Circulation of other respiratory viruses and viral co-infection during the 2009 pandemic influenza

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A B S T R A C T

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Coinciding with the pandemic wave of the influenza A(H1N1)pdm09 virus, other respiratory viruses have co-circulated in our area and were responsible for many acute respiratory infections and influenza-like illness (ILI). Apart from the pandemic virus that was responsible for most ILI cases, incidence rates of other viruses have varied among geographical areas. In general, human rhinovirus was the most frequent among individuals from the community, and respiratory syncytial virus among hospitalized patients. Detection rates of other respiratory viruses such as human metapneumovirus, adenovirus or parainfluenza viruses have been much lower. On the basis of an interference mechanism, human rhinovirus may contribute to modulate the pandemic wave, although available data are not conclusive to support this hypothesis. In contrast, the epidemic wave of respiratory syncytial virus during 2009-2010 was similar to previous seasons. Overall, incidence rates of respiratory viruses other than influenza did not change significantly during the pandemic season compared to other seasons. No association has been found between co-infection of pandemic influenza and other respiratory viruses with the prognosis of patients with influenza. The involvement of clinical virology laboratories in the etiological diagnosis of ILI cases has improved and has optimized diagnostic procedures.

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Circulación de otros virus respiratorios y coinfección viral durante la pandemia de gripe en 2009

R E S U M E N

Palabras clave:
Coinfección
Síndrome gripal
Gripe pandémica

Coincidiendo con la onda pandémica 2009 por el virus de la gripe A(H1N1)pdm09, otros virus respiratorios han circulado en nuestro medio, provocando numerosos casos de infección respiratoria aguda y de síndrome gripal (ILI, *influenza-like illness*). Aparte del virus pandémico, que fue responsable de la mayoría de los casos de ILI, la incidencia de otros virus ha sido diferente según la zona. En general, rinovirus fue el virus más frecuente en la comunidad y virus respiratorio sincitial en pacientes hospitalizados. Las tasas de detección de otros virus como metaneumovirus humano, adenovirus o virus parainfluenza han sido mucho menores. Sobre la base de un mecanismo de interferencia, la presencia de rinovirus pudo contribuir a modular la onda pandémica de gripe, aunque los datos existentes no apoyan esta hipótesis de modo concluyente, mientras que la onda de virus respiratorio sincitial en 2009-2010 se ha presentado de forma similar a otros años. En conjunto, la incidencia de los distintos virus respiratorios de gripe no varió significativamente durante la temporada de la pandemia con respecto a otros años. Por otro lado, no se ha asociado la coinfección por virus de la gripe con otros virus respiratorios con el pronóstico de los pacientes con gripe. La implicación de los laboratorios de virología clínica en el diagnóstico de ILI ha supuesto una mejora y una mayor optimización en los procedimientos diagnósticos.

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Introduction

The pandemic invasion of a new H1N1 influenza virus (flu) in 2009—A(H1N1)pdm09—provided a unique scenario to test and define diagnostic tools in laboratories of clinical virology to respond to the overwhelming demand for etiological diagnosis of viral acute respiratory infection (ARI) from respiratory samples. Moreover, the role of respiratory viruses (RV) other than flu in influenza-like illness (ILI) and the effect of viral co-detections and/or co-infections in the clinical course of patients with influenza have rarely been evaluated, and this pandemic offered an opportunity to perform these studies.

The availability of sensitive detection methods for influenza A(H1N1)pdm09 and other RV has allowed the evaluation of the influence of RV on the epidemiological course of the pandemic.

It has been suggested that a viral interference of human rhinovirus (HRV) could have delayed the epidemic evolution of influenza A(H1N1)pdm09 in early autumn of 2009 in some European countries. Similarly, influenza A(H1N1)pdm09 would have interfered with the epidemic evolution of respiratory syncytial virus (RSV) in the same period.

Cost-effective diagnostic algorithms for detecting RV must be used. The need for more or less complete virological studies depends not only on the resources and type of laboratory, but also on clinic-epidemiologic aspects such as patient age, severity of the disease, underlying conditions, etc.

This review analyzes the most relevant aspects of the co-circulation of RV other than influenza A(H1N1)pdm09 during the 2009 pandemic.

Clinical implications of the circulation of respiratory viruses other than influenza during the 2009 pandemic

From April 2009 to October 2010, influenza A(H1N1)pdm09 was the virus most frequently detected in the population. Coinciding with influenza A(H1N1)pdm09 circulation, other RV were also detected.

