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NOTE

Use of an innovative and non-invasive device for virologic sampling of cough aerosols in patients with community and hospital acquired pneumonia: a pilot study

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Filippo Patrucco[1], **Francesco Gavelli**[2], **Paolo Ravanini**[3], **Matteo Daverio**[1], **Giulia Statti**[2], **Luigi Mario Castello**[2], **Stefano Andreoni**[3] **and Piero Emilio Balbo**[1]

[1] Medical Department, Division of Respiratory Diseases, University of Piemonte Orientale, Maggiore della Carità Hospital, Novara, Italy
[2] Department of Translational Medicine, Emergency Medicine Unit, University of Piemonte Orientale, Maggiore della Carità Hospital, Novara, Italy
[3] Laboratory Medicine Department, Microbiology and Virology Unit, University of Piemonte Orientale, Maggiore della Carità Hospital, Novara, Italy

E-mail: filippo.patrucco@gmail.com

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Abstract

Background: The aetiology of lower respiratory tract infections is challenging to investigate. Despite the wide array of diagnostic tools, invasive techniques, such as bronchoalveolar lavage (BAL), are often required to obtain adequate specimens. PneumoniaCheck™ is a new device that collects aerosol particles from cough, allowing microbiological analyses. Up to now it has been tested only for bacteria detection, but no study has investigated its usefulness for virus identification. *Methods*: In this pilot study we included 12 consecutive patients with pneumonia. After testing cough adequacy via a peak flow meter, a sampling with PneumoniaCheck™ was collected and a BAL was performed in each patient. Microbiological analyses for virus identification were performed on each sample and concordance between the two techniques was tested (sensitivity, specificity and positive/negative predictive values), taking BAL results as reference. *Results*: BAL was considered adequate in 10 patients. Among them, a viral pathogen was identified by PneumoniaCheck™ 6 times, each on different samples, whereas BAL allowed to detect the presence of a virus on 7 patients (14 positivities). Overall, the specificity for PneumoniaCheck™ to detect a virus was 100%, whereas the sensitivity was 66%. When considering only herpes viruses, PneumoniaCheck™ showed a lower sensitivity, detecting a virus in 1/4 of infected patients (25%). *Conclusions*: In this pilot study PneumoniaCheck™ showed a good correlation with BAL for non-herpes virologic identification in pneumonia patients, providing excellent specificity. Further studies on larger population are needed to confirm these results and define its place in the panorama of rapid diagnostic tests for lower respiratory tract infections.

1. Introduction

Lower respiratory tract infections challenge the physician to investigate their aetiology, as the ideal specimen is difficult to collect from the site of infection and it is not uncommon to deal with poor quality samples. Nevertheless, the rapid and precise recognition of the pathogen still remains of cardinal importance, especially in immunosuppressed patients due to haematological, neoplastic or post-transplant conditions [1–3].

To date, oropharyngeal swab, induced or spontaneous sputum and bronchoalveolar lavage (BAL) are the main techniques that allow the clinician to perform microbiologic, cellular and immune-enzymatic evaluations on respiratory tract samples in the diagnostic pathway of either community acquired pneumonia (CAP) or hospital acquired pneumonia (HAP). Some of these techniques provide specimens that are adequate to detect viruses but often inadequate for other pathogens (i.e. nasopharyngeal swab), whereas other samples contain contaminants from the upper

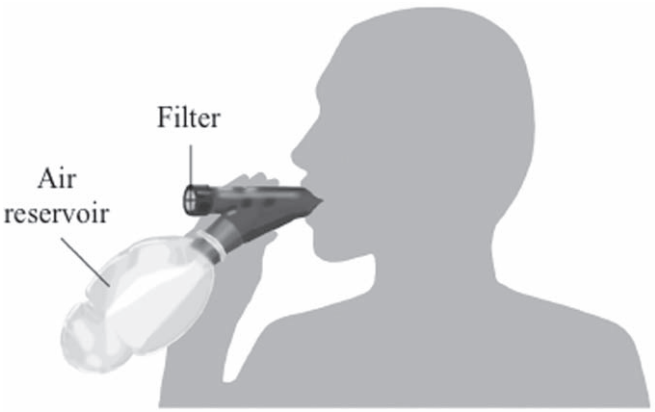

Figure 1. PneumoniaCheckTM structure. Reproduced from [29]. CC BY 4.0.

respiratory tract or do not allow to investigate the presence of specific pathogens (i.e. sputum), leading the physician to empirically treat the infection [4–7].

BAL allows to collect specimens directly from the lungs and is considered an important tool in the etiological diagnosis of pneumonia. Despite the fact that flexible bronchoscopy is a safe and usually well tolerated procedure [8], due to its invasive nature, it is still not routinely performed in every patient or in every centre. Furthermore, even with this technique the sample risks to be contaminated by pathogens from the upper respiratory tract [9–11]. To date, bronchoscopy and BAL are recommended for immunosuppressed patients or for patients in which the antibiotic therapy has failed to resolve the infection [12, 13]. It is estimated that CAP aetiology can be determined by endoscopic procedures in 44% of the patients, especially in those non responder to antibiotic therapy [14, 15].

