Brief report

Multiplex PCR Detects Respiratory Viruses at A Much Higher Rate in Kawasaki Disease

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Abstract: In Kawasaki disease (KD), convulsions are rare and mostly attributed to latent and coexisting encephalitis/encephalopathy due to KD itself. Therefore, we retrospectively investigated concomitant viral infections in patients with KD. Between January 2012 and December 2020, among 523 patients with KD, 7 (1.53%), 1 (0.19%), 6 and 1 (1.15% and 0.19%) were positive for adenovirus (AdV-Ag), respiratory syncytial virus (RSV-Ag), and influenza A or B (Flu A-Ag or Flu B-Ag) antigens, respectively. Among them, two were positive for both AdV-Ag and FluA-Ag. Seven patients with KD (1.3%) presented febrile convulsions, and only one was diagnosed with encephalitis/encephalopathy. Between January 2021 and August 2022, 57 patients with KD were newly diagnosed among whom FilmArray® respiratory panel (FARP) was applied on 24 patients, and 14 (58.3%) were positive for at least one virus. During the same period, 32 out of 45 patients (71.1%) with febrile convulsions examined using the FARP were positive for at least one virus. The trend of viruses in patients with KD and febrile convulsion reflected the regional epidemic viral infection. Respiratory virus detected at a much higher rate using the FARP suggests that most convulsions in patients with KD might be related to concomitant viral infection. The possible involvement and association of concomitant respiratory viral infections in patients with KD with prognosis or encephalopathy/encephalitis remain to be investigated.

Keywords: Kawasaki disease; febrile convulsions; respiratory virus; filmarray; multiplex PCR

1. Introduction

Kawasaki disease (KD) is an acute febrile vasculitis syndrome characterized by six major clinical manifestations of unknown etiology [1]. It is reported that KD mostly affects children aged <5 years and presents symptoms besides the six major ones, such as hepatitis, myositis, aseptic pyuria, pneumonia, and ileus, as a result of acute systemic inflammatory disease. Among these comorbidities, convulsions are relatively rare compared with that of the common frequency of febrile convulsions among children of the same age [2,3]. Febrile convulsions (seizures) are fits that can occur when a child has a fever and are most frequently noted between the ages of 6 months and 3 years. They usually last for <5 min and are harmless in nature. However, they may be signs of encephalopathy/encephalitis when they last or repeat in a short period. Convulsions in KD may be attributed to latent and coexisting encephalitis or encephalopathy due to KD itself [4].

We have previously reported a case of KD that presented with a series of febrile convulsions with bacteremia caused by *Streptococcus pneumoniae* [5]. Despite successful antibiotic treatment for bacteremia, the KD symptoms of the patient continued and disappeared only after two intravenous immunoglobulins with prednisolone and acetylsalicylic acid. Based on the clinical symptoms and course, a series of convulsions in this patient was attributed to bacteremia, but not the latent and coexisting encephalitis or encephalopathy of KD.

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In this context, we retrospectively analyzed patients with KD diagnosed between January 2012 and August 2022 to investigate the association of convulsion with the coexisting infection. Since January 2021, the multiplex polymerase chain reaction (PCR) assay FilmArray® respiratory panel (FARP) has been applied occasionally for patients with fever and/or respiratory symptoms or convulsions upon admission.

2. Materials and Methods

The patients' information were collected from the in-patient electronic medical records using the diagnostic terms "Kawasaki disease," "febrile seizure," "status epilepticus," "convulsion," "encephalopathy," "encephalitis," and a combination of each term. Each patient was validated by checking their medical records directly for eligibility for diagnosis. Diagnosis of KD was confirmed according to the Diagnostic Guidelines for Kawasaki Disease (5th revised edition) [6]. Patients already diagnosed with epilepsy and those who received antiepileptic medicines were also included if they had a fever and/or respiratory manifestations. The examinations for the possibly involved pathogens, such as blood culture, urine culture, liquor culture, and point-of-care antigen detection tests for influenza A/B (Flu A/B), adenovirus (AdV), group A *Streptococcus* (GAS), respiratory syncytial virus (RSV), and human metapneumovirus (hMPV), were performed based on the physician's discretion between January 2012 and December 2020. Furthermore, since January 2021, the FilmArray® respiratory panel (FARP; version 2.1, BioMerieux Japan, Tokyo) has been applied to patients with fever, respiratory symptoms, or convulsions upon admission based on the physician's discretion. This panel covers 18 viruses: AdV, coronavirus HKU1, 229E, OC43, NL63, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), influenza A, A/H1, A/H1 2009, A/H3, B, parainfluenza virus (PIV)-1, -2, -3, -4, RSV, rhinovirus/enterovirus (RV/EV), hMPV, and four other microorganisms: *Bordetella pertussis, Bordetella parapertussis, Chlamydia pneumonia, and Mycoplasma pneumonia*.

