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The Potential Role of Exhaled Breath Analysis in the Diagnostic Process of Pneumonia – A Systematic Review

Pouline M. P. van Oort[1*], Pedro Povoa[2,3], Ronny Schnabel[4], Paul Dark[5], Antonio Artigas[6], Dennis C.J.J. Bergmans[4], Timothy Felton[5], Luis Coelho[2,3], Marcus J. Schultz[1], Stephen J. Fowler[5] and Lieuwe D. Bos[1] on behalf of the BreathDx Consortium[+]

[1] Department of Intensive Care, Academic Medical Centre, Amsterdam, The Netherlands

[2] Hospital de São Fransisco Xavier, Centro Hospitalar Lisboa Ocidental, Lisbon, Portugal

[3] NOVA Medical School, CEDOC, New University of Lisbon, Lisbon, Portugal

[4] Maastricht University Medical Centre+, Maastricht, The Netherlands

[5] Division of Infection, Immunity and Respiratory Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, The University of Manchester and University Hospital of South Manchester, NHS Foundation Trust, Manchester UK

[6] Critical Care Department, CIBER Enfermedades Respiratorias, Corporacion Sanitaria Universitaria Parc Tauli, Sabadell, Spain

* Correspondence: Pouline M.P. van Oort, Dept of Intensive Care, Academic Medical Centre, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands. Email: pouline.vanoort@gmail.com

[+]A list of all members of the BreathDx Consortium: Waqar Ahmed, Antonio Artigas, Dennis C.J.J. Bergmans, Lieuwe D. J. Bos, Marta Camprubi, Luis Coelho, Paul Dark, Alan Davie, Emili Diaz, Gemma Goma, Timothy Felton, Stephen J. Fowler, Royston

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Goodacre, Hugo Knobel, Oluwasola Lawal, Jan-Hendrik Leopold, Ignacio Martin-Loeches, Tamara Nijsen, Pouline M. P. van Oort, Pedro Povoa, Craig Johnson, Nicholas J. W. Rattray, Guus Rijnders, Ronny Schnabel, Marcus J. Schultz, Ruud Steenwelle, Peter J. Sterk, Jordi Valles, Fred Verhoeckx, Anton Vink, Hans Weda, Tineke Winters, Tetyana Zakharkina

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Abstract

Diagnostic strategies currently used for pneumonia are time-consuming, lack accuracy and suffer from large inter-observer variability. Exhaled breath contains thousands of volatile organic compounds (VOCs), which include products of host and pathogen metabolism. In this systematic review we investigated the use of so–called 'breathomics' for diagnosing pneumonia.

A Medline search yielded 18 manuscripts reporting on animal and human studies using organic and inorganic molecules in exhaled breath, that all could be used to answer whether analysis of VOC profiles could potentially improve the diagnostic process of pneumonia. Papers were categorised based on their specific aims; the exclusion of pneumonia; the detection of specific respiratory pathogens; and whether targeted or untargeted VOC analysis was used.

Ten studies reported on the association between VOCs and presence of pneumonia. Eight studies demonstrated a difference in exhaled VOCs between pneumonia and controls; in the individual studies this discrimination was based on unique sets of VOCs. Eight studies reported on the accuracy of a breath test for a specific respiratory pathogen: five of these concerned preclinical studies in animals. All studies were valued as having a high risk of bias, except for one study that used an external validation cohort.

The findings in the identified studies are promising. However, as yet no breath test has been shown to have sufficient diagnostic accuracy for pneumonia. We are in need of studies that further translate the knowledge from discovery studies to clinical practice.

Key words: Exhaled breath analysis; Pneumonia; Diagnosis; Breathomics; Volatile Organic Compounds

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Introduction

The prevalence of community-acquired pneumonia (CAP)[1] and nosocomial pneumonia[2,3] is high with substantial impact on morbidity and mortality[4–10]. Treatment of pneumonia is a balance between optimal antibiotic therapy for the patient (*e.g.* a combination of antibiotics that effectively target the causative pathogen) and for the community (*e.g.* minimizing the duration of exposure to broad spectrum antibiotics to limit antimicrobial resistance)[11]. In the ideal world, this balance would be met through a diagnostic test that is not only quick, non-invasive, reliable and available in real-time at the bedside, but most of all (1) excludes pneumonia in order to withhold antibiotic treatment from patients without an infection; (2) enables targeting of antibiotic treatment of the causative pathogen; and (3) facilitates evaluation of the treatment response aiming to refine antibiotic de-escalation and duration of antibiotic treatment.