In Spain, as occurred in other countries, the capacity of most laboratories was strengthened for pandemic flu detection, but few centers were able to investigate other RV. Information on the detection rates of RV other than A(H1N1)pdm09 during the pandemic in our country was provided by reference regional laboratories, with sufficient infrastructure to investigate the most relevant RV.

Data from 4 Spanish regional laboratories (Hospital Donostia, Basque Country; Hospital Son Espases, Balearic Islands; Hospital Universitario Central, Asturias; and Hospital Universitario Virgen de las Nieves, Andalusia) reveal that, of a total of 18,893 respiratory samples, a virus was detected in 6,545 cases (34.6%). Flu was the most prevalent and was detected in 3,933 cases (60.1% of positives), 98% of which were subtyped as influenza A(H1N1)pdm09.

Apart from influenza A(H1N1)pdm09, another RV was detected in 39.9% of cases. Co-infection of two or more RV was observed in approximately 15% of patients. The highest detection rates were obtained for HRV and RSV, representing 43.7% and 31.4% of positive non-flu samples in this series, respectively (Fig. 1). However, some features characterized HRV and RSV positive cases: seasonality, type of patient (mild or severe cases) and patient age.

The temporal distribution of HRV vs. RSV throughout the pandemic season differed: HRV was the most frequently detected virus from May to October, 2009—85% of RV other than influenza A(H1N1) pdm09—whereas RSV detection was highest from December 2009 to March 2010: 65% of RV other than influenza A(H1N1)pdm09.

Among RV other than influenza A(H1N1)pdm09, RSV was involved in most hospital admissions (68%) during the pandemic, followed by HRV, and less frequently by human metapneumovirus (hMPV) and adenovirus (ADV) (Fig. 2). This feature has also been characteristic during prior flu epidemic seasons. The RSV detection rate was inversely proportional to patient age. It has been previously documented that RSV and hMPV affected younger children, causing lower tract respiratory infections that sometimes require oxygen therapy more frequently than flu, which usually affected the upper respiratory tract and older children[1].

However, the real incidence rates of other RV do not seem to have changed significantly with respect to previous years due to the presence of the pandemic virus[2].

