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Underdiagnosing of *Mycoplasma pneumoniae* infections as revealed by use of a respiratory multiplex PCR panel

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ABSTRACT

We compared a multiplex PCR diagnostic approach against specific PCR diagnosis for detection of *Mycoplasma pneumoniae* infection. Seventy-five percent of all *M. pneumoniae* infections were only detected "unintentionally" by the use of the multiplex PCR indicating underdiagnosing of *M. pneumoniae* due to absence of clinical suspicion.

Respiratory infectious diseases are often present with common symptoms, irrespective of the causative pathogen. In recent years, broad-spectrum multiplex PCR panels have been developed which are propagated as syndromic tests to diagnose respiratory infections (Popowitch et al., 2013). Whether highly multiplexed assays should be used as first-line tests is controversially discussed (Schreckenberger and McAdam, 2015). Whereas added clinical value of highly multiplexed assays is generally accepted, it remains unclear how exactly they are to be implemented in clinical routine (Dundas et al., 2011). A definite diagnosis can help in improved patient management, that is by avoidance of unnecessary antibiotic treatment (Schreckenberger and McAdam, 2015; Templeton, 2007). Underdiagnosing of specific infections has been put forward as another argument for use of highly multiplexed assays. Moreover, even for infectious diseases typically presenting with a combination of somehow specific symptoms, diagnosis in patients with comorbidities can be difficult (Campe et al., 2015).

Due to the organizational structure of the medical microbiology department in tertiary care University Hospital Heidelberg, with separated diagnostic units for virology and bacteriology, we recently were able to perform an unbiased evaluation of the diagnostic value of a multiplex PCR panel for aid in diagnosing infections by *Mycoplasma pneumoniae*. During a 6-month period from January 1 2015, to June 26 2015, all upper and lower respiratory tract, as well as sputum samples for which the clinicians requested a viral diagnostic test, were analyzed using the FTD-RP21 multiplex panel from Fast-track diagnostics (Esch-sur-Alzette, Luxembourg, Germany), run on the LightCycler 480 II. Nucleic acids were extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. This multiplex real-time PCR (Anderson et al., 2013; Bierbaum et al., 2014; Sakthivel et al., 2012) consists of 5 reaction mixtures (hydrolysis probes) which cover influenza A and B; influenza A (H1N1); coronaviruses NL63, 229E, OC43, and HKU1; parainfluenza 1, 2, 3, and 4; human metapneumovirus A and B; rhinovirus; respiratory syncytial viruses A and B; adenovirus; enterovirus; parechovirus; and bocavirus. Moreover, it also includes one bacterial target, *M. pneumoniae*. Detection of *M. pneumoniae* normally is not done at the virology department but has to be requested separately at the bacteriology department. Thus, we could analyze in an unbiased setup what the clinical value of a multiplex approach for detection of *M. pneumoniae* would be, as the request for diagnostic at the virology, the bacteriology or both departments was solely dependent on the clinicians' decision. Clinicians were not aware of the use of the panel PCR covering *M. pneumoniae* in samples with virologal request.

In total, $N = 2211$ respiratory samples were analyzed by the FTD-RP21 assay (Fig. 1A). Of those, $N = 23$ samples (1.04%), derived from 20 individual patients, were tested positive for *M. pneumoniae*. This is in the range of surveillance data from Germany (Weigl et al., 2007). Of the positive samples, 21 were confirmed in the bacteriology department by an *in house* PCR assay, the other 2 samples had high Ct values (Ct >35, indicative of low input) and were not available for retesting. Thus, the FTD-RP21 assay reliably detects *M. pneumoniae*. The 3 most prevalent results in the analyzed cohort were influenza A (positive detection rate 13.0%), RSV (6.9%), and coronavirus OC43 (2.8%) with an overall

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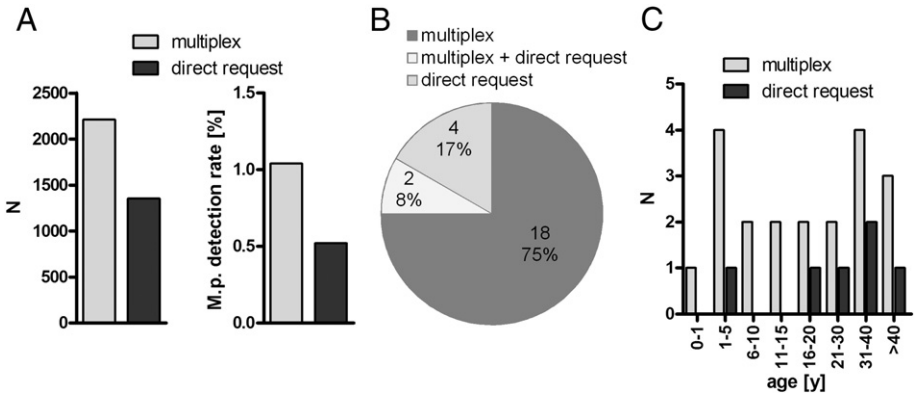

