TABLE 2 CD4+ CD8+ T-lymphocyte percentage values and the CD4+/CD8+ ratio in the study group				
	Mean $\pm$ SD	Median	Interquartile range	Range
CD4+ %	30.9 $\pm$ 10.5	28.0	23.8–38.1	19.9–54.6
CD8+ %	42.6 $\pm$ 8.9	41.3	37.3–51.9	27.7–56.7
CD4+/CD8+	0.78 $\pm$ 0.5	0.65	0.5–0.88	0.42–1.97

families. Therefore, the studied children may have come from communities with inadequate sanitation and living conditions.

Regarding macrophage counts, our results were similar to those found in other published studies. Also, CD4+/CD8+ ratio values found in this study were also similar to those described for healthy children [4, 8].

These peculiarities found in the BAL of healthy children should be considered when interpreting results from children with lung diseases. Thus, in our setting, the number of eosinophils and lymphocytes found in “normal” children’s BAL seems to be slightly elevated in relation to the results of studies conducted in developed countries. Therefore, we could accept ~2% eosinophils and ~14% lymphocytes as BAL reference values for our setting.

In our view, advancements in understanding cellular and immunological processes in normal children will provide the basis for acquiring greater knowledge of infant and childhood lung diseases, and a better understanding of the way in which, in later phases of life, such respiratory diseases are manifested.

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REFERENCES

- 1 de Blic J, Marchac V, Scheinmann P. Complications of flexible bronchoscopy in children: prospective study of 1,328 procedures. *Eur Respir J* 2002; 20: 1271–1276.
- 2 Midulla F, Villani A, Merolla R, *et al.* Bronchoalveolar lavage studies in children without parenchymal lung disease: cellular constituents and protein levels. *Pediatr Pulmonol* 1995; 20: 112–118.
- 3 Ratjen F, Bredendiek M, Brendel M, *et al.* Differential cytology of bronchoalveolar lavage fluid in normal children. *Eur Respir J* 1994; 7: 1865–1870.
- 4 Ratjen F, Bredendiek M, Zheng L, *et al.* Lymphocyte subsets in bronchoalveolar lavage fluid of children without bronchopulmonary disease. *Am J Respir Crit Care Med* 1995; 152: 174–178.
- 5 de Blic J, Midulla F, Barbato A, *et al.* Bronchoalveolar lavage in children. ERS Task Force on bronchoalveolar lavage in children. *Eur Respir J* 2000; 15: 217–231.
- 6 Tessier V, Chadelat K, Baculard A, *et al.* A controlled study of differential cytology and cytokine expression profiles by alveolar cells in pediatric sarcoidosis. *Chest* 1996; 109: 1430–1438.
- 7 Clement A, Chadelat K, Masliah J, *et al.* A controlled study of oxygen metabolite release by alveolar macrophages from children with interstitial lung disease. *Am Rev Respir Dis* 1987; 136: 1424–1428.
- 8 Riedler J, Grigg J, Stone C, *et al.* Bronchoalveolar lavage cellularity in healthy children. *Am J Respir Crit Care Med* 1995; 152: 163–168.
- 9 Koh YY, Jung da E, Koh JY, *et al.* Bronchoalveolar cellularity and interleukin-8 levels in measles bronchiolitis obliterans. *Chest* 2007; 131: 1454–1460.

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Clinical relevance of mixed respiratory viral infections in adults with influenza A H1N1

To the Editors:

Throughout the pandemic circulation of the 2009 influenza A H1N1 virus (A/H1N1p), concomitant detection of other respiratory viruses in the same patient was reported [1, 2],

but their clinical relevance remains unknown. Mixed respiratory viral infections in adults have scarcely been studied. The aim of this study was to assess both the incidence of multiple viral respiratory infections and their clinical significance in adult patients with confirmed A/H1N1p.

From October 1 to November 30, 2009, nasopharyngeal and oropharyngeal swabs were obtained from consecutive adults (>18 yrs of age) presenting with acute respiratory illness to the emergency department of Hospital Clinic (Barcelona, Spain). Specimens were placed in a tube containing viral transport medium (VTM) and were immediately sent to and processed at the microbiology laboratory for virological confirmation. Total nucleic acids were extracted from 200 µL of fresh specimen and eluted in 25 µL of RNase-free elution buffer using NucliSense easyMAG (BioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. The presence of A/H1N1p was confirmed by two specific one-step, multiplex, real-time RT-PCRs for influenza virus typing (A or B) and subtyping (H1, H3 or H5) as previously described [3, 4]. Laboratory results for A/H1N1p were provided to clinicians in <24 h. In those patients diagnosed with pneumonia, regular sampling for microbiological diagnosis was also performed as previously described [5].

