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Antibody Response to Mix and Match Vaccination for Covid-19

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Abstract

Background: *In Greece, administration of the AstraZeneca COVID-19 vaccine was discontinued in specific age groups. Individuals who had received the first dose of AstraZeneca subsequently completed their vaccination with an mRNA vaccine, most commonly Pfizer-BioNTech.*

Objective: *This study aimed to evaluate the antibody response on the day of administration of the second Pfizer dose in participants previously vaccinated with AstraZeneca, and to compare their antibody titers with those of individuals who received two Pfizer doses.*

Materials and Methods: *The study included healthy volunteers aged 19–32 years (mean 24.3), without underlying diseases or prior COVID-19 infection. Antibody titers were determined using the Roche Cobas-Pro analyzer with the same reagent lot. The study group (AstraZeneca first dose – Pfizer second dose) was compared with the control group (two Pfizer doses).*

Results: *According to Cobas 801 (Roche) standards (<0.8 AU/ml normal range), the maximum quantification capacity after 1:10 dilution was 2,500 AU/ml. The antibody response efficiency of the Pfizer-after-AstraZeneca scheme was 93.88%, while two Pfizer doses achieved 41.38%. The mean increase in antibody titer following the second Pfizer dose was 30.12-fold higher than after the AstraZeneca dose. Age and gender were not statistically significant factors. Statistical comparison using the two-way Wilcoxon test confirmed significant differences between groups ($p < 0.01$).*

Conclusions: *The heterologous ("mix-and-match") vaccination strategy demonstrated higher immunogenicity, potentially due to a longer dosing interval and complementary activation of immune mechanisms. AstraZeneca appears to elicit stronger T-cell responses, while Pfizer enhances antibody production, suggesting cumulative immune benefits when combined.*

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), produced unprecedented global morbidity and mortality and spurred an accelerated effort to develop effective vaccines. Vaccination campaigns worldwide have targeted both humoral and cellular arms of the adaptive immune response to reduce symptomatic disease, severe outcomes and transmission [1,2].

Multiple vaccine platforms were developed and deployed during the pandemic. These include nucleic-acid vaccines (mRNA), non-replicating viral vector vaccines, protein subunit vaccines, and inactivated whole-virus formulations. mRNA vaccines (e.g., BNT162b2) deliver lipid-encapsulated mRNA encoding the prefusion SARS-CoV-2 spike antigen into host cells, leading to intracellular antigen expression and robust induction of neutralizing antibodies and CD4+/CD8+ T-cell responses. Viral vector vaccines (e.g., ChAdOx1 nCoV-19/AstraZeneca) use engineered adenoviral vectors to express spike protein antigens, promoting both potent cellular immunity and antibody production through endogenous antigen presentation pathways. Protein subunit and inactivated vaccines present antigen directly and typically rely on adjuvants to enhance humoral responses [3-5].

Large clinical trials and subsequent real-world effectiveness studies demonstrated high initial efficacy of the leading mRNA vaccines in preventing symptomatic COVID-19 and severe disease, with early reports of vaccine efficacy near or above 90% in randomized trials. Viral vector vaccines also provided substantial protection against symptomatic disease and hospitalization, and their immunogenic profiles differed in the relative balance of T-cell versus neutralizing antibody responses. Importantly, measured vaccine effectiveness has varied by circulating variants, time since vaccination, and population characteristics, prompting widespread use of booster doses and heterologous strategies to sustain protection [5,6].

Heterologous prime-boost regimens, most commonly a viral vector prime followed by an mRNA boost, emerged as a pragmatic response in settings of changing vaccine recommendations or supply constraints. Accumulating evidence indicates that such "mix-and-match" schedules can elicit equal or greater spike-specific antibody titers and enhanced cellular responses compared with homologous regimens, without major safety concerns in most studied cohorts.[7] These immunological advantages have been attributed to complementary mechanisms of antigen presentation and differential priming of B- and T-cell compartments. Meta-analyses and randomized comparisons have generally supported the immunogenicity and acceptable reactogenicity profile of heterologous combinations [5-7].

Despite robust aggregate data, gaps remain regarding the durability of humoral and cellular immunity across platforms, the optimal interval between heterologous doses, and correlates of protection against evolving variants. Moreover, assay standardization challenges complicate direct quantitative comparisons of neutralizing antibody levels across studies and platforms.