Fig. 1. Improved detection of *Mycoplasma pneumoniae* employing the syndromic FTD-RP21 PCR panel. (A) Number of samples (left) received during a 6-month period for detection of respiratory viruses (analyzed with the multiplex FTD-RP21 panel) or directly requested for *M. pneumoniae* (analyzed by *in house* PCR). Positive detection rate of *M. pneumoniae* (right). (B) Distribution of the modes of detection of $N = 24$ patients testing positive for *M. pneumoniae*. Indicated is whether diagnosis was made using the broad-spectrum multiplex panel (multiplex, performed in the virology department) or the direct request for a specific PCR (direct request, done in the bacteriology department) or both (*N*; %). (C) Age distribution of the positively tested patients from Panel B, separately indicated for the multiplex PCR panel and the targeted PCR detection.

positivity rate for viral nucleic acid detection of 34.5%. Of the 20 patients positive for *M. pneumoniae*, 16 were male and 4 female.

Next, we asked whether for positively tested patients the bacteriology department had received a specific request for detection of *M. pneumoniae* by PCR as well. Only for 2 patients (10%) clinicians had specifically asked for a *M. pneumoniae*-specific PCR, leaving $N = 18$ patients that were only diagnosed "unintentionally" because of the use of the multiplex panel. Of the 20 patients positive for *M. pneumoniae,* only 3 showed also other viruses (H1N1, RSV, OC43, each once). Frequent codetection of viruses has been reported in children (Weigl et al., 2007); however, in adults suffering from community acquired pneumoniae, *M. pneumoniae* was often the only pathogen (Holter et al., 2015). Given the overall positivity rate of 34.5% for any virus by the FTD-RP21 assay, the isolated detection of *M. pneumoniae* argues for the clinical relevance of the result to explain the patients' symptoms. DNA positivity in *M. pneumoniae* infection has been reported to have a short median of 9.5 days (Gotoh et al., 2013). Thus, in a situation with clinical symptoms and no codetection of virus, the positive result for *M. pneumoniae* argues for an acute infection.

Next, we analyzed data of $N = 1353$ samples which during the same time period (Q1 and Q2/2015) had been sent to the bacteriology department with a specific request for *M. pneumoniae* (Fig. 1A). $N = 7$ samples (0.52%) from 6 individual patients tested positive by a real-time *in house* PCR (which is based on (Raggam et al., 2005)). Two of those patients had also been tested in parallel in the virology department and were also positive by the FTD-RP21 assay. Overall, from the altogether $N = 24$ patients tested positive in either of the departments in Q1 and Q2/2015, 75% were only diagnosed because of the use of the multiplexed FTD-RP21 assay (Fig. 1B). The FTD-RP21 found additional *M. pneumoniae* cases because in those samples, clinicians had not specifically asked for a targeted PCR. The data indicate underdiagnosing of *M. pneumoniae* infections due to missing clinical awareness or suspicion which might specifically occur when less severe or atypical symptoms are observed.

Analyzing further demographic and clinical data, we observed that for the FTD-RP21 assay 9/20 (45%) of the positive patients were younger than 16 years. In contrast, from the 6 patients positively tested by PCR in the bacteriology department (routine request), only 1 patient (17%) was in this age group (Fig. 1C), indicating that clinicians preferentially thought of viruses as causative agents in children and teenager and did not consider *M. pneumoniae*. Correspondingly, roughly half of the requests analyzed by FTD-RP21 came from pediatric wards, whereas these were rare senders in the bacteriology unit for the *M. pneumoniae* assay.

We conclude that *M. pneumoniae* is underdiagnosed in our hospital especially in the pediatric clinic. The main reason is an absence of clinical suspicion. Similar observations have been made for pediatric infections in Switzerland with *Coxiella burnetii* (Hackert et al., 2015). Multiplex, syndromic testing can overcome underdiagnosing of important respiratory pathogens thus, allowing improved, targeted therapy or application of appropriate infection control measures (Dundas et al., 2011; Gilca et al., 2014; Schreckenberger and McAdam, 2015). Thus, multiplex assays bear the potential to affect patient outcome, yet cost-effectiveness in general and clinically meaningful composition of such panels are still to be discussed (Schreckenberger and McAdam, 2015). Alternatively, targeted, directed diagnostics will depend on amendments in the preanalytics, including detailed knowledge on the regional and temporal epidemiology of the various infectious organisms, as exemplified in this study.

Conflict of interest

The authors declare no conflict of interest.

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