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Diagnostic performance of multiplex PCR on pulmonary samples versus nasopharyngeal aspirates in community-acquired severe lower respiratory tract infections

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\textbf{ARTICLE INFO}

\textbf{Keywords:}
Community-acquired infections
Respiratory pathogens
multiplex PCR

\textbf{ABSTRACT}

\textbf{Background:} PCR-based techniques for the diagnosis of community-acquired severe lower respiratory tract infections are becoming the standard of care. However, their relative ability to identify either atypical bacteria or viruses that cause LRTI from clinical samples from various sources is yet to be determined.

\textbf{Objectives and study design:} The aim of our study was to compare the diagnostic yield of nasopharyngeal aspirates with that of pulmonary samples for the etiological diagnosis of severe acute lower respiratory tract infections by multiplex PCR. Patients were adults with community-acquired pneumonia or acute exacerbation of chronic obstructive pulmonary disease.

\textbf{Results:} We obtained concordant results for 81 (79\%) of the 103 pairs of samples. In 14 of the 22 discordant results, more pathogens were evidenced in the lower respiratory tract samples.

\textbf{Conclusions:} Pulmonary samples had a similar diagnostic sensitivity for virus detection by multiplex PCR as nasopharyngeal aspirates. In contrast, in our study, the diagnostic efficacy of pulmonary samples for \textit{Legionella pneumophila} over simple aspirates was clearly superior.

\section{1. Background}

Acute lower respiratory tract infections (LRTI) are a major cause of morbidity and mortality worldwide. The identification of causative agents in severe community-acquired LRTI is mandatory for efficient clinical monitoring and treatment as clinical signs are poor etiological indicators. Until recently, two laboratory approaches have been used: one to identify bacterial infections from various pulmonary samples such as sputum, endotracheal aspirates, or bronchoalveolar lavage and one to identify respiratory viruses from nasopharyngeal aspirates, using generally low sensitivity assays, such as viral culture or antigen detection.

The development of multiplex real-time polymerase-chain reaction (PCR), to identify panels of viral pathogens and prevalent atypical respiratory bacteria has revolutionized the microbiological diagnosis of LRTI\textsuperscript{[1]} and expanded the range of pathogens that can be identified, including a large variety of viral agents\textsuperscript{[2]}. Several combinations of primers and probes are used by different manufacturers and commercial assays differ in their performances for each pathogen or group of pathogens\textsuperscript{[3,4]}. Validation of the method is not easy since multiplexing may hamper the performance of amplification as compared to single PCR\textsuperscript{[5]}. However, multiplex PCR have a better diagnostic yield for viruses than standard cell cultures with a specificity equal to or above 90\%\textsuperscript{[5]}.

PCR may prove useful for bacteria that are difficult or take a long time to culture\textsuperscript{[1]}. Thus numerous multiplex-PCR assays combine detection of viruses with that of a panel of fastidious bacteria in one run with a single clinical sample. However, their relative ability to identify either atypical bacteria or viruses that cause LRTI in various clinical samples of the upper or lower respiratory tract is yet to be determined.

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https://doi.org/10.1016/j.jcv.2018.08.001
Received 26 April 2018; Received in revised form 30 July 2018; Accepted 2 August 2018
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2. Objectives

The aim of our study was to compare the diagnostic yield of nasopharyngeal aspirates with that of pulmonary samples for the etiological diagnosis of severe LRTI in adults hospitalised in the intensive care unit at the University Hospital of Tours.

3. Study Design

3.1. Patients and samples

We enrolled a continuous series of adults between the ages of 20 and 89 years during their first two days following admission to the intensive care unit of the University Hospital of Tours for severe community-acquired pneumonia (CAP) or acute exacerbation of chronic obstructive pulmonary disease (COPD). Patients were recruited from January 2013 to January 2014. CAP was defined as the presence of a new pulmonary infiltrate by chest radiography associated with at least one of the following clinical symptoms: fever (> 38 °C) or hypothermia (< 35 °C), cough with or without sputum, shortness of breath, or cracking sounds on lung auscultation. Exacerbation of COPD was defined as symptomatic respiratory deterioration with an arterial pH < 7.35 or the need of oxygen therapy of over 3 L/min (Table 1).

Both nasopharyngeal aspirates (NPAs) and lower respiratory tract (LRT) samples were collected for each patient at the time of ICU admission. For NPAs, a disposable catheter connected to a mucus extractor was inserted into one nostril to a depth 5–7 cm and drawn back while applying gentle suction. LRT samples consisted of induced sputum, endotracheal aspirates, or bronchoalveolar lavage, depending on the clinical status of the patient. They were initially sent to the laboratory for bacterial culture. The intended time frame between collections was a maximum of 3 days.

The quality of all sputum and endotracheal samples was assessed by microscopic examination according to Bartlett’s cytologic criteria [8]. Samples with less than 25 squamous epithelial cells (magnification x100) were considered as representative of lower respiratory secretions. Eight samples that were not filling these criteria were discarded from the analysis.

Both sets of results were made available to the clinician and discrepant results discussed especially when specific treatment could be indicated for instance for influenza or Legionella infections.

3.2. Ethics statement

This was an observational and non-interventional study as no additional sampling was performed. Each patient or their legal representative received a written information letter of non-opposition. The study was approved by the ethics committee of the French society of intensive care, called SRLF for “Société de Réanimation de langue Française". Clinical and biological data were stored in an anonymized database.