'Breathomics' refers to the analysis of volatile compounds in exhaled breath that resulted from, or are affected by metabolism[12]. The complete human breathome consists of thousands of compounds[13–17]. The volatile organic compounds (VOCs) that are present in the exhaled breath have various origins. Exogenous VOCs are derived from the environment and are taken in through inhalation or ingestion (e.g. via food or drugs). VOCs that are produced within the body can emerge as products of physiological metabolic processes from the host, as products of metabolic processes from microbial pathogens, or results from of a host response to pathological processes such as infection or inflammation[18–20]. Changes, therefore, in host or microbial metabolism might lead to an impact on the composition of the exhaled breath profile.

In this systematic review we aim to investigate the potential role of exhaled breath analysis for diagnosing pneumonia, by providing: (1) sensitive detection of pneumonia; (2) specific detection of the causative organism(s); and (3) a tool to monitor the treatment response after the initiation of antibiotics (see Figure 1). We

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hypothesize that changed concentrations of VOCs in exhaled breath can be used to accurately discriminate patients with pneumonia from patients without pneumonia and may be used for specific identification of the causative pathogen.

Methods

Search

This is a systematic review following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, performed by two independent researchers. We searched Medline for potentially relevant articles up to March 7th 2017, using the following search terms: "(((Chromatography OR Spectrometry OR MS OR (Volatile AND Organic) OR Metabol*) AND breath) OR (volatile fingerprint*) OR (breathprin*) OR (electronic AND nose)) AND (pneumonia OR (lung infection*) OR (respiratory infection*) OR (lung bacteria*) OR (respirator* bacteria*))". There was no restriction with respect to human or animal studies; but articles written in a language other than English and studies performed in vitro were excluded. Two authors (PvO and LB) reviewed the abstracts and/or full-text manuscripts independently and selected those that were regarded to be relevant. No disagreement on selection of articles was seen between the two reviewers.

Selection criteria

Inclusion criteria were (1) human or animal studies that (2) studied volatiles in exhaled breath to (3) diagnose bacterial pneumonia or identify the causative organism of pneumonia. Objective 3 as mentioned in the introduction (the evaluation of the treatment effect in patients with pneumonia) was let go, due to a lack of studies specifically investigating this. We excluded in vitro studies and studies that focused on very specific atypical causative organisms (such as Aspergillus).

Reference test

The diagnosis of pneumonia could be based on clinical symptoms alone, or could be supported by chest radiography and/or microbiology testing (cultures of endotracheal

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aspirate (ETA), nondirected bronchial lavage (NBL or mini-BAL) or bronchoalveolar lavage (BAL)). For community-acquired pneumonia, the combination of clinical signs and symptoms with an evident infiltrate on the chest radiograph was considered a good reference test, while anything less was considered too nonspecific. For ventilator-associated pneumonia, clinical signs, laboratory parameters, an infiltrate on chest radiography and quantitative cultures of BAL or NBL were considered an appropriate reference standard.

Index test

Advances in chemical analytics have enabled the measurement of inorganic[21,22] and organic compounds[23–25] in biological matrices such as exhaled breath. Volatile molecules in breath can be studied via a targeted and an untargeted approach[12]. With the targeted approach the researcher identifies the molecules of interest beforehand and uses analytical assays to measure those compounds quantitatively. The untargeted approach entails analytical techniques that measure multiple molecules present in the breath. Untargeted analysis can be performed with mass-spectrometry based techniques aimed to identify a variety of VOCs[26] or with so-called electronic nose technology that is based on pattern recognition[14,27,28]. The analytical details of these techniques are discussed in detail in previous publications[27,29]. Figure 2 summarises the analytical methods that will be referred to in this systematic review. No single method is superior to the others, they provide different types of information, therefore the quality of the index test was assessed based on the use of an independent validation cohort, which has been shown to limit bias[27].

Methodological assessment and categorisation

The methodological quality of each selected full manuscript was evaluated using the QUADAS-2 tool by the same authors as describes above[30]. Risk of bias was assessed concerning patient selection, the interpretation or conduct of the index test, the interpretation or conduct of the reference standard and the patient flow. The

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papers were classified as either (1) studies concerning sensitive detection of the presence of pathogenic bacteria, either concerning studies investigating inorganic compounds, untargeted analysis of VOCs or eNose technology for discrimination between pneumonia and no pneumonia; or (2) studies investigating the use of VOC analysis for specific detection of pathogenic bacteria, in animals or in humans.

