Beyond airway disease: the value of breath tests in acute pulmonary embolism, a proof-of-principle study

Breathomics as a diagnostic tool for pulmonary embolism

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J Thromb Haemost; 8:2831-2833, 2010
ABSTRACT

Rationale. There is a need for a safe, fast, non-invasive and accurate diagnostic method to exclude the diagnosis of acute pulmonary embolism (PE) in order to decrease the proportion of patients that have to undergo chest imaging. We hypothesized that exhaled breath metabolomic profiling using an electronic nose can discriminate between PE-patients and non-PE patients, and that this discrimination is more pronounced in patients without relevant co-morbidity.

Methods. Patients with suspected PE based on elevated d-dimer or “likely” probability for PE according to the Wells rule were included in this proof-of-principle study. A breath sample was measured by electronic nose (Cyranose 320). PE was independently confirmed or excluded by a 64-slice CT scan.

Results. 40 patients with suspected PE were included, 20 patients with PE (52 ± 15 yr, 13 male) and 20 patients without PE (48 ± 19 yr, 8 male). Half of the subjects (7 PE, 13 no-PE patients) had co-morbidity known to influence the exhaled VOC-profile significantly: diabetes (n=4), cancer (n=12), renal insufficiency (n=2), and heart failure (n=4). Breathprints of PE and no-PE patients were separable in the non-comorbid group (accuracy 85%; p=0.008; PPV 0.86; NPV 0.83; AUC of ROC 0.81), but not in the co-morbid group (65%; p=0.78; AUC of ROC 0.55).

Conclusions. Breathomics assessment with an electronic nose can rule out PE accurately in patients with suspected PE without comorbidity. Breath analysis for PE may decrease the proportion of patients that require additional diagnostic imaging.
INTRODUCTION

Pulmonary embolism (PE) remains a serious and frequently occurring disease, with an incidence of 1-2 per 1000 per year in Western society [1]. Adequate diagnosis is mandatory to prevent PE-related mortality and morbidity on one hand, and unnecessary treatment on the other. Individual signs and symptoms have low accuracy and additional tests are also not sensitive or specific enough to rule PE in or out [2]. Preferably, excluding the diagnosis should be performed using safe, efficient and non-invasive diagnostic methods.

A breath test certainly is a candidate method to fulfill these conditions. Exhaled breath has been demonstrated to contain hundreds of volatile organic compounds (VOCs), derived from various metabolic pathways in the airways and elsewhere in the body. Samples of exhaled breath can be analyzed based on high-throughput assessment and pattern metabolomic recognition of molecular mixtures [3]. By means of ‘electronic noses’ (eNoses), the sampling of exhaled breath and its VOCs have become readily available, owing to their ability to discriminate biomarker profiles or ‘breathprints’ by composite nano-sensor arrays (‘breathomics’). Currently available eNoses include handheld devices using on-board pattern recognition software that is suitable for diagnostic classification without identifying the individual molecular components [3,4]. This provides the potential option of ‘on the spot’ diagnosis of diseases, as has been investigated in lung cancer [5], chronic obstructive pulmonary disease (COPD) and asthma [4,6].

The combination of a clinical decision rule (CDR) and D-dimer testing excludes PE in about 20-30% of patients [7]. This implies that the majority will undergo imaging tests, such as CT-scan. Since the minority of these latter patients will have PE, an increase in the number of patients where PE can be excluded without additional imaging is mandatory. The eNose is an interesting diagnostic tool in patients with suspected PE, especially in those without co-morbidity [8-11].

We hypothesized that exhaled breath molecular fingerprinting by eNose can differentiate between PE and no-PE in patients with suspected acute PE, who have a likely CDR or elevated D-dimer level, and that this differentiation is more pronounced in patients without relevant co-morbidity.
METHODS
The study was a prospective proof-of-principle study in patients with suspected PE. Patients with a diagnosis of PE were compared to patients in whom this diagnosis was excluded. Suspected PE was defined as sudden onset of dyspnea, deterioration of existing dyspnea, and/or sudden onset of pleuritic chest pain in combination with a “likely” clinical probability according to Wells or a d-dimer > 500 μg/L (Innovance® D-dimer quantitative immunoassay by Siemens) [12]. The diagnosis of PE was objectively confirmed by the presence of at least one filling defect in the pulmonary artery tree with a 64-slice CT scan. The institutional review board of the hospital approved the study protocol and informed consent was obtained from all patients.