Aerosols produced during coughs are a valid alternative to collect samples from the lower respiratory tract and alveolar spaces, as it is estimated that during a single cough, up to 66.000 aerosol particles can be generated [16–22]. According to the literature, to generate effective coughs it is required for the patient to reach 270 l min^{-1} (4.5 l s^{-1}) of peak cough expiratory flow (PCEF), measured by peak flow meter [23, 24]; normal values for a healthy adult are above 360 l min^{-1} [25]. Patients affected by lower respiratory tract infections can infect other people through the dispersion in the air of aerosol particles produced by coughs or sneezing; as a matter of fact, the concentration of pathogens from the lungs is higher in cough than in sneezing aerosols [26].

PneumoniaCheck™ (ARC Medical Inc.) is a new device designed to collect specimens from the lower respiratory tract exhaled with coughs; in particular, it retains aerosol particles in a dedicated filter, preventing contamination from the upper respiratory tract (figure 1). The filter is then analysed through molecular and microbiological tests, in order to detect the presence of different pathogens [27]. Fluid mechanics principles allow the filter to separate aerosol particles from the lungs and the air coming from the upper respiratory tract. When the patient coughs into the device, the air from the upper respiratory tract flows into the reservoir, which offers lower resistances compared to the filter. It should be reminded that normally, during exhalation, the anatomic dead space contaminates the first volume of air coming from the lungs; since the PneumoniaCheck™ reservoir has a volume of 250 ml, it ensures the separation of all the aerosols particles of the upper airways, with a 100 ml margin on the average dead anatomic space [28]. Furthermore, the reservoir bag is inelastic, forcing the subsequent exhaled air through the above-mentioned filter, allowing to collect only the lower respiratory tract aerosols (figure 2).

Two validation studies have tested the efficacy of this device on collecting samples during respiratory infections [30, 31]. To evaluate the concordance between biomolecular and microbiological results obtained by PneumoniaCheck™ and other traditional sampling methods in patients affected by pneumonia was, thus, the aim of this study.

2. Materials and methods

This prospective single-centre observational pilot study was conducted in accordance with STROBE statement for observational studies [32] and approved by our institutional review board. Informed consent was collected from each patient; possibility to withdraw it was given at any time. Patients older than 18 years with pneumonia (CAP or HAP) and non-productive cough, undergoing a diagnostic bronchoscopy with BAL were prospectively included. Exclusion criteria were a PCEF < 270 l min^{-1} (4.5 l s^{-1}) measured with peak flow meter (Vitalograph Peak Cough Flow, Medical Graphics Italia Srl, Milano, Italy) and pregnancy.

After the evaluation of PCEF, a sampling with PneumoniaCheck™ and a bronchoscopy with BAL

