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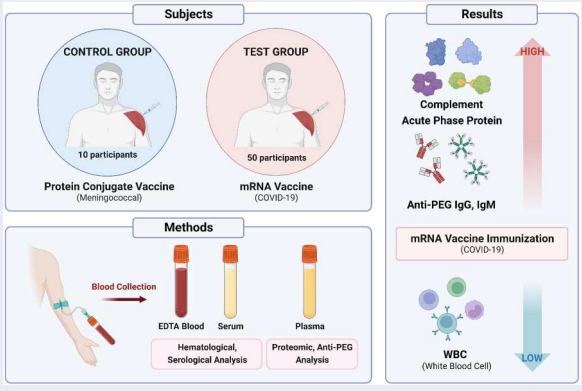
Comparative immunologic profiling of mRNA and protein-conjugated vaccines: acute inflammatory responses and anti-PEG antibody production

Gahyun Roh[a,b]*, Jisun Lee[a]*, Hyun-Mee Park[c]*, Woori Kwak[a,b]*, Hyo-Jung Park[a,b]*, Ayoung Oh[a,b]*, Yewon Na[d], Dahyeon Ha[a,b], Yu-Sun Lee[a,b], Seo-Hyeon Bae[a,b], Seonghyun Lee[a,b], Subin Yoon[a,b], Sowon Lee[a], Jaehun Jung[d], Jacob Lee[e] and Jae-Hwan Nam [a,b]

[a]Department of Medical and Biological Sciences, The Catholic University of Korea, Bucheon, Gyeonggi-do, Republic of Korea; [b]BK Four Department of Biotechnology, The Catholic University of Korea, Bucheon, Gyeonggi-do, Republic of Korea; [c]Advanced Analysis and Data Center, Korea Institute of Science and Technology, Seoul, Republic of Korea; [d]Department of Preventive Medicine, Gachon University College of Medicine, Incheon, Republic of Korea; [e]Division of Infectious Disease, Department of Internal Medicine, Kangnam Sacred Heart Hospital, College of Medicine, Hallym University, Seoul, Republic of Korea

ABSTRACT

Messenger ribonucleic acid (mRNA) vaccines have become a prevalent immunization method, even as the coronavirus disease 2019 (COVID-19) pandemic recedes. However, the potential adverse effects using mRNA vaccines need to be explored in this evolving landscape. In this study, 60 participants were randomly assigned to receive either an mRNA vaccine, specifically for COVID-19, or a conventional vaccine for meningococcal disease. Symptom records and blood samples were collected on Days 0, 3, and 7 after vaccination. Results showed that recipients of mRNA vaccines exhibited elevated levels of serum acute-phase proteins, such as haptoglobin and C-reactive protein, alongside decreased white blood cell counts compared to those receiving conventional vaccines. Proteomic analysis identified significant changes in nine proteins, including interactions involving complement component C9, haptoglobin, and alpha-1-acid glycoprotein, suggesting implications for complement activation and inflammatory responses. Furthermore, variability in anti-polyethylene glycol antibody levels was noted among mRNA vaccine recipients compared to conventional vaccine recipients. This research aims to provide useful information to help develop future vaccination strategies and shape research directions to mitigate individual adverse effects.



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Introduction

Messenger ribonucleic acid (mRNA) vaccines have become a focal point of discussion, particularly regarding their manufacturing techniques and encapsulation materials (Sahin et al. 2014; Hou et al. 2021). The swift development of mRNA vaccines during the

CONTACT Jae-Hwan Nam jhnam@catholic.ac.kr Department of Medical and Biological Sciences, The Catholic University of Korea, 14662, Bucheon, Gyeonggi-do, Republic of Korea; BK Four Department of Biotechnology, The Catholic University of Korea, 14662, Bucheon, Gyeonggi-do, Republic of Korea; Jacob Lee litjacob@hallym.or.kr Division of Infectious Disease, Department of Internal Medicine, Kangnam Sacred Heart Hospital, College of Medicine, Hallym University, 07440, Seoul, Republic of Korea
*These authors equally contributed to this work.
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coronavirus disease 2019 (COVID-19) pandemic represents a significant advancement (Pardi et al. 2018). Vaccines such as Pfizer-BioNTech's BNT162b2 and Moderna's mRNA-1273 were rapidly authorized for emergency use, playing a crucial role in saving countless lives during the peak of the pandemic (Watson et al. 2022; Callaway 2023). These vaccines are distinguished by their rapid development timelines and the ability to be manufactured under conditions with lower biosafety requirements (Moreira et al. 2022).

