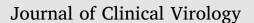
Contents lists available at ScienceDirect







journal homepage: www.elsevier.com/locate/jcv

Frequency of respiratory viruses among patients admitted to 26 Intensive Care Units in seven consecutive winter-spring seasons (2009–2016) in Northern Italy



Antonio Piralla^{a,1}, Bianca Mariani^{a,1}, Francesca Rovida^a, Fausto Baldanti^{a,b,*}

^a Molecular Virology Unit, Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
^b Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy

ARTICLE INFO

Keywords: respiratory viruses CAP lower respiratory tract infection molecular diagnostic respiratory infection

ABSTRACT

Background: The role of respiratory viruses in the etiology of community-acquired pneumonia (CAP) is still debated. The advent of molecular assays has improved the identification of viruses in patients with CAP and according to published studies, viruses account for 11-55% of adult CAP cases. *Objectives and study design*: In the present study, the frequency of respiratory viruses was evaluated in respiratory samples collected from 414 patients with CAP admitted to 26 ICUs in the Lombardy Region (10 million inhabitants) during seven winter-spring seasons (2009–2016). *Results*: In 226 (54.6%) patients one or more respiratory viruses were identified, while 188 (45.4%) patients ware negative A single virus infection was observed in 214/226 (94.7%) patients: while in 12/226 (5.3%) at

were negative. A single virus infection was observed in 214/226 (94.7%) patients; while, in 12/226 (5.3%) at least two respiratory viruses were detected. Influenza A was the most common virus in 140/226 patients (61.9%) followed by rhinoviruses (33/226, 14.6%), respiratory syncytial virus (13/226, 5.8%), influenza B virus (9/226, 4.0%), human coronaviruses (9/226, 4.0%), cytomegalovirus (9/226, 4.0%) and human metapneumovirus (1/226, 0.4%).

Conclusions: Viral infections are present in a consistent proportion of patients admitted to the ICU for CAP. Influenza A and rhinovirus accounted for three-quarters of all CAP in ICU patients. The use of lower respiratory instead of upper respiratory samples might be useful in the diagnosis of viral CAP.

1. Background

In the majority of patients with a poor outcome, who are admitted to intensive care units (ICUs), there is an association with severe respiratory distress syndromes [1]. Severe community-acquired pneumonia (CAP) is usually attributed to bacterial agents, but the introduction of broader diagnostic panels with increased sensitivity have improved the diagnosis of respiratory viruses with a relevant impact on outcomes for ICU-patients [2]. Indeed, based on recent reports, viruses account for 11–55% of the total infections in patients admitted to the ICU with severe respiratory distress [1,3–5]. However, many studies have only focused on the role of influenza A virus (e.g. H1N1pdm09) as an etiologic agent of acute respiratory failure [6,7]. Respiratory viruses other than influenza such as human rhinovirus (HRV), human parainfluenza viruses 1-4 (hPIV1-4), human respiratory syncytial virus (hRSV) and human coronaviruses (hCoVs) are not routinely investigated in respiratory samples of ICU patients, and their role as the cause of ICU admission might be under estimated. Understanding the etiologic agents of CAP may improve treatment decisions and patient outcome. However, despite technological advances, establishing the etiologic causes of pneumonia remains a challenge.

2. Objectives

The aim of this study was to investigate the prevalence of respiratory viruses in patients admitted to 26 ICUs in the Lombardy Region (nearly 10 million inhabitants) during seven consecutive winter seasons (2009–2016).

3. Study design

3.1. Study design and patients

Respiratory samples collected from patients admitted to 26 ICUs in

* Corresponding author at: Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy.

¹ These authors equally contributed to the development of the study.

http://dx.doi.org/10.1016/j.jcv.2017.05.004 Received 20 December 2016; Received in revised form 4 May 2017; Accepted 6 May 2017 1386-6532/ © 2017 Elsevier B.V. All rights reserved.

E-mail addresses: f.baldanti@smatteo.pv.it, fausto.baldanti@unipv.it (F. Baldanti).

the Lombardy Region with acute respiratory failure, severe pneumonia or acute respiratory distress syndrome assisted by non-invasive or mechanical ventilation or by extra-corporeal membrane oxygenation were analyzed during seven consecutive winter seasons, between November 1st and April 31st (2009-2016). Respiratory samples were collected at admission to the ICU following standard protocols and according to the physician's orders. Severe respiratory syndrome was defined as acute onset (≤ 1 week) respiratory failure, with hypoxemia $(pO_2/FiO_2 ratio < 300 mmHg while on positive end-expiratory pres$ sure or noninvasive CPAP \geq 5 cmH₂O) and bilateral opacities at chest imaging [8]. Respiratory samples were sent to the Molecular Virology Unit at the Fondazione IRCCS Policlinico San Matteo, Pavia according to the Influenza Regional Surveillance and Preparedness Plan (DGR IX/ 1046, 22 Dec. 2010 and DGR 5988, 30 Jun 2011). Local Ethics Committee approval was not required as the study was conducted under the auspices of the Influenza Regional Surveillance and Preparedness Plan (DGR IX/1046, 22 Dec. 2010 and DGR 5988, 30 Jun 2011).

