Brazilian Journal of Microbiology (2021) 52:1173–1179
https://doi.org/10.1007/s42770-021-00536-w

BACTERIAL, FUNGAL AND VIRUS MOLECULAR BIOLOGY - SHORT COMMUNICATION

Rapid multiplex PCR assays in patients with respiratory viral infections: is semi-quantitative data useful? A pilot study

Pierre Gazeau[1] · Sophie Vallet[2,3] · Séverine Ansart[1,4] · Clémence Beauruelle[3,5] · Adissa Tran-Minoui[2] · Christopher Payan[2,3] · Léa Pilorgé[2]

Received: 15 March 2021 / Accepted: 26 May 2021 / Published online: 9 June 2021

Abstract

Viral respiratory rapid multiplex PCR assays FilmArray® (FA) and ePlex® (eP) provide qualitative results which may not reflect clinical relevance. In a pilot study, we report retrospectively whether the semi-quantitative PCR assay R-GENE® would have facilitated clinical interpretation. Forty-four patients were hospitalized for various respiratory manifestations; all of them have benefited from a respiratory sample during acute symptoms. Among the 44 patients, FA detected 23 positive samples including 31 viruses, 26 of them gave high or moderate R-GENE® scores (cycle threshold < 35), and all but one were consistent with clinical history. Semi-quantitative scores would have allowed for critical interpretation of the results; those are a key additional element for an optimal exploitation of the rapid multiplex PCR assays power.

Keywords Respiratory tract · Multiplex PCR · Semi-quantitative results · Viral respiratory panel

Introduction

Rapid multiplex PCR assays (RMPA) have recently been developed to detect viral respiratory pathogens in a very short time with high sensitivity and specificity [1]. Detection strategies that allow multiple agents to be simultaneously detected with a reduced laboratory turnaround time may have a significant impact on infectious disease management [2].

Analytical performances of RMPA FilmArray® Respiratory Panel 2 Plus (RP2 +) (FA) (BioFire-bioMérieux, Marcy-l'Étoile, France) and ePlex® (eP) (GenMark Diagnostics, Carlsbad, USA) for the rapid simultaneous detection of 20 pathogens have already been evaluated, showing equivalent performances except for rhinovirus/enteroviruses and common human coronaviruses (types 229E, NL63, OC43, and HKU1) [3]. However, most RMPA provide qualitative results only and a semi-quantitative result would provide more useful information, for example, helping to monitor the viral infection or to discriminate a clinically significant viral load [4].

In this retrospective descriptive pilot study, viral results obtained with FA and eP were supplemented by semi-quantitative data obtained from our local routinely used real-time duplex PCRs which detect 14 pathogens (R-GENE®, bioMérieux, Marcy-l'Étoile, France). The study's objective was to determine whether complementing FA and eP with a semi-quantitative assay could have improved clinical relevance of test results and hence patient outcome.

Responsible Editor: Luiz Henrique Rosa

✉ Léa Pilorgé
lea.pilorge@chu-brest.fr

1 Unité Des Maladies Infectieuses Et Tropicales, Centre Hospitalier Régional Et Universitaire de Brest, Brest, France

2 Unité de Virologie, Département de Bactériologie-Virologie-Parasitologie-Mycologie-Hygiène, Pôle de Biologie-Pathologie, Centre Hospitalier Régional Et Universitaire de Brest, Brest, France

3 Univ Brest, Inserm, EFS, UMR 1078, GGB Génétique, Génomique Fonctionnelle Et Biotechnologies, Brest, France

4 Laboratoire de traitement de l'information médicale, LaTIM-UMR 1101, INSERM, Université de Bretagne Occidentale, Brest, France

5 Unité de Bactériologie, Département de Bactériologie-Virologie-Parasitologie-Mycologie-Hygiène, Pôle de Biologie-Pathologie, Centre Hospitalier Régional Et Universitaire de Brest, Brest, France