RSV circulated with relative frequency during the pandemic[3,4]. In the study carried out by Lovato et al., RSV was the main pathogen responsible for hospitalizations in young children[5]. Parainfluenza viruses (PIVs) were also detected in a high percentage of hospitalized patients (Nissii et al, 2010) as well as in outpatients[6,7].
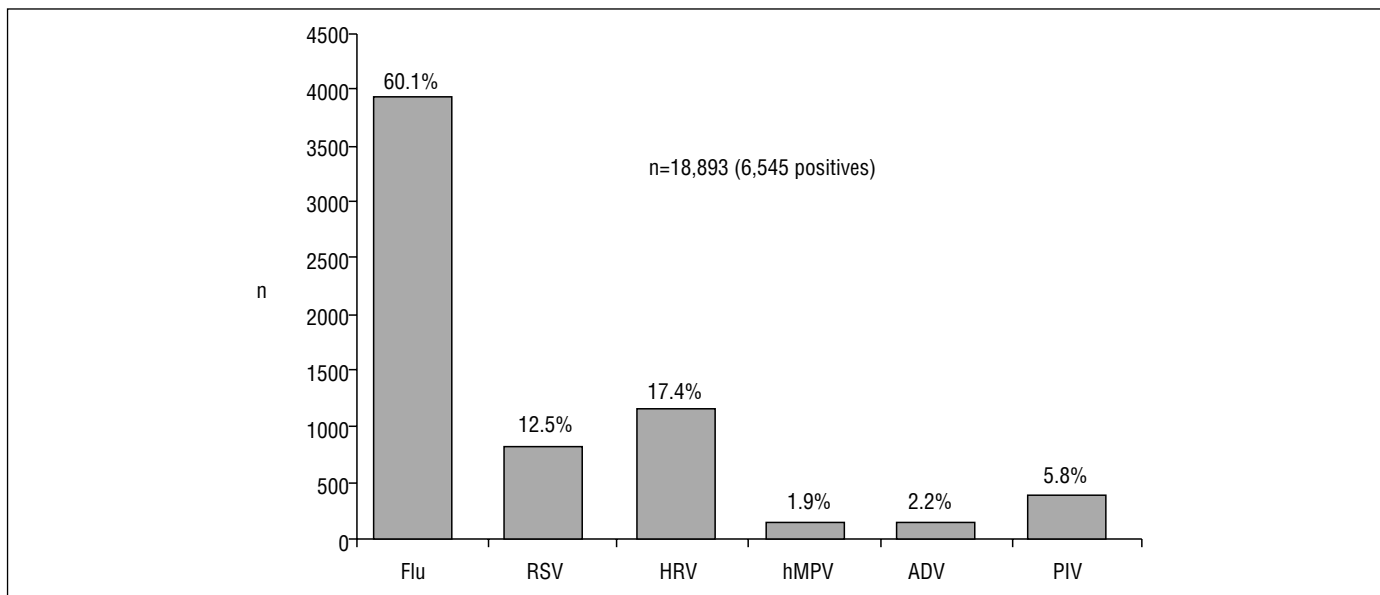
Figure 1. Viruses detected in respiratory samples from May 2009 to March 2010. Source: regional laboratories of clinical virology from Hospital Donostia (Basque Country), Hospital Son Espases (Balearic Islands), Hospital Central Universitario (Asturias) and Hospital Virgen de las Nieves (Andalusia).
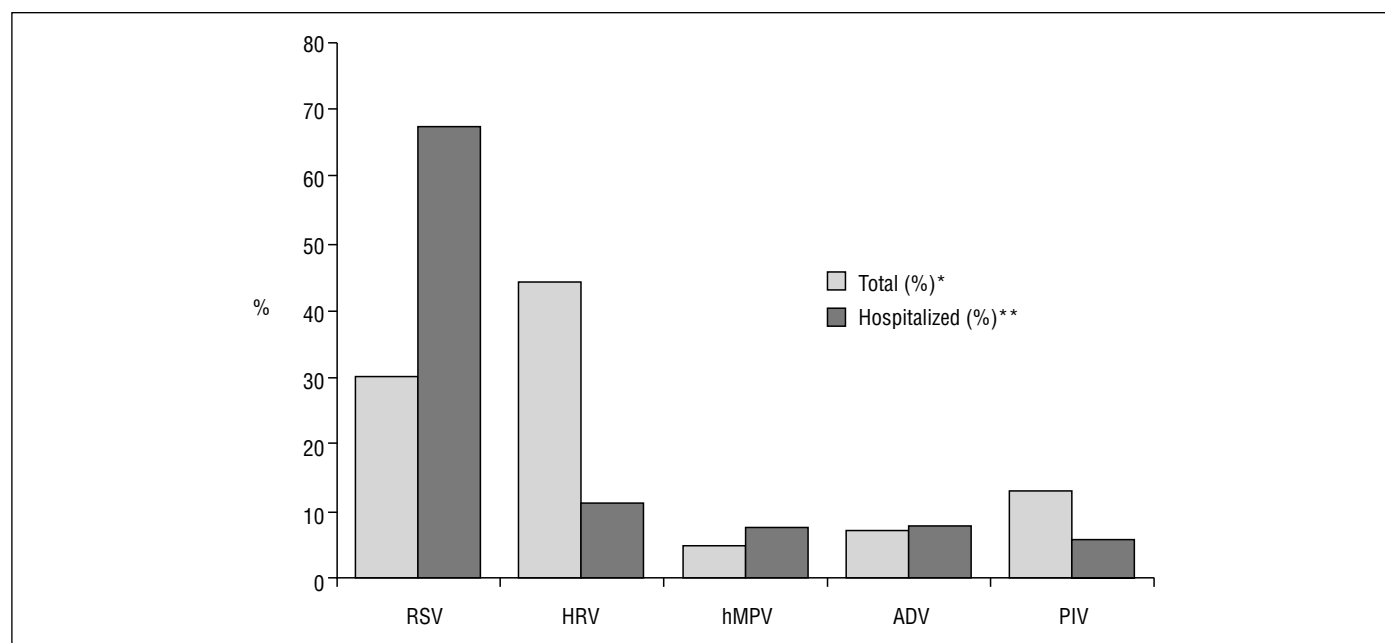
Figure 2. Viruses other than A(H1N1)pdm09 detected in respiratory samples from May 2009 to March 2010. Source: *regional laboratories of virology from Hospital Donostia (Basque Country), Hospital Son Espases (Balearic Islands) and Hospital Virgen de las Nieves (Andalusia); **regional laboratory of virology from Hospital Central Universitario (Asturias) and Hospital Virgen de las Nieves (Andalusia).

