DOI:10.1111/irv.12062
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Short Article

Influenza C virus–associated community-acquired pneumonia in children

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Accepted 30 October 2012. Published Online 07 December 2012

To evaluate the impact of influenza C (ICV) infection in children with community-acquired pneumonia (CAP), all of the children consecutively seen during 4 influenza seasons with respiratory symptoms and radiographically confirmed CAP were prospectively evaluated. ICV was identified in the respiratory secretions of five of 391 patients (1·3%). In children with ICV-associated CAP, clinical data were similar to those observed in children with IAV-associated CAP and worse than those observed in children with IBV-associated. The phylogenetic tree showed that the sequenced strains clustered in two of the six ICV lineages. These findings highlight that ICV can be a cause of CAP of children and that this can be severe enough to require hospitalization.

Keywords Children, community-acquired pneumonia, influenza, influenza C, influenza C virus.

Please cite this paper as: Principi *et al.* (2013) Influenza C virus–associated community-acquired pneumonia in children. Influenza and Other Respiratory Viruses 7(6), 999–1003.

Despite its widespread circulation, influenza C virus (ICV) is traditionally considered a scarcely virulent infectious agent because, unlike influenza A (IAV) and B viruses (IBV), it is thought to be associated with infections that, when symptomatic, are mild and mainly involve the upper respiratory tract.[1–3] However, the frequency and clinical picture of ICV-associated pediatric community-acquired pneumonia (CAP) have not been defined, nor which virus lineages more frequently cause the disease. This prospective study was designed to evaluate the incidence and clinical relevance of ICV infection in children with radiographically confirmed CAP aged <15 years.

The study was approved by the Institutional Review Board of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, and was carried out in Pediatric Clinic 1 of the University of Milan between 1 November and 31 March of four consecutive winter seasons (2008–2009, 2009–2010, 2010–2011, and 2011–2012). The written informed consent of a parent or legal guardian was required, and children older than 8 years were asked to give their assent. All of the otherwise healthy children aged between 1 month and 14 years seen in our Emergency Room because of fever and signs and symptoms of lower respiratory tract involvement whose chest radiograph was consistent with CAP were considered eligible for the study. The chest radiographs were evaluated by an independent expert radiologist who classified the findings as alveolar, interstitial, or no pneumonia in accordance with the World Health Organization (WHO) criteria.[4] Upon admission, respiratory secretions were taken from each child using a pernasal flocked swab and stored in a tube of UTM-RT (Kit Cat. No. 360c, Copan Italia, Brescia, Italy).

Viral tests for the identification of influenza viruses were performed for study purposes and were always available after the patient's discharge. Upon enrollment, detailed information regarding the patients' demographics, clinical history, and disease characteristics was collected, together with a blood sample for the evaluation of laboratory variables including white blood cell (WBC) counts, C-reactive protein (CRP) and procalcitonin (PCT) levels, and blood cultures. First drug treatment as well as decision on when to hospitalize was chosen by attending pediatrician in charge on the basis of the guidelines of the Italian Society of Pediatrics.[5] None of the children was treated with antivirals. All of the enrolled children (whether they were hospitalized or sent home immediately after enrollment) were reevaluated 15 ± 2 days later by means of interviews and clinical examinations carried out by trained investigators using standardized questionnaires.

Each sample underwent real-time polymerase chain reaction (PCR) to identify IAV (and its subtypes) and IBV as previously described.[6] ICV was identified by amplifying the

non-structural protein (NS) gene using the method described by Matsuzaki *et al.*[7] The laboratory-confirmed IAV-, IBV- and ICV-positive samples were then tested for coinfections with respiratory syncytial virus (RSV)-A and RSV-B, parainfluenza virus 1, 2, 3, and 4, adenovirus, human metapneumovirus (hMPV), coronaviruses 229E, NL63, OC43, and HKU1, enterovirus/rhinovirus, and human bocavirus using the Luminex x TAG Respiratory Virus Panel (RVP) fast assay (Luminex Molecular Diagnostics Inc., Toronto, ON, Canada) in accordance with the manufacturer's instructions.[6]

