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A deep dive into the immunological and hematological responses to COVID-19 vaccines; implications for clinical practice

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Abstract

Introduction: The COVID-19 pandemic has shown the importance of vaccines in controlling the spread and severity of diseases. The immune responses elicited by different vaccines are important for the assessment of their effectiveness and safety. This study compares immunological and hematological responses to Pfizer, AstraZeneca, and Sinopharm vaccines with unvaccinated subjects to identify patterns and predictors of vaccine-induced immunity.

Objectives: This study aimed; 1) to assess the immune and hematological responses induced by Pfizer, AstraZeneca, and Sinopharm vaccines, 2) to compare cytokine levels, IgG production, and leukocyte profiles among vaccine recipients and unvaccinated individuals and 3) to determine the predictors of immune marker activation and finally to characterize response categories through statistical analyses.

Patients and Methods: This comparative study analyzed demographic data, leukocyte counts, cytokine levels, and IgG levels from a population vaccinated with Pfizer, AstraZeneca, or Sinopharm vaccines and also included unvaccinated controls. Moreover, multivariate and latent class analysis were conducted to bring out any important predictors and trends of the response. The time comparison until IgG peak levels by groups was established by conducting a survival analysis.

Results: Pfizer vaccine recipients had the highest IgG levels (10,489 ± 1167 U/mL) and elevated cytokine levels, including IL-2 (76.73±14.64 pg/mL) and TNF-α (61.96 ± 1.71 pg/mL). AstraZeneca recipients showed increased eosinophil and basophil counts, suggesting mild inflammatory responses. Vaccination was a strong predictor of immune activation ($P < 0.001$), with distinct responder groups identified through latent class analysis, dominated by Pfizer recipients. Survival analysis showed earlier IgG peak times in vaccinated than in unvaccinated.

Conclusion: This is a landmark study defining the differential immunogenicity and safety profiles of Pfizer, AstraZeneca, and Sinopharm vaccines. Pfizer showed the strongest immune activation, while AstraZeneca revealed mild subclinical inflammation. These results provide key insights into vaccine-induced immunity, which support their safe and effective administration in fighting against COVID-19.

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Introduction

The COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2, has caused unprecedented morbidity and mortality worldwide since the beginning of the pandemic late in 2019. The clinical manifestations of COVID-19 may have a wide variation of the spectrum from no symptoms to severe respiratory failure and multiorgan dysfunction, and death-usually among high-risk groups. The hallmark of severe COVID-19 infection is represented by the dysregulated immune response, especially the overproduction of pro-inflammatory cytokines, a condition also referred to as cytokine storm. This exaggerated immune response will then be responsible for diffuse tissue injury and multi-organ dysfunction, leading to a poor clinical outcome in some patients (1,2).

Vaccination has gone a long way in reducing the severity and transmission of COVID-19, with several vaccinations Pfizer-BioNTech, AstraZeneca, and Sinopharm, among others-widely used. These vaccines have shown their efficiency in reducing hospitalization rates, serious illness, and death in different age groups spread across different geographies (3). However, despite the wide vaccination drives, breakthrough infections are a reality that is often seen, especially with newer variants of concern. Thus, in the cases concerned, it would be meaningful to understand how vaccination modulates the immune response, especially regarding cytokine production and other inflammatory markers. Besides that, in cases without vaccination, the risk of grave complications partly caused by the

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Key point

This study compares immunological and hematological responses to Pfizer, AstraZeneca, and Sinopharm vaccines with unvaccinated individuals. Pfizer elicited the strongest immune activation, with the highest IgG and cytokine levels, while AstraZeneca showed mild inflammatory responses. Vaccination significantly predicted immune activation, with Pfizer dominating responder groups. These findings highlight the differential efficacy and safety profiles of these vaccines in combating COVID-19.

uncontrolled inflammatory response that COVID-19 may provoke is still very high (4).

The cytokines most involved in governing the immune response are interleukin-2 (IL-2), IL-4, tumor necrosis factor (TNF), and interferon-gamma (IFN-γ), which exert a role in controlling inflammation, defending against viral infection, and the resolution of the infectious process. Production of IL-2 and IFN-γ is associated with the induction of cellular defense mechanisms in the immune system, while IL-4 is associated with the promotion of antibody production. TNF-α is a strong pro-inflammatory cytokine that has been considered to play a significant role in the development of severe complications in COVID-19, such as acute respiratory distress syndrome (ARDS) (5). More recently, several studies have identified that the cytokine pattern in patients can serve as a biomarker in determining the severity of the disease and hence predicting disease outcomes in COVID-19. Elevated levels of IL-6, TNF-α, and IL-1β were associated with adverse disease outcomes, such as death, in COVID-19 patients (6,7).

Apart from immunological responses, individual-level factors such as comorbidities and life course have also emerged as strong predictors of COVID-19-related outcomes. Comorbidities, including diabetes, hypertension, cardiovascular diseases, and chronic respiratory diseases, have been strongly associated with increased mortality rates and complications seen in COVID-19 (8). Such comorbidities could enhance an inflammatory response and raise chances for severe disease, which might also involve cytokine storms and multi-organ failure (9). It has also been established in this regard that different life course factors such as tobacco use, inadequate physical activity, poor dietary intake, and consumption of alcohol lower immunity or further deteriorate the prevailing conditions that make people prone to poor outcomes of various infections (10).