Results

The search was last updated on March 7th 2017 and yielded 321 articles, of which 18 were selected after screening on title/abstract and full text (Figure 3). Of these, 13 studies were in humans and five were performed in murine models. Eight studies dealt with the detection of specific pathogenic bacteria, the others focused on discrimination between patients with and without pneumonia. One of the studies discussed treatment response. Table 1 demonstrates the areas of interest for each study and summarises the methodology used.

The studies were critically appraised and risk of bias was assessed regarding patient selection, index test, reference standard and flow and timing (Table 2). The domain 'patient selection' was considered not applicable in the five animal studies. For most studies the risk of bias was valued as high, except for one that used a validation cohort[31], resulting in a low risk of bias regarding the index test.

Discrimination between patients with and without pneumonia

Detection of volatile inorganic compounds

NO was not increased in the breath of a small group of patients admitted with pneumonia, when compared with control patients[32]. As expected it was elevated in patients with an exacerbation of asthma. This result was in contrast to the results of a larger study at less risk of bias (Table 2) in which exhaled NO was measured in tracheal and nasal gas in patients ventilated within 72 hours of ICU admission[31].

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Some of these patients were later diagnosed with VAP and this was used as the reference standard. A validation cohort consisting of similar patients to the first group was used to determine sensitivity and specificity of the NO threshold that was calculated in the preceding group. NO concentrations were measured at multiple sampling points in the airway as well as in the nasal cavity, and significantly higher NO levels were found at all points in patients with pneumonia. Of these, the maximum (end-expiratory) tracheal NO values resulted in the highest sensitivity and specificity for the diagnosis of pneumonia: 88% and 76% respectively (see Table 1). Results from one study with an imperfect reference test, namely subjective symptoms of lower respiratory infection, suggested a possible relationship between elevated exhaled CO levels and the clinical presence of pneumonia[33]. Notably, the exhaled CO concentration followed similar trends as the patients' symptoms after antimicrobial treatment.

Untargeted analysis of VOCs

The abundance of particular VOCs seems to be different in the breath of mechanically ventilated patients with pneumonia compared with those without pneumonia[34–36]. The results of studies using gas chromatography and mass spectrometry (GC-MS, see Figure 2), however, were not uniform. The described VOCs differed between studies and two compounds that were identified as being associated with VAP (ethanol and heptane) showed conflicting results in two studies (as shown in Table 3). Differences between studies regarding investigated cohorts, reference standards and outcome measures (sensitivity, specificity and/or accuracy) can be found in Table 1. Nevertheless, breath tests showed promising discrimination between patients with and without pneumonia in the included clinical studies. The most frequently isolated pathogens in these studies were *Staphylococcus aureus, Haemophilus influenzae, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae*[34–36].

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Electronic nose technology

Preliminary results indicated a potential correlation between chest CT scans[37] or Clinical Pulmonary Infection Score (CPIS)[38] and the subsequent eNose sensor responses in mechanically ventilated patients. The eNose (see Figure 2) seemed to distinguish patients with and without bacterial infection[39] (Table 1). When specifically focusing on diagnosis of VAP, the eNose appeared to have good accuracy, moderate sensitivity and a rather poor specificity[40].

Specific detection of pathogens by VOC analysis

Secondary electrospray ionization – mass spectrometry (SESI-MS, see Figure 2) breathprint analysis was used to investigate the ability to identify respiratory infection caused by strains of *Haemophilus influenzae, Klebsiella pneumoniae, Legionella pneumophila, Moraxella catarrhalis, Pseudomonas aeruginosa, Staphilococcus aureus* or *Streptococcus pneumoniae* in mice[41–44]. Overall SESI-MS breathprints seemed to be able to distinguish between respiratory infection and no infection in mice and enabled differentiation between strains of aforementioned pathogens. A high degree of variation was seen when translating *in vitro* experiments to the *in vivo* VOC fingerprints[42]. The relative contribution of bacterial metabolism and host response on the exhaled breath profile could be inferred through an experiment in which mice were exposed to bacterial cell lysates[45]. This experimental set-up, using these bacterial cell lysates, allows for host and pathogen derived metabolites to be differentiated. The obtained SESI-MS breathprints changed over time after lysate exposure and appeared to 1) correlate to the host immune response; and 2) distinguish active infections of *P. aeruginosa* or *S. aureus* from cell lysate exposure.

Also using GC-MS specific VOCs in the exhaled breath seemed to reflect the presence of particular microorganisms in the respiratory tract and, in line with the use of SESI-MS, direct translation of biomarkers from the *in vitro* to the *in vivo* setting

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proved difficult[46,47]. In clinical studies, the evidence for specific detection of particular causative pathogens seemed speculative; as the published papers did not provide data on the accuracy of such measurements[46]. The only study that reported a high diagnostic accuracy for the identification of a causative pathogen focused on *Acinetobacter baumannii*. A set of eight compounds resulted in excellent separation of patients with *A. baumannii* pneumonia, colonization with the same bacterium and controls. The major limitation of the described studies was that they did not evaluate the diagnostic accuracy of a breath test in the clinical scenario where such a test would be used; e.g. in patients with a clinical suspicion of VAP.