A segment of the *hemagglutinin-esterase* (*HE*) gene was sequenced in the ICV-positive samples to determine the lineages of the circulating viruses. One segment was amplified using two published primers.[8] The sequences identified in this study, together with previously published[7,9,10] representative sequences of each lineage downloaded from the GenBank database, were aligned using ClustalX v.2.0.12[11] and default parameters. A phylogenetic tree based on the nucleotide sequences was reconstructed as previously described.[12]

The five nucleotide sequences identified in this study were submitted to the GenBank database and assigned accession numbers JX129180, JX129181, JX129182, JX129183, and JX129184.

The study involved 391 children with radiographically confirmed CAP (180 males; mean age ± SD 2·9 ± 2·7 years): 156 enrolled in the 2008–2009 season, 132 in 2009–2010, 66 in 2010–2011, and 37 in 2011–2012. Influenza C virus was identified in the respiratory secretions of five patients (1·3%) only in the 2008–2009 (two cases; prevalence 1·3%) and 2009–2010 seasons (three cases; prevalence 1·3%). Influenza A and B viruses were identified in 26 and three children, respectively (seven A/H3N2 and one B in 2008–2009; 13 A/H1N1/2009 and one B in 2009–2010; six A/H1N1/2009 and one B in 2010–2011; none in 2011–2012).

Table 1 summarizes the characteristics of the five children with ICV infection: four aged <3 years, the fifth aged 14 years. No other virus was found in any of these cases. All of them were hospitalized but a significant improvement in clinical signs and symptoms of the disease took place in few days in all the patients. Post-discharge check-ups showed that all of the children were completely cured, and none experienced any recurrence. These characteristics were similar to those observed in children with IAV-associated CAP and worse than those observed in children with IBV-associated CAP (none of the IBV-positive cases was hospitalized) (Table 2).

The phylogenetic tree constructed using the sequences of the five ICVs identified in this study, and previously described ICVs showed that the five sequenced strains clustered in two of the six classic ICV lineages (Figure 1). The two strains isolated during the 2008–2009 season (C/Milan/550/2008 and C/Milan/1101/2009) were located

Table 1. Characteristics and outcomes of children hospitalized because of CAP and ICV infection

Patient	Gender	Age (years)	Peak of fever (°C)	Symptoms	Signs	SaO_2 (%)	Breath rate (breath/minutes)	WBC (cells/μl)	CRP (mg/l)	PCT (ng/ml)	Blood culture and other viral tests	Type of CAP at chest X ray	Duration of hospitalization (days)
1	F	2·2	39·5	Cough and rhinitis	Wheezing and rales	96	42	18 600	3·4	0·1	Negative	Alveolar	3
2	M	1·6	39	Cough and rhinitis	Wheezing and rales	90	44	16 900	0·5	0·01	Negative	Alveolar	5
3	M	1·3	38·3	Cough and rhinitis	Wheezing and rales	90	39	15 800	0·3	0·01	Negative	Alveolar	5
4	F	1	40	Cough and rhinitis	Rales	98	37	9800	3·7	0·16	Negative	Interstitial	3
5	F	14	38·2	Cough and rhinitis	Rales	100	18	21 700	0·1	0·01	Negative	Interstitial	3

CAP, community-acquired pneumonia; CRP, C-reactive protein; PCT, procalcitonin; WBC, white blood cell count.

Table 2. Comparisons of children with radiographically confirmed CAP infected by different influenza viruses