Consequently, context-specific evaluations (age, interval, prior infection status, assay used) are essential to interpret immunogenicity data and guide public-health policy. The present study aims to contribute to this evidence base by quantifying antibody responses on the day of the second Pfizer dose in young, healthy individuals who received an initial AstraZeneca dose and comparing them to homologous two-dose Pfizer recipients, using a single standardized analytical platform [8].

In Greece, the administration of AstraZeneca's vaccine was abolished in certain age groups. Those who had the first dose with AstraZeneca and belong to these age groups, completed their vaccination with an mRNA vaccine. The aim of the study is to evaluate the antibody response on the day of application of the second dose with the Pfizer vaccine, in a group of patients that had received the Astra vaccine as the first dose, as well as to measure their antibody titer.

Materials and Methods

The study included participants aged between 19 and 32 years (mean value 24.3), without underlying diseases, who had not been infected by COVID-19. All measurements were performed at the Roche Hellas SA Cobas-Pro analyzer, using the same reagent lot. The patient group (First dose Astra Zeneca-Second dose Pfizer) was compared to the control group (2 doses Pfizer).

Results

Vaccine titer efficiency, based on Cobas 801 (Roche) – normal values < 0.8 Au/ml, is considered as the maximum discrimination capacity of the specific analyzer after 1:10 dilution = 2,500 Au/ml. The efficiency of a dose of Pfizer after 1 dose of AstraZeneca equals to 93.88%. The mean increase from the Astra dose to the second Pfizer dose in antibody titer is 30.12 times greater. The efficiency of two doses of Pfizer equals to 41.38%. Age and gender are not statistically significant factors. In the multiple histogram with logarithmic antibody titers are shown side by side the frequencies of titers for those examined when applying a second dose of Pfizer after a first dose of Astra (mix and match-green color), a second dose of Pfizer after a first dose of Pfizer (blue color), and the titers concerning the first dose of Astra on the day of vaccination with a second dose of Pfizer (pink color), Figure 1. The multiple violin BoxPlot of the logarithmic indications as of the groups in the previous diagram, presents the antibody performance of the vaccines and indicates the difference in performance of one method (mix and match) from the other (2 Pfizer doses) which is also verified by the 2-way Wilcoxon test ($p < 0.01$), Figure 2.

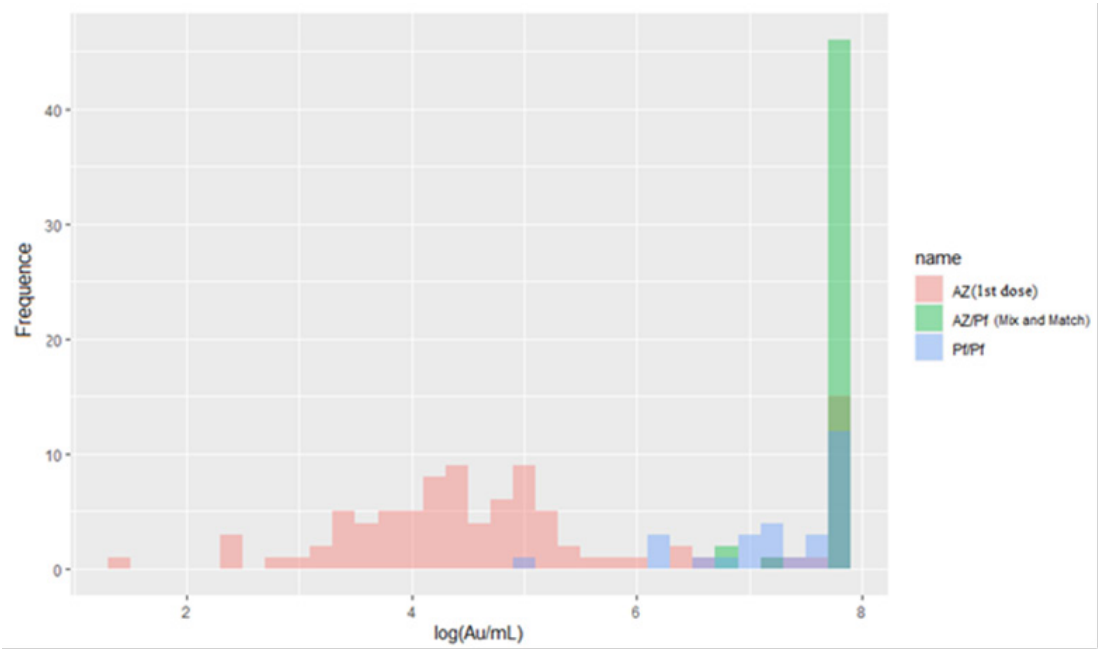

Figure 1: Comparison of logarithmic antibody titers across different vaccination schemes.

