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Clinical and epidemiological characteristics of human parainfluenza virus infections of children in southern Taiwan

Kai-Wen Wu [a], Shih-Min Wang [a,b,d], Ching-Fen Shen [a], Tzong-Shiann Ho [a,b], Jen-Ren Wang [c,d,e], Ching-Chuan Liu [a,d,*]

[a] *Department of Pediatrics, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan City, Taiwan*
[b] *Department of Emergency Medicine, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan City, Taiwan*
[c] *Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng Kung University, Tainan City, Taiwan*
[d] *Center of Infectious Disease and Signaling Research, National Cheng Kung University, Tainan City, Taiwan*
[e] *Department of Pathology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan City, Taiwan*

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Abstract *Background:* Human parainfluenza viruses (HPIV) 1–4 had been analyzed as being one of the most frequent causes of hospitalizations for young children with respiratory tract illnesses.
Methods: This retrospective study was performed from children virologically confirmed as HPIV infection through throat swab or nasopharyngeal aspirates at a tertiary care university hospital, between January 2012 and December 2014. HPIV4 was not checked and analyzed, due to not include in the commercial kit. The demographic, epidemiological, clinical presentations, diagnosis, treatment, outcomes, and laboratory data were analyzed.
Results: Totally 398 cases were enrolled, including 39 (9.8%) of HPIV1, 67 (16.8%) of HPIV2, and 292 (73.4%) of HPIV3. The mean age of HPIV-infected children was 2.9 year-old, and 50.5% were among one to three year-old. A total of 56.8% HPIV3-infected children were among one to three years old, however, no HPIV2-infected children was younger than one year-old. The HPIV1-infected patients were more common to develop wheezing and diagnose as acute bronchiolitis. HPIV2-infected children were more likely to have hoarseness (23.9%), and were

* Corresponding author. Department of Pediatrics, National Cheng Kung University Hospital, No.138, Sheng-Li Road, Tainan City 70403, Taiwan. Fax: +886 6 2753083.
E-mail address: liucc@mail.ncku.edu.tw (C.-C. Liu).

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associated with croup (25.4%). HPIV3 was isolated from two fatal cases, with neurological underlying diseases.
Conclusion: The impact caused by HPIVs infections is significant in hospitalized children. In the current study, our results contribute to the epidemiologic, clinical and laboratory information of HPIV infection in children in the important areas of respiratory tract infection that could support the development of optimization management.

Introduction

Acute respiratory tract infection (ARTI) is the major cause of morbidity and mortality among children worldwide.[1] Respiratory tract infections are responsible for in excess of 3.1 million deaths per year.[2] ARTI causes approximately 20% of deaths in pre-school children via systematic analysis.[3] There are many pathogens cause ARTI, such as bacteria and viruses. Viral pathogens are the most common etiological agents of ARTI.[4,5] Common viral respiratory pathogens include respiratory syncytial virus, influenza types A and B, human parainfluenza virus (HPIV), human rhinovirus, human metapneumovirus, adenoviruses, WU polyomavirus, and human bocavirus. HPIVs were first described in the 1950s,[6] and although resembling influenza virus, their poor growth in embryonated eggs and different antigenic sites placed them into a new family of viruses, Paramyxoviridae. HPIVs are enveloped non-segmented, negative, single-stranded RNA viruses.[7,8] There were five major serotypes of HPIV (HPIV1, 2, 3, 4a, and 4b) that were defined by complement fixation and hemagglutinating antigens.[9–11] This study was designed to evaluate the epidemiology and clinical characteristics of the three major types (type 1, 2 and 3) of the HPIV infections in children in a medical center of southern Taiwan during a period of three consecutive years.