Discussion

Based on our systematic review, the presence of certain profiles or patterns of volatile molecules in the exhaled breath appeared to be associated with pneumonia. However, the precise identity of these volatile biomarkers remains largely unknown. Furthermore, none of the studied breath tests delivered results with sufficient clinical diagnostic accuracy that would likely impact on clinical decisions. Most of the available studies provided feasibility or proof of concept data with a substantial risk of bias and did not test a clear, pre-defined hypothesis.

There are two leads to follow in the diagnosis of pneumonia: measurement of the host response or direct identification of the pathogen[48,49], both important establishing the ideal diagnostic test. In vitro results suggested that different pathogenic bacteria produced different volatile molecules, which might be used for identification[20]. One of the major challenges is that bacterial growth and metabolism are influenced by the chosen culture media, timing and the selection of particular strains and, therefore, may not be representative of growth in vivo[20]. A sterile inflammatory response altered the VOC release in several animal models of lung injury[21]. Thus, pneumonia may be recognized through exhaled breath analysis by detection of molecules produced either directly by the pathogen or through an altered host metabolism associated with the host response. Animal studies might offer

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advantages enabling the investigation of 1) a single bacterial infection, 2) the influence of timing on disease progression and 3) post-mortem histology for the gold-standard diagnosis of respiratory infection.

This systematic literature review demonstrates that certain volatile molecules could be useful as possible biomarkers for the diagnosis of pneumonia. One of them is nitric oxide (NO), a compound that has a bronchodilating and vasodilating effect in the respiratory tract and plays a key role in local inflammatory response[22]. NO is relatively easy to measure and thus forms an attractive candidate for diagnostic purposes[50,51]. In the airways NO is produced by endothelial, epithelial and inflammatory cells. Generation of NO involves the oxidation of the aminoacid L-arginine, a process that is catalysed by the enzyme NO synthase[52]. An increased concentration of exhaled NO is seen in asthma, bronchiectasis and sepsis[53], and has also been associated with rhinitis, active pulmonary sarcoidosis and viral respiratory illnesses[52]. Table 3 shows other biomarkers of potential interest regarding the discrimination of patients with and without pneumonia. However, hardly any overlap is seen between the different VOCs reported in separate studies and they also show conflicting results for heptane and ethanol. Two studies found an association between pneumonia and a decrease in exhaled breath acetone. Generally, acetone is present in large quantities in the exhaled breath. Its decrease in the breath of pneumonia patients might be explained by a reduced ketogenesis that is seen during inflammation or infection[34].

As soon as a breath test fulfils the requirements for a diagnostic test for pneumonia, it shall be able to fulfil a role alongside the currently available and frequently used alternatives[54,55] and can either compete with them, or complement them. The diagnosis of pneumonia relies on a combination of physical examination and chest radiography[56], potentially accompanied by measurement of inflammatory markers in plasma, urinary antigen testing[54], repeated determination of C-reactive protein (CRP)[57] and collecting airway samples for microbiology cultures[58]. Current

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diagnostics lack clinical accuracy[59] and have high inter-observer variability[60]. Microbiology results take 48-72 hours to become positive. The unnecessary prescription of antimicrobial treatment increases antimicrobial resistance[61–63], whereas applying the wrong antibiotics is likely to increase mortality[64–66]. In order to withhold antibiotics, the CPIS[67] combines clinical and physiological data, pulmonary radiography and microbiology results into a numeric score that can be used to exclude pneumonia with moderate accuracy due to substantial inter-observer variability[68,69]. Additionally, biomarkers like pulmonary interleukin-1β (IL-1β) and interleukin-8 (IL-8) measured in BAL fluid have shown promising results as discriminators for VAP[70,71]. In the near future Polymerase Chain Reaction (PCR) of respiratory samples might be used to identify the causative pathogen rapidly and specifically[72–74] and serum procalcitonin has been proposed as an attractive candidate for determining antibiotic duration[75,76]. How would exhaled breath analysis compete with these alternatives? In contrast to blood or BAL samples, breath can be collected completely non-invasive and it is continuously available. A breath test could also provide results rapidly and cost-effectively, which is important in the setting of pneumonia. A breath test with the right test characteristics could thus provide real opportunities for improved real-time diagnostic utility, patient acceptability and cost effectiveness.