Despite the widespread adoption and success of Pfizer-BioNTech and Moderna's mRNA vaccines, their initial deployment during the pandemic has set a new standard in vaccine development and emergency response strategies (Chaudhary et al. 2021). However, despite their perceived safety and efficacy, concerns persist among the public due to sporadic reports of severe side effects associated with mRNA vaccines. These include anaphylaxis, viral infections in immunocompromised individuals, facial nerve palsy, and in rare cases, fatalities (Chen et al. 2021). Additionally, mRNA-based BNT162b2 has been linked to hematological abnormalities like thrombocytopenia and immune thrombocytopenic purpura, suggesting potential triggers for immune system responses (Kim et al. 2021; Mingot-Castellano et al. 2022). Therefore, comprehensive post-vaccination monitoring is essential to assess the risks of adverse reactions across diverse populations.

Moreover, the immune response elicited by mRNA vaccine platforms, combined with adjuvant materials, plays a critical role in vaccine efficacy and safety (Krammer 2020; Tregoning et al. 2020). The primary components of conventional vaccines are antigenic proteins (Dolgin 2021). Therefore, it is essential to comprehensively evaluate different responses between recipients of protein-based conventional vaccines and mRNA vaccines. The results of these comparative analyses are expected to deepen our understanding of mRNA vaccine safety, efficacy, and overall performance, guiding ongoing efforts to refine and optimize immunization strategies.

Furthermore, mRNA vaccines have raised concerns regarding immune responses related to polyethylene glycol (PEG) and the production of anti-PEG antibodies (Ju et al. 2022). PEG, a widely used biocompatible polymer, enhances the stability and circulation of therapeutic molecules, including those in mRNA vaccine formulations (Harris and Chess 2003; Khurana et al. 2021). In these vaccines, PEG is incorporated as a PEGylated lipid, a key component of lipid nanoparticles (LNPs) that encapsulate and protect the mRNA cargo. The PEGylated lipid stabilizes the LNP structure, prolongs circulation time, and facilitates cellular uptake of the mRNA. However, PEG-containing LNPs have also been implicated in immune responses, particularly in individuals with pre-existing anti-PEG antibodies, which may lead to hypersensitivity reactions, including allergic responses and anaphylaxis (Lee et al. 2023; Tenchov et al. 2023; Wang et al. 2023). Pre-existing anti-PEG antibodies are found in individuals who have been previously exposed to PEGylated therapeutics, cosmetics, or pharmaceuticals. In these individuals, PEG in mRNA vaccines may trigger immune complex formation or complement activation, potentially leading to rapid clearance of the vaccine components, reduced vaccine efficacy, or adverse allergic reactions. Understanding the prevalence and immunological impact of pre-existing anti-PEG antibodies is crucial for identifying at-risk individuals and developing strategies to mitigate potential hypersensitivity reactions associated with mRNA vaccination.

Thus, this study aimed to assess mRNA vaccine safety through comprehensive analyses of hematological, serological, and protein-level parameters in blood samples from mRNA or protein-vaccinated individuals and to mitigate individual adverse effects and inform future vaccination strategies and research directions. Our findings provide valuable insights into how pre-existing anti-PEG antibodies may interact with mRNA vaccine components, potentially affecting immune responses and vaccine outcomes.

Materials and methods

Supplementary materials and methods

The details regarding hematological and serological analysis and preparation of samples for liquid chromatography-mass spectrometry (LC-MS) proteomics analysis are included in the Supplementary Materials.