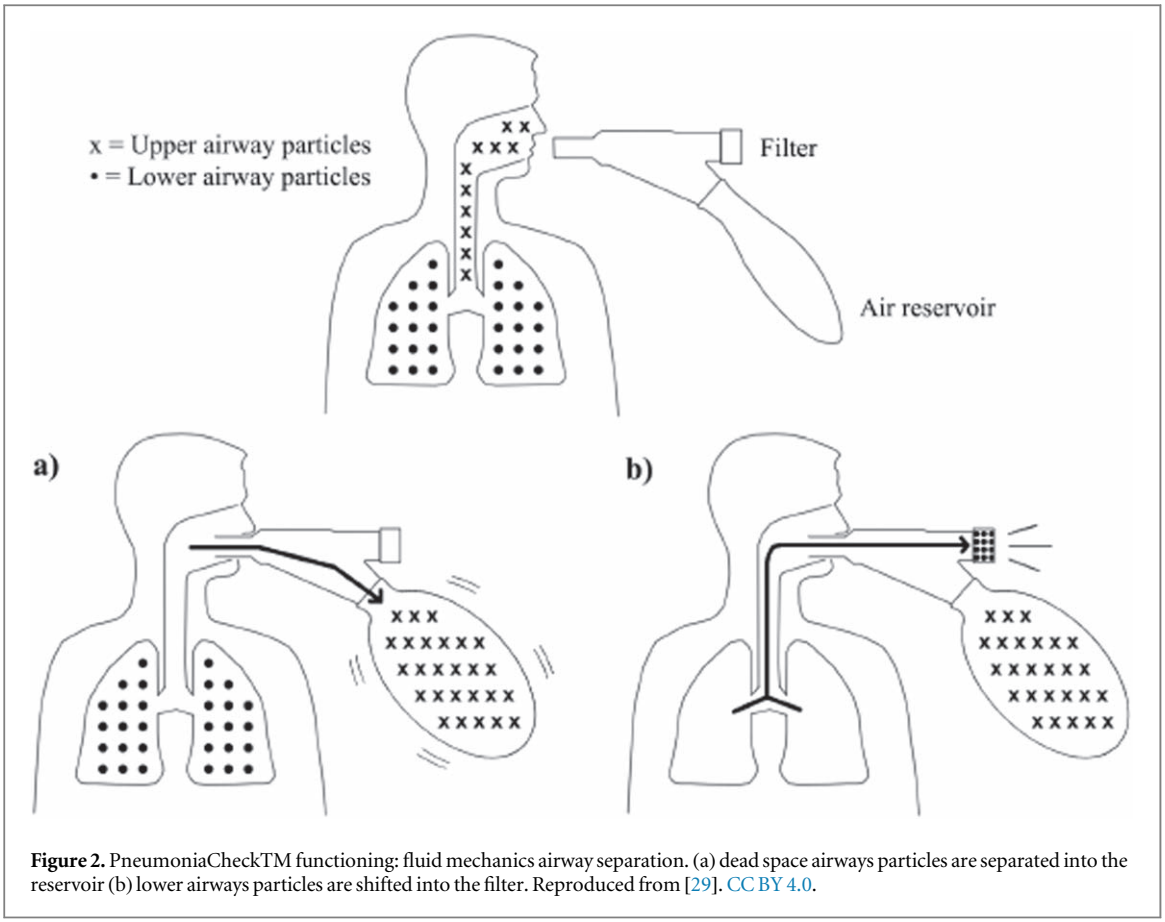

Figure 2. PneumoniaCheckTM functioning: fluid mechanics airway separation. (a) dead space airways particles are separated into the reservoir (b) lower airways particles are shifted into the filter. Reproduced from [29]. CC BY 4.0.

were performed in each patient. After the cough procedure in the device, the filter was simply removed with sterile pincers and immediately put in a eNAT test tube containing 1 ml of virological conservative fluid (Copan Italia SpA, Brescia, Italy). A first aliquot of 300 μl was used for a molecular multiplex determination in Real-Time Polymerase Chain Reaction (RT-PCR) with the TOCE^{TM} Technology, using the extractor Microlab Nimbus IVD (Hamilton Company) and the Anyplex II RV16 kit (Seegene Inc.). The Anyplex II RV16 System allows to find specific viral pathogens such as Influenza A and B, Respiratory Syncytial Viruses A and B, Adenovirus, Bocavirus, Rhinovirus, Parainfluenza (1, 2, 3 and 4), Respiratory Enteroviruses, Coronavirus (NL63, 229E, OC43) and Metapneumovirus. The remaining 700 μl were used for RT-PCR tests using the Elite MGB Kits (ELITechGroup Inc.) for Herpesviruses. Through the Elite MGB Kits, it was possible to detect Herpes Simplex Virus-1, Herpes Simplex Virus-2, Varicella-Zoster Virus, Epstein-Barr Virus, Human CMV and Human Herpes Virus-6. Subsequently, a bronchoscopy with a BAL was performed on each patient and the same determinations were done in the same way on specimens obtained by BAL. Each BAL and its adequacy were performed and evaluated in accordance with international guidelines [33]; for each patient we recorded the volume of fluid recovery and its adequacy. The amount of BAL fluid destined to microbiological and virological analysis was 5 ml. For the virological testing with RT PCRs, we used 1 ml of BAL fluid. After collection, this amount of specimen was immediately separated in two aliquots and stored at −20 °C until processed.

We evaluated the concordance of results obtained with PneumoniaCheck^{TM} and BAL. We also calculated sensitivity, specificity, positive and negative predictive values for viral infections for PneumoniaCheck^{TM}. Data are expressed as mean ±SD. Statistical analysis was performed with MedCalc 18.2.1 software (Mariakerke, Belgium).

3. Results

Between December 2017 and February 2018 twelve consecutive patients underwent PneumoniaCheck^{TM} sampling, followed by bronchoscopy for pneumonia in the Interventional Pulmonary Unit of a medium size teaching hospital. Five patients were hospitalized whereas seven were outpatients. Demographic characteristics, smoking habits and comorbidities are listed in table 1. We included 3 HAP and 9 CAP: among the patients with CAP, 2 were immunocompromised for onco-hematological disorders and 1 for renal transplant. At the CT scan, the pneumonia radiological patterns encountered were consolidative (50%), pure ground-glass (25%), consolidative and ground-glass (10%) and tree-in-bud (5%). The vast majority (83%) of patients were or had been recently treated with antibiotics, 2 were taking acyclovir as prophylaxis

Table 1. Patient characteristics. C: cardiologic, E: endocrine, R: respiratory, K: nephrologic, O: onco-hematologic, N: neurologic, H: hepatic, T: trasnplant; CAP: community-acquired pneumonia; HAP: hospital-acquired pneumonia; CT: computed tomography; ATB: antibiotic; ATV: antiviral (numbers after ATB are referred to the day of ongoing treatment).

