 2. baseline FENO levels in chronic smokers are lower than in non-smokers and 3. sputum induction by hypertonic saline reduces FENO levels in both study groups, without affecting lung function.

INTRODUCTION

Sputum induction by hypertonic saline (SI) and fractional exhaled nitric oxide (FENO) are validated, commonly used non-invasive biomarker sampling methods of the lower airways [1;2]. FENO measurements are increasingly applied for diagnosis and monitoring of asthma [3]. Furthermore, both methods are often used as complementary research tools to assess the airway inflammation in response to interventions with (novel) anti-inflammatory therapeutic modalities [4]. However, there is evidence that sampling methods sometimes interfere and thus may affect the levels of biomarkers [5]. So far, two published studies have addressed the effect of SI on FENO values in asymptomatic atopic subjects and asthmatic patients and showed a maximal decrease in FENO directly post-induction with still a substantial decrease up to 4 hours after SI [6;7]. In this study population, FENO levels were reproducible and unrelated to the initial SI-induced decrease in FEV1 [6;7]. So far, few data have been published on chronic smokers [8;9]. Therefore, we tested the reproducibility and differences in FENO levels between asymptomatic chronic smokers and healthy non-smokers. Furthermore, we investigated the effect of SI on FENO levels in both study groups.

METHODS

SUBJECTS The study population consisted of two groups: 16 asymptomatic chronic smokers with a smoking history of at least 10 pack-years (8F/8M; 32-52 years) and 16 healthy non-smokers (8F/8M; 30-49 years) who had not smoked for at least 12 months prior to study enrolment and who had a total smoking history of less than 5 pack-years. For the smoker group, the last cigarette was smoked at least one hour before any study procedure. All subjects had no history of relevant lung disease or any respiratory tract infection for at least 4 weeks before the start of the study. All subjects gave written informed consent. The study was approved by the Ethics Committee of Leiden University Medical Centre, Leiden, Netherlands.

STUDY DESIGN The study comprised two study days, 4 to 10 days apart. On each study day, FENO was measured approximately 55 minutes before and 30 minutes after the SI-procedure. All assessments were performed at the same time of the day (± 2 H). This study was conducted as part of a larger biomarker study; the focus of this manuscript is on methodological issues related to the interaction of SI on FENO levels.

FRACTIONAL EXHALED NITRIC OXIDE (FENO) ❦ FeNO measurements were performed by a chemiluminescence analyser (Ecomedics CLD88sp, Ecomedics, Duernten, Switzerland) according to current guidelines [1]. Briefly, after a deep inhalation of NO-free air, subjects exhaled for approximately 10 seconds against a resistance at a stable flow of approximately 50 mL/s. The mean of the first three technically acceptable measurements (within 10%) were included in the analysis and expressed in parts per billion (PPB).

PULMONARY FUNCTION TESTS ❦ Spirometry was performed according to standardized protocols by a calibrated spirometer (Vmax Spectra Sensor Medics; Cardinal Health, Houten, The Netherlands) [10] connected to a personal computer. The mean of the two out of three (within 5%) highest, technically satisfactory forced expiratory volume in 1 second (FEV1) measurements was included in the analysis.

HYPERTONIC SALINE SPUTUM INDUCTION ❦ Sputum was induced by hypertonic saline (4.5% NaCl) nebulised by an ultrasonic nebulizer (DeVilbiss Ultra NEB 2000, Somerset, PA, USA) according to current guidelines during three periods of 5 MIN each [2]. Spirometry was performed 7 minutes after each SI-period.

ANALYSIS ❦ The reproducibility of the FeNO levels in both study groups was assessed on log-transformed data by a Mixed Analysis of Variance (ANOVA) to estimate the intra-subject Coefficient of Variation (CV). The differences in FeNO levels between both study populations were analyzed with a Student's t-test and the effect of inhaled NaCl 4.5% on FeNO and FEV1 within both study groups was analysed with the paired Student's t-test. Results were back-transformed to ratios and expressed as percentage difference.

RESULTS

STUDY SUBJECTS ❦ The study groups were well-matched with no statistically significant differences in baseline characteristics between the two groups (*Table 1*).

REPRODUCIBILITY AND DIFFERENCE IN FENO BETWEEN STUDY GROUPS ❦ The intra-subject mean CV for baseline FeNO measurements was 19.2% and 27.2% for non-smokers and smokers, respectively (Table 2). Mean FeNO was significantly lower in smokers compared with non-smokers.

EFFECT OF SPUTUM INDUCTION ON FENO AND FEV1 ❦ On day 1, SI decreased FeNO levels in non-smokers by on mean 35% (95% CI: -57%, -0.6%; p=0.047) and in smokers by on mean 37% (95% CI:-53%, -14%; p = 0.0045) (Table 2). SI did not affect FEV1 in either study group.

DISCUSSION

In line with previous observations in allergic asthmatics, we found reproducible FeNO levels in asymptomatic chronic smokers and healthy non-smoking controls. In smokers, FeNO levels were generally lower than in non-smokers and within similar ranges as previously reported [11]. Similarly to previous observations in allergic asthmatics [6], hypertonic saline decreased FeNO levels in both study groups without affecting FEV1. Therefore, our findings confirm and extend previous data (5-7;12).

The sputum inductions in our study were performed according to standardized procedures [1] in age- and gender-matched populations, while in the smokers the time between smoking and any measurements was kept within the same ranges [12]. Hence, the lack of statistical significance between both study groups and pre- and post-SI on study day 2 is most probably due to a larger variability of the FeNO values in a small sample size, possibly caused by external factors.