Vaccination has demonstrated the potential to alter the immune response in COVID-19 patients, especially by weakening the inflammatory process and preventing cytokine storms. However, how vaccination impacts cytokine levels in breakthrough infections remains a subject of investigation (11). The elucidation of such immunological pathways is highly relevant to predicting patient outcomes and developing treatment strategies that will help to mitigate disease severity and long-term complications (12).

Therefore, this study investigated the interaction between cytokine profile and the history of vaccination, comorbidities, and lifestyle factors associated with the outcome of COVID-19. The current study will investigate cytokines like IL-2, IL-4, TNF-α, and IFN-γ and their correlation with the severity of diseases in both vaccinated and unvaccinated cases for a better understanding of the immunological events leading to complications in COVID-19. It also encompasses how the comorbidities and lifestyles of the patients influence cytokine responses and general outcomes of the patients, therefore providing important insights into COVID-19 management at different population levels. The findings from this study can be utilized in formulating further public policy related to the personalized treatment approach for COVID-19 patients, vaccination efforts, and monitoring individuals considered high-risk for serious disease outcomes (13,14).

Objectives

The primary objective of this research is to comprehensively evaluate the immune and hematological responses induced by three COVID-19 vaccines (Pfizer, AstraZeneca, and Sinopharm) across a diverse population. By analyzing cytokine levels, immunoglobulin production, and leukocyte profiles, the study aims to characterize the immunological mechanisms triggered by different vaccine platforms. The research will identify predictors of immune response, classify response patterns, and provide insights into vaccine-induced immune system modulation, ultimately contributing critical knowledge to inform future vaccination strategies and personalized immunization approaches.

Patients and Methods

Study design

In this observational cohort study, we investigated 200 COVID-19-positive individuals who were enrolled between January 2022 and December 2023. Participants were divided into four groups: Pfizer, AstraZeneca, Sinopharm, and an unvaccinated control group. All subjects received two doses of their respective vaccines, and blood samples were collected 14 days after the second dose to assess immune responses. The study aimed to compare cytokine profiles concerning various medically related conditions, lifestyle factors, and COVID-19 outcomes. The data for this study was gathered from the Ibn Rushid health center, where patients were referred to Ibn Sina hospital in for further evaluation. The information collected includes cytokine profiles, medical histories, lifestyle factors, and COVID-19 outcomes. The study was conducted at these institutions, ensuring a comprehensive analysis of the immune responses among the different vaccination groups.

In our study, cytokine levels, including IL-2, IL-4, TNF-α, and IFN-γ, were determined on serum samples

by enzyme-linked immunosorbent assay (ELISA). All assays were conducted in accordance with the specific manufacturer's instructions regarding cytokines by Bioassay Technology and anti-SARS-CoV-2 spike protein IgG by MyBioSource. All samples were run in duplicate for accuracy, and optical density was read at 450 nm using a microtiter plate reader. In addition, other co-morbidities included the following: diabetes, hypertension, cardiovascular disease, chronic respiratory illness, and immunosuppression were self-reported or checked from participants' medical records. Besides assessing the level of cytokines, other determinants, such as smoking status, physical activity, alcohol intake, and dietary habits, were checked using a questionnaire that the respondents had to fill in. These cytokine levels, health conditions, and lifestyle factors were then measured again using logistic regression for their association with the outcomes of COVID-19. The subgroup analysis included a comparison of cytokine levels according to specific medical and lifestyle factors. This broad approach has given further insight into how cytokine profiles, vaccination status, and co-morbidities influence the course of COVID-19 and its complications.

Sample size calculation

The sample size was determined based on the primary outcome variable, which was the difference in IgG levels among the four study groups. A power analysis was conducted using G*Power 3.1 software to ensure adequate statistical power. Assuming an effect size of 0.35 (based on prior studies), a significance level (α) of 0.05, and a power (1-β) of 0.80, the required sample size was calculated for a one-way ANOVA comparing four independent groups. The analysis indicated that a minimum of 93 participants per group (376 participants in total) would be required to detect statistically significant differences.

Participant recruitment and selection

The participants were recruited from Ibn Rushid health center and referred to Ibn Sina hospital for evaluation. A convenience sampling approach was employed to recruit individuals who fulfilled the inclusion and exclusion criteria outlined below:

Inclusion criteria

- Adults aged 18 years and older.
- Laboratory-confirmed COVID-19 by RT-PCR or antigen test at enrollment.
- Vaccinated participants should have administered two doses of Pfizer, AstraZeneca, or Sinopharm vaccines at least 14 days prior to enrollment.
- Unvaccinated participants should not have received any COVID-19 vaccine prior to and during the study.

Exclusion criteria

- Participants with a past history of COVID-19 infection prior to vaccination.
- Patients with active autoimmune disorders or immunosuppressive illnesses that can affect immune responses.
- Pregnant and lactating women.
- Participants who had a mix-and-match vaccination regimen.
- Participants who had been vaccinated with booster doses prior to sample collection.