Although the co-circulation of flu viruses other than A(H1N1)pdm09 during the same period was a rare event, Raboni et al.[4] found 11% of seasonal flu A and Lee et al.[8] found 7% of flu A(H3N2) and 32.8% of flu B.

Although viral co-infection was not common in influenza A(H1N1)pdm09-infected patients, some studies showed a prevalence of about 12% and 17%[6,9], mainly in pediatric patients and in those with underlying diseases.

It has been reported that viral co-infections may be involved in a torpid clinical course[10]. However, few data on the influence of co-detection of A(H1N1)pdm09 and other RV in the severity of the disease have been published. The relationship between bacterial co-infection and the severity of the disease has been documented elsewhere. A recent study carried out on 100 biopsies from fatal A(H1N1)pdm09 cases demonstrated bacterial co-infection in 26%, mainly by *Streptococcus pneumoniae*, and no other RV was detected in these specimens[11]. In Spain, a case of myocarditis by PIV 3 was reported in an immunocompetent child, occurring within 2 weeks following influenza A(H1N1)pdm09 infection that was apparently resolved[12].

Another aspect of the controversy is the adequacy of correlating clinical criteria of ILI with a confirmed flu case. The predominant pathogen of ILI, according to the CDC[13], is flu; nevertheless, ILI can be attributed to a wide range of RV and therefore it is clinically impossible to distinguish between one virus and another. A multivariate analysis carried out in France revealed that ILI symptoms such as cough, dyspnea, hyperemia and chills were significantly associated with flu etiology[7]. In this study, only 28% of ILI were laboratory-confirmed flu, and other RV were detected, i.e., HRV, enterovirus (EV), hMPV, ADV, PIVs and human coronavirus (hCoV) OC43. Whereas the association of ILI with flu is usually found in older children and adults, it is more unlikely to occur in children under 5 years old[14].

Infections by other RV have been observed concomitantly with the spread of influenza A(H1N1)pdm09. Two studies report similar percentages of influenza A(H1N1)pdm09 and other RV; 39% vs. 36%, and 26.9% vs. 21.3%, respectively, in each study[4,5]. Curiously, in both studies, co-infection occurred more frequently among children and elderly hospitalized patients.

From documented data, the finding of high rates of HRV during the pandemic period is noteworthy. Although in most cases HRV followed a typically mild course of ILI[7], its implication in more severe disease has been recognized, principally in immunocompromised adults. Kraft et al.[15] reported that hospital admission rates, intensive care unit admissions, and mortality were not statistically different between the HRV and influenza A(H1N1)pdm09 groups. A similar result has been published by Chan et al.,[3] showing a similar ICU admission rate for patients infected with influenza A(H1N1)pdm09 and HRV; however, mortality was higher in influenza A(H1N1)pdm09–infected patients.

Epidemiology of viral co-circulation: interference of respiratory viruses other than influenza in the epidemic wave of 2009 pandemic flu

Viral interference is a phenomenon defined as the protection of host cells against a specific virus when it is infected by another virus. Research in this field led to the discovery of interferon in 1957[16]. Some diagnostic procedures using cell cultures are based on this phenomenon. For example, the presence of rubella virus is detected in Vero cells by the absence of enterovirus-specific cytopathic effects due to the interference of the rubella virus, which protect cells from enteroviral infection.

Some authors have postulated that a possible interference of other RV, HRV and RSV, with influenza A(H1N1)pdm09 during the pandemic period, could have caused a change in the epidemic wave of pandemic flu[17-22]. This conclusion may be based on previous observations that RSV and flu epidemic peaks do not usually coincide within the same period, and on the fact that co-infections of HRV and other RV are less common than expected[20,23].

In Europe, the peak incidence of A(H1N1)pdm09 took place in autumn 2009, although the epidemiological week when this maximum was reached varied among countries[24].