Many different methods for breath sampling have been described in literature, including but not limited to: glass syringes, needle traps[77] steel or glass tubes filled with sorbent material and/or breath gas bags (e.g. Tedlar bags). Pre-concentration of the breath sample could be established through the absorption of the VOCs using for instance organic polymers (e.g. Tenax TA), graphitized carbon, activated charcoal or carbon molecular sieves[78]. A challenge in the process of breath sampling is the humidity of exhaled breath – especially true for mechanically ventilated patients – which possibly affects pre-concentration, separation and detection of individual compounds[16]. The use of storage containers such as Tedlar Bags has been linked to

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loss of analytes or contamination of samples[77]. The lack of standardization of analytical methods leads to a wide variation of results among studies. Application of a standardised method of exhaled breath analysis would lead to comparable results, thereby facilitating the potential use of breath biomarkers in the future[79].

Based on the results from the studies included in this review, we can conclude that the VOCs that are measurable in exhaled breath are altered during pneumonia and can derive from the bacterial metabolism as well as the host response. However, these results do not yet allow us to link specific compounds to particular pathogens or disease states, nor does it allow us to pool data from different experiments or studies due to bias and heterogeneity in experimental procedures. Future studies should utilize this understanding and not only focus on VOCs produced by bacteria or the host, but should also combine these two for optimal diagnostic accuracy. Additionally, a more stringent approach towards the methodological design of the studies is recommended. This includes following the STARD guidelines for reporting studies on diagnostic accuracy to limit the amount of bias[80,81]. Previous reviews[27,82] properly summarised the necessary steps to validate preliminary results in breath research. Importantly, future studies should focus more on the clinical application of a breath test. As advocated in this review such a test would 1) exclude pneumonia in order to withhold antibiotic treatment from patients without an infection; 2) enable targeting of antibiotic treatment to the causative pathogen; and/or 3) facilitate evaluation of the treatment response aiming to refine or stopping antibiotics. To date, most focus has been on VAP rather than on community-acquired pneumonia, implicating that currently most evidence is available for this particular respiratory infection aetiology. Therefore, this might also be the clinical problem that might require direct focus in the forthcoming years of breath research.

This systematic review of the literature has several strengths and weaknesses. We chose to apply wide inclusion criteria in order to fully cover the

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literature in this relatively nascent field of research. Naturally, this resulted in a wide diversity of selected articles and made it impossible to pool data due to the underlying heterogeneity, which can be seen as a limitation of our review. In general one can also wonder to what extent the results provided by animal experiments can be translated to the human situation. This study also has several strengths: clinical and pre-clinical studies with multiple analytical devices were included and the results were clustered into the clinical perspective of three scenarios where a biomarker could alter clinical decision-making.

This review demonstrates that a relationship exists between respiratory infection and the presence of particular VOCs in the exhaled breath. Presently, no available breath test is accurate enough to qualify for a role within the diagnostic process of pneumonia. Future studies should focus on clinical scenarios in which a breath test could impact on antimicrobial stewardship and should limit bias by strictly adhering to the latest guidelines.

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Author Contributions PvO and LB conceptually designed the manuscript and performed the literature search. PvO and LB prepared the initial version of the paper and PP, RS, PD, AA, DB, TF, LC, MS and SF advised on the composition of the subsequent final manuscript. All authors approved the submitted version of this article. PvO can be regarded the guarantor of the paper, taking responsibility for the

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integrity of the work as a whole, from incepton to published article.

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Table Legends

Table 1. Full text selected articles
The selected articles either concern clinical (C) or pre-clinical (P) studies. Investigated inorganic compounds include nitric oxide (NO) and carbon monoxide (CO). The investigated study population could be on ICU patients in general, or could involve a specific target group, e.g. patients with COPD (Chronic obstructive pulmonary disease), Community-acquired pneumonia (CAP) or Ventilator-associated pneumonia (VAP). As a reference test a protected specimen brush (PSB), bronchoalveolar lavage (BAL) or endotracheal aspirate (ETA) was used. The used index test to analyse Volatile Organic Compounds (VOCs) could involve secondary electrospray ionization – mass spectrometry (SESI-MS), Clinical Pulmonary Infection Score (CPIS), eNose technology or gas chromatography – mass spectrometry (GC-MS): either sensitivity (sens) and specificity (spec), or accuracy has been displayed in the Table.
*For details: see Table 3.

Table 3. VOCs identified by GC-MS: The VOCs are either increased **(**↑) or decreased (↓) in the exhaled breath of pneumonia patients when compared to patients without pneumonia.

