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Cross-protection to new drifted influenza A(H3) viruses and prevalence of protective antibodies to seasonal influenza, during 2014 in Portugal

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ABSTRACT

Introduction: Immune profile for influenza viruses is highly changeable over time. Serological studies can assess the prevalence of influenza, estimate the risk of infection, highlight asymptomatic infection rate and can also provide data on vaccine coverage. The aims of the study were to evaluate pre-existing cross-protection against influenza A(H3) drift viruses and to assess influenza immunity in the Portuguese population.

Materials and methods: We developed a cross-sectional study based on a convenience sample of 626 sera collected during June 2014, covering all age groups, both gender and all administrative health regions of Portugal. Sera antibody titers for seasonal and new A(H3) drift influenza virus were evaluated by hemagglutination inhibition assay (HI). Seroprevalence to each seasonal influenza vaccine strain virus and to the new A(H3) drift circulating strain was estimated by age group, gender and region and compared with seasonal influenza-like illness (ILI) incidence rates before and after the study period.

Results: Our findings suggest that seroprevalences of influenza A(H3) (39.9%; 95% CI: 36.2–43.8) and A (H1)pdm09 (29.7%; 95% CI: 26.3–33.4) antibodies were higher than for influenza B, in line with high

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ILI incidence rates for A(H3) followed by A(H1)pdm09, during 2013/2014 season. Low pre-existing cross-protection against new A(H3) drift viruses were observed in A(H3) seropositive individuals (46%). Both against influenza A(H1)pdm09 and A(H3) seroprotection was highest in younger than 14-years old. Protective antibodies against influenza B were highest in those older than 65 years old, especially for B/Yamagata lineage, 33.3% (95% CI: 25.7–41.9). Women showed a high seroprevalence to influenza, although without statistical significance, when compared to men. A significant decreasing trend in seroprotection from north to south regions of Portugal mainland was observed.

Conclusions: Our results emphasize that low seroprotection increases the risk of influenza infection in the following winter season. Seroepidemiological studies can inform policy makers on the need for vaccination and additional preventive measures.

1. Introduction

The pattern of influenza virus circulation is unpredictable and varying between each flu season, thus changing the baseline age specific immunity in the population after each influenza epidemic period. Serological surveillance provides estimates of population immunity level against vaccine preventable diseases [1]. Seroepidemiology data monitor the gradual accumulation of susceptible people, changes in age-specific risk of infection and potential risk of outbreaks [1]. Data from seroepidemiological studies can guide intervention actions concerning vaccination programmes and other preventive measures, especially in high-risk groups. Influenza seroprevalence studies are important contributors to estimate the true incidence and determine the vulnerable populations to disease. Data on population immunity is even more important in pandemic scenarios, being essential to assess pre-existing susceptibility, true infection attack rates, exposure to circulating viruses, estimate asymptomatic infection rates and inform on vaccine coverage [2,3]. Moreover, seroprevalence surveys are the most practical method for accurately estimating the infection attack rate (IAR) in an epidemic such as influenza [4]. However, seroepidemiology data adds important and valuable information to influenza surveillance and could support decision-making in target groups for vaccination, as only a few National Influenza Surveillance Programmes at European level integrate routinely the serological studies in National Influenza Surveillance Systems [5–8]. In Portugal, this is the first seroepidemiological study in the scope of influenza surveillance that aims at assessing the cross-protection to the new drift influenza A(H3) viruses determining the prevalence of seasonal influenza protective antibodies by age, gender and health region as well as considering the relationship with influenza- like illness (ILI) incidence rates observed on seasons before and after the sample collection.

2. Materials and methods

To study influenza immunity in the Portuguese population, a non-probabilistic sample was used. Samples were collected from people attending to hospital laboratories for other reasons aside from influenza infection. We developed a cross-sectional study based on a convenience sample of 626 sera collected during June 2014. Sera were selected from all age groups (0–4; 5–14; 15–64 and ≥65 years old) and both genders, in equal proportion, at 11 hospital laboratories from the Portuguese Laboratory Network for the Diagnosis of Influenza Infection [9], covering all administrative health regions (HR) of Portugal: Norte, Centro, Lisboa e Vale do Tejo, Alentejo, Algarve and including also the Açores (São Miguel and Terceira islands) and Madeira islands. For each HR, an equal representation of all age groups was guaranteed, with the exception of Algarve that didn't select samples from individuals under 5. A remaining volume (minimum selected volume 250 μl) of recently collected sera that comes to laboratory for serological analysis, excluding influenza, were selected for the present study. Sera were randomly selected taking only into account the patient age. Data regarding vaccination or influenza previous infection weren't recorded, because this information weren't available at patient hospital admission registries. All samples were anonymized and data regarding district of residence or sample collection, gender and age were recorded.

The present investigation follows the international ethical guidelines, and was approved by the Health Ethics Committees of the National Institute of Health Dr. Ricardo Jorge (ref. 17/3/2014) and by the Hospital of Divino Espírito Santo of Ponta Delgada (ref. 582/2014).

In all sera, the antibody titers to the influenza virus strains recommended for the tri and quadrivalent vaccines (northern hemisphere, 2014/2015) were assessed: A/California/7/2009 (AH1pdm09), A/Texas/50/2012 (AH3), B/Massachusstes/02/2012 (B/Yamagata lineage), and B/Brisbane/60/2008 (B/Victoria lineage) [10]. Sera from all age groups, with protective antibody titers against A/Texas/50/2012 equal or higher than 80, were tested for the presence of cross reactive antibodies to the new A(H3) drift viruses, antigenically different from vaccine strain: A/Switzerland/9715293/2013 (3C.3a subclade) and A/Hong Kong/5738/2014 (3C.2a subclade) [11]. The selected 150 sera were distributed across all age groups and regions, conforming to the distribution of all A(H3) seropositive samples.

Serum antibody titers were evaluated in duplicate by the technique of hemagglutination inhibition (HI) according to the standard methodology [12] in laboratory biosafety level 2 conditions. Sera were pre-treated with receptor destroying enzyme [RDE (II) "SEIKEN"; Denka Seiken Co. Ltd.] and tested in two fold serial dilutions starting at 1:10 to a final dilution of 1:1280, using guinea pig red blood cells. HI endpoint titer was assessed as the reciprocal of the highest dilution of serum that completely inhibits hemagglutination. HI titer ≥40 were considered protective against tested virus strain [13] and in titers <10, 5 were assigned to enable geometric means titer (GMT) calculation. The WHO Collaborating Centre in London kindly provided the influenza reference virus strains and antiserums. Viruses were grown in Mardin Darbin canine kidney (MDCK) cells and in Mardin Darbin Canine Kidney Sialic Acid Over-Expression cells (MDCK-Siat1) [12]. Reference antiserum and homologous virus strains were tested in each assay.

Seroprevalence estimates are presented with respective 95% confidence intervals (95% CI). Differences between groups (gender, age and region) regarding the proportion of sera with antibody titer considered protective (titer ≥40) were tested using the chi-square test, while differences in titers between groups were evaluated using the Kruskal-Wallis test. The level of significance was set at 5%. All statistical analysis was performed in R version 3.0.3.

Seasonal ILI incidence rates were estimated for each age group for 2013/2014 and 2014/2015 influenza seasons using data

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