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A. Bal[1,2], JS. Casalegno[1,2], C. Melenotte[3], F. Daviet[4,5], L. Ninove[6] , S. Edouard[3], F. Morfin[1,2], M. Valette[1,2], X. De Lamballerie[6], B. Lina[1,2], L. Papazian[4,5], A. Nougairède[6], S. Hraiech[4,5]

[1]Laboratoire de Virologie, Institut des Agents Infectieux, Groupement Hospitalier Nord, Hospices Civils de Lyon, Lyon, France

[2]Univ Lyon, Université Lyon 1, CIRI, Inserm U1111 CNRS UMR5308, Virpath, Lyon, France

[3]Aix Marseille Univ., IRD, AP-HM, MEPHI, IHU Méditerranée Infection, Marseille, France

[4]Service de Médecine Intensive - Réanimation, APHM, Hôpital Nord, Marseille, France

[5]CEReSS - Center for Studies and Research on Health Services and Quality of Life EA3279, Aix-Marseille University, France

[6]Unité des Virus Emergents (UVE: Aix- Marseille Univ., IRD 190, INSERM 1207, IHU Méditerranée Infection), Marseille, France

*** Correspondance:** Sami Hraiech, M.D, Ph.D.

Service de Médecine Intensive - Réanimation, APHM, Hôpital Nord, Marseille, France
CEReSS - Center for Studies and Research on Health Services and Quality of Life EA3279, Aix-Marseille University, France

Email: sami.hraiech@ap-hm.fr

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Objectives

We aimed to describe bacterial co-infections and acute respiratory distress (ARDS) outcomes according to influenza type and subtype.

Methods

A retrospective observational study was conducted from 2012 to 2016 in patients admitted to the respiratory intensive care unit (ICU) of Marseille university hospital for influenza-induced ARDS. Microbiological investigations, including multiplex molecular respiratory panel testing and conventional bacteriological cultures, were performed as part of the routine ICU care on the bronchoalveloar lavage collected at admission. Bacterial co-infections, ICU mortality, and respiratory function were investigated according to virus type and subtype.

Results

Among the 45 ARDS-patients included, A(H1N1)pdm09 was the most frequent influenza virus identified (28/45 A(H1N1)pdm09, 8/45 A(H3N2), and 9/45 influenza B). Bacterial co-infections involving a total of 23 bacteria were diagnosed in 16/45 patients (36%). A(H1N1)pdm09 patients presented fewer bacterial co-infections (17.9% *vs*. 50.0% for A(H3N2) patients and 77.8% for B patients; $p<0.01$). Overall, mortality at 90 days post-admission was 33.3% (15/45), and there was no significant difference between influenza type and subtype. The need for extracorporeal membrane oxygenation was more frequent for A(H1N1)pdm2009 (20/28, 71.4%) and B patients (7/9, 77.8%) as compared to the A(H3N2) subtype (1/8, 12.5%; $p<0.01$). A(H1N1)pdm09-ARDS patients were associated with fewer ventilation-free days at day 28 (median [IQR]: 0[0-8] day) as compared with other influenza-ARDS patients (15 [0-25] days, $p<0.05$).

Conclusions

In a population of influenza-induced ARDS, A(H1N1)pdm09 was associated with fewer bacterial co-infections but poorer respiratory outcomes. These data underline the major role of A(H1N1)pdm09 subtype on influenza disease severity.

Introduction

According to the World Health Organization (WHO), seasonal influenza may result in 290,000-650,000 deaths annually [1]. Influenza surveillance networks estimated that approximately 15% of hospitalized patients required admission to an intensive care unit (ICU), and the mortality could be up to 45% in case of acute respiratory distress syndrome (ARDS) [2–4]. ARDS is a life-threatening form of acute lung injury characterized by diffuse alveolar damage and lung capillary endothelial injury [5]. The development of bacterial co-infections also contributes to the severity of influenza infections, although their frequency and impact may differ according to the circulating influenza virus [6]. It was reported that ARDS encompassed heterogenic sub-phenotypes associated with different clinical outcomes [7]. However, the outcomes of ARDS according to influenza type and subtype have been poorly studied thus far. In this study we aimed to describe bacterial co-infections and ARDS outcomes by influenza type/subtype.