In line with previous studies we found lower FeNO levels in smokers compared with non-smokers [12]. It appears that smoking inhibits NO formation from inducible nitric oxide synthase in epithelial lung cells [13]. Furthermore, NO synthesis may be reduced by negative feedback as a result of high NO-concentrations in cigarette smoke [12], NO oxidation or interaction with other molecules present in tobacco smoke [14].

In conclusion, FeNO levels in chronic smokers were found to be reproducible and generally lower than in healthy non-smokers. Sputum induction reduced FeNO in both study populations without affecting FEV1. Our data extend previous observations in allergic asthmatics to chronic smokers. In view of the interference of sputum induction with FeNO measurements: FeNO should be measured before sputum induction.

Table 1 Subjects' baseline characteristics

	Non-smokers	Smokers
Number of subjects	16	16
Age (years)	41 (30-49)	41 (32-52)
Gender (M/F)	8M/8F	8M/8F
Height (meters)	1.75 (1.56-1.93)	1.73 (1.58-1.85)
Weight (KG)	77.5 (51.8-110.2)	73.1 (46.5-100.5)
BMI (KG/M2)	25.1 (21.1-29.5)	24.3 (18.6-30.0)
FEV1 (L)	3.66 (2.75-5.16)	3.55 (2.38-4.64)
FEV1 (% predicted)	102.9 (78-119.6)	102.7 (82.1-121.1)
FVC (L)	4.78 (3.21-6.42)	4.46 (3.15–6.38)
FVC (% predicted)	105.1 (82-131)	111.7 (76-127)

Values presented as mean (range)

Table 2 Effect of S1 on FENO and FEV1 values

Study population / parameters	Day 1			
	Pre NACL 4.5%	**30 min post NACL 4.5%**	**Difference (95% CI)**	**p-value**
NON-SMOKERS				
FENO (PPB)	18.4	12.0	-34.9% (-57.3%, -0.6%)	p = 0.047
FEV1 (L)	3.66	3.68	0.02 (-0.38, 0.41)	p = 0.94
SMOKERS				
ENO (PPB)	10.6	6.7	-36.6% (-53.2%, -14.2%)	p =0.0045
FEV1 (L)	3.56	3.55	-0.01 (-0.52, 0.49)	p = 0.95
Study population / parameters	**Day 2**			
	Pre NACL 4.5%	**30 min post NACL 4.5%**	**Difference (95% CI)**	**p-value**
NON-SMOKERS				
FENO (PPB)	15.9	12.3	-22.6 % (-50.5%, 21.1%)	P = 0.25
FEV1 (L)	3.56	3.68	0.12 (-0.31, 0.57)	P = 0.56
SMOKERS				
FENO (PPB)	11.1	8.5	-23.9% (-43.5%, 2.6%)	P = 0.072
FEV1 (L)	3.49	3.53	0.04 (-0.44, 0.52)	P = 0.86
Coefficient of variation (CV%) for FENO (pre NACL 4.5%)	non-smokers		19.2%	
	smokers		27.2%	

CI = confidence interval. FENO values in geometric means; CV = coefficient of variation

REFERENCE LIST

1 ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am.J.Respir.Crit Care Med. 2005; 171: 912-930
2 Djukanovic R, Sterk PJ, Fahy JV, Hargreave FE. Standardised methodology of sputum induction and processing. Eur.Respir.J.Suppl 2002; 37: 1s-2s
3 Kharitonov SA, Barnes PJ. Exhaled biomarkers. Chest 2006; 130: 1541-1546
4 Diamant Z, Boot D, Kamerling I, Bjermer L. Methods used in clinical development of novel anti-asthma therapies. Respir.Med. 2008; 102: 332-338
5 Deykin A, Massaro AF, Coulston E, Drazen JM, Israel E. Exhaled nitric oxide following repeated spirometry or repeated plethysmography in healthy individuals. Am J Respir.Crit Care Med 2000; 161: 1237-1240
6 Beier J, Beeh KM, Kornmann O, Buhl R. Sputum induction leads to a decrease of exhaled nitric oxide unrelated to airflow. Eur.Respir.J 2003; 22: 354-357
7 Piacentini GL, Bodini A, Costella S, Vicentini L, Suzuki Y, Boner AL. Exhaled nitric oxide is reduced after sputum induction in asthmatic children. Pediatr. Pulmonol. 2000; 29: 430-433
8 Sutherland ER, Martin RJ. Airway inflammation in chronic obstructive pulmonary disease: comparisons with asthma. J.Allergy Clin.Immunol. 2003; 112: 819-827
9 Comandini A, Rogliani P, Nunziata A, Cazzola M, Curradi G, Saltini C. Biomarkers of lung damage associated with tobacco smoke in induced sputum. Respir.Med 2009; 103: 1592-1613
10 Miller MR, Hankinson J, Brusasco V et al. Standardisation of spirometry. Eur.Respir.J. 2005; 26: 319-338
11 Travers J, Marsh S, Aldington S et al. Reference ranges for exhaled nitric oxide derived from a random community survey of adults. Am J Respir.Crit Care Med 2007; 176: 238-242
12 Kharitonov SA, Robbins RA, Yates D, Keatings V, Barnes PJ. Acute and chronic effects of cigarette smoking on exhaled nitric oxide. Am.J.Respir.Crit Care Med. 1995; 152: 609-612
13 Hoyt JC, Robbins RA, Habib M et al. Cigarette smoke decreases inducible nitric oxide synthase in lung epithelial cells. Exp Lung Res 2003; 29: 17-28
14 Gaston B, Drazen JM, Loscalzo J, Stamler JS. The biology of nitrogen oxides in the airways. Am.J.Respir. Crit Care Med 1994; 149: 538-551