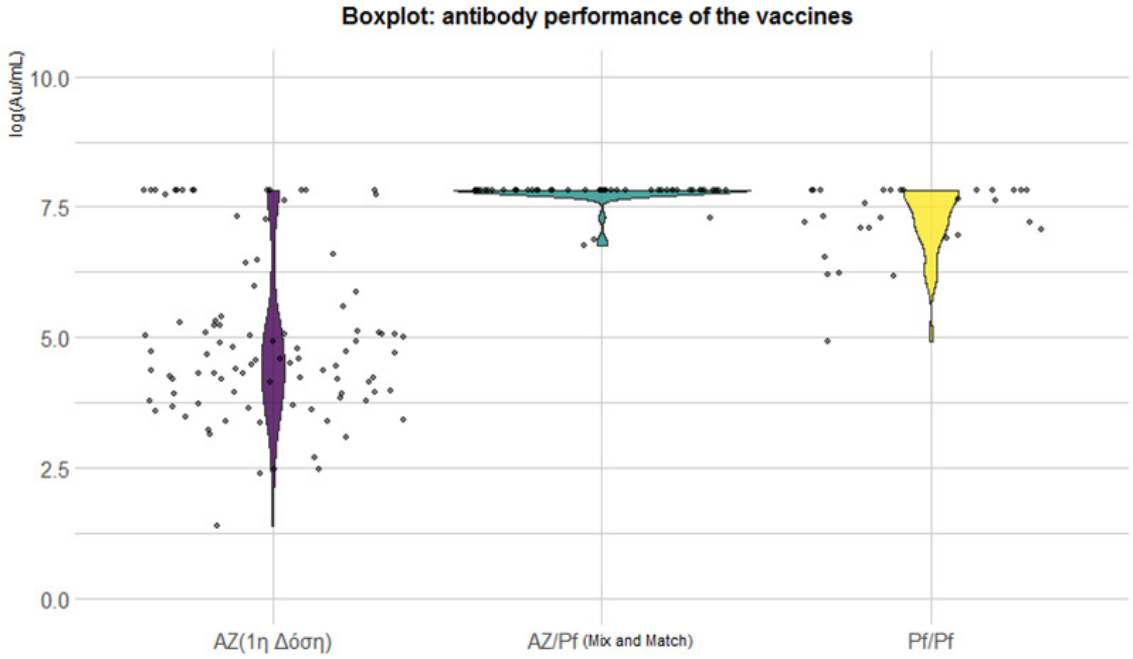

Figure 2: Comparison of antibody titers between vaccination schemes (log scale).

Discussion

Comparative Findings from the Literature

Several studies have evaluated the immunogenicity and effectiveness of heterologous ("mix-and-match") COVID-19 vaccination regimens, comparing them with homologous schedules. A recent meta-analysis including 28 studies with over 5,800 participants reported that heterologous vaccination significantly enhanced immune responses compared with homologous schedules. The geometric mean titers (GMTs) of neutralizing antibodies were 1.65-fold higher in heterologous combinations versus 1.27-fold in homologous regimens, while anti-RBD IgG levels were 1.85-fold vs. 1.15-fold, respectively [9].

Another study directly compared three groups—heterologous (ChAdOx1-S/BNT162b2) and two homologous regimens—showing that the heterologous group produced a statistically significant higher serological response ($p < 0.0001$), with IgG anti-S levels mainly influenced by the vaccine combination rather than age or sex [10].

In a cohort of ≈300 participants primed with ChAdOx1-S and boosted with BNT162b2, compared with ≈140 individuals receiving two mRNA doses, anti-spike IgG titers declined over time in all groups but remained significantly higher in the heterologous group at three months post-boost. Neutralizing activity was also superior in the mix-and-match group compared to homologous mRNA vaccination [11].

Longitudinal data also indicate differences in antibody waning kinetics. A study monitoring neutralizing antibodies for up to 16 weeks after booster vaccination found that participants primed with two BNT162b2 doses and boosted with Ad26.COV2.S reached peak neutralizing titers of 1,018 AU/ml (IQR 699–1,646) at week 2, declining 6.9-fold by week 16. In contrast, homologous BNT162b2 boosters peaked at 859 AU/ml (IQR 467–1,838) at week 4 and declined only 2.1-fold by week 16 [12].