Methods

Study population and outcome measures

We conducted a retrospective observational study from the 2012-2013 to the 2015-2016 influenza seasons in the respiratory ICU of the Marseille university hospital (extracorporeal membrane oxygenation, ECMO, reference centre). Inclusion criteria were the association of an ARDS with a positive influenza polymerase chain reaction (PCR) test performed on the bronchoalveloar lavage (BAL) collected at admission. Exposure variables were the influenza type/subtype. Primary outcome was mortality at day 90. Secondary outcomes were the need for ECMO, the duration of mechanical ventilation (MV), the number of ventilation-free days

(VFD) calculated from day 1 to day 28, and the length of ICU stay. Bacterial co-infections were described according to influenza type/subtype. Clinical characteristics and demographic data were retrospectively collected from electronic medical records.

Microbiological diagnosis

All microbiological investigations were performed on the BAL fluid collected at admission as part of our ICU routine care. The main respiratory bacteria and viruses were tested using a multiplex PCR assay (FTD respiratory pathogens 33; fast-track diagnostics, Luxembourg). Influenza A subtyping, conventional bacteriological culture, specific PCR and pneumococcal urinary antigen test (Alere Inc, MA, USA) were also performed. Respiratory bacterial co-infections were defined as procalcitonin level ≥0.25 µg/L associated with molecular and/or culture detection of respiratory pathogen, and/or urinary detection of pneumococcal antigen. See supplementary data for additional information.

Ethics

In accordance with the national regulations, patients or relatives were informed at ICU admission of the possible use of anonymous healthcare data collected retrospectively for clinical research. They were also informed of the possibility to refuse the use of this data. The study received approval from the French data protection authority (*Commission Nationale de l'Informatique et des Libertés*) and is registered with the national data protection agency (number 2019-57).

Statistical Analysis

Continuous variables are presented as means ± standard deviations (SD) or medians with interquartile ranges [IQR] and compared using non-parametric Kruskal-Wallis or Mann–Whitney tests. Proportions were compared using the Chi-squared or Fisher's exact test as appropriate. A p-value of <0.05 was regarded as statistically significant. Statistical tests were

performed using SPSS (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY, USA).

Results

Study population

A(H1N1)pdm2009 subtype represented the most frequent influenza virus detected (28/45 A(H1N1)pdm2009, 8/45 A(H3N2) subtype and 9/45 B type). Mean±SD age differed according to influenza type/subtype (A(H3N2): 69.4±9.8 years; A(H1N1): 55.4±14.1 years; B patients: 49.7±19.1 years, $p<0.05$). Most patients had pre-existing comorbidities (35/45, 77.8%), but vaccination rate was only 11.1% (5/45; Table 1). Empirical antibiotic therapy was initiated with or adjusted to a broad spectrum β-lactam and macrolide for all patients. Of note, approximately 90% of patients had received antibiotics prior to ICU admission. Antiviral treatment (oseltamivir) was administered to all patients. None received intravenous neuraminidase inhibitors. No viral co-infection was diagnosed in this cohort.

Bacteriological results at admission according to influenza type and subtype

A bacterial co-infection was detected in 16 patients (35.6%). A(H1N1)pdm09 patients presented fewer bacterial co-infections than patients infected with another type/subtype (17.9% *vs*. 50.0% for A(H3N2) patients and 77.8% for B patients; $p<0.01$). A total of 23 respiratory bacteria were identified, mainly *Staphylococcus aureus* (9/23, 39%) and *Streptococcus pneumoniae* (7/23, 30%, table 1). Six of the 23 (26.1%) bacteria responsible of co-infections were detected using conventional cultures.