Study design

Sixty healthy individuals with a mean age of 37.2 ± 9.4 years (28 males and 32 females) were enrolled in the study. The test group included 22 males and 28 females (mean age: 40.36 ± 2.1 years) who received the SARS-CoV-2 mRNA vaccines (BNT162b2 or mRNA-1273), whereas the control group included 6 males and 4 females (mean age: 22.8 ± 1.1 years) who received Menveo® (GlaxoSmithKline Biologicals SA) (Supplementary Table 1), which is a meningococcal (groups A, C, Y, and W-135) oligosaccharide diphtheria CRM197 conjugate vaccine (GlaxoSmithKline Biologicals SA). BNT162b2 and mRNA-1273 are LNP-formulated nucleoside-modified mRNA vaccines. The participants answered self-report questionnaires to monitor adverse effects 7 days after vaccination. Local and systemic adverse events (AEs) were assessed according to

the severity evaluation guidelines for AEs in vaccine clinical trials. The participants who received the mRNA vaccine were categorized into two groups based on the severity of their side effects according to our predefined criteria for quantifying local and systemic AEs; grade 1 included individuals with mild or no side effects, and grade 2 included those with moderate side effects (Supplementary Table 1).

This study was approved by the Institutional Review Board (IRB) of Hallym University Kangnam Sacred Heart Hospital (Approval No. 2022-02-014). Informed consent was obtained from all participants after the nature and possible consequences of the study had been fully explained to them.

LC-MS proteomics analysis

A preprocessed sample of 5 µL was injected into a nanoLC system (Thermo Scientific™ Ultimate™ 3000 RSLC nano System). The injected sample was loaded onto a trap column (Thermo Acclaim PepMap™ C18 nanoViper, 100 A, 75 µm × 2 cm, 3 µm) at a flow rate of 5 µL/min of 95% A solvent. After 4 min, the sample was separated on an analytical column (Thermo PepMap™ RSLC C18 ES803A, 100 A, 75 µm × 50 cm, 2 µm) by changing the mobile phase from 5–90% B (0.1% formic acid in acetonitrile) at a flow rate of 300 nL/min, and this separation was performed for 150 min. The Orbitrap mass spectrometer (Thermo Scientific™ Orbitrap Eclipse Tribrid™ Mass Spectrometer) was operated in data-dependent acquisition mode, alternating between MS and MS2 scans, with a total of 20 scans. The following mass analysis parameters were used: mass accuracy of 10 ppm, ion spray voltage of 1850V, capillary temperature of 275 °C, resolution of 120,000 for full scans (m/z 375–1575), 35% normalized collision energy for HCD activation scans, quadrupole isolation window of 1.4 Da, and resolution of 30,000 for MS/MS orbitrap scans. The raw data were annotated using the SequestHT algorithm in Proteome Discoverer 2.4 with the Uniport Human database (as of September 29, 2022). Filtering was applied with a high confidence in false discovery rate (FDR) and a minimum requirement of two unique peptides.

Data analysis for proteome profile

Data normalization was performed for the proteome profiles of 267 proteins identified from Thermo Proteome Discoverer 2.4 using NormalyzerDE (Willforss et al. 2019) with the variance stabilizing normalization method. NA filtering and imputation were performed using the Promor package (Ranathunge et al. 2023). After filtering and normalization, the T12_3d sample, which was expected to be an outlier in the MDS plot, was excluded from downstream analysis. To identify significantly different proteins between the groups, the Kruskal–Wallis test was performed using R, and the *p*-value was adjusted using BH FDR. Boxplots were created using ggplot2 to visualize protein expression (Wickham 2011). To identify the related function of the proteins with significantly different expressions, Reactome pathway analysis (Fabregat et al. 2018) was performed with the default parameter.