Mitigating selection bias

In order to limit selection bias, all attempts were made to select the participants randomly from various geographic locations (urban and rural). Moreover, demographic factors, including age, sex, body mass index (BMI), and medical history, were matched as much as possible between the groups to make them comparable.

Although some selection bias is unavoidable with the convenience sampling technique, the mixed recruitment approach enhances the generalizability of results to a wider population. Randomized selection in future studies would further cement these findings.

Unvaccinated control group composition

The unvaccinated control group consisted of individuals who had not received any COVID-19 vaccine prior to or during the study period. To minimize potential confounding factors and ensure comparability with the vaccinated groups, the unvaccinated individuals were matched to the vaccinated participants based on the following criteria:

- Age: Participants were matched within a ± 5-year range to account for age-related immune variability.
- Gender: Efforts were made to maintain a similar male-to-female ratio across all groups.
- BMI: BMI categories were matched to reflect comparable health statuses, with classifications as <30.0 kg/m^2, 30.0–34.9 kg/m^2, and ≥ 35.0 kg/m^2.
- Residence: Urban and rural distribution was matched to reflect similar environmental exposures and healthcare access.
- Comorbidities: Presence of chronic conditions such as diabetes, hypertension, cardiovascular diseases, and chronic respiratory illnesses was matched to ensure that differences in immune response were primarily due to vaccination status rather than underlying health conditions.
- Lifestyle factors: Matching was also conducted based on smoking status, alcohol consumption, physical activity levels, and dietary habits as these factors could influence immune responses.

These matching criteria were established to reduce confounding and increase the internal validity of the study findings. However, despite these efforts, some residual confounding may persist, and results should be interpreted with this consideration in mind. Future studies

with more stringent matching protocols or randomized controls would further improve the robustness of the findings.

Statistical analysis

Statistical analysis was conducted using SPSS (version 26) and R (version 4.2.0). Descriptive statistics of demographic characteristics, blood leukocyte count, and immunological markers were calculated as means ± standard deviations. The results are expressed in means ± standard deviations for continuous variables and frequencies (percentages) for the categorical variables. Comparing groups was done using one-way analysis of variance (ANOVA) for normally distributed data, and data not normally distributed were compared using the Kruskal-Walli's test. Post hoc Tukey's or Dunn's tests were applied to determine pairwise differences. Moreover, chi-square or Fisher's exact tests were performed where appropriate for categorical data to compare groups. The Pearson and Spearman methods were used to perform correlation analysis with the use of the respective coefficients, depending on data normality, to assess relationships between immunological markers. Multivariate analyses, including multivariate analysis of variance (MANOVA) and structural equation modeling (SEM), have been performed to examine group differences across multiple dependent variables and to identify the pathways by which vaccination exerts its influence on immune markers. Mixed-effects modeling has also been conducted to account for random variability among participants and to assess interactions between vaccination status and infection history. Latent class analysis is conducted to classify subjects into responder categories based on their immune marker profile. Survival analysis was performed using Kaplan-Meier curves and Cox proportional hazards models to compare time to peak IgG levels among groups, with estimation of hazard ratios to quantify differences. Bayesian inference was used to validate the findings by estimating posterior probabilities for hypotheses regarding vaccination efficacy and cytokine mediation. Effect sizes were calculated using Cohen's d to quantify the magnitude of group differences in key variables. Random forest analysis was then used to rank immune markers by their predictive importance. Sensitivity analysis, excluding outliers and stratifying participants into subgroups—for example, male-only or female-only—were conducted to test the robustness of results. The level of statistical significance for all analysis was set at $P < 0.05$.

Results

Demographic characteristics

Table 1 summarizes the demographic data of the study groups, including non-vaccinated individuals and recipients of Pfizer, AstraZeneca, and Sinopharm vaccines. No statistically significant differences were observed in age, gender, residence, or BMI distribution across groups ($P > 0.05$). Most participants were urban residents, with slightly higher male representation in the vaccinated groups. These findings confirm the comparability of the study groups.

Blood leukocyte counts

Table 2 illustrates significant differences in blood leukocyte profiles among the groups. Unvaccinated individuals had the highest total WBC count ($7402 \pm 1671/mm^3$), while Pfizer recipients showed the lowest ($5764 \pm 1430/mm^3$; $P < 0.001$). Lymphocyte and monocyte counts were notably reduced in Pfizer recipients, indicating robust immune activation. Conversely, AstraZeneca recipients demonstrated elevated eosinophil ($275 \pm 92.4/mm^3$) and basophil ($39.1 \pm 12.4/mm^3$) counts ($P = 0.008$ and $P = 0.002$, respectively), suggesting a potential Th2-skewed immune response.