Two studies carried out in France and Sweden suggested that HRV might have interfered with the epidemic wave of influenza A(H1N1)pdm09 HRV during the early weeks of autumn 2009. Temporal distribution of HRV- and A(H1N1)pdm09-positive cases in these
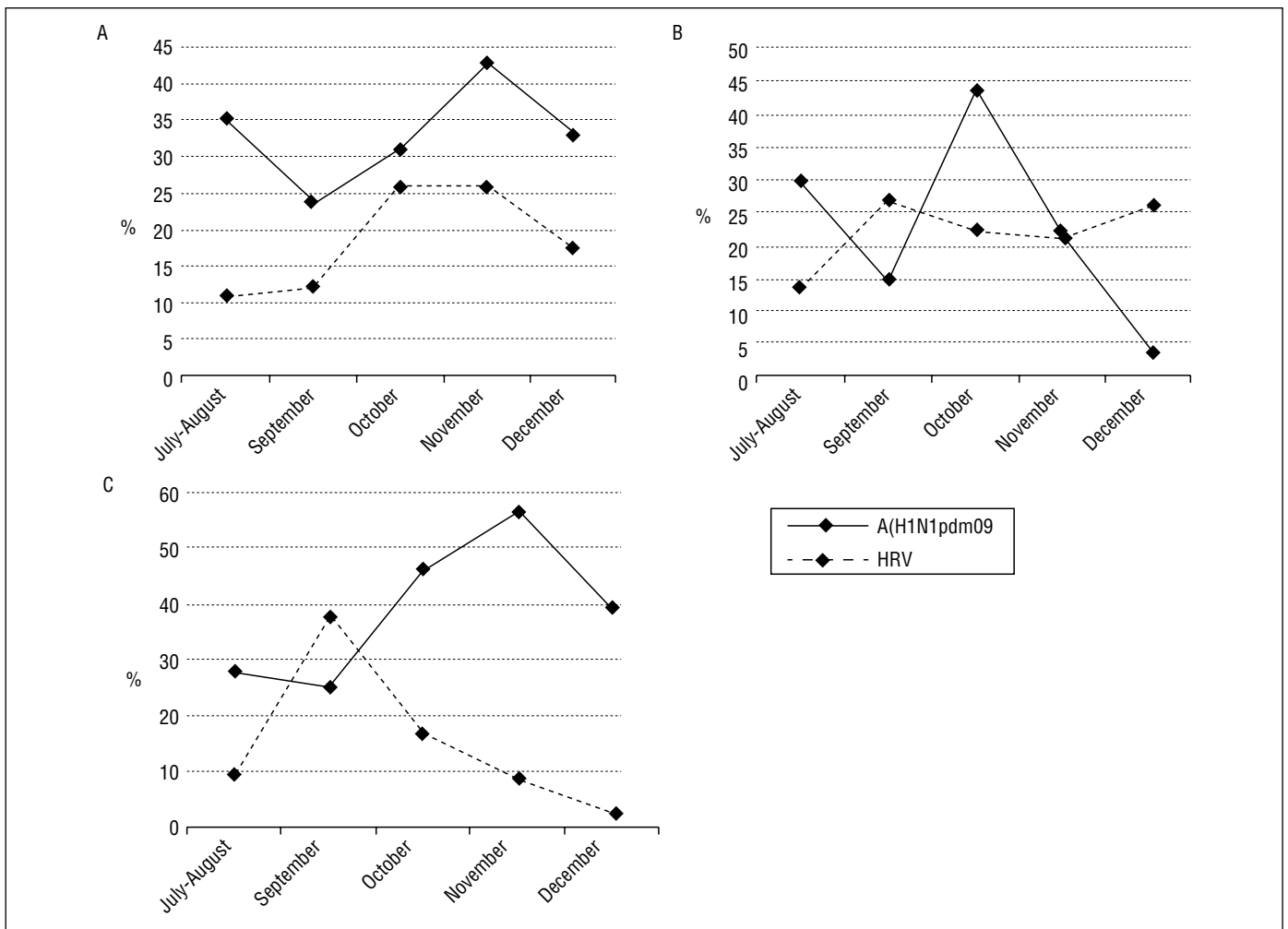
Figure 3. Temporal distribution of human rhinovirus and A(H1N1)pdm09 in summer-autumn 2009, Spain. Data obtained from regional laboratories for influenza surveillance from Andalusia (A), Basque Country (B) and Asturias (C).

studies suggested that changes in behavior, mainly those related to the beginning of the school period in mid-September when A(H1N1)pdm09 cases were expected to rise, favored an outbreak of HRV infection that delayed the epidemic by influenza A(H1N1)pdm09. Epidemiological results were explained on the basis of an interference phenomenon between both viruses. Thus, in these countries, most ILI cases within these weeks would have been due to HRV[18,19].

In Spain, data obtained from three laboratories participating in this review, reveal differences in HRV and influenza A(H1N1)pdm09 temporal distributions. In Andalusia and the Basque Country, both HRV and pandemic flu seem to follow an independent course during September-December 2009. As expected, HRV cases increased when the school period began but coincided with an increase of A(H1N1)pdm09 cases as well, which reached their peak in late October in the Basque Country and in November in Andalusia. In contrast, in Asturias, the situation was similar to that reported in the French and Swedish studies (Fig. 3).