Materials and methods

The study included all patients for whom both eP and FA assays had been performed on the same respiratory sample between January and March 2018 at the virology laboratory of Brest University Hospital, France. As the number of RMPA tests available in the laboratory was limited for this period, this work is a pilot study, in preparation for a larger and prospective study. Respiratory samples had been collected using either a nasopharyngeal (NP) flocked swab placed in universal transport medium (FLOQSwabs™, Copan, Brescia, Italy), NP aspiration, or bronchoalveolar lavage (Table 1) and were tested by viral and/or bacterial screening with RMPA FA and eP. Residual volumes of respiratory samples were stored at – 80 °C for retrospective analyses with semi-quantitative specific duplex real-time PCR assays including influenza A virus and influenza B virus, human metapneumovirus and respiratory syncytial virus, human parainfluenza viruses including types 1, 2, 3, and 4 and common human coronaviruses including types 229E, NL63, OC43, and HKU1, human rhinoviruses and/or enteroviruses, and one simplex real-time PCR for adenoviruses (R-GENE®, bioMérieux, Marcy-l'Étoile, France). Cell control was also performed with a semi-quantitative specific real-time PCR assay (R-GENE®, bioMérieux, Marcy-l'Étoile, France). A semi-quantitative scoring system of the real-time PCR assays was defined as follows: very high (Ct (cycle threshold) < 25), high (Ct between 25 and 30), moderate (Ct between 30 and 35), and low (Ct > 35) viral load.

FilmArray® and eP also provide information concerning bacteria: *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Bordetella pertussis*, and *Bordetella parapertussis* for FA and *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* for eP. No bacterial culture was performed from those samples.

Organization of the Brest virology lab was such that RMPA was mostly carried out for intensive care unit (ICU) patients (47.7%), immune-compromised patients (20.5%), and children < 15 years old (50%), with 82.6% infants for various respiratory manifestations (Table 1). Since this was our routine method, only the FA results were given to clinicians.

Standardized clinical, biological, and radiological data were collected for each patient. Diagnoses were classified into six categories according to Rogers et al. [5], before the additional information given by the semi-quantitative assay. Adequacy between microbiological results and clinical history was analyzed by our local infectious disease specialist with strong experience in respiratory tract infections management.

Continuous data are described as median ± first and third quartile (Q1–Q3). Data were analyzed using GraphPad Prism 6.0 (La Jolla, CA) and Microsoft Excel 14.4.0 (Redmond, WA). The 95% confidence intervals (CI) for percent agreements were calculated with the Clopper-Pearson method.

Results/discussion

Viral respiratory rapid multiplex PCR assays are sensitive and specific and may contribute to the improvement of patient care procedures [2]. However, most of these tests only provide qualitative results, namely, "positive" or "negative," whereas semi-quantitative methods like real-time RT-PCR can provide useful additional data. Indeed, for some viruses, disease severity has been associated with high viral load [6]. Moreover, in codetection of multiple viruses, viral load determination could help to determine the viruses which potentially contributed to the disease [7].

A total of 44 patients were included in the present study. Population characteristics are summarized in Table 1. Sample quality was checked by amplifying the hypoxanthine phosphoribosyltransferase (HPRT1) gene with the real-time PCR assay R-GENE®; all of the 44 respiratory samples were considered to be of high quality for PCR (Ct value of HPRT1 < 30). Unfortunately, such a target is not yet available in RMPA.

Virus-positive samples (23 for FA with 31 viruses, including 6 viral coinfections; 19 for eP with 23 viruses, including 3 viral coinfections) were retrospectively analyzed with a semi-quantitative method (R-GENE®) (Table 2). Concordant positive screening results between the two RMPA concerned 23 detected viruses. All the 21 negative samples with FA were also negative with eP. One sample which tested positive in FA failed to be identified using eP, but re-testing was not attempted. Of note, no bacterial infections have been detected in the 44 samples tested with FA and eP. Overall the results of the present study give a positive percent agreement (PPA) between FA and eP of 76.6% (57.7–90.1), a negative percent agreement (NPA) of 100% (83.9–100), and an overall percent agreement of 86.3% (73.7–94.3) for virus detection, with a 95% confidence interval. Discordant screening results between the two RMPA corresponded to detected viruses, presence/absence, type, and/or number (Table 2) and concerned six respiratory samples from six patients. FA detected seven more viruses than eP. In three samples, discrepancies were linked with multiple virus detections, with FA demonstrating one or two more viruses than eP (P2, P18, and P39; Table 2). One virus was detected with only FA in each of the other three samples (P4, P10, and P30; Table 2).