Measurement of anti-PEG antibody

Anti-PEG immunoglobulin (Ig)G and IgE enzyme-linked immunosorbent assay (ELISA) Maxisorp 96-well microplates (NUNC) were coated with 100 µg/mL 8-arm PEG-NH_2 (JENKEM Technology). After washing plates with phosphate-buffered saline (PBS) (GenDEPOT) and blocking the wells with 5% bovine serum albumin solution, the obtained plasma samples were incubated in four-fold dilutions (1:4). Horseradish peroxidase (HRP)-conjugated goat anti-human IgG (BETHYL) was added at 1:2000 dilution to detect specific PEG IgG antibodies. Specific PEG IgM antibodies were detected by incubating the samples first with an HRP-conjugated rabbit anti-human IgM (BETHYL) antibody at 1:1000 dilution. After a final wash with PBS, substrate buffer containing TMB (Thermo scientific) was added, and subsequently, the reaction was stopped using 2 N H_2SO_4. The plates were read at a wavelength of 405 nm using a microplate reader (GloMax® Explorer Multimode Microplate Reader, Promega). Plasma concentrations of specific IgG and IgM antibodies to PEG were interpolated from a standard curve constructed using anti-PEG human IgG and anti-PEG human IgM, respectively (SOFTmax PRO 4.3 LS).

Statistical analysis

The Wilcoxon non-parametric test was performed to examine the difference in concentrations between the test and control groups for each measurement point. Repeated-measures analysis of variance was performed to confirm the differences in the concentration changes during the observation period. All statistical analyses were performed using SAS Enterprises Guide 7.1 (SAS Institute Inc., Cary, NC, USA).

Results

AEs

Local adverse reactions, including injection site pain, erythema, and swelling, were compared between the

test (BNT162b2 or mRNA-1273) and control (Menveo) groups. Injection site pain was reported by 40 of 50 COVID-19 mRNA vaccine recipients (80%) in the test group, whereas only three of 10 meningococcal protein conjugate vaccine recipients (30%) in the control group reported such pain. Erythema was noted in 12 of 50 individuals (24%) in the test group and three of 10 participants (30%) in the control group, with no significant difference observed. Similarly, swelling occurred in seven of 50 participants (14%) in the test group and two of 10 participants (20%) in the control group, showing no substantial variance (Figure 1A).

Systemic AEs, including fever, vomiting, diarrhea, headache, fatigue, muscle pain, joint pain, and chills, were assessed in all participants. Notably, participants in the test group reported a higher incidence of these AEs than those in the control group. Fatigue was the most frequently reported symptom (32 participants, 64%), followed by muscle pain (31, 62%). Fever, vomiting,