Methods

Patients and definition

The medical chart was reviewed retrospectively from all children less than 18 years old who were confirmed virologically as the HPIV infection via throat swab or nasopharyngeal aspirates at National Cheng Kung University Hospital (NCKUH), a university-affiliated medical center in southern Taiwan, between January 2012 and December 2014. The demographic, epidemiological, clinical presentations, diagnosis, treatment courses, clinical outcomes, and laboratory findings were retrieved. Pharyngotonsillitis was defined as the symptoms of cough, sore throat, and fever, together with inflammation confined to the pharynx or tonsils. Croup was defined as the symptoms of infectious upper airway obstruction, such as barking cough and inspiratory stridor. Acute bronchiolitis is characterized by the onset of rapid breathing and wheezing. Respiratory distress was including nasal flaring, tachypnea, intermittent cyanosis, and retractions. Bronchopneumonia was usually a generalized process involving multiple lobes of the lung. Lobar pneumonia was defined as the pneumonia localized to one or more lobes of the lung in which the affected lobe or lobes are completely consolidated. The Clinical Research Ethics Committee of the National Cheng Kung University Hospital approved the study protocol (A-ER-104-324).

Specimens collection and transport

Throat swabs or nasopharyngeal aspirates were collected into transport medium containing 2 ml of Eagle's minimum essential medium (EMEM) (pH 7.2) with gelatin (5 mg/L), penicillin (400 U/L), streptomycin (400 mg/L), gentamicin (50 mg/L) and amphotericin B (Fungizone) (1.25 mg/L). Specimens were placed on ice and transported to the NCKUH virology laboratory immediately after collections.[12]

Virus isolation and identification

Specimens were inoculated onto Madin–Darby canine kidney (MDCK), A549, rhabdomyosarcoma (RD), and green monkey kidney (GMK) cells. These culture tubes were incubated at 35 °C and examined for cytopathic effect (CPE) daily for 10–14 days. Viral identification was done by immunofluorescent staining with virus-specific monoclonal antibody (D^3 Ultra 8™ DFA Respiratory Virus Screening & Identification Kit, Ohio, USA).[12] HPIV 4a and 4b were not checked and analyzed due to the commercial kit did not include.

Statistical analysis

All analyses were performed with a Statistical Package for the Social Sciences version (SPSS) 18.0. The data in this study were analyzed by the chi-square test, Fisher's exact test, Analysis of variance (ANOVA), and post hoc analysis. A p-value < 0.05 was considered to be statistically significant.

Results

Demographic data

Overall 398 HPIV confirmed cases were enrolled during the study period. Thirty-nine (9.8%) cases were the HPIV1, 67

(16.8%) cases were the HPIV2, and 292 (73.4%) cases were the HPIV3, so HPIV3 was the leading pathogen. The mean age of the HPIV-infected children was 2.9± 2.8 years old. HPIV infections commonly infected children among one to three year-old (50.5%, 201/398). The male to female ratio was 1.47:1. About 40% of the HPIV-infected patients had contact history with an index case, and the mean febrile day before visiting was 2.1 days.

The mean age of the HPIV1-infected children was older than the age of the HPIV3-infected patients (3.8± 3.8 year-old v.s. 2.3± 1.8 year-old, $p = 0.001$). The mean age of the HPIV2-infected patients was older than the HPIV3 and HPIV1-infected patients (5.2± 4.0 year-old v.s. 2.3± 1.8 year-old, $p < 0.001$; 5.2± 4.0 year-old v.s. 3.8± 3.8 year-old, $p < 0.05$). Among the patients under one year old, HPIV1 and HPIV3-infected patients were more than HPIV2-infected patients (9/65 v.s. 0/65, $p = 0.005$; 56/65 v.s. 0/65, $p < 0.001$). Among the patients between one and three-year old, HPIV3-infected patients were more than HPIV1 and HPIV2-infected patients (166/201 v.s. 10/201, $p = 0.001$; 166/201 v.s. 25/201, $p = 0.01$). Among the patients more than five-year old, the percentage of HPIV3-infected patients was less than the percentage of HPIV1 and HPIV2-infected patients (4.5% v.s. 23.1%, $p = 0.001$; 4.5% v.s. 37.3%, $p < 0.001$). Most of the HPIV3-infected children were among one to three year-old (56.8%, 166/292). Majority (95.5%, 279/292) of HPIV3-infected children were younger than five years old, and 37.3% (25/67) of HPIV2-infected children were older than 5 years old. All of the HPIV2-infected children were older than one year old (100%, 67/67) (Table 1).