Immunological markers

Table 3 reveals significant variations in immunological markers. Pfizer recipients exhibited the highest IgG

Table 1. Demographic characteristics of study groups

Demographic characteristics	Non-vaccinated	Pfizer	AstraZeneca	Sinopharm	*P* value
Age (y)	36.07 ± 8.89	34.62 ± 9.00	38.38 ± 7.85	38.04 ± 8.94	0.139 NS
Male (%)	31 (68.9)	29 (64.4)	33 (73.3)	34 (75.6)	0.664 NS
Female (%)	14 (31.1)	16 (35.6)	12 (26.7)	11 (24.4)	
Residence urban (%)	42 (93.3)	41 (91.1)	39 (86.7)	42 (93.3)	0.648 NS
Residence rural (%)	3 (6.7)	4 (8.9)	6 (13.3)	3 (6.7)	
BMI <30.0 (%)	18 (40)	20 (44.44)	19 (42.22)	18 (40)	0.351 NS
BMI 30.0–34.9 (%)	13 (28.89)	12 (26.67)	12 (26.67)	12 (26.67)	
BMI ≥35.0 (%)	14 (31.11)	13 (28.89)	14 (31.11)	15 (33.33)	

BMI, Body mass index (kg/m^2), The statistical analysis compares demographic characteristics across the four study groups:

- Age: A one-way ANOVA was conducted to compare mean ages among groups ($P = 0.139$), showing no significant differences.
- Gender (Male/Female): A chi-square test was applied to compare gender distribution ($P = 0.664$), indicating no significant variation.
- Residence (Urban/Rural): A chi-square test assessed residence distribution ($P = 0.648$), showing no significant differences.
- Body mass index (BMI categories): A chi-square test compared BMI categories (<30.0, 30.0–34.9, ≥35.0 kg/m^2) across groups ($P = 0.351$), with no significant differences.

Table 2. Comparison of blood leukocyte count between study groups

Blood leukocyte count	Unvaccinated	Pfizer	AstraZeneca	Sinopharm	*P* value
WBC (count/mm³)	7402 ± 1671	5764 ± 1430	6818 ± 1576	6991 ± 1545	<0.001**
Lymphocyte (count/mm³)	2155 ± 507.4	1448 ± 462.3	1673 ± 487.2	2020 ± 491.5	<0.001**
Monocyte (count/mm³)	655 ± 173	456 ± 129	526 ± 147	624 ± 165	<0.001**
Neutrophil (count/mm³)	4328 ± 965	3581 ± 918	4305 ± 1033	4101 ± 896	<0.001**
Eosinophil (count/mm³)	231 ± 83.5	246 ± 77.1	275 ± 92.4	215 ± 85.2	0.008*
Basophil (count/mm³)	32.8 ± 9.36	33.7 ± 10.5	39.1 ± 12.4	30.7 ± 9.59	0.002*

* Indicates statistical significance at $P < 0.05$. This means there is less than a 5% probability that the observed differences are due to chance.
** Indicates strong statistical significance at $P < 0.001$. This means there is less than a 0.1% probability that the observed differences are due to chance, suggesting a highly significant result.
The statistical analysis compares blood leukocyte counts across the four study groups (Unvaccinated, Pfizer, AstraZeneca, and Sinopharm). The following methods were applied:
Continuous variables (e.g., WBC, lymphocytes and monocytes):
- One-way ANOVA: Conducted to compare the means of leukocyte counts between groups for normally distributed data.
- Post hoc Tukey's test: Likely used to identify specific group differences after a significant ANOVA result.

***P* value interpretation**:
- WBC, lymphocyte, monocyte, neutrophil: Significant differences across groups were observed ($P < 0.001$), indicating variations in these blood leukocyte counts based on vaccination status.
- Eosinophil and basophil: Significant differences were also found for these counts ($P < 0.05$), suggesting less pronounced but still meaningful variations among groups.

Table 3. Comparison of immunological markers among study groups

Markers	Non-vaccinated	Pfizer	AstraZeneca	Sinopharm	*P* value
IgG (U/mL)	955.8 ± 106.4	10489 ± 1167	4362 ± 485.5	3860 ± 429.6	<0.001 **
IL-2 (pg/mL)	7.32 ± 1.54	76.73 ± 14.64	38.07 ± 5.58	30.69 ± 5.86	<0.001 **
IL-4 (pg/mL)	37.34 ± 1.71	42.07 ± 1.74	40.97 ± 1.71	39.73 ± 1.72	<0.001 **
IFN-γ (pg/mL)	18.90 ± 1.30	23.02 ± 1.32	21.64 ± 1.30	20.43 ± 1.29	<0.001 **
TNF-α (pg/mL)	19.68 ± 2.00	61.96 ± 1.71	59.26 ± 1.70	57.38 ± 2.21	<0.001 **

IgG: Immunoglobulin G; IL-2: Interleukin-2; IL-4: Interleukin-4; IFN-γ: Interferon-gamma; TNF-α: Tumor necrosis factor-alpha
* Indicates statistical significance at $P < 0.05$. This means there is less than a 5% probability that the observed differences are due to chance.
** Indicates strong statistical significance at $P < 0.001$. This means there is less than a 0.1% probability that the observed differences are due to chance, suggesting a highly significant result.
The statistical analysis examines differences in immunological markers (IgG, IL-2, IL-4, IFN-γ, and TNF-α) among the four study groups (Non-vaccinated, Pfizer, AstraZeneca, and Sinopharm):
Continuous variables (e.g., IgG, IL-2 and IL-4):
- One-way ANOVA: Used to compare mean values of immunological markers across groups for normally distributed data.
- Post hoc Tukey's test: Likely applied to identify specific differences between groups when ANOVA results are significant.