3.3. Detection of respiratory pathogens by multiplex PCR

3.3.1. Nucleic acid extraction

Samples were pre-treated to avoid problems linked to viscosity and prevent non-specific inhibition of the PCR reaction. NPAs were diluted with an equal volume of sterile isotonic saline solution. Sputum and endotracheal aspirates were pre-treated with an equal volume of Digest-EUR™ (Eurobio, France) for 15 min at room temperature and then centrifuged for 15 min at 1500 g. The supernatant was discarded and replaced by an equivalent volume of sterile water before vortexing. Both types of samples were then frozen until use.

DNA and RNA were extracted using an EZI Advanced XL automatic extractor (Qiagen, Germany). Nucleic acids were extracted from 200 μL pre-treated samples and eluted in a final volume of 90 μL.

3.3.2. Multiplex PCR

All samples were analysed using the CE-marked multiplex molecular assay RespiFinder*SMART 22 (PathoFinder, The Netherlands). This assay is based on a multiplex ligation-dependent probe amplification technique [6]. It allows the simultaneous qualitative detection of four bacteria (Legionella pneumophila, Chlamydia pneumoniae, Mycoplasma pneumoniae, and Bordetella pertussis) and 18 respiratory viruses: influenza A, B, and A-H1N1pdm2009 virus, respiratory syncytial virus A and B, parainfluenza viruses 1–4, coronavirus OC43, 229E, NL63, and HKU1, rhinovirus/enterovirus, human metapneumovirus, Adenovirus, and human bocavirus. Assay manufacturer recommendations indicate that “a variety of specimen was suitable for the diagnosis of viral and/or bacterial infections of the respiratory tract” and a list included: nasopharyngeal aspirates, sputum, endotracheal aspirates and bronchoalveolar lavage.

Assays were performed on a LightCycler 480 (Roche, Switzerland), according to the manufacturer’s instructions.

3.4. Statistical analysis

Categorical variables were compared by using a Chi-square or Fisher’s exact test when appropriate. A p-value < 0.05 was considered as statistically significant. Frequencies, percentages, and sensitivities were manually calculated.

4. Results

103 eligible patients had available paired nasopharyngeal aspirate and pulmonary samples and were included in the study. Eighty-one patients were hospitalised for CAP and 22 for exacerbation of COPD. The primary clinical characteristics of the patients are shown in Table 1.

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Table 1

| Characteristics | No. of patients (%) |
|-----------------|---------------------|
| Demographic features | 103 |
| Sex (Male/Female) | 72 (70) /31(30) |
| Age mean (years ± SD) | 61.6 ± 14.8 |
| Age range (years) | 20-89 |
| Diagnosis |  |
| CAP | 81 (78.5) |
| Exacerbation of COPD | 22 (21.5) |
| Underlying conditions |  |
| Obesity | 37 (36) |
| Diabetes | 19 (18.5) |
| Heart failure | 14 (13.5) |
| COPD | 34 (33) |
| Chronic alcoholism | 23 (22.5) |
| Smoking | 40 (39) |
| Immune depression | 27 (26) |
| Chest radiography |  |
| New infiltrate on chest X-ray | 81 (78.5) |
| Interstitial opacity | 10 (10.3) |
| Alveolar opacity | 55 (68.0) |
| Interstitial and alveolar opacity | 16 (19.8) |
| Clinical feature and outcome |  |
| Simplified index of gravity (IGS II score: mean ± SD) | 40.6 ± 16 |
| Invasive mechanical ventilation | 65 (63) |
| Mortality | 9 (8.5) |

SD: standard deviation; COPD: Chronic obstructive pulmonary disease.
CAP: Community acquired pneumonia.
IGS II = Indice de gravité simplifié (Simplified gravity score) used in intensive care units.
Adapted from the Simplified Acute Physiology Score (SAPS II) as defined by Le Gall et al. [7].
We found that LRT specimens had a similar diagnostic sensitivity for virus detection by multiplex PCR as NPAs. In contrast, the diagnostic efficacy of pulmonary samples for Legionella pneumophila over NPAs was clearly superior. Some authors have suggested that sputums were even preferable for the molecular detection of viruses [11,13]. However, sampling methods need to be standardised to obtain LRT specimens of good quality, in terms of contamination with saliva, along with pretreatment procedures before extraction.

Development of accurate point-of-care tests for respiratory viruses has been listed as a priority by World Health Organization to replace empirical antimicrobial use and thus prevent emergence of resistance. Syndromic approaches in laboratory assays has driven the development of multiplex. PCR-based techniques that associate the detection of a large panel of viruses along with several atypical bacteria. PCR-based techniques can be performed in a few hours and are much more sensitive than previous assays based on antigenic detection. In many
instance, there are no specific recommendations pertaining to the type of samples to be used with such assays, although it appears that upper and lower respiratory tract samples perform differently for some pathogens.

In the same way that we have highlighted the superiority of pulmonary samples for the detection of L. pneumophila, comparable studies are needed to define the optimal sample and the detection limits for each respiratory pathogen of interest in LRTI. This should help to refine existing panels of respiratory pathogens, based on the type of patients and samples, and improve etiologic diagnosis.

Author contributions

SR conducted the viral experiments with the help of CLB. CL coordinated the collection of clinical specimens with the help of AL and JM. SR and CL contributed equally to the analysis.

DG and AG initiated the study, planned the experimental design and contributed to write the paper. All authors read and approved the final manuscript.

Conflict of interest

None.

Funding

None.

Ethical approval

This was an observational and non-interventional study as no additional sampling was performed. Each patient or their legal representative received a written information letter of non-opposition. The study was approved by the ethics committee of the French society of intensive care, called SRLF for “Société de Réanimation de langue Française”. Clinical and biological data were stored in an anonymized database.

Randomized controlled trial

NA.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Appendix A. Supplementary data

Supplementary material related to this article can be found in the online version, at doi:https://doi.org/10.1016/j.jcv.2018.08.001.

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