In the HPIV-infected patients, 18.6% (74/398) had underlying conditions, such as prematurity (8.8%, 35/398), immunocompromised status (6%, 24/398), neurologic diseases (4.5%, 18/398), congenital heart diseases (1.3%, 5/398), asthma (1.0%, 4/398), and chronic lung disease (0.3%, 1/398). More HPIV1-infected patients had underlying disease than the HPIV2 and HPIV3-infected patients (25.6% v.s. 23.9%, 16.4%) (Table 1).

There were two patients co-detection with enteroviruses, one with influenza A virus, three with adenoviruses, and one with respiratory syncytial virus. There was no patient co-detection with different serotypes of HPIVs.

Seasonality

HPIV1-infected patients were identified frequently in March. A peak of the HPIV2-infected patients were noted in October in 2013. HPIV3 was isolated through the study period with a peak in June and July, especially in 2012. The peak of HPIV3-infected cases number was lower in 2013 and 2014, in comparison with that in 2012 (Fig. 1).

Clinical presentations

The most common symptoms of the HPIV-infected children were cough (72.6%, 289/398), rhinorrhea (49.5%, 197/398), and vomiting (26.1%, 104/398). Overall 10.1% (40/398) of the HPIV-infected children had hoarseness, and 5.3% (21/398) of the HPIV-infected children had wheezing and stridor. Rash was observed in 6.5% (26/398) of the HPIV-infected children. The HPIV2-infected patients were more frequently to develop hoarseness than the HPIV3-infected patients (23.9% v.s. 5.5%, $p < 0.001$). The HPIV1-infected patients were more common to develop wheezing (10.3%, 4/39), headache (10.3% v.s. 1.4%, $p = 0.003$) and abdominal pain (15.4% v.s. 4.1%, $p = 0.011$) than the HPIV3-infected patients. The HPIV2-infected patients were more tend to develop myalgia than the HPIV3-infected patients (7.5% v.s. 2.1%, $p = 0.031$) (Table 2).

Table 1 Demographic data and underlying diseases of children with human parainfluenza virus infections.

	HPIV1 (n = 39)	HPIV2 (n = 67)	HPIV3 (n = 292)	Total (n = 398)
Age (years)[abc]	3.8 ± 3.8	5.2 ± 4.0	2.3 ± 1.8	2.9 ± 2.8
0−<1[ac]	9 (23.1%)	0 (0%)	56 (19.2%)	65 (16.3%)
≥1−<3[bc]	10 (25.6%)	25 (37.3%)	166 (56.8%)	201 (50.5%)
≥3−<5	11 (28.2%)	17 (25.4%)	57 (19.5%)	85 (21.4%)
≥5[bc]	9 (23.1%)	25 (37.3%)	13 (4.5%)	47 (11.8%)
Male sex	20 (51.3%)	40 (59.7%)	177 (60.6%)	237 (59.5%)
Contact history	13 (33.3%)	26 (38.8%)	121 (41.4%)	160 (40.2%)
Febrile days	2.0 ± 1.3	2.5 ± 2.0	2.0 ± 1.7	2.1 ± 1.7
Underlying diseases	10 (25.6%)	16 (23.9%)	48 (16.4%)	74 (18.6%)
Immunocompromised	3 (7.7%)	8 (11.9%)	13 (4.5%)	24 (6%)
Prematurity	5 (12.8%)	4 (6.0%)	26 (8.9%)	35 (8.8%)
CHD	0 (0%)	1 (1.5%)	4 (1.4%)	5 (1.3%)
Chronic lung disease	0 (0%)	0 (0%)	1 (0.3%)	1 (0.3%)
Asthma[b]	2 (5.1%)	1 (1.5%)	1 (0.3%)	4 (1.0%)
Neurologic disease	2 (5.1%)	3 (4.5%)	13 (4.5%)	18 (4.5%)

CHD = Congenital heart disease.
[a] $p < 0.05$ is used to compare the HPIV1 and the HPIV2.
[b] $p < 0.05$ is used to compare the HPIV1 to the HPIV3.
[c] $p < 0.05$ is used to compare the HPIV2 to the HPIV3.