Patient	Gender	Age	Comorbidities	Pneumonia	CT pattern	Treatment—day
1	M	78	C, E	HAP	Tree-in-bud	ATB-4
2	F	69	C, E	CAP	Consolidative	ATB-7
3	M	58	C, R, N	HAP	Ground glass	ATB-8; ATV
4	M	36	O	CAP	Consolidative and ground glass	ATV
5	M	79	C, R, K	CAP	Consolidative	ATB-10
6	F	70	E, R	HAP	Consolidative	ATB-12
7	M	56	C, K, T	CAP	Consolidative	ATB-5, ATV
8	F	74	C, N	CAP	Consolidative	ATB-10
9	M	22	O	CAP	Consolidative and ground glass	None
10	F	67	R	CAP	Ground glass	ATB-7
11	M	71	C, R	CAP	Ground glass	ATB-10
12	F	74	R, K, H	CAP	Consolidative	ATB-12

Table 2. PneumoniaCheckTM and bronchoalveolar lavage (BAL) results. PCEF: peak cough expiratory flow; HSV1: herpes simplex virus 1, FluA: influenza A virus, RSV: respiratory syncytial virus; EBV: Ebstein-Barr virus; CMV: cytomegalovirus; HHV6: human herpesvirus-6

Patient	PCEF ($l\,min^{-1}$)	PneumoniaCheck™	BAL—Virologic	BAL—Bacteria	BAL fluid recovery
1	>270	HSV1	Rhinovirus, HSV1, EBV, CMV, HHV6	*Pseudomonas aeruginosa*	55 ml
2	>270	Negative	EBV	Negative	60 ml
3	>270	FluA	FluA, EBV, HHV6	Negative	50 ml
4	>270	Parainfluenzae	Parainfluenzae	Negative	70 ml
5	>270	Negative	Coronavirus NL63, EBV	*Klebsiella pneumoniae*	50 ml
6	>270	Negative	Negative	*Moraxella catarralis*	35 ml
7	>270	Parainfluenzae	Invalid	Invalid	60 ml
8	>270	Negative	Invalid	Invalid	50 ml
9	>270	RSV	RSV	Negative	65 ml
10	>270	Negative	Negative	Negative	50 ml
11	>270	Rhinovirus	Rhinovirus	Negative	45 ml
12	>270	Negative	Negative	*Pseudomonas aeruginosa*	70 ml

therapy (acyclovir 400 mg) and none of them was in treatment with antifungal drugs. Details of days of treatment are reported in table 1. Five patients had previously received seasonal influenza vaccination and one patient pneumococcal vaccination.

All the patients had a PCEF $\geqslant 270\,l\,min^{-1}$ ($4.5\,l\,s^{-1}$) measured with peak flow meter and the specimen collection via PneumoniaCheck™ was correctly completed by all of them. The sample obtained with bronchoscopy was considered adequate in 10 patients (83%) and the mean recovery BAL volume was 55 ± 10.4 ml. No complications related to bronchoscopy were observed. A viral pathogen was identified by PneumoniaCheck™ 6 times, each on different samples, whereas BAL allowed to detect the presence of a virus on 7 patients (14 positivities). The list of pathogens identified by each method is reported in table 2.

Patients with an inadequate BAL were not entered in the statistical analysis, since this technique is considered the gold standard for comparison analysis. Concordance rate for non-herpes viruses was 66% (4/6): in four cases PneumoniaCheck™ and BAL identified the same virus, while in two cases BAL identified a virus which was not detected by PneumoniaCheck™ (Rhinovirus and Coronavirus NL63). Furthermore, in one case PneumoniaChecK™ detected a virus when BAL was considered as inadequate (Parainfluenza virus).

PneumoniaCheck™ sensitivity and specificity for non-herpes viruses were 66% and 100% respectively, and predictive positive and negative values were 100% and 66% respectively (data are referred to table 2 results). Herpes viruses were detected only once by PneumoniaCheck™ and BAL, on the same patient. Overall, PneumoniaCheck™ was able to identify 1 of 4 patients with at least one Herpes virus detected with BAL (sensitivity 25%). When considering all Herpes viruses detections on BAL, the sensitivity dropped to 12,5% (1/8) (table 2).

Finally, the microbiological analysis on BAL specimens allowed to identify 4 bacterial pathogens on different patients (table 2).

4. Discussion

Our pilot study shows that PneumoniaCheck™ is a useful non-invasive tool for detecting non-herpetic viral pathogens in patients with effective cough. The concordance between PneumoniaCheck™ and BAL samples shows a high specificity (100%) and a good sensitivity (66%). In case of Herpesvirus the concordance was weaker. To our best knowledge, this is the first study that has specifically tested PneumoniaCheck™ for virus detection.