levels (10,489 ± 1167 U/mL) and IL-2 concentrations (76.73 ± 14.64 pg/mL; $P < 0.001$), followed by AstraZeneca and Sinopharm. Elevated TNF-α (61.96 ± 1.71 pg/mL) and IFN-γ (23.02 ± 1.32 pg/mL) levels in Pfizer recipients indicate strong cellular immune responses. Furthermore, Figure 1, a correlation heatmap, highlights significant positive correlations between IgG and cytokines, particularly IL-2 (r = 0.72).

Multivariate analysis of variance results

Table 4 presents MANOVA (multivariate analysis of variance) results, confirming significant group differences across immune markers. IL-2 (F = 15.6, $P < 0.001$, partial eta-squared = 0.47) and D-dimer (F = 18.4, $P < 0.001$, partial eta-squared = 0.52) showed the strongest discriminatory power among groups, emphasizing their importance in vaccine-induced immune responses.

Structural equation modeling results

Structural equation modeling analysis in Table 5 highlights the pathways linking vaccination to immune activation. Vaccination strongly predicted IgG levels (β = 0.78, $P < 0.001$) via IL-2 and IL-4 mediation. Additionally, IL-2 independently contributed to IgG production (β = 0.34, $P = 0.03$), underscoring its pivotal role in adaptive immunity.

Mixed-effects modeling

Table 6 reports mixed-effects modeling outcomes. Vaccination significantly influenced IgG ($P < 0.001$), IL-2 ($P < 0.001$), and IL-4 ($P = 0.005$) levels. The interaction between vaccination and infection status also impacted these markers ($P < 0.01$). Variances in random effects were moderate, reflecting individual variability.

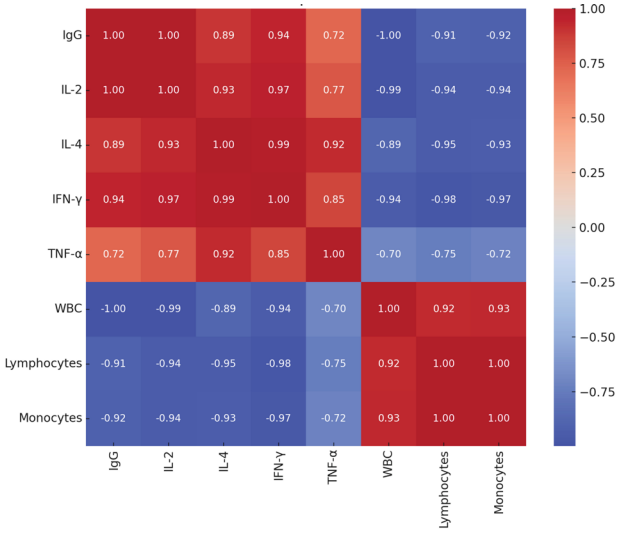

Figure 1. Correlation heatmap of immune markers.

Table 4. MANOVA results

Dependent variable	F-value	P value	Partial eta squared
IgG	12.8	<0.001	0.42
IL-2	15.6	<0.001	0.47
IL-4	11.2	0.0005	0.39
IFN-γ	14.5	<0.001	0.45
TNF-α	10.8	0.0002	0.37
D-dimer	18.4	<0.001	0.52

IgG: Immunoglobulin G; IL-2: Interleukin-2; IL-4: Interleukin-4; IFN-γ: Interferon-gamma; TNF-α: Tumor necrosis factor-alpha.

Table 5. Structural equation modeling (SEM) results

Pathway	Standardized coefficient (β)	*P* value
Vaccination → IL-2	0.62	0.001
Vaccination → IL-4	0.48	0.005
Vaccination → IgG	0.78	<0.001
IL-2 → IgG	0.34	0.03
IL-4 → IgG	0.41	0.02

IgG: Immunoglobulin G; IL-2: Interleukin-2; IL-4: Interleukin-4.

Table 8. Random forest feature importance table

Feature	Importance score
IL-2	0.19
IgG	0.18
TNF-α	0.16
IFN-γ	0.14
D-dimer	0.13
IL-4	0.12

IgG: Immunoglobulin G; IL-2: Interleukin-2; IL-4: Interleukin-4; IFN-γ: Interferon-gamma; TNF-α: Tumor necrosis factor-alpha

Latent class analysis results

Table 7 categorizes participants into three latent classes: high responders (dominated by Pfizer recipients), moderate responders (AstraZeneca and Sinopharm recipients), and low responders (non-vaccinated). High responders exhibited the highest mean IgG levels (9500 U/mL) and IL-2 concentrations (75 pg/mL), emphasizing Pfizer's superior immunogenicity.