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Table 1. Full text selected articles

Type	Study	Population	Sample size	Index test	Disease under study; Reference test	Sens	Spec	Accuracy	Comments
C	31	ICU patients	49	NO analyser	VAP; Clinical signs + culture of unknown specimen	88%	76%		
C	32	General ward	42	NO analyser	CAP; Clinical signs + X-ray				Quantitative; lower NO concentration
C	47	Outpatient clinic	72	CO analyser	CAP; Clinical signs			~90%	Accuracy estimated from figure
C	80	ICU patients	76	eNose	VAP; Clinical signs			~90-100%	
C	45	ICU patients	28	GC-MS	VAP; Culture of BAL / ETA			Unknown	Quantitative
C*	34	ICU patients	46	GC-MS	VAP; Culture of ETA	98%	98%		Invasively ventilated patients at risk for VAP
C	46	ICU patients	60	GC-MS	VAP; Clinical signs + radiology + culture of BAL / PSB / ETA			88-89%	
C	36	ICU patients	25	eNose	VAP; Chest CT scan			91-100%	
C	37	ICU patients	44	eNose	VAP; CPIS > 6			70%	
C*	33	ICU patients	100	GC-MS	VAP; Clinical signs + radiology + culture of BAL	76%	73%		Only patients that were suspected of VAP
C	39	ICU patients	125	eNose	VAP; Clinical signs + radiology + culture of BAL	76%	56%		Only patients that were suspected of VAP
C	38	COPD patients	43	eNose	CAP; Sputum cultures	73%	76%		
C*	35	ICU patients	93	GC-MS	VAP; Clinical signs + radiology + culture of ETA	75%	72%	73%	
P	43	Mice	12	SESI-MS	Instillation of MRSA or MSSA			100%	Experimental model with *Staphylococcus*
P	44	Mice	87	SESI-MS	Instillation of alive or lysated bacteria			100%	Experimental model with alive or dead bacteria
P	41	Mice	?	SESI-MS	Instillation of *Pseudomonas* or *Staphylococcus*			Unknown	Quantification of difference between in vitro and in vivo
P	40	Mice	42	SESI-MS	Instillation of seven different bacterial strains			Unknown	Quantification of difference between pathogens
P	42	Mice	?	SESI-MS	Instillation of *Pseudomonas* or *Staphylococcus*			Unknown	Quantification of dynamic changes in breath VOCs

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Table 2. QUADAS-2 (adapted version)

Study		Risk of Bias			
	Year	Patient selection	Index test	Reference standard	Flow and timing
Adrie[31]	2001	No	No	No	No
Ali-Ali[32]	2001	Yes	Yes	Yes	No
Bean[44]	2014	N/A	Yes	No	No
Bean[45]	2015	N/A	Yes	No	No
Biernacki[33]	2001	Yes	Yes	Yes	No
Chiu[83]	2014	Yes	Yes	Yes	Unclear
Filipiak[46]	2015	Yes	Yes	No	No
Fowler[35]	2015	Unclear	Yes	No	No
Gao[47]	2016	Yes	Yes	No	No
Hockstein[37]	2004	Yes	Yes	Yes	No
Hockstein[38]	2005	Yes	Yes	Yes	No
Schnabel[34]	2015	Unclear	Yes	No	No
Schnabel[40]	2015	No	Yes	No	No
Van Geffen[39]	2016	Yes	Yes	No	No
Van Oort[36]	2017	No	Yes	No	No
Zhu[42]	2013	N/A	Yes	No	No
Zhu[41]	2013	N/A	Yes	No	No
Zhu[43]	2013	N/A	Yes	No	No

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Table 3. VOCs identified by GC-MS: increased (↑) or decreased (↓) in breath of pneumonia vs. no pneumonia patients

Classification	Volatile Organic Compound	Reporting article		
		Schnabel[34]	Fowler[35]	Van Oort[36]
Ketone	Acetone	↓		↓
	2-methyl cyclopentanone		↓	
	Methylisobutylketone			↓
Aldehyde	Acrolein	↓		
	Nonanal		↑	
	Tetradecanal	↑		
Ether	Sevoflurane			↓
	Tetrahydrofuran	↓		
Alkane	2-methyl butane	↑		
	2-ethoxy-2-methyl propane			↓
	Carane	↑		
	Dodecane	↓		
	Heptane	↑	↓	
	Tetradecane	↑		
	2,6,11,15-tetramethyl-hexadecane		↑	
Alkene	Cyclohexene			↓
Terpene	3-carene		↑	
Alcohol	Ethanol	↑	↓	
	Isopropyl Alcohol	↓		
	Hexafluoroisopropanol			↓
	1-propanol			↓
Arene	Ethylbenzene	↑		
Ester	N-butyric acid 2-ethylhexyl ester		↑	
Sulfide	Carbon disulfide			↓
Amide	N-cyclohexyl-N'(2-hydroxyethyl)thio-urea		↓	

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Figure Legends

Figure 1. The aspired contribution of breath analysis regarding antibiotic stewardship for pneumonia (↑: increase)

Figure 2.