Among these three single virus detection discrepancies, eP did not detect one influenza A virus, one rhinovirus/enterovirus, and one influenza B virus, with the influenza A virus positive (moderate) by real-time RT-PCR and the latter

Table 1 Population characteristics

	Overall (n = 44)	Patients with at least one positive result (n = 23)	Patients < 15 yo with at least one positive result (n = 19)	Patients with both RMPA[a] negative results (n = 21)	Patients < 15 yo with both RMPA[a] negative results (n = 4)
Children (% < 15 yo)	23 (0.5)	19 (82.6)	–	4 (19)	–
Age, years (med, Q1–Q3)	15 (0.3–63)	0.4 (0.1–12)	0.25 (0.1–0.6)	57 (21.5–68.5)	2 (0.6–9)
Sex (male) (%)	29 (65)	17 (73.9)	13 (68.4)	12 (57)	3 (75)
Sampling technique					
Swab (%)	22 (50)	7 (30)	5 (26.3)	15 (71.4)	2 (50)
Bronchoalveolar lavage (%)	5 (11.4)	2 (8.7)	0 (0)	3 (14.3)	0 (0)
Nasopharyngeal aspiration (%)	17 (38.6)	14 (60.8)	14 (73.7)	3 (14.3)	2 (50)
Delay between result and end of hospitalization (days) (med, Q1–Q3)	2.5 (1–8)	1 (1–6)	1 (1–5.3)	7 (1–10)	6.5 (2–9.5)
Hospitalization unit					
Conventional care unit	17 (38.6)	11 (47.8)	12 (63.2)	6 (28.5)	3 (75)
Onco/hematology	6 (13.6)	1 (4.3)	0 (0)	5 (23.8)	0 (0)
Intensive care unit	21 (47.7)	11 (47.8)	7 (36.8)	10 (47.6)	1 (25)
Clinical presentation and management					
Intensive care unit required during hospitalization (%)	22 (50)	10 (43.5)	7 (36.8)	12 (57.1)	2 (50)
Immunocompromised (%)	9 (20.5)	1 (4.3)	0 (0)	8 (38)	0 (0)
Respiratory condition history (%)	7 (15.9)	2 (8.7)	1 (5.3)	5 (23.8)	1 (25)
Initial nasosinusal symptoms (%)	15 (34.1)	13 (56.5)	12 (63.2)	2 (9.5)	0 (0)
Respiratory symptoms at testing (%)	34 (77.3)	19 (82.6)	16 (84.2)	15 (71.4)	4 (100)
Respiratory distress syndrome (%)	30 (68.2)	12 (52.2)	7 (36.8)	11 (52.4)	2 (50)
Oxygen support required (%)	25 (56.8)	13 (56.5)	10 (52.6)	12 (57.1)	3 (75)
Fever at time of testing (%)	20 (45.5)	10 (43.5)	7 (36.8)	10 (47.6)	2 (0.5)
Normal chest X-rays (%)	15/34 (44.1)	10/17 (58.8)	9/13 (62.2)	5/17 (29.4)	0/2 (0)
Clinical diagnosis					
Isolated viral infection (bronchiolitis, viral pneumonia) (%)	18 (40.9)	16 (69.6)	15 (78.9)	2 (9.5)	1 (25)
Incidence of chronic respiratory disease (%)	3 (6.8)	1 (4.3)	1 (5.3)	2 (9.5)	0 (0)
Bacterial pneumonia (confirmed or not) (%)	11 (25)	2 (8.7)	0 (0)	9 (42.9)	2 (50)
Acute distress respiratory syndrome (%)	2 (4.5)	1 (4.3)	0 (0)	1 (4.8)	0 (0)

Table 1 (continued)

	Overall (n = 44)	Patients with at least one positive result (n = 23)	Patients < 15 yo with at least one positive result (n = 19)	Patients with both RMPA[a] negative results (n = 21)	Patients < 15 yo with both RMPA[a] negative results (n = 4)
Isolated fever or ENT disease (%)	8 (18.2)	3 (13)	3 (15.8)	5 (23.8)	1 (25)
Other (%)	2 (4.5)	0 (0)	0 (0)	2 (9.5)	0 (0)
Death	7 (15.9)	0 (0)	0 (0)	7 (33.3)	1 (25)
Directly related to infection (%)	2 (4.5)	0 (0)	0 (0)	2 (9.5)	0 (0)
Indirectly related to infection (%)	5 (11.3)	0 (0)	0 (0)	4 (19)	1 (25)
Unrelated to infection (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Data are expressed as median (interquartile range)

[a]FilmArray® Respiratory Panel 2 Plus and ePlex® Respiratory Pathogen panel

ENT ear, nose, and throat

two not detected by either real-time PCR. The viral loads of these targets could be at the limit of sensitivity of the assays. The possibility of false positive results with FA related to contamination should also be considered. In multiple virus detection discrepancies, most of the discordant targets had a moderate or even a high signal, which suggests that eP might not correctly detect some viruses, particularly some coronaviruses or rhinovirus/enteroviruses, as was previously reported [3].