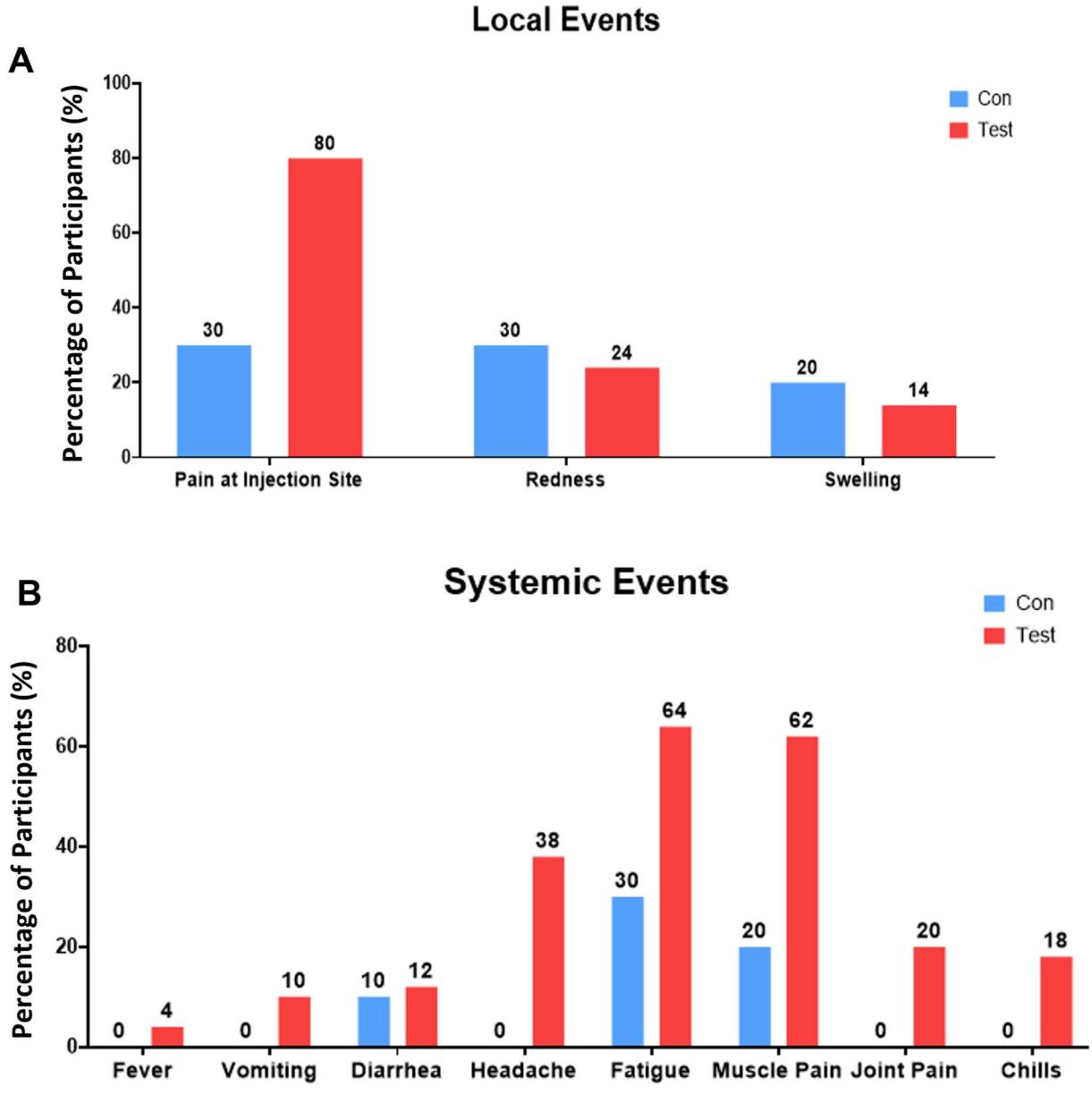


Figure 1. Adverse reactions reported 7 days after the administration of the mRNA and conventional vaccines. Percentages of participants with adverse reactions observed 7 days after administering messenger ribonucleic acid (mRNA) vaccines (coronavirus disease 2019, COVID-19) and the conventional protein conjugate vaccine (meningococcal vaccine). Sixty participants were divided into the test (n = 50) and control groups (n = 10). (A) Percentage of local events (pain at the injection site, redness, and swelling). (B) Percentage of systemic events (fever, vomiting, diarrhea, headache, muscle pain, joint pain, and chills). CON, Meningococcal protein conjugate vaccine (Menveo) immunized group; TEST, COVID-19 mRNA vaccine (BNT162b2 or mRNA-1273) immunized group.

headache, joint pain, and chills were exclusively reported in the test group and were absent in the control group. Specifically, headache, joint pain, chills, vomiting, and fever were reported by 19 (38%), 10 (10%), nine (18%), and two (4%) participants, respectively, in the test group. The two groups did not differ significantly in diarrhea reports (6 participants [12%] in the test group and 1 [10%] in the control group) (Figure 1B). Although mRNA vaccines are generally considered safe and effective, our findings indicate a higher frequency of systemic AEs following mRNA vaccination than protein vaccination.