Exhaled breath analysis for the prediction of pneumonia: several available techniques and accompanying analytical principles.

eNose: Conventional electronic noses consist of an array of gas sensors using transducer principles (e.g. through metal oxide sensors, conducting polymer sensors or surface or bulk acoustic wave sensors)[84], whereas new approaches involve optical sensor systems and colorimetric sensors[17]; GC-MS: Gas chromatography – mass spectrometry is currently seen as the preferred method for separation, detection and identification of individual VOCs; SESI-MS: Secondary electrospray ionization – mass spectrometry enables rapid detection of VOCs without the need for sample pretreatment[85]. After introduction into the SESI reaction chamber the sample passes through an electrospray cloud that ionizes the volatiles, after which the ionized VOCs are detected in the mass spectrometer. The method cannot be used to quantify individual VOCs, as many volatiles have similar molecular weights and the methodology relies on pattern recognition. GC-MS and SESI-MS can both be used for targeted and untargeted analysis.

Figure 3. Flow diagram of article selection.

VOC: Volatile Organic Compound; GC-MS: Gas chromatography – mass spectrometry; SESI-MS: Secondary electrospray ionization – mass spectrometry.

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Figures

Figure 1

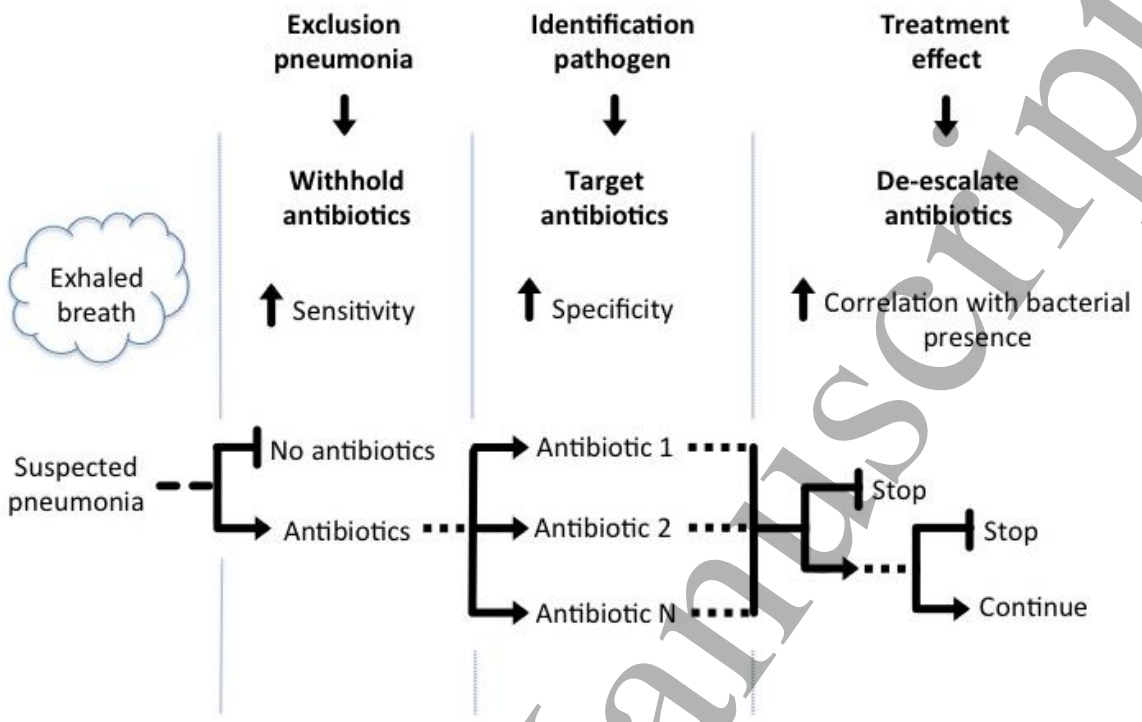

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Figure 2.