This study was conducted in accordance with the tenets of the Declaration of Helsinki, and the study protocol was approved by the Musashino Red Cross Hospital Clinical Research Ethics Committee (registration number: 4046).

3. Results

Between January 2012 and December 2020, 523 patients were newly diagnosed with KD at our institute (Table 1).

Table 1. KD patients between 2012 and 2020.

n	Flu-Ag	RSV-Ag	AdV-Ag	bacteremia	encephalopathy
523	7	1	7	1	1
	1.3%	0.2%	1.3%	0.2%	0.2%

A throat swab for adenovirus antigen (AdV-Ag) was positive in seven patients (1.53%) and group A *Streptococcus* antigen was positive (GAS-Ag) in seven patients (1.34%). Nasopharyngeal swab for respiratory syncytial virus antigen (RSV-Ag) was positive in one patient (0.19%), and influenza A or B antigen (Flu A-Ag or Flu B-Ag) was positive in six patients and one, respectively (1.15% and 0.19%). Among them, two patients were positive for both AdV-Ag and FluA-Ag, and one was positive for both RSV-Ag and GAS-Ag. Seven patients with KD presented febrile convulsions (1.3%), as shown in Table 2.

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Table 2. The profile of 7 KD patients who presented febrile convulsions between 2012 and 2020.

age (y)	sex	RSV-Ag	AdV-Ag	Flu-Ag	GAS-Ag	blood culture	liquor culture	pleocytosis
3	m	n.d.	negative	negative	negative	negative	negative	negative
1	m	n.d.	negative	n.d.	n.d.	negative	negative	negative
1	f	negative	negative	negative	negative	negative	negative	negative
1	f	n.d.	negative	n.d.	negative	positive	negative	negative
1	m	n.d.	negative	n.d.	negative	negative	n.d.	n.d.
1	m	n.d.	negative	negative	negative	negative	n.d.	n.d.
3	m	positive	negative	negative	positive	negative	negative	negative

n.d. : not done

None of the patients had an episode of febrile convulsions. One patient was positive for blood culture with *S. pneumoniae*, as described above, and one was positive for both RSV-Ag and GAS-Ag. The patient positive for both RSV-Ag and GAS-Ag was later diagnosed with encephalopathy because of repeated convulsions with persistent disturbance of consciousness and was treated with intravenous immunoglobulin, steroid pulse therapy, infliximab administration, and plasma exchange. The other six patients were diagnosed with febrile convulsions but not encephalopathy/encephalitis from the clinical course. The frequency of encephalopathy/encephalitis in patients with KD in this cohort was 0.19% (1/523), almost equal to that reported in a national survey study in Japan [7].

Between January 2021 and August 2022, 57 patients with KD were newly diagnosed, in which FARP was applied on 24 patients, and 14 (58.3%) were positive for at least one virus. During this period, no patients presented convulsions. In addition, ADV-Ag was positive in 1 out of 31 patients, and GAS-Ag was positive in 1 out of 40 patients (Table 3).

Table 3. FARP detected concomitant respiratory viruses at a higher rate than point-of–care antigen detection tests in KD patients between January 2021 and August 2022.