Along these lines, the European Centre for Disease Control and Prevention (ECDC) published an explanatory communication in April 2010[25], reporting that epidemiological studies were suggestive but not conclusive of a possible interference of HRV against A(H1N1)pdm09 in early autumn as observed in France and Sweden. ECDC based their conclusions on discrepancies in the French study and other previous studies and on discrepancies between the epidemiological data and virological aspects of respiratory infections.

First, the ECDC reported that the French study was biased: the population groups were not comparable since the mean ages of HRV and A(H1N1)pdm09 patients were significantly different (2.4 and 5.6 years old, respectively). Only the pediatric population was studied, so this effect was not evaluated in adults in whom influenza A(H1N1)pdm09 caused high infection rates and in whom HRV is the main causal agent of the common cold. Moreover, other studies dispute the interference between HRV and flu due to the discrepant results[23]. Whereas HRV infection was associated with a lesser probability of flu A infection, in 24% of HRV cases another RV was co-detected.

Secondly, the epidemiological data are not supported by a virological basis for various reasons: 1. the duration of an antiviral status against a new RV infection when cells of the respiratory tract are infected by another virus lasts a few hours; 2. Flu A viruses may exert an antagonist effect against the IFN-mediated immune response by its NS1 protein; 3. The cellular receptors for HRV and flu A in the respiratory tract differ[26-28].

Finally, based on the data from the ECDC weekly influenza surveillance report in May 28, 2010[24], overall levels of positive specimens in Europe reached their maximum during weeks 46-47 (see Figs. 1 and 2 of the ECDC report), which was similar to the positivity peak for influenza A(H1N1)pdm09 in France and Sweden that took place in weeks 48 and 47, respectively, as observed in the graphs of the epidemiological overview for both countries within this report.

In light of the data obtained from Spain and other European countries and from ECDC comments and reports, it is difficult to conclude with certainty that HRV interfered with the influenza A(H1N1)pdm09 epidemic wave in early autumn. More studies in other countries with similar weather and during the same months of other year-periods of flu surveillance would provide more results to reach clearer conclusions.

The same French authors have postulated that a similar mechanism of interference between RSV and influenza A(H1N1)pdm09 occurred during the pandemic season[17]. The authors observed that RSV peak incidence was delayed with respect to previous year periods, and argue that this might have been due to a viral interference by pandemic flu that produced an epidemic peak earlier than in previous periods. However, the pandemic situation created by influenza A(H1N1)pdm09 hampers a comparison of the epidemic wave caused by the new virus with those of previous seasonal flu A viruses.

Data from Spain reveal a different situation. As annual surveillance reports show, incidence peaks of RSV and flu coincided during the 2008-2009 season[29], whereas the influenza A(H1N1)pdm09 peak came earlier than RSV during 2009-2010[30] (see Fig. 6 of both reports). However, if we focus on RSV, incidence peaks during the pandemic and the previous period do not vary[31]. These data suggest that the incidence evolution of both RSV and A(H1N1)pdm09 in Spain are independent.

Finally, it is surprising that influenza A(H1N1)pdm09 has almost disappeared from the Northern Hemisphere during 2011-2012 in contrast to the predominance of this subtype in 2010-2011. In 2011-2012, most cases have been due to flu A H3N2 and to a lesser extent, to flu B. This feature is different from what occurred in previous pandemics. flu A subtypes responsible for other pandemics have replaced seasonal flu A subtypes circulating during previous years. Whether the intrinsic genetic and antigenic characteristics of A(H1N1)pdm09 (no subtype change took place compared to previous pandemics) or the rapid control measures adopted by health systems worldwide may have influenced this particular evolution of the last pandemic is still to be determined. Nevertheless, the epidemiological behavior of flu in previous pandemics suffered great variations depending on the geographic area[32].

Cost-effective diagnosis of acute respiratory infections of viral etiology

During periods of influenza outbreak in the community, it has been reported that clinicians have a low threshold for suspecting, diagnosing, and treating the infection according to the recommended guidelines[33]. Accordingly, many patients with ARI who received medical care during the influenza A(H1N1)pdm09 pandemic were treated with oseltamivir; however, many of these infections might have been due to other RV.