3.2. Identification of respiratory viruses

Respiratory samples were tested with a panel of laboratory-developed real-time RT-PCR or real-time PCR able to detect and quantify the following viruses [9,10]: influenza virus A and B, including subtype determination, hPIV-3, hRSV types A and B, hCoV types -OC43, -229E, -NL63, and -HKU1, hMPV, HRV and CMV (in a subset of samples).

4. Results

During the study period, a total of 414 (266 male and 148 female) patients were hospitalized in 26 ICUs with severe respiratory distress. Of these, 242/414 (58.2%) patients were adults (18–65 years), 134/414 (32.4%) patients aged > 65 years, while 39/414 (9.4%) were children (Table 1). Overall, the median age was 56 years (range: 1 mos–86 yrs). In two seasons (2011–2012 and 2014–2015) the overall median age was higher than 60 years, while in one season (2013–2014) it was lower than 50 years. The majority of ICU patients for each year were adults (18–65 yrs), ranging from 45.5% to 66.7% of total patients, over the seven seasons. Of note, in one season (2011–12), patients aged > 65 years were the most frequently observed reaching 47.7% of the total patients analyzed (Table 1).

Respiratory viruses were detected in clinical samples from the lower respiratory tract (LRT) (Broncho alveolar lavage or Broncho aspirate) during 179/414 (43.2%) CAP episodes; while respiratory viruses were detected in samples from the upper respiratory tract (URT) (nasal swab or nasopharyngeal aspirate) during 225/414 (56.8%) episodes.

A mean of 59 CAP episodes were analyzed yearly with a minimum of 36 episodes in the 2013–14 season and a maximum of 88 episodes in the 2014–2015 season. In 226/414 (54.6%) patients one or more respiratory viruses were detected, while 188/414 (45.4%) patients resulted negative. The mean positive frequency was 52.4% with a broad

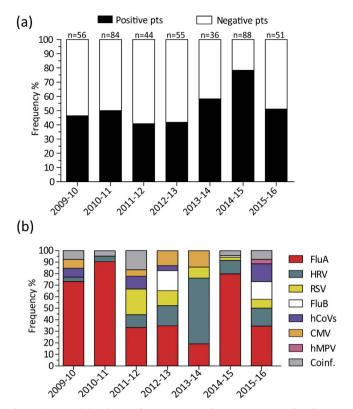


Fig. 1. (a) Seasonal distribution of virus-positive and virus-negative episodes of CAP in ICU-admitted patients. The total number of CAP episodes analyzed yearly are reported above each column. (b) Seasonal distribution of respiratory virus detection.

difference between seasons (Fig. 1a). Indeed, the highest positive frequency (78.4%) was observed in the 2014-15 season, while the lowest (40.9%) was observed in the 2011–12 season (Fig. 1a).

A single virus infection was observed in 214/226 (94.7%) patients, while in 12/226 (5.3%) at least two respiratory viruses were identified. Overall, influenza A was the most common virus identified (140/226 patients, 61.9%) followed by HRV (33/226, 14.6%), hRSV (13/226, 5.8%), influenza B virus (9/226, 4.0%), hCoVs (9/226, 4.0%), CMV (9/ 226, 4.0%) and human metapneumovirus (hMPV) (1/226, 0.4%). No hPIVs were detected during the study period. In mixed infections (n = 12), HRV was the most frequently detected virus (7/12, 58.3%) followed by hCoV (6/12, 50.0%), influenza A virus (5/12, 41.7%), hRSV (3/12, 25.0%), CMV (3/12, 25.0%) and influenza B virus (2/12, 16.7%) (data not shown). Influenza A virus accounted for at least 70.0% of virus-positive infections in three seasons (2009-10, 2010-11 and 2014-15) and in another three (2011-12, 2012-13 and 2015-16) seasons it was the most frequently detected virus (33.3-34.8%) (Fig. 1b). In one season (2013-14), HRV was the most frequently detected virus (57.1% Fig. 1b).

In pediatric patients (n = 39; 9.4% of total), 19/39 (48.7) had a

Table 1

Age distribution of ICU-patients included in the study according to season.

	All seasons $(n = 414)$	2009–10 (n = 56)	2010–11 (n = 84)	2011–12 (n = 44)	2012–13 (n = 55)	2013–14 (n = 36)	2014–15 (n = 88)	2015–16 (n = 51)
Median age (range)	56	52	52	63	57	47	62	59
	(1 mos-86)	(3-86)	(1 mo-84)	(1-83)	(9 mos-84)	(1-84)	(3 mos-84)	(1 mos-86)
Age group (%)								
< 1 year	8 (1.9)	0 (0.0)	3 (3.6)	0 (0.0)	1 (1.8)	0 (0.0)	2 (2.3)	2 (3.9)
1–5 years	14 (3.4)	1 (1.8)	3 (3.6)	1 (2.3)	5 (9.1)	3 (8.3)	1 (1.1)	0 (0.0)
6–18 years	17 (4.1)	4 (7.1)	2 (2.4)	2 (4.5)	4 (7.3)	2 (5.6)	1 (1.1)	2 (3.9)
19-65 years	242 (58.2)	37 (66.1)	56 (66.7)	20 (45.5)	29 (52.7)	23 (63.9)	46 (52.3)	30 (58.8)
> 65 years	134 (32.4)	14 (25.0)	20 (23.8)	21 (47.7)	16 (29.1)	8 (22.2)	38 (43.2)	17 (33.3)

Mo, month.

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