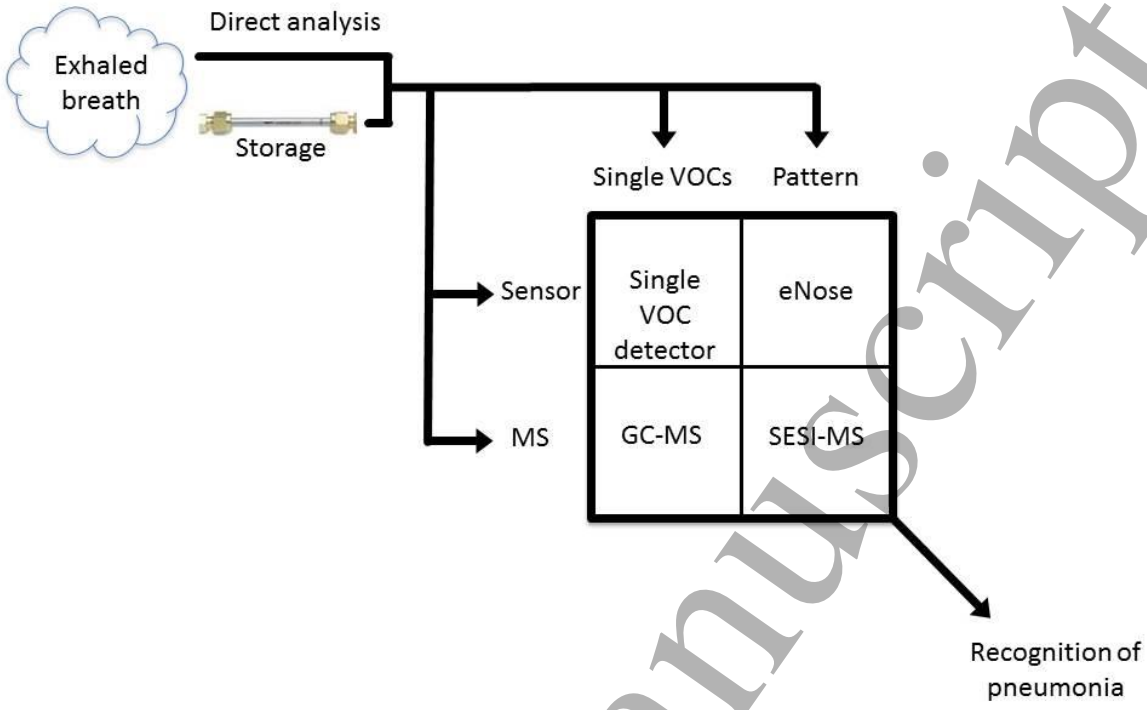

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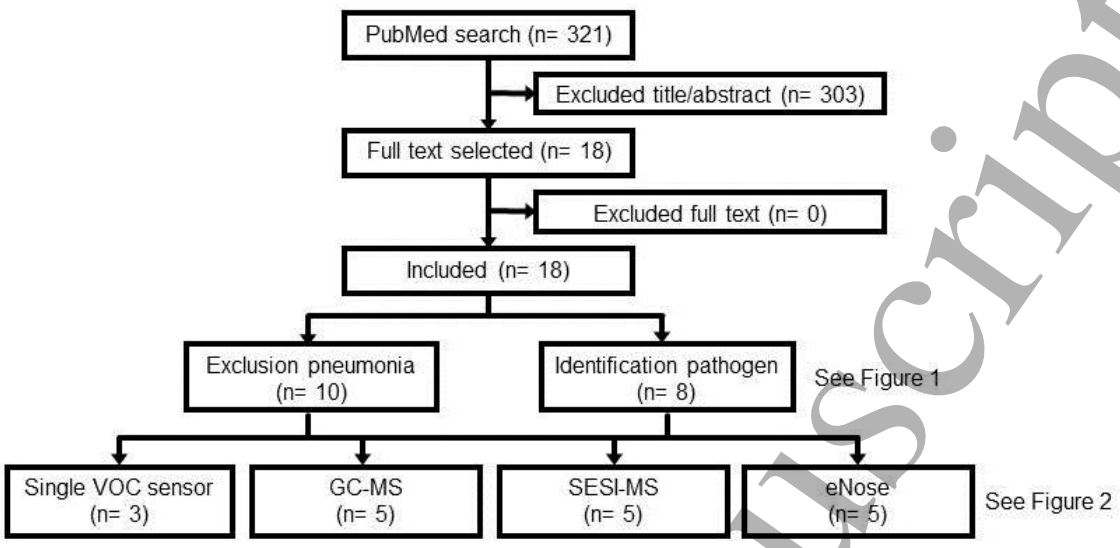
Figure 3.



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