Comparative Analysis of the Present Findings with Published Literature

The present study demonstrated a marked enhancement of humoral immune response in the heterologous vaccination group (AstraZeneca first dose followed by Pfizer second dose), showing an efficiency of 93.88% and a 30.12-fold increase in antibody titers compared with the response following the first AstraZeneca dose. In contrast, the homologous Pfizer–Pfizer group exhibited a lower efficiency of 41.38%, indicating that the mix-and-match strategy induced substantially

higher antibody production. These results are consistent with findings from several international studies that have reported superior immunogenicity for heterologous vaccination schemes combining viral-vector and mRNA vaccines.

Barocci et al. observed that participants who received the ChAdOx1-S/BNT162b2 combination developed significantly higher anti-S IgG titers than both homologous ChAdOx1-S and BNT162b2 groups ($p < 0.0001$), confirming that the antibody response was primarily influenced by vaccine type rather than demographic factors [10].

Similarly, the meta-analysis from Cheng et al. of 28 studies reported that heterologous regimens produced 1.65-fold higher neutralizing antibody GMTs and 1.85-fold higher anti-RBD IgG levels compared with homologous schedules [9].

Furthermore, a recent longitudinal study from Kohmer et al. found that heterologous ChAdOx1-S/BNT162b2 vaccination maintained significantly higher neutralizing activity after three months than homologous mRNA vaccination, despite a general decline over time [11]. These observations align closely with our findings, suggesting that the combined use of a viral-vector prime and an mRNA boost provides a broader and more durable immune stimulation.

In addition, data on antibody waning dynamics indicate that while neutralizing titers decrease in both homologous and heterologous schemes, the heterologous approach may produce a stronger initial peak [12]. This is consistent with our results, which show a robust antibody surge following the second Pfizer dose in the AstraZeneca–Pfizer group.

Taken together, our findings reinforce the growing evidence that heterologous vaccination elicits synergistic activation of both humoral and cellular immunity [13]. The enhanced antibody titers observed may reflect the complementary mechanisms of immune priming induced by adenoviral-vector and mRNA platforms. The absence of significant age or gender effects in our data further supports prior observations that immune response magnitude is primarily determined by vaccine composition and timing rather than demographic parameters.

In summary, our study demonstrates that heterologous vaccination with an initial AstraZeneca dose followed by a Pfizer booster elicits markedly higher antibody titers and overall immunogenic efficiency compared with homologous two-dose Pfizer regimens. These findings align closely with international evidence, including meta-analyses and cohort studies, which consistently report superior humoral and cellular responses in mix-and-match vaccination schemes [9,10]. The immunological advantage of heterologous regimens likely arises from complementary mechanisms: the viral-vector prime promotes robust T-cell priming and memory formation, while the mRNA boost amplifies neutralizing antibody production and B-cell maturation. Collectively, these data suggest that heterologous vaccination strategies may provide more durable and broad-spectrum protection, particularly in contexts of evolving SARS-CoV-2 variants or vaccine supply limitations. Our results further support the adoption of flexible vaccination schedules that optimize immune responses while maintaining safety and tolerability, reinforcing the potential public health benefit of heterologous COVID-19 vaccination strategies.

Conclusions

The higher efficiency in the mix and match method may be attributed to the fact that the second dose after AstraZeneca was performed over a longer period of time than the first, compared to the 2 Pfizer doses, resulting in a more efficient expansion of memory β-cells. The evidence shows that the initial dose with AstraZeneca generates a stronger T-cell response compared to Pfizer, while Pfizer generates greater antibody titers. The vaccines therefore seem to work cumulatively.

Conflict of Interest

The authors declare no conflicts of interest.

Authors' Contributions

- Study Conception and Design: Marilena Stamouli, Ioannis Kotsiaras, Athina Michopoulou, Emmanouil Konstantakis
- Data Collection: Marilena Stamouli, Ioannis Kotsiaras, Athina Michopoulou
- Laboratory Analysis: Marilena Stamouli, Athina Michopoulou

- Data analysis and Interpretation: Marilena Stamouli, Christina Seitopoulou
- Manuscript Drafting: Antonia Mourtzikou, Marilena Stamouli
- Critical Revision of the Manuscript for Important Intellectual Content: Antonia Mourtzikou, Marilena Stamouli, Christina Seitopoulou
- Supervision: Emmanouil Konstantakis, Maria Kimouli
- All authors have Read and Approved the Final Manuscript.

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