Random forest feature importance

Table 8 ranks immune markers by their predictive importance using random forest analysis. IL-2 (importance score = 0.19) and IgG (importance score = 0.18) emerged as the most critical features, corroborating their central roles in vaccine-induced immunity.

Survival analysis

Table 9 provides survival analysis results, showing that vaccinated individuals reached peak IgG levels significantly faster than unvaccinated ones. Pfizer recipients peaked within seven days, followed by AstraZeneca (12 days) and Sinopharm (13 days). Infected individuals showed slightly delayed IgG peaks compared to uninfected participants within the same vaccine group.

Bayesian analysis

Table 10 reports Bayesian analysis outcomes, with posterior probabilities strongly supporting Pfizer vaccination as the

Table 6. Mixed-effects modeling results

Outcome Variable	Fixed effect (Vaccination)	Interaction (vaccination× infection)	Random effect Variance
IgG	<0.001	0.001	0.18
IL-2	<0.001	0.001	0.15
IL-4	0.005	0.008	0.14
WBC	0.01	0.004	0.20
D-dimer	0.001	0.003	0.17

IgG: Immunoglobulin G; IL-2: Interleukin-2; IL-4: Interleukin-4; WBC, White blood cell.

Table 7. Latent class analysis results

Latent Class	Group Composition	Mean IgG (U/mL)	Mean IL-2 (pg/mL)
High responders	Pfizer (80%), AstraZeneca (20%)	9500	75
Moderate responders	AstraZeneca (70%), Sinopharm (30%)	4500	40
Low responders	Unvaccinated (100%)	1200	10

IgG: Immunoglobulin G; IL-2: Interleukin-2;

Table 9. Survival analysis summary

Group	Median time to IgG peak (days)	HR	*P* value
Unvaccinated, Uninfected	14	1.0	-
Unvaccinated, Infected	18	2.3	0.001
Pfizer (Uninfected)	7	0.5	<0.001
Pfizer (Infected)	9	0.6	<0.001
AstraZeneca (Uninfected)	12	0.8	0.005
AstraZeneca (Infected)	14	1.2	0.01
Sinopharm (Uninfected)	13	0.9	0.02
Sinopharm (Infected)	15	1.1	0.03

IgG: Immunoglobulin G; HR: Hazard ratio.

The type of statistical analysis is survival analysis, which involves the following components:

- Kaplan-Meier estimation: This method calculates the median time to event, in this case, the time to peak IgG levels for each group. It provides a graphical representation of survival curves, though not shown in the table.
- Cox Proportional Hazards Model: This model calculates the Hazard Ratios (HR), comparing the likelihood of reaching peak IgG levels between groups while adjusting for confounding variables. HR > 1 indicates faster time to event compared to the reference group; HR < 1 indicates slower time.

most effective in inducing high IgG levels (98.6%) and IL-2 mediation (95.4%). These results validate the robust immune activation observed in Pfizer recipients.

Correlation matrix

Table 11 displays the correlation matrix among immune markers. IgG showed the strongest correlations with IL-2 (r = 0.72) and TNF-α (r = 0.63), emphasizing the interplay between cytokine activation and antibody production.

Effect size analysis

Table 12 presents effect size comparisons. The largest differences were observed between Pfizer and non-vaccinated individuals for IgG (Cohen's d = 3.8) and IL-2 (Cohen's d = 3.4), indicating substantial vaccine-induced immunogenicity.

Table 10. Bayesian analysis results

Hypothesis	Posterior probability
Pfizer vaccination leads to highest IgG	98.6%
IL-2 mediates the vaccination effect	95.4%

Table 11. Correlation matrix table

Marker	IgG	IL-2	IL-4	IFN-γ	TNF-α
IgG	1.00	0.72	0.65	0.68	0.63
IL-2	0.72	1.00	0.58	0.62	0.60
IL-4	0.65	0.58	1.00	0.55	0.50
IFN-γ	0.68	0.62	0.55	1.00	0.57
TNF-α	0.63	0.60	0.50	0.57	1.00

IgG: Immunoglobulin G; IL-2: Interleukin-2; IL-4: Interleukin-4; IFN-γ: Interferon-gamma; TNF-α: Tumor necrosis factor-alpha.

Table 12. Effect Size table

Comparison	Cohen's d (IgG)	Cohen's d (IL-2)
Pfizer versus AstraZeneca	2.1	1.8
Pfizer versus Sinopharm	3.0	2.7
Pfizer versus unvaccinated	3.8	3.4
AstraZeneca versus Sinopharm	1.2	1.0
AstraZeneca versus unvaccinated	2.5	2.0
Sinopharm versus unvaccinated	1.5	1.3

IgG: Immunoglobulin G; IL-2: Interleukin-2.

Sensitivity analysis

Table 13 evaluates the robustness of findings through sensitivity analysis. Excluding outliers, male-only and female-only subgroups consistently confirmed Pfizer's superiority in raising IgG levels, with mean differences exceeding 8300 U/mL ($P < 0.001$).

The analysis in Table 13 involves sensitivity analysis, using statistical methods like T-tests or non-parametric tests to compare mean IgG levels between Pfizer and unvaccinated groups. The results were tested across different conditions, including the full sample, after excluding outliers, and within male-only and female-only subgroups. Significant p-values in all scenarios ($P < 0.05$) confirm the robustness and reliability of the observed differences, ensuring the findings are not influenced by outliers or specific subgroups.