Table 1. Patient characteristics by influenza type and subtype

	Total n=45	A(H1N1) n=28	A(H3N2) n=8	B n=9	p-value
Clinical features					
Age, mean value, years ± SD	56.7±15.6	55.4±14.1	69.4±9.8	49.7±19.1	<0.05
Male sex, n (%)	27 (60.0)	17 (60.7)	5 (62.5)	5 (55.6)	ns
Influenza vaccination, n (%)	5 (11.1)	4 (14.3)	1 (12.5)	0 (0.0)	ns
No comorbidity, n (%)	10 (22.2)	6 (21.4)	2 (25.0)	2 (22.2)	ns
Smoking, n (%)	23 (51.1)	16 (57.1)	5 (62.5)	2 (22.2)	ns
Heart disease, n (%)	16 (35.6)	11 (39.2)	3 (37.5)	2 (22.2)	ns
Diabetes, n (%)	13 (28.9)	10 (35.7)	2 (25.0)	1 (11.1)	ns
Obesity, n (%)	12 (26.7)	9 (32.1)	1 (12.5)	2 (22.2)	ns
COPD, n (%)	11 (24.4)	6 (21.4)	3 (37.5)	2 (22.2)	ns
Immunosuppression, n (%)	8 (17.8)	4 (14.3)	3 (37.5)	1 (11.1)	ns
Alcoholism, n (%)	6 (13.3)	2 (7.1)	2 (25.0)	2 (22.2)	ns
Pregnancy, n (%)	1 (2.2)	0 (0.0)	0 (0.0)	1 (11.1)	ns
Patient pathway, n (%)					
Admission from other ICU	24 (53.3)	18 (64.3)	2 (25.0)	4 (44.4)	ns
Admission from emergency department	11 (24.4)	4 (14.3)	5 (62.5)	2 (22.2)	ns
Admission from other unit	7 (15.6)	5 (17.9)	1 (12.5)	1 (11.1)	ns
Other	3 (6.7)	1 (3.6)	0 (0.0)	2 (22.2)	ns
Bacteriological results					
Bacterial co-infection, n (%)	16 (35.6)	5 (17.9)	4 (50.0)	7 (77.8)	<0.01
S. aureus	9 (20.0)	5 (17.9)	0 (0.0)	4 (44.4)	ns
S. pneumoniae	7 (15.6)	1 (3.6)	3 (37.5)	3 (33.3)	<0.05
S. pyogenes	2 (4.4)	0 (0.0)	0 (0.0)	2 (22.2)	<0.05
H. influenzae	5 (11.1)	0 (0.0)	1 (12.5)	4 (44.4)	<0.01

SD: standard deviation; ns: non-significant; COPD: chronic obstructive pulmonary disease

Patient outcomes according to influenza type and subtype

Overall, mortality at 90 days post-admission was 33.3% (15/45), and there was no significant difference between influenza type and subtype. Twenty-six patients (58%) presented with severe ARDS. The need for ECMO was more frequent for A(H1N1)pdm2009 (20/28, 71.4 %) and B patients (7/9,77.8%) as compared with A(H3N2) subtype (1/8, 12.5%; p<0.01). The median [IQR] VFD at day 28 was significantly different between influenza type and subtype (A(H1N1)pdm09 patients: 0[0-8] days; B patients: 9[0-18] days; A(H3N2) patients: 27[0.0-28]

days, p<0.05; Table 2). A(H1N1)pdm09-ARDS patients were associated with fewer ventilation-free days at day 28 (median [IQR]: 0[0-8] day) as compared with other influenza-ARDS patients (15[0-25] days, p<0.05).