For the purpose of the study, samples with confirmed A/H1N1p were retrospectively assessed by multiplex PCR using the xTAG® RVP FAST Assay (Luminex–Abbott Molecular, Wiesbaden, Germany) according to the manufacturer's instructions for qualitative detection of influenza virus A and B, respiratory syncytial virus, human coronavirus (strains 229E, OC43, NL63 and HKU1), parainfluenza types 1, 2, 3 and 4, human metapneumovirus, rhinovirus/enterovirus, adenovirus, and human bocavirus. In those samples positive for rhinovirus/enterovirus, an RT-PCR assay was further performed to distinguish between the two [6].

A single real-time RT-PCR for direct quantification of influenza A virus RNA, with a set of primers and probes specific for the matrix protein (M1) gene, was performed as previously described [4, 7]. A/H1N1p could not be quantified in 25 (14%) out of 181 respiratory samples due to insufficient sample material.

The following parameters were collected at admission: age, sex, pregnancy, underlying disease (autoimmune diseases,

malignancies, diabetes mellitus, solid organ transplant, HIV infection, chronic obstructive pulmonary disease and asthma), presence of cough, diarrhoea, fever $\geq 37.8^{\circ}\text{C}$ and pneumonia (defined as a new infiltrate on a chest radiograph). In our study, we also recorded whether hospital admission had been required, and in such cases, the duration of hospital stay and mortality. The study was approved by the ethics committee of our institution.

We compared epidemiological and clinical characteristics and nasopharyngeal viral load of A/H1N1p between patients with and without respiratory virus co-infection. Fisher's exact or Chi-squared tests for categorical variables, and Mann–Whitney U-tests or paired t-tests for continuous variables were used. A two-tailed p-value <0.05 was considered significant. All analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

181 patients with confirmed A/H1N1p were included. 21 (12%) out of 181 patients had co-infection with at least one of the respiratory viruses assessed. Characteristics of the patients are described in table 1. 113 (62%) patients were female, of whom 22 (19%) were pregnant. Mean age was 39 yrs. Mean time from onset of symptoms to hospital admission was 2.7 days. 83 (46%) patients had underlying diseases, the most common being asthma (30%), followed by HIV infection (19%), diabetes (12%), obesity (7%) and chronic obstructive pulmonary disease (6%). 37 (21%) patients required hospital admission. Only one patient was admitted to the intensive care unit and required mechanical ventilation; this patient had been receiving corticosteroid therapy, had no viral co-infection and showed a favourable prognosis. 111 (61%) patients received oseltamivir. Pneumonia was detected in 30 (17%) patients. Of these, three were co-infected with *Streptococcus pneumoniae* and one patient died. Age, sex, pregnancy status, time from onset of symptoms, clinical presentation and underlying conditions were similar in both groups of patients. Nevertheless, patients with viral co-infection showed a trend toward higher rates of

TABLE 1 Comparison of epidemiological and clinical characteristics with nasopharyngeal viral load of pandemic influenza A H1N1 (A/H1N1p) between patients with and without respiratory virus co-infection

Characteristic	A/H1N1p only	A/H1N1p with other respiratory viruses	p-value
Patients	160 (88)	21 (12)	
Age yrs	38 ± 13	40 ± 14.2	0.63
Females	100 (63)	13 (62)	0.95
Underlying conditions	73 (46)	10 (48)	0.86
Pregnancy	21 (13)	1 (5)	0.27
Time after onset of symptoms days	2.7 ± 1.87	2.5 ± 1.93	0.66
Fever $\geq 37.8^{\circ}\text{C}$	133 (84)	18 (86)	0.85
Cough	123 (78)	18 (86)	0.40
Diarrhoea	17 (11)	2 (10)	0.87
Pneumonia	25 (16)	5 (24)	0.34
Requirement for hospital admission	30 (23)	7 (33)	0.12
Hospitalisation time days	0.6 ± 1.6	2.3 ± 5.4	0.0019
Treatment with oseltamivir	99 (62)	12 (57)	0.67
Viral load log₁₀ RNA copies per mL VTM	5.08 ± 1.4	5.6 ± 1.4	0.108

Data are presented as n (%) or mean ± SD, unless otherwise stated. VTM: viral transport medium.

pneumonia and hospital admission requirement. In addition, the duration of hospitalisation in patients with viral co-infection was significantly longer than in those without co-infection. Among patients admitted, average duration of hospitalisation was 9, 4 and 1 day for patients co-infected with rhinovirus, enterovirus and parainfluenza virus 3, respectively.

All samples were tested by both RT-PCR techniques, multiple real-time and xTAG® RVP FAST Assay. There was a good correlation for the detection of A/H1N1p using both techniques, except for three samples that tested positive only by multiple real-time with threshold cycle values of >35, indicating low viral loads in discordant samples.