Viral type	No. of cases	Males (%)	Median age (range), yrs	Hospitalized for clinical reasons, No. (%)	Wheezing, No. (%)	$SaO_2 < 92\%$, No. (%)	Median WBC (range), cells/μl	Median CRP (range), mg/l	Median PCT (range), ng/ml	Viral coinfections, No. (%)	Alveolar CAP, No. (%)
Influenza A	26	13 (50·0)	4·1 (0·6–14)	18 (69·2)*	9 (34·6)	9 (34·6)	16 800 (7600–24 500)	2·8 (0·1–7·3)	0·09 (0·01–2·3)	5 (19·2)	12 (46·2)
A/H1N1/2009	19	9 (47·4)	5·0 (0·6–14)	15 (78·9)*	6 (31·6)	7 (36·8)	15 900 (7900–24 500)	2·4 (0·1–5·4)	0·06 (0·01–0·16)	1 (5·2)	10 (52·6)
A/H3N2	7	4 (57·1)	1·8 (0·7–14)	3 (42·9)	3 (42·9)	2 (28·6)	19 200 (7600–22 440)	3·8 (0·1–7·3)	0·14 (0·01–2·3)	4 (57·1)	2 (28·6)
Influenza B	3	1 (33·3)	4·4 (1·6–14)	0 (0·0)	0 (0·0)	0 (0·0)	14 700 (6700–19 800)	2·3 (0·1–2·8)	0·01 (0·01–0·14)	1 (33·3)	1 (33·3)
Influenza C	5	2 (40·0)	1–14 (1·6)	4 (80·0)*	3 (60·0)	2 (40·0)	16 900 (9800–21 700)	0·1–3·7 (0·5)	0·01 (0·01–0·16)	0 (0·0)	3 (60·0)

CAP, community-acquired pneumonia; CRP, C-reactive protein; PCT, procalcitonin; WBC, white blood cell count.
*$P < 0.05$ in differences in hospitalization rates between influenza A, influenza A/H1N1/2009, and influenza C versus influenza B; no other significant between-group differences groups.

in the Kanagawa/1/76 and Sao Paulo/378/82 lineages, respectively. Of the three ICVs identified in the 2009–2010 influenza season, one (C/Milan/128/2009) was located in the Kanagawa/1/76 lineage and two (C/Milan/976/2010 and C/Milan/1130/2010) in the Sao Paulo/378/82 lineage.

To the best of our knowledge, this is the first study specifically designed to evaluate the importance of ICV in pediatric CAP. The collected data seem to indicate that ICV can be considered a possible cause of CAP in children, particularly in younger subjects. Our results also suggest the need for further prospective studies that should be carried out throughout the year to provide conclusive results. Previous studies have shown that ICV infection in temperate zones does not have the marked seasonal nature of IAV or IBV infections as it has been in patients with respiratory infections during winter (concomitantly with other influenza viruses) as well as during late spring and early summer.[1] It is therefore possible that the global importance of ICV as a cause of CAP in children is greater than that found in our study, which only involved the winter months.

The greater frequency of ICV than IBV in patients with respiratory infections has previously reported by Calvo *et al.*[2] and Antón *et al.* in Spain,[3] who studied children and the general population and analyzed subjects with any type of respiratory tract infection. The clinical characteristics of ICV-associated CAP were not different from those found in cases of IAV-associated CAP, but our IAV- and ICV-positive cases required hospitalization significantly more often than the cases of IBV-associated CAP. However, the number of patients with influenza viruses is too small to allow comprehensive comparisons.

Similarities in the clinical pictures of diseases due to IAV and ICV have been found by other authors in studies evaluating the global clinical features of influenza virus disease.[1] However, ours are the first data exclusively regarding CAP and, given the importance of this disease in younger children, merit consideration.

We found that ICV was a possible cause of CAP only during the first 2 years of enrollment. This may have been due to its different circulation rates year by year or to the relatively small number of children with CAP enrolled in the last 2 years of the study. As the incidence of ICV-associated CAP in the 2 years with positive cases was 1·3% and 2·3%, respectively, it is possible that enrolling fewer than 100 cases of CAP per year could have led to negative results even if ICV was circulating.