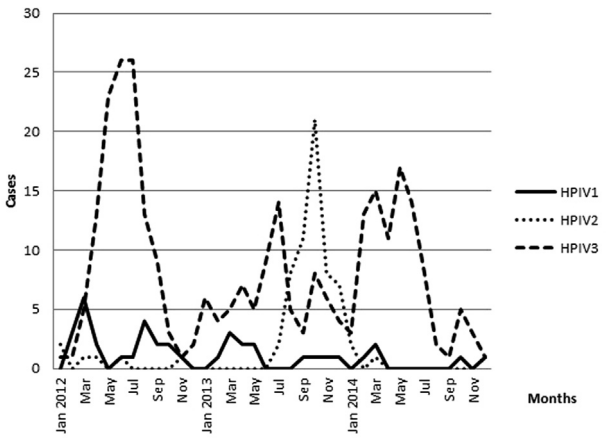

Figure 1. Monthly distribution of the human parainfluenza virus infections, 2012—2014.

Clinical diagnosis

The most common clinical diagnosis of HPIV infections were pharyngotonsillitis (50.3%, 200/398), bronchopneumonia (23.9%, 95/398), and croup (11.6%, 46/398), following by acute bronchiolitis (7.8%, 31/398). The HPIV3-infected patients were more common to have pharyngotonsillitis than HPIV2-infected children (53.8% v.s. 35.8%, $p = 0.029$), and HPIV3 could also cause lower respiratory tract infections, including acute bronchiolitis, bronchopneumonia, and lobar pneumonia. The HPIV2-infected patients were more common to associate with croup than the HPIV3-infected patients (25.4% v.s. 7.5%, $p < 0.001$). The HPIV2-infected patients more frequently (32.8%) to diagnosis as bronchopneumonia than the HPIV1 and HPIV3-infected patients. More HPIV1-infected patients were had acute bronchiolitis (10.3%) (Table 2).

Laboratory data

There were 193 patients were bled for the lab tests, including 23 HPIV1-infected patients, 37 HPIV2-infected patients, and 133 HPIV3-infected patients. The mean white blood cell (WBC) count in HPIV-infected children was $8.772 \pm 4.551 \times 10^9$/L. The mean platelet count was $258 \pm 144 \times 10^9$/L. The mean levels of C-reactive protein (CRP) was 22 ± 26 mg/L, and 37.3% (72/193) of the HPIV-infected patients was in the normal range. The percentage of band form of WBC is higher in HPIV2-infected patients than the HPIV3-infected patients (12% v.s. 8%, $p = 0.012$). The percentage lymphocyte is higher in HPIV3-infected patients than that in HPIV2-infected patients (34% v.s. 25%, $p = 0.03$) (Table 3).

Treatment course and outcomes

The hospitalization rate was 28.4% (113/398), with a mean duration of 1.8 days, and ICU admission rate was 4.0% (16/398) (Table 4). HPIV1-infected children had high hospitalization rate (41%, 16/39). Totally 35.4% (141/398) of the HPIV-infected patients received antibiotics treatment, and 5.8% (23/398) had bronchodilators administration. The O_2 requirement rate of HPIV-infected children was 3.5% (14/398), and 0.8% (3/398) was intubated for mechanical ventilation, including one had congenital heart disease and two due to acute myocarditis and subsequently expired. All the intubated patients were infected by HPIV3. One patient

Table 2 Clinical presentations and diagnosis of children with human parainfluenza virus infections.