Table 2. Patient severity and outcome according to influenza type and subtype

	Total **n=45**	**A(H1N1)** **n=28**	**A(H3N2)** **n=8**	**B** **n=9**	**p-value**
Severity at ICU admission					
Mild ARDS, n (%)	4 (8.9)	1 (3.6)	2 (25.0)	1 (11.1)	ns
Moderate ARDS, n (%)	15 (33.3)	10 (35.7)	3 (37.5)	2 (22.2)	ns
Severe ARDS, n (%)	26 (57.8)	17 (60.7)	3 (37.5)	6 (66.7)	ns
SOFA, mean ± SD	9.0 ± 4.2	8.9 ± 4.4	7.5 ± 3.2	11.0 ± 3.9	ns
SAPS II, mean ± SD	48.2 ± 14.7	46.3 ± 13.4	49.3±13.2	53.3 ± 19.6	ns
Pa02/Fi02 (mmHg) mean ± SD	98.9 ± 41.7	99.6 ± 40.7	109.0 ± 49.2	87.9 ± 38.3	ns
Patient outcomes					
90-day mortality, n (%)	15 (33.3)	10 (35.7)	3 (37.5)	2 (22.2)	ns
ECMO, n (%)	28 (62.2)	20 (71.4)	1 (12.5)	7 (77.8)	<0.01
MV duration, days, median [IQR]	21.6 [6.0-33.0]	22.5 [13.8-41.8]	1.5 [0.0-12.8]	10.0 [6.0-17.0]	<0.01
VFD at day 28, median [IQR]	0 [0-18]	0 [0-8]	27 [0-28]	9 [0-18]	<0.05
Length of ICU stay, days, median [IQR]	23.3 [8.0-32.0]	25.0 [13.8-42.3]	9.0 [5.0-18.5]	15.0 [6.0-17.0]	<0.05

ARDS was categorized as mild, moderate, or severe according to the Berlin conference definition [5]. ICU: intensive care unit, ns: non-significant; ARDS: acute respiratory distress syndrome, SOFA: sequential organ failure assessment, SD: standard deviation, SAPS: simplified acute physiology score, ECMO: extracorporeal membrane oxygenation, IQR: interquartile range, VFD: ventilation free-days.

Discussion

In the present study of influenza-induced ARDS, A(H1N1)pdm09 was found to be predominant and was associated with fewer bacterial co-infections and poorer respiratory outcomes than A(H3N2) or B-induced ARDS.

Since 2009, A(H1N1)pdm09 has also been reported to be the most frequently detected in ICU patients underscoring the particular virulence of this influenza subtype [4,8,9]. Töpfer et al. found that A(H1N1)pdm09-ARDS led to a more important impairment of gas exchange as compared to non-A(H1N1)pdm09-ARDS; interestingly, the rate of bacterial co-infection between the two groups was not different [10]. These results are consistent with initial reports from the 2009 pandemic during which A(H1N1)pdm09 subtype was responsible for extremely severe infections mostly without the development of bacterial co-infections [2,11]. Several studies have, however, found greater morbidity and mortality rates in case of bacterial co-infections associated with A(H1N1)pdm09 indicating that viral pneumonia alone is not exclusively responsible for the severity of influenza infections [4,12,13].

It has been reported that A(H3N2) particularly affected elderly people, as was found herein, leading to a high mortality and ICU admission rates in this population [9]. Although considered as less severe and mainly responsible for mild presentation in children, B infections can also cause severe and fatal cases [14], notably due to the development of bacterial co-infections [15]. In the present study, most of influenza B-induced ARDS patients presented severe forms of ARDS and were more frequently associated with bacterial co-infections at ICU admission.

The main limitation of this retrospective study is the number of patients included which did not allow the investigation of the role of co-infections on respiratory function for each influenza type and subtype. However, a high number of influenza-induced ARDS patients might be

155 difficult to achieve and comprehensive descriptive data regarding the microbial epidemiology
156 and ARDS outcomes related to the three circulating influenza viruses are lacking.
157 To conclude, the present study underlines the major role of A(H1N1)pdm09 on influenza
158 disease severity. The detection of this subtype at admission should alert clinicians to the
159 potential evolution towards an extremely severe case requiring long ICU stay.

160 **Conflicts of interest**

161 The authors declare that the study was conducted in the absence of any commercial or financial
162 relationships that could be construed as a potential conflict of interest.

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165 **Author contributions**

166 AB, J-SC, BL, XD, LP, AN, SH conceived the study. AN, MV, LN, FM, SE performed
167 microbiological investigations. CM, FD, LP, SH are the guarantors for clinical data and sample
168 collection. AB and JS-C were the main writers of the manuscript. All authors reviewed and
169 approved the final version of the manuscript.

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