Lower respiratory tract infections are one of the leading causes of morbidity and mortality worldwide [34, 35]. The incidence of viral pathogens in this context is highly variable among the studies and it is often influenced by the diagnostic techniques [36]. Nevertheless, a prompt and definitive diagnosis remains the cornerstone for the management and treatment of viral respiratory infections [37]. In CAP, some of the most important viruses involved are Influenza and Parainfluenza, Respiratory Syncytial Virus, Adenovirus, Coronavirus and Rinhovirus [36, 38] and they can be detected through analyses of serum samples, cultures, rapid diagnostic testing with enzyme immunoassay or immunofluorescence [39, 40]. Nevertheless, the introduction of PCR has increased the diagnostic yields, compared to the conventional diagnostic procedures [41]. Despite all these tools, it remains difficult in some cases to determine whether one of these pathogens is involved in the development of the infection. As a matter of fact, it is considered that only the detection of the Influenza virus can be seen as an actual etiologic factor and not as colonisation. Thus, prudence is required in interpreting the results, as up to 15% of healthy people are carriers of respiratory tract viruses [42].

PneumoniaCheck™ is a new device that collects microbiologic samples from the lower respiratory tract, bypassing the upper respiratory tract and avoiding possible contaminations. A recent study has demonstrated that more than 99% of bacteria and viruses can be retained by the microbial filter and when the device was tested on healthy volunteers, it showed the absence of contaminants, even when the sample was collected after stimulation with 15 ml of liquid [43]. Moreover, by analysing different alcohol and oxygen levels, it has been demonstrated that PneumoniaCheck™ is able to efficaciously separate gas from the upper and the lower airways ($p < 0.0001$) [43]. The same study evaluated the collection proprieties of the filter by testing the viral filtration efficiency, which resulted to be 99.9975%, with a mean particle size of 2.8 μm. With this results the authors confirmed both that the filter used in the PneumoniaCheck™ is able to capture 99.99% of viruses in aerosol particles and that PCR analysis on bacterial DNA could be performed (notably, the authors evaluated the efficacy of RT-PCR only for bacteria and not for viruses). Moreover, the A-M System Inc. who produced the VBMax filter incorporated in PneumoniaCheck™, used the Bacteriophage X174 to test the viral filtration efficiency; due to the diameter of the Bacteriophage X174, we can presume that the filter of PneumoniaCheck™ is actually able to collect a large quality of viruses screened by RT-PCR system (only Rhinoviruses, Enteroviruses and Bocaviruses have dimension similar to the Bacteriophage X174).

Recently, Ku *et al* examined lower tract infections through PneumoniaCheck™ in cystic fibrosis (CF) patients. According to their results, 65% of the samples collected via PneumoniaCheck™ was positive for CF-related bacteria and none of them showed contamination from commensal bacteria (compared to 100% of contamination in the sputum samples) [29]. Nevertheless, the patients' medical history was peculiar and well-known by the physicians and tests were performed for bacteria very likely to be present in the airways. In this study we decided to focus our attention on viral pathogens, as they are more difficult to detect compared to the bacterial ones, and since the biological samples are more likely to be naive of antimicrobial treatment, reducing this risk of bias. According to the literature, the prevalence of virus isolations on BAL fluid in patients with CAP or HAP is about 17%, but it is estimated to be higher in case of transplanted patients [44, 45]. One of the drawbacks of BAL is the risk to obtain an inadequate specimen, due to the variability of the sampling procedure [46]. As a matter of fact, in our cohort, 2 of the 12 BAL samples were considered as inadequate for a definitive diagnosis. Conversely, all PneumoniaCheck™ collections were adequate for virological analysis, showing good ability of this device in obtaining good samples. The array of viruses isolated through PneumoniaCheck™ is in line with community acquired respiratory viruses: Herpes Simplex Virus 1, Virus Influenzae A, Epstein-Barr Virus, Human Herpes Virus 6, Virus Parainfluenzae, Rhinovirus and Syncitial Respiratory Virus. The only case of Influenza Virus present in our cohort was detected by both BAL and PneumoniaCheck™.

In our cohort we identified a large number of herpes virus positivities on BAL but only one on PneumoniaCheck™ samples. On the one hand, we must consider that BAL gives a large amount of sample for molecular multiplex determinations, whereas the filter of the PneumoniaCheck™ has 1 ml of conservative fluid and 700 μl are used only for the RT PCR tests for herpes viruses. On the other hand, it is necessary to highlight the role of this family of viruses in the pathogenesis of lower respiratory tract infections both in the immunocompromised and in the immunocompetent host. In the latter group, the infection usually has an asymptomatic or mononucleosis-like syndrome course but, occasionally, a primary cytomegalovirus (CMV) infection can evolve into more severe organ-specific manifestations [47–52]. Furthermore, as previously mentioned, many patients may display a colonisation from herpes viruses and, thus, a positive

PCR might not be sufficient to distinguish asymptomatic colonisation from an active infection. Conversely, quantification of the viral load on BAL fluid could potentially differentiate between these two conditions [53].