	HPIV1 (n = 39)	HPIV2 (n = 67)	HPIV3 (n = 292)	Total (n = 398)
Symptoms				
Cough	26 (66.7%)	50 (74.6%)	213 (72.9%)	289 (72.6%)
Rhinorrhea	13 (33.3%)	31 (46.3%)	153 (52.4%)	197 (49.5%)
Vomiting	8 (20.5%)	17 (25.4%)	79 (27.1%)	104 (26.1%)
Diarrhea	3 (7.7%)	4 (6.0%)	39 (13.4%)	46 (11.6%)
Hoarseness[a]	8 (20.5%)	16 (23.9%)	16 (5.5%)	40 (10.1%)
Stridor[b]	6 (15.4%)	6 (9.0%)	9 (3.1%)	21 (5.3%)
Wheezing	4 (10.3%)	4 (6.0%)	13 (4.5%)	21 (5.3%)
Hypoxia	0 (0.0%)	1 (1.5%)	10 (3.4%)	11 (2.8%)
Respiratory distress/failure	2 (5.2%)	5 (7.5%)	7 (2.4%)	14 (3.6%)
Headache[b]	4 (10.3%)	4 (6.0%)	4 (1.4%)	12 (3.0%)
Abdominal pain[b]	6 (15.4%)	6 (9.0%)	12 (4.1%)	24 (6.0%)
Myalgia[a]	3 (7.7%)	5 (7.5%)	6 (2.1%)	14 (3.5%)
Skin rash	2 (5.1%)	3 (4.5%)	21 (7.2%)	26 (6.5%)
Diagnosis				
Pharyngotonsillitis[a]	19 (48.7%)	24 (35.8%)	157 (53.8%)	200 (50.3%)
Croup[a]	7 (17.9%)	17 (25.4%)	22 (7.5%)	46 (11.6%)
Bronchiolitis	4 (10.3%)	1 (1.5%)	26 (8.9%)	31 (7.8%)
Bronchopneumonia	6 (15.4%)	22 (32.8%)	67 (22.9%)	95 (23.9%)
Lobar pneumonia	1 (2.6%)	1 (1.5%)	5 (1.7%)	7 (1.8%)

[a] $p < 0.05$ is used to compare the HPIV2 to the HPIV3.
[b] $p < 0.05$ is used to compare the HPIV1 to the HPIV3.

Table 3 The laboratory data of children with human parainfluenza virus infections.

	HPIV1 (n = 23)	HPIV2 (n = 37)	HPIV3 (n = 133)	Total (n = 193)
WBC ($\times 10^9$/L)	7.373 ± 3.357	7.811 ± 4.073	9.282 ± 4.783	8.772 ± 4.551
Band (%)[a]	9 ± 10	12 ± 8	8 ± 7	9 ± 8
Segment (%)	42 ± 24	51 ± 16	46 ± 20	46 ± 20
Lymphocyte (%)[a]	36 ± 22	25 ± 16	34 ± 19	33 ± 19
Platelet ($\times 10^9$/L)	287 ± 289	213 ± 108	266 ± 110	258 ± 144
CRP (mg/L)	23 ± 31	22 ± 26	21 ± 25	22 ± 26
<7	8 (34.8%)	14 (37.8%)	50 (37.6%)	72 (37.3%)
>7	15 (65.2%)	23 (62.2%)	83 (62.4%)	121 (62.7%)

[a] $p < 0.05$ is used to compare the HPIV2 to the HPIV3.
WBC = white blood cell.
CRP = C-reactive protein.

Table 4 Treatment courses and outcomes of children with human parainfluenza virus infections.

	HPIV1 (n = 39)	HPIV2 (n = 67)	HPIV3 (n = 292)	Total (n = 398)
Hospitalization	16 (41%)	17 (25.4%)	80 (27.4%)	113 (28.4%)
Hospitalization days	2.2 ± 3.2	1.4 ± 2.9	1.9 ± 2.5	1.8 ± 4.2
ICU admission	2 (5.1%)	1 (1.5%)	13 (4.5%)	16 (4.0%)
Antibiotics use	16 (41.0%)	29 (43.3%)	96 (32.9%)	141 (35.4%)
Bronchodilator	1 (2.6%)	3 (4.5%)	19 (6.5%)	23 (5.8%)
O2 use	1 (2.6%)	1 (1.5%)	12 (4.1%)	14 (3.5%)
Intubation[a]	0	0	3 (1.0%)	3 (0.8%)
Mortality[b]	0	0	2 (0.7%)	2 (0.5%)
Neurologic complication[c]	0	0	1 (0.3%)	1 (0.3%)

[a] One had congenital heart disease, and was intubated due to respiratory failure; two were intubated due to acute myocarditis and respiratory failure.
[b] Two were expired due to suspicion of viral sepsis and acute myocarditis. One had epilepsy, and the other had cerebral palsy.
[c] Neurologic complication includes acute necrotizing encephalopathy.
ICU = intensive care unit.

had HPIV3-associated acute necrotizng encephalopathy (ANE).