Phylogenetic analysis of the *HE* gene of the ICVs identified in this study revealed the simultaneous co-circulation of C/Kanagawa/1/76- and C/Sao Paulo/378/82-related lineages in the influenza seasons 2008–2009 and 2009–2010. As there are no data concerning the circulation of ICV in Italy during previous or subsequent years, it is not possible to establish

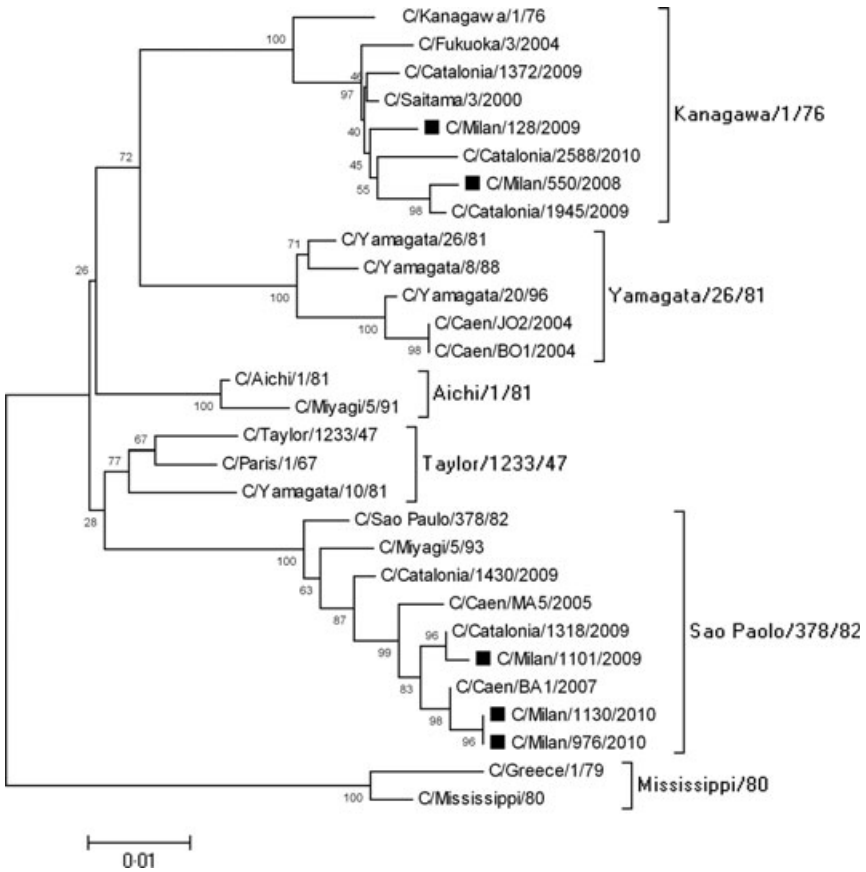

Figure 1. Phylogenetic tree of influenza C viruses based on a region of about 1000 bp of the *HE* gene. The viruses isolated in this study (Milan, Italy) are marked by a black symbol. The other sequences have been described elsewhere.[7,9–11] The values at the nodes are bootstrap supported on the basis of 500 replicates.

which was the dominant genetic lineage, or whether a new dominant group was replacing a previously more important lineage. Data collected in France from 2004 to 2007[10] and in Spain in 2009–2010[3] indicate that strains belonging to the same lineages as those found in this study were co-circulating there immediately before or simultaneously with those identified in Italy. Moreover, four of the five ICVs identified in this study had 99% nucleotidic identity with strains first identified in France[10] and Spain.[3] This confirms that spontaneous variations in the amino acid sequences of circulating ICV strains occur slowly and that continuous virological surveillance of ICV may contribute to improving our knowledge of the importance of the different strains in causing disease.

In conclusion, our findings suggest that ICV can be an important cause of CAP of children and could have considerable consequences. Further studies aimed at defining the peak season of ICV infection in different countries, the real role of ICV in causing severe respiratory disease, and establishing which strains are more epidemiologically important in different countries and different years are urgently needed.

Acknowledgements

The authors would like to thank Dr. Yoko Matsuzaki, Department of Infectious Diseases, Yamagata University Faculty of Medicine, Japan, for providing the influenza C-positive cDNA control and primer sequences for *HE* amplification. This study was supported by a grant from the Italian Ministry of Health (Bando Giovani Ricercatori 2007). None of the authors has any potential conflict of interest. All of the authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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