	FARP	GAS-Ag	AdV-Ag	RSV-Ag	blood culture
test (n)	24	40	31	4	9
positive (n)	14	1	1	0	0
positive ratio	58.3%	2.5%	3.2%	0.0%	0.0%

During the same period, 91 pediatric patients were admitted due to febrile convulsions in our institute, and two were diagnosed with encephalopathy/encephalitis (2.2%), in which one was positive for PIV3 and one for both PIV1 and RV/EV, which were detected using the FARP. The FARP was applied to 45 patients, and 32 (71.1%) were positive for at least one virus. As previously reported in our study, at least one virus was detected in approximately 80% of children aged under 5 years admitted due to a fever and/or respiratory symptoms who were examined using the FARP [8]. There was no difference in the trend of respiratory virus detected between patients with KD, febrile convulsion, and all pediatric patients aged under 5 years who were examined using the FARP in our institute (Table 4).

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Table 4. The trend of respiratory viruses detected in KD, FC and age<5 patients by FARP between January 2021 and August 2022.

	n	RV/EV	RSV	CoV-2	PIV1	PIV3	PIV4	hMPV	AdV	OC43	NL63	HKU-1
KD	24	9	4	1	0	1	1	0	0	1	0	0
		37.5%	16.7%	4.2%	0.0%	4.2%	4.2%	0.0%	0.0%	4.2%	0.0%	0.0%
FC	45	19	7	2	2	6	0	2	1	1	1	1
		42.2%	15.6%	4.4%	4.4%	13.3%	0.0%	4.4%	2.2%	2.2%	2.2%	2.2%
age < 5y	377	148	81	22	9	33	6	12	17	12	5	2
		39.3%	21.5%	5.8%	2.4%	8.8%	1.6%	3.2%	4.5%	3.2%	1.3%	0.5%

In the KD group, three patients were positive for the two viruses. In FC, four patients were positive for two viruses, and three patients were positive for three viruses.

This indicates that the respiratory viruses detected in patients with KD and febrile convulsion reflect the regional epidemic viral infection. Notably, the influenza virus was not detected during this period.

4. Discussion

Seasonal influenza epidemics were not observed worldwide from 2020 to 2021 because of the SARS-CoV-2 pandemic [9,10]. Studies in the United States reported that respiratory viruses were detected only in 8.8% of patients with KD [11], in which a less sensitive viral culture or fluorescent antibody method was used to detect viral infection. Between January 2012 and December 2020, 684 pediatric patients were admitted to our institute due to febrile convulsion. Eighteen patients were diagnosed with encephalopathy/encephalitis, of which two were positive for RSV-Ag, five were positive for Flu-Ag, and two were related to exanthema subitum. As shown in Table 5, point-of-care viral antigen detection tests for Flu-Ag, RSV-Ag, hMPV-Ag, and AdV-Ag available in Japan could detect respiratory viruses only in as low as 15.1% of patients with febrile convulsion in total.

Table 5. Point-of-care antigen detection tests and blood culture detected pathogenic microorganisms at a low rate in FC patients between 2012 and 2020.

n	Flu-Ag	RSV-Ag	hMPV-Ag	AdV-Ag	bacteremia	Ext. subitum	encephalopathy
684	62	20	10	11	8	58	18
	9.1%	2.9%	1.5%	1.6%	1.2%	8.5%	2.6%

Exanthema subitum was diagnosed clinically based on the typical systemic rash manifestation after fever resolution. There were no double-positive cases in point-of-care tests.

There were 6 cases of bacteremia with Streptococcus pneumoniae, one with Streptococcus oralis and one with Staphylococcus aureus.

Recently, it has been reported that multiplex PCR can detect respiratory viruses in 70–80% of pediatric patients with febrile convulsion [12,13]. Respiratory viruses were detected in patients with KD and common febrile convulsions at a much higher rate using the FARP than that of the previously used method. In our study, most convulsions in patients with KD might be related to concomitant viral infection, although it does not underestimate the convulsion due to the encephalopathy/encephalitis caused by KD itself. Interestingly, Kawasaki-like disease has been reported to develop during the SARS-CoV-2 epidemic in Italy [14].

The limitation of this study is that examination tests for respiratory viruses, such as point-of-care antigen tests and FARP, were performed based on the physicians' discretion. It is possible that asymptomatic or mild respiratory viral infections were overlooked.

5. Conclusion

Respiratory viruses were detected in patients with KD at a much higher rate using the FARP than that of the previously used method.

The possible involvement and association of concomitant respiratory viral infections in patients with KD with prognosis or encephalopathy/encephalitis remain to be investigated.

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