Diagnoses of clinicians, who had the FA results, showed that they always considered the detected viruses to be the cause of acute respiratory issues or organ failure (Table 2). Among the 23 virus infected patients, 16 (69.9%) had respiratory symptoms related to an isolated viral infection (Table 1). The median length of hospital stay after the RMPA result (Table 1) was not significantly shorter for the patients who were positive for at least one virus than for negative patients (1 day [1–6] versus 7 [1–10], p = 0.082). Interestingly, only 25% of adults who had a positive result had their antibiotics continued versus 47% among those with a negative result. However, this difference did not reach significance (p = 0.056). It is noteworthy that all of the patients with a positive result and normal chest X-rays were no longer receiving antibiotics after 48 h. Most of the positive cases had been associated with an antibiotic discontinuation after 48 h (time of antibiotherapy reevaluation) (17/23) and were all pediatric cases. A reduction of antibiotic prescription following a positive test result has already been reported in adults [9].

Of the 17 positive samples with a single viral target with FA, all but five had a high or very high RT-PCR signal, which is indicative of an active virus replication and likely cause of patients symptoms (Tables 1 and 2). Indeed, among the five remaining cases, three were serious influenza A virus or parainfluenza virus 3 infection, whose course had begun at least a week earlier, which might explain the moderate or low signals [10]. The influenza B virus detected only with FA (P30) from a child in ICU is more questionable, as the course was not typical of a flu infection. Moreover, influenza B virus viral load has been described as higher in ICU patients than in those in ambulatory settings [11]. This is an example of situation where a complementary semi-quantitative PCR could have been of significant value in aiding clinician decision and overall management of the patient health. The rhinovirus/enterovirus detection (P10) is likely a real positive case with a very low signal because the clinical context was evocative of a viral bronchiolitis, and the next sampling (10 days later) showed a very high signal (data not shown). A semi-quantitative result would have indicated the start of the infection. Of note, the single freeze–thaw cycle to which the samples were subjected is not likely to be the cause of these five remaining lower viral loads, which are consistent with clinical data.

In patients with multiple virus detections, the viruses associated with higher qPCR scores were likely involved in active infection according to the clinical contexts (Table 2) [7]. The other ones may be associated with ending infections or carriage and would not confer increased severity. Influenza virus, metapneumovirus, respiratory syncytial virus, and parainfluenza virus 3 are often presented as "true pathogens," detected with high signals in samples with or without coinfection, whereas adenovirus, rhinovirus, or coronavirus have often been detected in association with lower signals in symptomatic persons [7, 12]. In our study, not all viral codetections implicated a "true pathogen" (P29 and P39), and in other cases, the "true pathogen" did not always have the highest signal (P7 and P18). None of these

Table 2 Patients with at least one positive RMPA result for at least one viral target

