

Peer Review Report

Review Report on Prevalence of circulating antibodies against hemagglutinin of influenza viruses in epidemic season 2021/2022 in Poland

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EVALUATION

Q 1 Please summarize the main findings of the study.

The study aimed to assess the presence of anti-hemagglutinin antibodies in the serum of patients during the 2021/2022 epidemic season in Poland. A total of 700 serum samples, categorized into seven age groups, were tested using the haemagglutination inhibition assay (OZHA). The investigation focused on antigens recommended by the World Health Organization (WHO) for that season. The results confirmed the presence of anti-hemagglutinin antibodies for specific influenza virus strains, revealing variations in antibody levels across age groups. Given the low vaccination rate in Poland (6.90% in the studied season), the findings suggest the observed immune responses are likely linked to previous influenza virus infections rather than vaccination given the low percentage of vaccinated individuals in certain age groups.

The study found that children under 14 generally had higher average antibody titers, except for a specific influenza strain. Children aged 0–14 more frequently reached a protective antibody level against certain strains, while adults over 14 exhibited higher protection against a different strain. Notably, a significant portion of children under 14 lacked antibodies against a specific strain. The study suggests that the observed protection levels may result from past influenza infections rather than vaccination, highlighting the influence of previous infections on immune responses, especially in the context of a low vaccination rate in certain age groups.

Q 2 Please highlight the limitations and strengths.

Limitations of the study.

- 1) The study focuses on a specific population in Poland during the 2021/2022 epidemic season. The findings may not be fully representative of global or even national trends, as factors such as population demographics, healthcare practices, and influenza virus strains can vary.
- 2) The study highlights a low percentage of vaccinated individuals in Poland (6.90% in the 2021/2022 season). However, it does not delve deeply into the specific reasons for low vaccination rates or consider potential variations in vaccine effectiveness.
- 3) The study captures a snapshot of antibody levels during a specific epidemic season. Long-term trends, seasonal variations, or changes in influenza virus strains over time are not extensively explored.
- 4) While the study focuses on antibody titers, it does not assess other aspects of immune response, such as cellular immunity or memory responses. A comprehensive understanding of immunity may require a broader set of measures.
- 5) The study suggests that observed protection levels may result from past infections. However, relying solely on antibody levels may not provide a complete picture of previous exposure, as other aspects of immune memory could contribute to protection.
- 6) The study employs the haemagglutination inhibition assay (OZHA) to determine antibody levels. While widely used, the assay has its limitations, and other complementary methods could provide additional insights. Understanding these limitations is crucial for appropriately interpreting the study's findings and for informing future research endeavors in the field of influenza immunity.

Strengths of the study:

- 1) The study has a clear and specific focus on determining the levels of anti-hemagglutinin antibodies during the 2021/2022 epidemic season in Poland. The well-defined aim enhances the study's relevance and applicability.
- 2) The inclusion of a substantial number of serum samples (700) and the stratification of samples into different age groups (0–4, 5–9, 10–14, 15–25, 26–44, 45–64, and ≥ 65 years) adds depth to the analysis. This allows for a more nuanced understanding of antibody levels across various age demographics.
- 3) The study aligns with World Health Organization (WHO) recommendations by testing for antibodies against specific influenza virus strains recommended for the 2021/2022 epidemic season. This connection to international guidelines enhances the study's significance in a global context.
- 4) The study not only reports on the presence of antibodies but also compares the antibody levels across different age groups. This comparative analysis provides valuable insights into potential age-related differences in immune responses.
- 5) The study proposes that the observed protection levels may be a result of past infections, contributing to the understanding of the role of natural immunity. This insight has implications for public health strategies and vaccination campaigns.
- 6) The summary of the findings is presented in a clear and structured manner, facilitating understanding for both scientific and non-specialist audiences.

Overall, these strengths enhance the study's credibility and contribute valuable information to the broader field of influenza immunity, potentially informing public health strategies and vaccination efforts.

Q 3 Please comment on the methods, results and data interpretation. If there are any objective errors, or if the conclusions are not supported, you should detail your concerns.

The methodology describes the multiplication of the virus on chicken embryos, which is a common method for propagating influenza viruses. However, the specific details of this process, such as the virus strains used, incubation conditions, and quality control measures, are not provided. These details are crucial for ensuring the reliability of the virus used in subsequent analyses.

The use of the hemagglutination inhibition assay (HAI), particularly the haemagglutination inhibition assay (OZHA), is a standard method for assessing anti-hemagglutinin antibodies. However, the specifics of the testing conditions (e.g., concentrations, incubation times) and the inactivation process are not fully detailed. These details are essential for reproducibility and standardization.

The methodology refers to the use of adopted standards (Tyrell & Horsfall et al., 1952; WHO, 2011) for sample inactivation. While these references are commonly accepted, the specific procedures followed should be explicitly outlined to ensure transparency and reproducibility.

The definition of the protection rate refers to individuals with anti-hemagglutinin antibodies at a level $\geq 1:40$, which is considered protective. This is a standard criterion, but the methodology could benefit from explicitly stating the rationale for choosing this specific threshold.

Additionally, transparency in reporting enhances the study's credibility and facilitates the evaluation of potential errors or biases.

In summary, the discussion effectively communicates the study's main findings and implications. Addressing minor repetitions and enhancing the structure could further improve the clarity of the discussion. Additionally, providing more specific data points and encouraging a critical analysis of limitations could add depth to the interpretation of the results. Consider enhancing the overall structure and formatting of the discussion for better readability. Breaking down longer paragraphs into smaller, focused sections can improve the flow and comprehension. Streamlining the content of the discussion could enhance clarity.

The conclusions drawn from the study align with the study's objectives, the methods employed, and the presented findings. They provide a clear and concise summary of the key outcomes, contributing to the overall coherence and support for the study.

Check List

Q 4 Please provide your detailed review report to the editor and authors (including any comments on the Q4 Check List)

The study aims to evaluate the levels of anti-hemagglutinin antibodies in sera samples collected from patients across different age groups during the 2021/2022 influenza season in Poland. The study investigates the circulation of four influenza virus strains included in the vaccine, the age-related differences in antibody levels, and the implications of low vaccination rates on immunity. The study successfully achieves its objectives by assessing anti-hemagglutinin antibodies in sera samples across various age groups during the 2021/2022 influenza season. Clear presentation of results, alignment of conclusions with objectives, and relevance to public health make this study a valuable contribution to the understanding of influenza immunity dynamics. Addressing the suggested improvements would enhance the study's overall impact and robustness.

Q 5 Is the English language of sufficient quality?

Yes.

Q 6 Is the quality of the figures and tables satisfactory?

Yes.

Q 7 Does the reference list cover the relevant literature adequately and in an unbiased manner?

Yes.

Q 8 Are the statistical methods valid and correctly applied? (e.g. sample size, choice of test)

Yes.

Q 9 Are the methods sufficiently documented to allow replication studies?

Yes.

Q 10 Are the data underlying the study available in either the article, supplement, or deposited in a repository? (Sequence/expression data, protein/molecule characterizations, annotations, and taxonomy data are required to be deposited in public repositories prior to publication)

Not Applicable.

Q 11 Does the study adhere to ethical standards including ethics committee approval and consent procedure?

Not Applicable.

Q 12 Have standard biosecurity and institutional safety procedures been adhered to?

Not Applicable.

QUALITY ASSESSMENT

Q 13 Originality



Q 14 Rigor



Q 15 Significance to the field



Q 16 Interest to general audience



Q 17 Quality of the writing



Q 18 Overall quality of the study