Exhaled breath of patients was analyzed using the Cyranose 320 electronic nose (Smiths Detection, Pasadena, USA), a handheld portable chemical vapor analyzer, containing a 32 polymer nano-composite sensor array [13]. Patients breathed normally through a mouthpiece, connected to a three-way-non-re-breathing valve and an inspiratory VOC-filter (A2, North Safety, The Netherlands) for 5 minutes. After a deep inspiration the patient exhaled a vital capacity volume into a Tedlar bag connected to the expiratory port [4,6]. Within 30 minutes the eNose was connected to the Tedlar bag, followed by 1 minute sampling of the exhaled air providing a breathprint. The breathprints were analyzed using SPSS software (version 17.0). Double cross-validatory implementation of linear discriminant analysis on principal component reduction was performed and ROC curves were constructed. The technician was unaware of the result of the CT-scan of the patients.

RESULTS
40 Patients with suspected PE and an indication for CT scanning based on either a likely CDR or abnormal D-dimer level were analyzed. We compared 20 patients with PE (age 52 ± 15 yr, 13 male) and 20 patients in whom PE was excluded (48 ± 19 yr, 8 male). Half of the subjects (7 PE, 13 no-PE patients) had co-morbidity known to influence the exhaled VOC-profile significantly: diabetes (n=4), cancer (n=12), renal insufficiency (n=2), and heart failure (n=4). These patients were analyzed separately.

Breathprints of PE and no-PE patients were separable in the non-comorbid group (accuracy 85%; 17/20 correctly classified; p=0.008), but not in the co-morbid group (65%; p=0.78). For the non-comorbid group, PPV was 0.86 and NPV 0.83. ROC analysis of breathprints for PE showed an AUC of 0.81 (p=0.02; 95% CI 0.56-1.00) in the group without co-morbidity, whereas for the co-morbid group this was 0.55 (Figure 1). Notably, in none of the patients in the co-morbid group PE could be correctly diagnosed using breathomics.

We further combined the probabilities of PE diagnosis based on breathprints and on Wells score into one diagnostic algorithm. The predictive value in the non-comorbid group increased to AUC 0.90 (p=0.006; 95% CI 0.74-1.05) (Figure 1). In the patients with co-morbidity, AUC decreased to 0.51.
DISCUSSION

In this proof-of-principle study we showed for the first time that exhaled breath molecular profiling using a portable electronic nose can adequately identify or exclude PE in patients without relevant comorbidity in a non-invasive way. All patients had either a likely CDR or abnormal D-dimer, necessitating the performance of CT-scan. The eNose could specifically increase the proportion of patients in whom PE can be excluded without diagnostic imaging. However, due to the small sample size of this proof-of-principle study, results should be interpreted with caution. Furthermore, in patients with co-morbidity associated with distinctive breath VOC-profiles the eNose was unsuccessful to discriminate between PE and non-PE, suggesting that the signal associated with PE is masked by other diseases. To overcome this limitation, a solid and large training set consisting of ‘pure’ PE patients without comorbidity should provide the gold standard PE breath fingerprint [14]. Subsequently, this diagnostic model should be tested in a newly recruited validation set [14,15], followed by a diagnostic management study.

In conclusion, in patients with suspected PE who have a likely CDR or abnormal D-dimer level, breathomics assessment with the eNose seems to provide accurate discrimination of patients with confirmed PE versus patients in whom PE could be excluded. Exhaled breath analysis in PE may increase the exclusion of high risk PE patients without additional diagnostic imaging.

Figure 1 ROC curves of the predictive value of eNose breathprints, Wells score and the combination of eNose breathprints and Wells score for diagnosing PE in subjects (a.) without and (b.) with co-morbidity.
REFERENCES