Moreover, herpes viruses transmission is a clinical challenge, in particular for clinician who are involved in transplant protocols: in these situations the majority of infections are the consequence of reactivation of a latent infection. Herpes viruses transmission occurs usually through inhalation of oral secretion. In literature, studies evaluating virus colonization of droplets are lacking and, for this reason, details regarding the patient-to-patient transmission of viruses are matter of discussion [54]. However, we know that PneumoniaCheck^{TM} collects particles originating from lower airways (mainly over the dead space) giving sample of the colonizing viruses. Furthermore, we must consider that different viruses have different targets in the airways: in fact non-herpes viruses are responsible for the bigger part of viral pneumonia, colonizing the bronchial surface and sometimes the alveolar one. On the contrary, herpes viruses usually colonize the upper airways (rarely the lower ones); this could partially explain our results, since the upper airways are excluded from the sampling [55]. In addition, since BAL fluid can collect a higher number of viruses and cells infected, by washing the alveolar and bronchial surface, the possibility to evaluate the presence of viruses through Q-PCR leads to higher quantitative results.

We believe that the use of PneumoniaCheck^{TM} in a specific population make easier the identification of specific agents but risks to ignore the presence of other ones. Compared to the study of Ku *et al* [29], we included 12 consecutive patients with pneumonia who underwent bronchoscopy in a University Hospital Pulmonary Unit. The population of the study was not known, and the diagnostic pathway required a larger displacement of diagnostic tools. Thus, to narrow down the list of the possible implicated pathogens and to improve the accuracy of our results, we decided to test PneumoniaCheck^{TM} specifically for virus detection. We found that this device has a high specificity, which facilitates the detection of non-infected patients, and a lower sensitivity. Nevertheless, the latter may be influenced by the small number of subjects included in our study and the type of virus. As a matter of fact, compared to non-herpes viruses, herpes viruses were more difficult to detect, lowering the sensitivity of the device for this sub-population.

Two are the crucial and mainstay points of our study. First, all patients were tested with peak flow meter before PneumoniaCheck^{TM} use in order to verify the presence of an effective cough, a precaution that was not taken in the study of Ku *et al* which allows us to look at the 100% of adequacy of PneumoniaCheck^{TM} samples as an indirect validation. Second, we compared this new device with BAL, which is at the time the gold standard for the diagnosis.

Our study has some limitations. First, the small number of subjects included may have influenced the results, in particular the sensitivity of PneumoniaCheck^{TM}. Nevertheless it is a pilot study and further patients inclusions as well as new trials could clarify this issue. Second, the microbiological analyses were limited to virological samples, excluding standard bacterial and fungal cultures. Nevertheless, the restricted amount of biologic sample collected into the filter of PneumoniaCheck^{TM} would have not been sufficient for a complete investigation of a wide range of possibly involved bacteria in a non-selected population. Conversely, this issue could become a strength of the device, as it could allow a rapid identification of bacteria and viruses in specific and highly-selected populations, as lung transplanted and CF patients. Finally, another limit of the device is the filtering power of the filter used in the PneumoniaCheck^{TM}: the great portion of aerosol size in exhaled air ranges from 0.3 to 0.5 μm and for this reason some negative PneumoniaCheck^{TM} results are in contrast with those positive obtained with BAL [56]. Nevertheless, as demonstrated by Lindsay *et al* a high number of influenza RNA could be found also in particles with an aerodynamic diameter greater than 4 μm [57]. For this reason we think that, even if the filter has a collecting efficiency of 3 μm, a significant number of particles carrying viruses can be collected.

5. Conclusions

PneumoniaCheck^{TM} is a new device, safe and easy to use, that collects lower airways pathogens from cough aerosols; it shows a good correlation with BAL for non-herpes virologic identification in patients with pneumonia, providing excellent specificity and good sensitivity. Further studies on larger population are needed to confirm our results and to find the correct place of PneumoniaCheck^{TM} in the panorama of rapid diagnostic tests in patients with lower tract respiratory infections.

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The authors declare that they have no conflict of interest.

ORCID iDs

Filippo Patrucco https://orcid.org/0000-0002-4794-8734
Francesco Gavelli https://orcid.org/0000-0002-0581-245X

References

[1] Dela Cruz C S *et al* 2018 Future research directions in pneumonia. NHLBI working group report *Am. J. Respir. Crit. Care Med.* **198** 256–63

[2] Costa C *et al* 2012 Clinical impact of HSV-1 detection in the lower respiratory tract from hospitalized adult patients *Clin. Microbiol. Infect Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis.* **18** E305–7

[3] Gambarino S *et al* 2009 Lower respiratory tract viral infections in hospitalized adult patients *Minerva Med.* **100** 349–55

[4] Burns M W 1968 Precipitins to Klebsiella and other enterobacteria in the serum of patients with chronic respiratory disorders *Lancet* **1** 383–5

[5] Goddard A F *et al* 2012 Direct sampling of cystic fibrosis lungs indicates that DNA-based analyses of upper-airway specimens can misrepresent lung microbiota *Proc. Natl Acad. Sci. USA* **109** 13769–74