Mean viral load in the whole population was $5.15 \pm 1.41 \log_{10}$ copies per mL of VTM. There were no significant differences between patients without (5.08 ± 1.4) and patients with (5.6 ± 1.4) respiratory viral co-infection ($p=0.108$).

The most common viruses concomitantly detected with A/H1N1p were rhinovirus in 14 patients (67%), followed by enterovirus in five (24%) patients, and bocavirus, coronavirus OC43 and parainfluenza virus 3 in one case each. One patient presented A/H1N1p, rhinovirus and enterovirus concomitantly, and another patient presented A/H1N1p, rhinovirus and *S. pneumoniae*; the latter patient died.

During the most recent A/H1N1p pandemic, some studies reported an absence or low incidence of co-epidemic respiratory viruses, a fact that the investigators attributed to the interference between influenza and other respiratory viruses [8]. We have shown that 12% of adult patients with A/H1N1p were co-infected with other respiratory viruses, in accordance with other investigators [1, 2].

Mixed respiratory viral infections in adults have scarcely been studied, and therefore their clinical relevance remains unknown. Available data from children suggest that respiratory viral co-infections show more severe clinical features than single infections [9]. In adults, there are data suggesting that co-infection with bacteria (most commonly *S. pneumoniae*) increases morbidity and mortality of patients with influenza [10]. Our data shows that A/H1N1p-infected adults with respiratory viral co-infections had longer hospital stays despite similar epidemiological characteristics, clinical features at presentation and A/H1N1p viral load. This fact suggests that co-infection with additional respiratory viruses in patients with A/H1N1p infection could result in a more protracted, and perhaps more severe, clinical course.

However, our study had some limitations. The number of patients included was small and prognostic variables after hospital admission could not be adequately studied. Although a longer hospital stay may reflect a worse clinical situation, this conclusion cannot be definitively established. Diagnosis of respiratory viral co-infection in our study was PCR-based, but this test might detect low amounts of viral genome from past infections, therefore, not proving the presence of active infection.

In summary, we found that 12% of adults with confirmed A/H1N1p infection showed evidence of co-infection with other respiratory viruses and that these patients had a longer hospitalisation, possibly reflecting a more severe clinical

course. Prospective studies incorporating a more comprehensive prognostic assessment and cell cultures in addition to quantitative PCR to diagnose active co-infections will help to clarify the clinical relevance of multiple respiratory viral infections in adults.

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REFERENCES

- 1 Nisii C, Meschi S, Selleri M, *et al.* Frequency of detection of upper respiratory tract viruses in patients tested for pandemic H1N1/09 viral infection. *J Clin Microbiol* 2010; 48: 3383–3385.
- 2 Renois F, Talmud D, Huguenin A, *et al.* Rapid detection of respiratory tract viral infections and coinfections in patients with influenza-like illnesses by use of RT-PCR DNA microarray systems. *J Clin Microbiol* 2010; 48: 3836–3842.
- 3 Antón A, Marcos MA, Martínez MJ, *et al.* Double (V27A/S31N) mutant 2009 pandemic influenza A (H1N1) virus isolated from adamantane non-treated immunocompetent child. *Diagn Microbiol Infect Dis* 2010; 67: 114–115.
- 4 Suwannakarn K, Payungporn S, Chieochansin T, *et al.* Typing (A/B) and subtyping (H1/H3/H5) of influenza A viruses by multiplex real-time RT-PCR assays. *J Virol Methods* 2008; 152: 25–31.
- 5 Angeles Marcos M, Camps M, Pumarola T, *et al.* The role of viruses in the aetiology of community acquired pneumonia in adults. *Antivir Ther* 2006; 11: 351–359.
- 6 Corias MT, Aguilar JC, García ML, *et al.* Simultaneous detection of fourteen respiratory viruses in clinical specimens by two multiplex reverse transcription nested-PCR assays. *J Med Virol* 2004; 72: 484–495.
- 7 Bermejo-Martin JF, Martin-Loeches I, Rello J, *et al.* Host adaptive immunity deficiency in severe pandemic influenza. *Crit Care* 2010; 14: R167.

- 8 Casalegno JS, Ottmann M, Duchamp MB, *et al.* Rhinoviruses delayed the circulation of the pandemic influenza A (H1N1) 2009 virus in France. *Clin Microbiol Infect* 2010; 16: 326–329.
- 9 Fabbiani M, Terrosi C, Martorelli B, *et al.* Epidemiological and clinical study of viral respiratory tract infections in children from Italy. *J Med Virol* 2009; 81: 750–756.
- 10 Morens DM, Taubenberger JK, Fauci AS. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. *J Infect Dis* 2008; 198: 962–970.

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