Hematological and serological analysis of human blood following mRNA or conventional vaccine administration

To comprehensively assess the impact of mRNA and conventional vaccines on hematological and serological factors, participant blood and serum samples were collected at three-time points, before vaccination and on Days 3 and 7 post-vaccination. Although most studied factors were within normal ranges, significant differences were observed between the control and test groups (Figure 2). In the hematological analysis, parameters were within normal limits, including red blood cells, hemoglobin, hematocrit, platelets, mean corpuscular volume, mean cell hemoglobin, and mean cell hemoglobin concentration (Supplementary Figure 1). However, white blood cell (WBC) counts were significantly reduced in the test group on Day 3 compared to those in the control group (p-value = 0.02; p for trend = 0.03). Additionally, although the percentage of monocytes did not show statistical significance, there was a trend toward an increase in the test group on Day 3 compared to the control group (p-value = 0.29; p for trend = 0.28).

Serological parameters such as albumin, bilirubin, calcium, chloride, phosphate, sodium, alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels were also within normal ranges (Supplementary Figure 2). However, acute-phase proteins such as haptoglobin and C-reactive protein (CRP) showed significant elevation on Day 3 in the test group compared to that in the control group (p = 0.02 and <0.01; p for trend <0.0001 and <0.01, respectively). Furthermore, IgE levels exhibited a significant increasing trend in the test group on Day 3, which persisted through Day 7. In contrast, the control group showed a decrease on Day 3, followed by an increase and recovery by Day 7 (p for trend < 0.01) (Figure 2 and Supplementary Table 2). Our findings underscore distinct hematological and serological responses following mRNA and conventional vaccine administration.

Correlation between the alteration of hematological and serological factors and grades of AEs

To explore the relationship between parameter changes and the severity of AEs following COVID-19 mRNA vaccine administration, the participants were categorized into two grades based on the intensity of their side effects. No participants reported serious side effects requiring emergency room visits. Consequently, individuals reporting mild or no side effects were classified under Grade 1 (n=25), whereas those reporting moderate side effects were categorized under Grade 2 (n=25). Figure 3 illustrates that no significant abnormalities were observed in the analyzed factors, with consistent trends across most parameters regardless of the severity of the side effects.

Consistent with our previous hematological and serological analysis, most parameters remained within the normal ranges. However, some variations in AEs were observed. WBC count showed a significant decrease in the Grade 2 group compared with that in the Grade 1 group on Day 3 (p = 0.01; p for trend = 0.06). Additionally, the Grade 2 group displayed a more substantial increase in the mean percentage of monocytes on Day 3, which then decreased by Day 7 (p for trend = 0.11). Furthermore, the mean percentage of basophils, though lacking statistical significance, exhibited a slight decrease until Day 7 in the Grade 2 group, whereas the Grade 1 group experienced marginal recovery by Day 7 (p for trend = 0.21).

In the serological analysis, potassium levels on Day 3 were significantly higher in the Grade 2 group than in the Grade 1 group. Additionally, although haptoglobin and CRP showed no statistical significance based on AEs, the Grade 2 group exhibited a more pronounced increase on Day 3 than the Grade 1 group (Figure 3 and Supplementary Table 3). These findings indicate the relationship between hematological and serological changes and the severity of AEs following COVID-19 mRNA vaccination.

Analysis of anti-PEG antibody levels following mRNA or conventional vaccine administration and their associations with AEs

To explore the potential association between AEs and anti-PEG antibodies, the levels of anti-PEG IgG and IgM antibodies in human plasma were assessed using an established ELISA method. Plasma samples were collected from the 60 participants just before vaccination with the mRNA-LNP vaccine and on Days 3 and 7 post-vaccination and were analyzed for anti-PEG IgG and