[6] Lentino J R and Lucks D A 1987 Nonvalue of sputum culture in the management of lower respiratory tract infections *J. Clin. Microbiol.* **25** 758–62

[7] Rosenfeld M *et al* 1999 Diagnostic accuracy of oropharyngeal cultures in infants and young children with cystic fibrosis *Pediatr. Pulmonol.* **28** 321–8

[8] Gavelli F, Patrucco F, Statti G and Balbo P E 2018 Mild-to-moderate haemoptysis: a diagnostic and clinical challenge *Minerva Med.* **109** 239–47

[9] Gilchrist F J, Salamat S, Clayton S, Peach J, Alexander J and Lenney W 2011 Bronchoalveolar lavage in children with cystic fibrosis: how many lobes should be sampled? *Arch. Dis. Child.* **96** 215–7

[10] Willner D *et al* 2012 Spatial distribution of microbial communities in the cystic fibrosis lung *ISME J.* **6** 471–4

[11] Wimberley N, Faling L J and Bartlett J G 1979 A fiberoptic bronchoscopy technique to obtain uncontaminated lower airway secretions for bacterial culture *Am. Rev. Respir. Dis.* **119** 337–43

[12] Arancibia F *et al* 2000 Antimicrobial treatment failures in patients with community-acquired pneumonia: causes and prognostic implications *Am. J. Respir. Crit. Care Med.* **162** 154–60

[13] van der Eerden M M, Vlaspolder F, de Graaff C S, Groot T and Jansen HMBoersma W G 2005 Value of intensive diagnostic microbiological investigation in low- and high-risk patients with community-acquired pneumonia *Eur. J. Clin. Microbiol. Infect. Dis. Off. Publ. Eur. Soc. Clin. Microbiol.* **24** 241–9

[14] Ortqvist A, Kalin M, Lejdeborn L and Lundberg B 1990 Diagnostic fiberoptic bronchoscopy and protected brush culture in patients with community-acquired pneumonia *Chest* **97** 576–82

[15] Costantini E, Allara E, Patrucco F, Faggiano F, Hamid F and Balbo P E 2016 Adherence to guidelines for hospitalized community acquired pneumonia over time and its impact on health outcomes and mortality *Intern. Emerg. Med.* **11** 929–40

[16] Couch R B, Cate T R, Douglas R G, Gerone P J and Knight V 1966 Effect of route of inoculation on experimental respiratory viral disease in volunteers and evidence for airborne transmission *Bacteriol. Rev.* **30** 517–29

[17] Couch R B *et al* 1965 Production of illness with a small-particle aerosol of coxsackie A21 *J. Clin. Invest.* **44** 535–42

[18] Gerone P J, Couch R B, Keefer G V, Douglas R G, Derrenbacher E B and Knight V 1966 Assessment of experimental and natural viral aerosols *Bacteriol. Rev.* **30** 576–88

[19] Lee N *et al* 2009 Viral loads and duration of viral shedding in adult patients hospitalized with influenza *J. Infect. Dis.* **200** 492–500

[20] Wainwright C E *et al* 2009 Cough-generated aerosols of Pseudomonas aeruginosa and other gram-negative bacteria from patients with cystic fibrosis *Thorax* **64** 926–31

[21] Knibbs L D *et al* 2014 Viability of pseudomonas aeruginosa in cough aerosols generated by persons with cystic fibrosis *Thorax* **69** 740–5

[22] Costa C, Bucca C, Bergallo M, Solidoro P, Rolla G and Cavallo R 2011 Unsuitability of exhaled breath condensate for the detection of herpesviruses DNA in the respiratory tract *J. Virol. Methods* **173** 384–6

[23] Bach J R, Ishikawa Y and Kim H 1997 Prevention of pulmonary morbidity for patients with Duchenne muscular dystrophy *Chest* **112** 1024–8

[24] Mier-Jedrzejowicz A, Brophy C and Green M 1988 Respiratory muscle weakness during upper respiratory tract infections *Am. Rev. Respir. Dis.* **138** 5–7

[25] Bach J R *et al* 1993 Airway secretion clearance by mechanical exsufflation for post-poliomyelitis ventilator-assisted individuals *Arch Phys. Med. Rehabil.* **74** 170–7

[26] Bischoff W E, Swett K, Leng I and Peters T R 2013 Exposure to influenza virus aerosols during routine patient care *J. Infect. Dis.* **7** 1037–46

[27] Liljemark W F and Gibbons R J 1972 Proportional distribution and relative adherence of Streptococcus miteor (mitis) on various surfaces in the human oral cavity *Infect. Immun.* **6** 852–9

[28] Orzalesi M M, Hart M C and Cook C D 1965 Distribution of ventilation in normal subjects from 7 to 45 years of age *J. Appl. Physiol.* **20** 77–8

[29] Ku D N *et al* 2016 Ability of device to collect bacteria from cough aerosols generated by adults with cystic fibrosis *F1000Res.* **5** 1920