Patient number	Clinician diagnosis	FilmArray®	ePlex®	Semi-quantitative R-GENE® score
P1	Children Viral bronchiolitis, NAR	Adenovirus	Adenovirus	High
		Coronavirus NL63	Coronavirus NL63	High
		Parainfluenza virus 2	Parainfluenza virus 2	Very high
P2	Children Viral bronchiolitis, NAR, recovery	Coronavirus HKU1	Negative	High
		Metapneumovirus	Metapneumovirus	High
P4	Adult Acute distress respiratory syndrome associated with influenza A virus Antibiotics added without documentation, recovery	Influenza A virus	Negative	Moderate
P7	Children Viral bronchiolitis, NAR	Rhinovirus/enterovirus	Rhinovirus/enterovirus	Very high
		Respiratory syncytial virus	Respiratory syncytial virus	Moderate
P10	Children Viral bronchiolitis associated with decompensated chronic cardiac condition, NAR, recovery	Rhinovirus/enterovirus	Negative	Negative
P11	Children Viral infection due to RSV	Respiratory syncytial virus	Respiratory syncytial virus	Very high
P12	Adult Bacterial pneumonia associated with influenza A virus	Influenza A virus	Influenza A virus	Moderate
P14	Children ENT symptoms, NAR	Rhinovirus/enterovirus	Invalid	Very high
P18	Children Respiratory infection due to 3 viruses, NAR	Coronavirus NL63 and OC43	Coronavirus NL63 and OC43	Very high
		Metapneumovirus	Negative	Moderate
		Rhinovirus/enterovirus	Negative	High
P19	Children Viral infection due to RSV, NAR	Respiratory syncytial virus	Respiratory syncytial virus	High
P21	Children Viral infection complicated with bacterial pneumonia	Respiratory syncytial virus	Respiratory syncytial virus	Very high
P22	Children Viral bronchiolitis, NAR	Rhinovirus/enterovirus	Rhinovirus/enterovirus	High
P23	Children Viral bronchiolitis, NAR	Rhinovirus/enterovirus	Rhinovirus/enterovirus	Very high
P25	Children Viral bronchiolitis, NAR	Rhinovirus/enterovirus	Rhinovirus/enterovirus	High
P29	Children Viral bronchiolitis, NAR	Coronavirus OC43	Coronavirus OC43	Very high
		Rhinovirus/enterovirus	Rhinovirus/enterovirus	Low
P30	Children Influenza B virus associated with bacterial pneumonia, recovery	Influenza B virus	Negative	Negative
P31	Children Viral bronchiolitis, NAR	Rhinovirus/enterovirus	Rhinovirus/enterovirus	High
P32	Children Atypical viral infection associated with cutaneous rash	Rhinovirus/enterovirus	Rhinovirus/enterovirus	Very high
P34	Adult Viral infection complicated with acute pulmonary edema	Parainfluenza virus 3	Parainfluenza virus 3	Low
P35	Children ENT symptoms, NAR	Rhinovirus/enterovirus	Rhinovirus/enterovirus	Very high
P38	Viral infection complicated with bacterial pneumonia	Respiratory syncytial virus	Respiratory syncytial virus	Very high

Table 2 (continued)

Patient number	Clinician diagnosis	FilmArray®	ePlex®	Semi-quantitative R-GENE® score
P39	Children Epilepsy due to a virus in an epileptic patient, no other infection, normal lumbar puncture	Coronavirus NL63 Rhinovirus/enterovirus	Coronavirus NL63 Negative	High Negative
P44	Children Isolated, non-severe, influenza A virus, NAR	Influenza A virus	Influenza A virus	High

NAR, no antibiotic required; *ENT*, ear, nose, and throat; in bold, discordances

coinfections were associated with clinical severity as previously described [13].

A reduced antibiotic use in patients with positive viral testing and chest imaging without infiltrates may or may not be sufficient evidence for clinicians to withhold or stop antibiotherapy with regard to potential bacterial coinfection [14].

One limitation to our pilot study is the low number of patients with 44 participants. It is too small for a comparison of assay performance; thus, the objective of this study was rather to look at whether using a semi-quantitative method would improve patient care. Our results support this point but they will need to be confirmed in a prospective study with larger target population size: we would ask the clinicians to interpret a positive/negative result first, and we would then ask them to reconsider or not their decision based on semi-quantitative data.

In conclusion, this work points out the high consideration clinicians have of positive RMPA results regardless of the number of pathogens or the atypical clinical presentation. Semi-quantitative data allows for more critical biological interpretation of the results and thus an appropriate use of them in clinic. In our study, these data could have warned physicians of a mismatch between result and clinical situation, or helped them to monitor some viral infections. The combination of qualitative multiplex testing and semi-quantitative real-time PCR in routine use for positive samples is not feasible because it would negate the benefits related to the rapidity of multiplex testing and would generate a significant additional cost. The ideal solution seems to be the development of RMPA tests that also can produce semi-quantitative results. This is the case of the QIAstat Respiratory Panel (QIAstat RP, Qiagen, Hilden, Germany) which has been recently commercialized.

Author contribution Each of the authors acknowledges that he or she participated sufficiently in the work to take public responsibility for its content and agrees to the contents of the manuscript in its submitted form.

Data availability Available.

Code availability Available.

Declarations

Ethics approval This study (29BRC18.0233) was approved by the Brest University Hospital ethics committee no. 2018CE.48.

Consent to participate/consent for publication An information letter was sent to each patient so they could have opposed inclusion or publication if they wished. No opposition was registered.

Competing interests The authors declare no competing interests.

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