Discussion

In this study, the mean age of the HPIV2-infected patients was the oldest (5.2 ± 4.0 years old), and more frequently to develop croup (25.4%) and bronchopneumonia (32.8%). The major presentation was pharyngotonsillitis in all HPIV-infected patients. In other studies, Hsieh et al. found the mean age of the HPIV2-infected children was 30.9 ± 18.8 months old, which was younger than our findings (5.2± 4.0 year-old).[14] Knott et al. found that the major clinical manifestations of HPIV1 and HPIV2 infections were croup and pharyngitis. Upper respiratory tract infection caused by HPIV3 was predominant in all age groups.[15] Yang et al. reported the HPIV1 was accounted for all of the cases of croup.[13] The HPIV2 was reported to cause 60% of croup cases in the community in years during which the HPIV1 was not endemic.[9,18] Yang et al. found 87.2% of the HPIV-infected children were younger than three years old,[13] and Hsieh et al. found 95% of the hospitalized HPIV-infected children were under five years of age.[14] In our study, 66.8% (266/398) of the HPIV-infected children are younger than three years old. Among hospitalized HPIV-infected children, 87.6% (99/113) were younger than five years old. Age between one to three years old was the most common group of HPIV infection.

Biennial pattern of the HPIV1 and HPIV2 had been reported in New York, Tennessee and Beijing,[9,15–17] and the HPIV3 appeared yearly with a peak activity in spring or summer.[9,14,15] Yang et al. failed to show seasonality of HPIV1 and HPIV2 in Taiwan.[13] Hsieh et al. found HPIV1 could be identified throughout the year; HPIV2 tended to cluster in the late summer and autumn.[14] In our study, the HPIV1 did not show the biennial pattern; however, it was more common to detect in spring. The HPIV2 did not occur yearly, and no biennial pattern was noted from 2012 to 2014. The HPIV3 appeared yearly with a peak activity in late spring and summer period in southern Taiwan. The seasonality was noted both in HPIV1 and HPIV3 in southern Taiwan, which was located in the tropics.

Hsieh et al. found HPIV2 was associated with high percentage of lower respiratory infection, but no significant difference among serotypes of HPIV among the hospitalized children.[14] In our study, among the HPIV-infected patients, HPIV2 was also associated with highest percentage of lower respiratory tract infection (35.8%, 24/67). HPIV3 was found to cause more severe respiratory complications than the HPIV1 and the HPIV2.[9,16,19–22] In our studies, hospitalization rate was the highest in HPIV1-infected patients (41%),

however, the HPIV3 infection may associate with severe complications, including acute myocarditis and ANE. In ICU-admitted patients, 31.3% (5/16) had underlying disease, such as prematurity, congenital heart disease and cerebral palsy. The HPIVs are also a common cause of fever and neutropenia in children with cancer.[18] In our studies, 6% of HPIV-infected patients were in immunocompromised conditions, including children with cancer under chemotherapy.

Hsieh et al. found no significant differences in mean WBC and serum levels of CRP among the serotypes of HPIV infection.[14] In our study, the percentage of band form of WBC was higher in the HPIV2-infected patients than in HPIV3-infected patients, but the percentage of lymphocyte was higher in the HPIV3-infected patients than in HPIV2-infected patients. Serum levels of CRP was no significant differences among HPIV1, HPIV2 and HPIV3-infected patients.

Currently, a phase I clinical trial of the experimental live-attenuated HPIV1 vaccine was conducted in the United States of America.[23] A safety and immunogenicity of an intranasal sendai virus-based HPIV1 vaccine was also tested in adults and 3- to 6-year-old children.[24] Phase I study of a live attenuated HPIV3 vaccine was well-tolerated in seronegative young children.[25] Future efforts to conduct the clinical trials of HPIV vaccine are warranted.

This study also had some limitations. First, this was a retrospective study, so the information and data in the medical records might be incomplete. Second, because of HPIV 4a and 4b were not included in the commercial kit, so not all types of HPIVs could be analyzed. Third, the case number of the HPIV1-infected children was low, so there was limitation for statistical analysis.

In conclusion, the HPIVs were found to affect children between one to three years old, and contribute to various clinical manifestations and also cause diversity of respiratory symptoms from asymptomatic to lobar pneumonia. Further epidemiological and virological surveillance and investigations of different HPIVs are needed in the community.

Conflicts of interest

All authors declare no conflicts of interest.

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