[30] Eccles S, Pincus C, Higgins B and Woodhead M 2014 Guideline development group. Diagnosis and management of community and hospital acquired pneumonia in adults: summary of NICE guidance *Brit. Med. J.* **349** g6722

[31] Mandell L A *et al* 2007 Infectious diseases society of America/ American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **44** S27–72

[32] von Elm E, Altman D G, Egger M, Gotzsche P C and Vandenbroucke J P 2007 STROBE initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies *Lancet* **370** 1453–7

[33] Haslam P L and Baughman R P 1999 Report of ERS task force: guidelines for measurement of acellular components and standardization of BAL *Eur. Respir. J.* **14** 245–8

[34] Wunderink R G and Waterer G W 2014 Clinical practice community-acquired pneumonia *New Engl. J. Med.* **370** 543–51

[35] Monti S *et al* 2011 H1N1 2009 influenza vaccine prevention: a comparison between the italian press and the scientific recommendations *Ital. J. Public Health* **8** 49–59

[36] Cesario T C 2012 Viruses associated with pneumonia in adults *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **55** 107–13

[37] Rappo U *et al* 2016 Impact of early detection of respiratory viruses by multiplex PCR assay on clinical outcomes in adult patients *J. Clin. Microbiol.* **54** 2096–103

[38] Costa C *et al* 2011 Detection of human rhinoviruses in the lower respiratory tract of lung transplant recipients *Arch. Virol.* **156** 1439–43

[39] Lieberman D, Shimoni A, Shemer-Avni Y, Keren-Naos A, Shtainberg R and Lieberman D 2010 Respiratory viruses in adults with community-acquired pneumonia *Chest* **138** 811–6

[40] Gaydos C A 2013 What is the role of newer molecular tests in the management of CAP? *Infect. Dis. Clin. North Am.* **27** 49–69

[41] Ruuskanen O, Lahti E, Jennings L C and Murdoch Dr 2011 Viral pneumonia *Lancet* **377** 1264–75

[42] Jartti T *et al* 2008 Identification of respiratory viruses in asymptomatic subjects: asymptomatic respiratory viral infections *Pediatr. Infect. Dis. J.* **27** 1103–7

[43] Scholz T L, Midha P A, Anderson L J and Ku D N 2010 PneumoniaCheck: a device for sampling lower airway aerosols *J. Med. Devices* **4** 041005

[44] Garbino J *et al* 2009 Respiratory viruses in bronchoalveolar lavage: a hospital-based cohort study in adults *Thorax* **64** 399–404

[45] Potena L, Solidoro P, Patrucco F and Borgese L 2016 Treatment and prevention of cytomegalovirus infection in heart and lung transplantation: an update *Expert Opin. Pharmacother.* **17** 1611–22
[46] Baughman R P 1997 The uncertainties of bronchoalveolar lavage *Eur. Respir. J.* **10** 1940–2
[47] Reid G E *et al* 2016 Herpesvirus respiratory infections in immunocompromised patients: epidemiology, management, and outcomes *Semin. Respir. Crit. Care Med.* **37** 603–30
[48] Uhlin M, Mattsson J and Maeurer M 2012 Update on viral infections in lung transplantation *Curr. Opin. Pulm. Med.* **18** 264–70
[49] Assink-de Jong E *et al* 2013 Clinical correlates of herpes simplex virus type 1 loads in the lower respiratory tract of critically ill patients *J Clin. Virol. off. Publ. Pan. Am. Soc. Clin. Virol.* **58** 79–83
[50] Taplitz R A and Jordan M C 2002 Pneumonia caused by herpesviruses in recipients of hematopoietic cell transplants *Semin. Respir. Infect.* **17** 121–9
[51] Cohen J I and Corey G R 1985 Cytomegalovirus infection in the normal host *Medicine* **64** 100–14
[52] Grilli E, Galati V, Bordi L, Taglietti F and Petrosillo N 2012 Cytomegalovirus pneumonia in immunocompetent host: case report and literature review *J. Clin. Virol. off. Publ. Pan. Am. Soc. Clin. Virol.* **55** 356–9
[53] Tachikawa R *et al* 2014 Detection of herpes viruses by multiplex and real-time polymerase chain reaction in bronchoalveolar lavage fluid of patients with acute lung injury or acute respiratory distress syndrome *Respir. Int. Rev. Thorac. Dis.* **87** 279–86
[54] Azevedo L S *et al* 2015 Cytomegalovirus infection in transplanted recipients *Clinics* **70** 515–23
[55] Galvan J M, Rajas O and Aspa J 2015 Review of non-bacterial infections in respiratory medicine: viral pneumonia *Arch. Bronconeumol.* **51** 590–7
[56] Fabian P *et al* 2008 Influenza virus in human exhaled breath: an observational study *PLoS ONE* **3** e2691
[57] Lindsley W G *et al* 2010 Measurements of airborne influenza virus in aerosol particles from human coughs *PLoS ONE* **5** e15100