Etiological diagnosis of viral ARI is crucial in order to avoid unnecessary antibiotic use, to establish the appropriate use of antiviral drugs and to maintain a comprehensive cohort of hospitalized patients that minimizes the risk of nosocomial transmission. In addition, diagnoses provide epidemiological information for an early release of recommendations for prevention and treatment, and reduce the overall costs derived from patient management.

Samples received for the diagnosis of ARI of viral etiology have significantly increased during and after the 2009 pandemic, in part due to a greater awareness among health institutions and clinicians of the importance of diagnosing these processes. The early published news about influenza A(H1N1)pdm09 showed an elevated mortality rate, especially in some vulnerable groups such as pregnant women[34,35].

This sample overflow has led to a need to optimize the use of available diagnostic tools. Counting on the same human resources in most cases, each laboratory has been encouraged to reduce costs and the time needed to report a result, without a decrease in the quality of the diagnostic procedure.

In previous years, some authors analyzed the usefulness of distinguishing flu and other RV[36,37]. Whereas "classical" direct detection methods, e.g. antigen detection and viral culture, are still useful for the detection of "classical" RV in clinical samples, recently reported RV, such as hMPV, new hCoV and human bocavirus, are mainly detected by nucleic acid amplification techniques (NAATs). However, we must never forget that viral culture is useful for epidemiological studies and for the genetic and antigenic characterization of new viruses.

Although available rapid diagnostic tests based on chromatographic immunoassays that detect viral antigens in nasopharyngeal samples demonstrated an excellent cost-effectiveness in the past[38,39], they were not able to detect the new virus with acceptable sensitivity[40,41]. Accordingly, only NAATs could reach these objectives (speed, sensitivity and a favorable cost/benefit ratio), such as real-time PCR[42]. The use of NAATs was a success for the appropriate management of patients with respiratory infectious disease by influenza virus, because a significant decrease in the response time was associated with reductions in mortality, hospital stay, prolonged use of antivirals and inappropriate use of antibiotics[43], and with a reduction in additional costs. Indeed, NAATs could be performed to discriminate infections due to RV other than flu, since clinical signs and symptoms of viral ARI are usually indistinguishable.

Molecular multiplex NAATs have recently emerged as a potent diagnostic tool for detection of virus involved in acute respiratory infections by providing insights into their epidemic profile[44]. Some platforms using multiplex NAATs report a better workflow, which reduces laboratory costs and improves efficiency[45]. These platforms are attractive but we are not sure about the need for wide and indiscriminate use. The main inconvenience of NAATs is the higher cost compared to the former methods, which continuously increases with the number of pathogens included in the assay.

The optimal use of the diagnostic tools implies that, together with the technical aspects mentioned above, other clinic and epidemiologic criteria must be considered (patient age, underlying diseases, type of sample, prevalence of specific RV in a certain area and study period).

Clinical laboratories must take into account all these points and the availability of infrastructure and resources, to organize their workflow and portfolio related to the diagnosis of viral ARI. Moreover, each laboratory has been encouraged to reduce costs and time needed for reporting results, without a decrease in the quality of the diagnostic procedure.