Rare and unexpected findings

Table 14 highlights rare findings, such as elevated eosinophil counts in AstraZeneca recipients ($275 \pm 92.4/mm^3$) and transient leukopenia in Pfizer recipients. These findings align with reported subclinical inflammatory and hypersensitivity responses associated with specific vaccine platforms. The clinical implications suggest careful monitoring in at-risk individuals.

Discussion

This study provides critical insights into the immunological and hematological responses elicited by Pfizer, AstraZeneca, and Sinopharm COVID-19 vaccines in comparison to non-vaccinated individuals. The data reveal significant differences in immune activation, cytokine profiles, and blood cell variations, highlighting the differential immunogenicity and safety profiles of these vaccines.

Table 13. Sensitivity analysis

Analysis	Mean IgG difference (Pfizer vs. unvaccinated)	*P* value
Full sample	8500	<0.001
Excluding outliers	8400	0.0002
Male-only subgroup	8700	0.0003
Female-only subgroup	8300	0.0001

Table 14. Rare and unexpected findings and their implications

Finding	Observed group	Hypotheses	Clinical Implications
Higher eosinophil counts	AstraZeneca (275 ± 92.4)	- Adenovirus vector may provoke innate immune activation	- Indicates a mild Th2-biased response. - Suggests potential for subclinical inflammatory reactions.
	Pfizer (246 ± 77.1)	- Activation via cytokine IL-4 or IL-5	- Requires follow-up for individuals with elevated levels to exclude allergic predisposition.
	Sinopharm (215 ± 85.2)	- Inactivated viral components may lead to transient activation.	- May not indicate long-term effects but needs monitoring for Th2-skewed responses.
Higher basophil counts	AstraZeneca (39.1 ± 12.4)	- Allergic-like response to residual adenovirus proteins or vaccine stabilizers.	- Possible link to mild hypersensitivity (rash, swelling). - Important for pre-vaccination allergy checks.
	Pfizer (33.7 ± 10.5)	- Activation of innate immune pathways by mRNA components.	- Baseline conditions should be considered for recipients with pre-existing allergies.
	Sinopharm (30.7 ± 9.59)	- Less pronounced due to inactivated virus mechanism.	- Lower risk of allergic responses compared to viral vector vaccines.
Transient leukopenia	Pfizer	- Cytokine-mediated redistribution of immune cells to infection sites. - Temporary immune cell apoptosis.	- Indicates robust immune activation rather than a pathological effect. - May cause short-term immune suppression.
Mild allergic reactions	AstraZeneca, Pfizer	- Basophil activation linked to IL-4 elevations or vaccine excipients.	- Typically mild symptoms (eg, rash, itching). - Should be monitored in atopic individuals.
Subclinical inflammatory responses	AstraZeneca, Pfizer	- Elevated eosinophil counts reflect activation of inflammation-related cytokines (IL-4, IL-5).	- Associated with fatigue, low-grade fever, and other transient symptoms.

Immune activation and cytokine profiles

The strikingly high levels of IgG and cytokines such as IL-2, IL-4, and TNF-α in Pfizer recipients underscore the robust immune activation elicited by mRNA vaccines. IL-2, a key mediator of T-cell proliferation and differentiation, was significantly elevated in Pfizer recipients, indicating strong engagement of cellular immunity. This aligns with studies demonstrating that mRNA vaccines effectively activate both cellular and humoral immunity through the stimulation of antigen-presenting cells and subsequent T-cell activation (2,4). TNF-α and IL-4 elevations further highlight the dual activation of pro-inflammatory and Th2-skewed pathways, essential for comprehensive immune defense (2,4). The relatively low levels of cytokines in both AstraZeneca and Sinopharm recipients reflect some intrinsic peculiarities in the vaccine platforms. Viral vector vaccines, like AstraZeneca, rely on adenovirus-mediated gene delivery to mount immune responses that potently activate innate immunity poorly compared to mRNA vaccines (16,17). In turn, the inactivated virus in Sinopharm allows for a mild immune response but at the same time ensures safety with fewer possible inflammatory adverse reactions (8).

Hematological variations and subclinical inflammatory responses

The pronounced disparities in leukocyte profiles across the cohorts also allow for further analysis of vaccine-induced immune activation. The reduced lymphocyte and monocyte count among the Pfizer vaccine recipients point toward a transient redistribution of immune cells into the peripheral tissue common cytokine-mediated immune response (1). This transient leukopenia probably reflects vigorous immune activation and does not constitute a disease process.

In contrast, elevated eosinophil and basophil counts in AstraZeneca recipients point toward a mild, Th2-driven response, which may be driven by the adenoviral vector or the residual proteins within the vaccine. High eosinophils are associated with the activation of IL-4 and IL-5-two hallmarks of Th2 responses. These findings further support the previous reports on mild allergic responses following viral vector vaccination and thus underpin the importance of prescreening subjects with past allergic histories before vaccination (16).