Figure 4 represents a possible stepwise algorithm of respiratory sample processing for viral detection that may give an efficient response to the current demand. *First step:* viral diagnosis in outpatients with mild ARI is not probably cost-effective, and should not be routinely performed, except for studies from Surveillance Programs or of epidemiological interest. *Second step:* antigen detection methods for RSV and/or flu may be enough for patients attended at emergency units that do not fulfill severity clinical criteria for hospitalization, except for pregnant women, patients with severe underlying conditions and other special situations. *Third step:* samples from patients requiring hospitalization with negative RSV or flu antigen results should be subjected to extended virological study including viral culture assays and/or NAAT targeted at more or less RV depending on the severity of the disease and the need for a rapid result. With this premise, NAAT for detection of RSV and flu (at the type and subtype level) in patients with less severe ARI who do not require admission at intensive care units (ICU) should be carried out first. *Fourth step:* on certain patients, multiplexed methods of low complexity could be performed including hMPV, HRV and PIV detection, depending on the patient's age and/or underlying diseases.
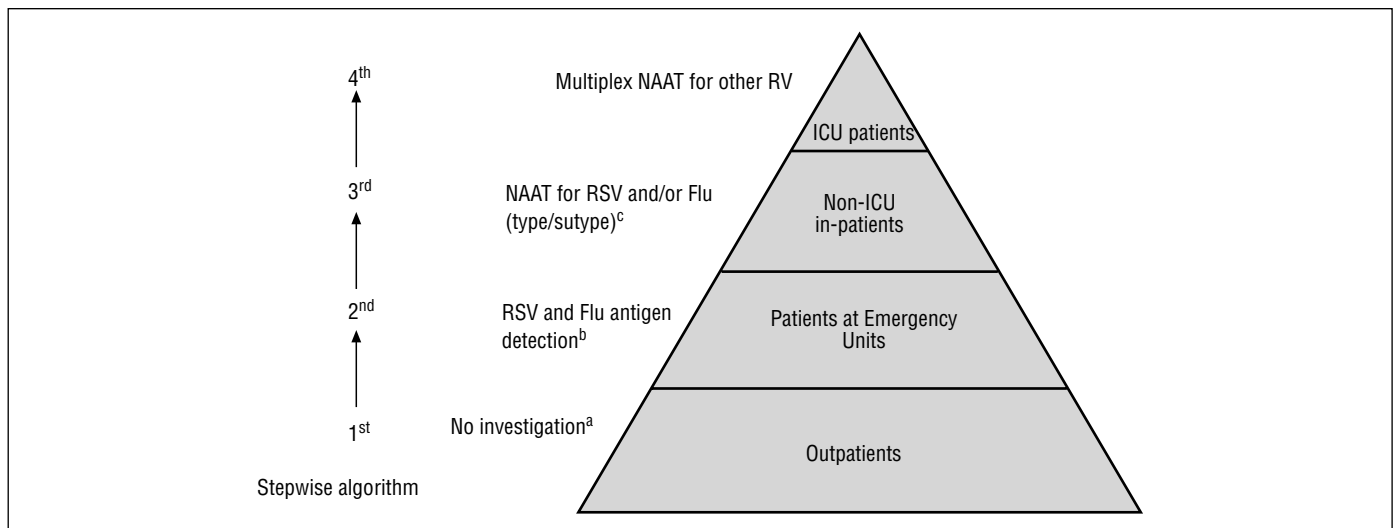
Figure 4. Proposal of a cost-effective and rational stepwise algorithm for respiratory virus (RV) investigation. [a]Except for patients included in virological surveillance within the national influenza surveillance network. [b]Except special situations such as pregnancy, predisposing underlying diseases, etc. [c]NAAT for other RV (HRV, hMPV, PIV) can be added in patients with high risk of severe respiratory illness.

More complex NAAT that detect other less frequent RV should be reserved for certain situations such as more severe cases (ICU patients) in which previous NAAT for the most prevalent viruses have yielded negative results[44-46].

Conclusions

The pandemic flu caused by influenza virus A(H1N1)pdm09 led to a significant increase in the number of respiratory specimens processed in laboratories at that time. However, data obtained in 2009 on the participation of other RV in these conditions do not suggest a significant change in their real incidence.

The 2009 pandemic altered significantly the detection rates of RV in recent years, establishing flu viruses as the major pathogens involved in ARI worldwide, whereas flu detections had always been below RSV in previous seasons. Seasonal flu generally shows wide variations in incidence depending on the epidemic season and the antigenic characteristics of the circulating strains. In contrast, yearly incidence rates for RSV are fairly stable.

The state of health alert caused by the pandemic led to an increased surveillance of ARI. This increase meant that large numbers of samples were processed for RV investigation during the pandemic period; however, the global percentages of positivity against all RV did not increase significantly. In 2009, the main virus involved in ARI was influenza A(H1N1)pdm09, with detection rates varying among different geographical areas.

Epidemiological studies suggested that a mechanism of viral interference could have altered the epidemic waves of the pandemic flu and RSV during the 2009-2010 season in certain countries. However, neither a virological basis nor the data obtained from Spain comparing different year-periods support this hypothesis.

Thus, although viral interference is plausible, results obtained from epidemiological studies in various periods are not enough to support this phenomenon.

Due to the higher costs and resources these tools suppose, rational stepwise diagnostic algorithms should be used, considering other clinical and epidemiological data.

In conclusion, in the year of the pandemic flu and despite the absolute predominance of A(H1N1)pdm09, other RV presented with an epidemiological pattern similar to previous years. Continuous surveillance is necessary to detect other RV in order to increase understanding of their epidemiological dynamics. A rapid diagnostic screening of a large panel of respiratory pathogens may provide critical information for patient management.

Conflicts of interest

All authors declare that they have no conflicts of interest in this article.

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