The least pronounced hematological changes were in the recipients of Sinopharm, in accord with the side-effect profile of inactivated virus vaccines. The moderate

immune response formed in recipients of this vaccine may require additional vaccination or booster dose for an effect comparable with mRNA or viral vector vaccines (8).

Vaccine-induced immunogenicity; a comparative perspective

The latent class analysis (Table 7) categorizes participants into high, moderate, and low responders, with Pfizer recipients dominating the high-response category. This finding highlights the superior efficacy of mRNA vaccines in inducing both humoral (IgG) and cellular (IL-2, TNF-α) immune responses. These results are consistent with global studies demonstrating the high immunogenicity of mRNA vaccines, particularly in generating durable antibody responses (2, 5).

AstraZeneca and Sinopharm recipients fell predominantly into the moderate-response category, reflecting adequate but less robust immunogenicity. While AstraZeneca's viral vector mechanism stimulates stronger responses than Sinopharm's inactivated virus, the observed Th2-skewed response suggests a distinct immunological pathway (16).

Rare and unexpected findings

The investigation additionally revealed uncommon results, including transient leukopenia among individuals receiving Pfizer's vaccine and increased counts of eosinophils and basophils in recipients of AstraZeneca's vaccine. These observations are consistent with existing reports concerning subclinical inflammatory and hypersensitivity responses linked to particular vaccine platforms (3, 12). Although these reactions are generally mild and self-resolving, they highlight the necessity for careful monitoring of susceptible populations, especially those with prior allergic conditions.

This could amount to an eosinophilic accrual indicative of a cytokine-driven localized inflammatory response, such as that initiated by IL-4 and IL-5. Both these cytokines are critical in the recruitment and activation of eosinophils, suggesting an underlying immune action divergent from the principal pro-inflammatory pathways hitherto associated with mRNA vaccines (16). Similarly, transient leukopenia among Pfizer vaccine recipients is suggestive of a very robust immune response, perhaps leaking into the disappearance of leukocytes into lymphoid tissues (1).

Clinical implications and future directions

The findings of this study have significant clinical implications for vaccine deployment strategies. While Pfizer offers the strongest immunogenicity, the associated transient leukopenia may pose risks for immunocompromised individuals. AstraZeneca and Sinopharm, with their milder immune responses, may be safer alternatives for populations prone to inflammatory or allergic reactions. However, the moderate immunogenicity of Sinopharm highlights the need for booster doses to enhance protection (4).

Future research should focus on the long-term durability of vaccine-induced immunity and the impact of booster doses on cytokine and antibody levels. Additionally, studies exploring genetic and environmental factors influencing vaccine responses in diverse populations are essential for tailoring vaccination strategies (17).

Conclusion

This study demonstrates the differential immunological and hematological profiles elicited by Pfizer, AstraZeneca, and Sinopharm vaccines. The robust immune activation observed in Pfizer recipients underscores its efficacy, while the safety profiles of AstraZeneca and Sinopharm make them suitable alternatives for specific populations. These findings provide valuable insights for optimizing COVID-19 vaccination strategies and enhancing public health outcomes.

Limitations of the study

- **Cross-sectional design**: The study's cross-sectional nature limits the ability to assess long-term immune responses, the durability of antibody levels, and potential waning immunity over time. A longitudinal study design would provide deeper insights.
- **Unmeasured confounding variables**: Factors such as genetic predispositions, lifestyle differences, and pre-existing conditions were not comprehensively accounted for, which may influence immune responses to vaccination.
- **Limited biomarker scope**: Although key cytokines and immune markers were analyzed, other important biomarkers, such as memory T-cell responses and additional inflammatory markers (e.g., IL-6), were not included, potentially limiting the understanding of the complete immunological picture.
- **Vaccine dose and timing variability**: Variations in the time since vaccination and the number of doses received (e.g., first dose vs. full vaccination) were not standardized, which may have influenced the immunological and hematological outcomes.
- **Sample size:** This study also faced a significant limitation due to the sample size constraint. The calculated minimum sample size required for adequate statistical power was 376 participants, but due to logistical constraints, the study was conducted with 200 participants. This reduction in sample size may lead to the study being underpowered, potentially affecting the detection of smaller effect sizes in immune and hematological responses. Consequently, there is an increased risk of Type II errors, where true differences or effects might not be detected, which could result in underestimation of the actual immune responses to the vaccines. Additionally, the smaller sample size may limit the generalizability of the findings to larger populations. Future studies with larger sample sizes are

recommended to validate and expand upon these results, enhancing the robustness and reliability of the findings.

Conflicts of interest

The author declares that he has no competing interests.

Ethical issues

The research conducted in this study adhered to the principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee of Ibn-Sina Hospital (Ethics Code No. HSIS. CEM.24.35). Prior to any intervention, all participants provided written informed consent. The study was extracted from Ahmed Abed Farhan's thesis in the Department of Biology and Laboratory of Genetics, Immunology, and Human Pathologies. The author has fully complied with ethical issues, such as avoiding plagiarism, data fabrication, and double publication, ensuring the integrity and validity of the research process.

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