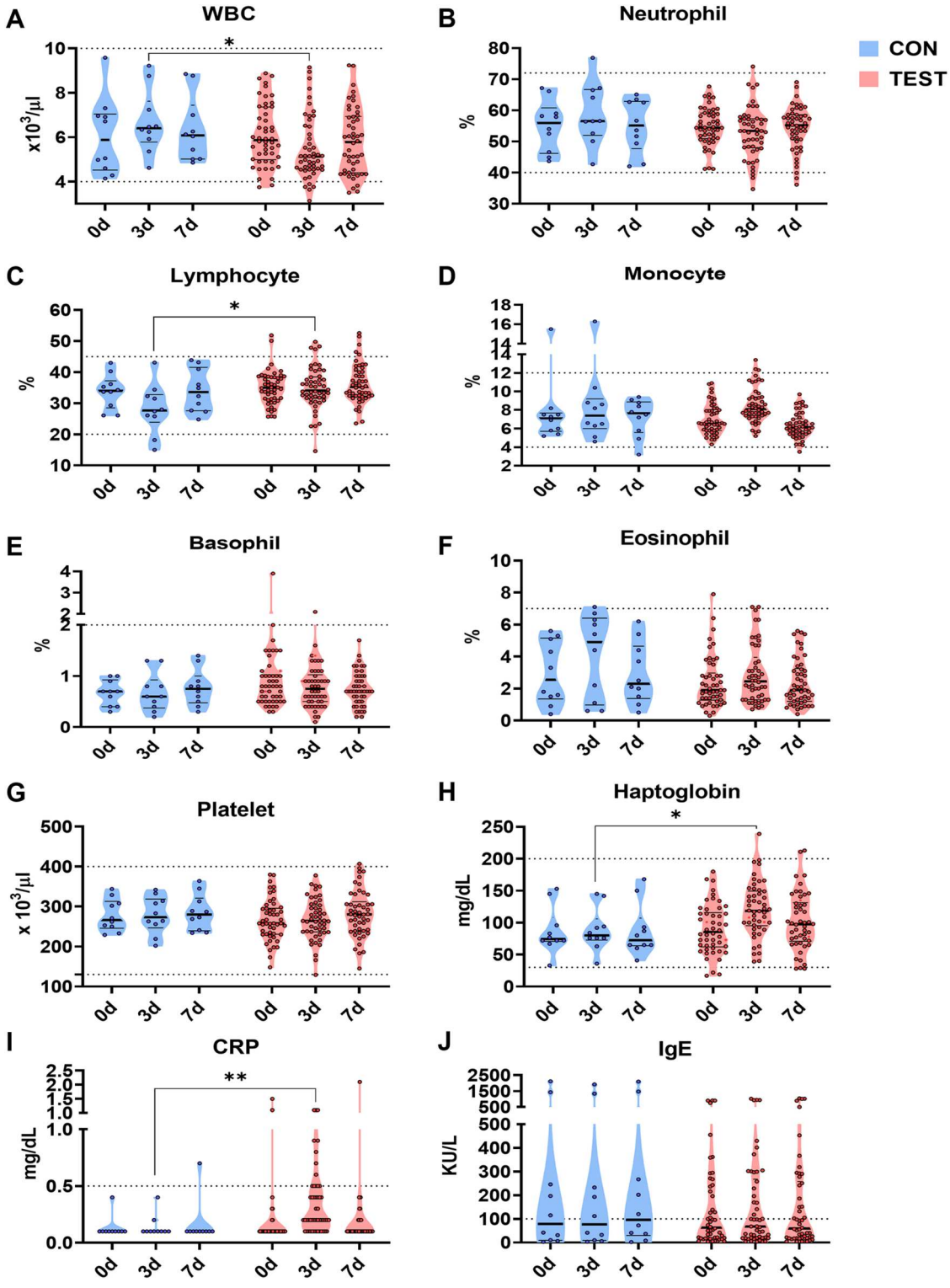


Figure 2. Hematological and serological parameters distribution and median values for mRNA and protein vaccine groups. The analysis used EDTA blood and serum samples from 60 healthy participants before and 3 and 7 days after vaccination who were divided into a test (n = 50) and control group (n = 10). CON, Meningococcal protein conjugate vaccine (Menveo) immunized group; COVID-19, coronavirus disease 2019; mRNA, messenger ribonucleic acid; TEST, COVID-19 mRNA vaccine (BNT162b2 or mRNA-1273) immunized group. Data are shown as the mean ± SEM. Statistical significance was analyzed using the Wilcoxon non-parametric test for comparisons between groups (*$p < 0.05$, **$p < 0.01$ vs CON). Analyses were performed using SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, NC, USA).

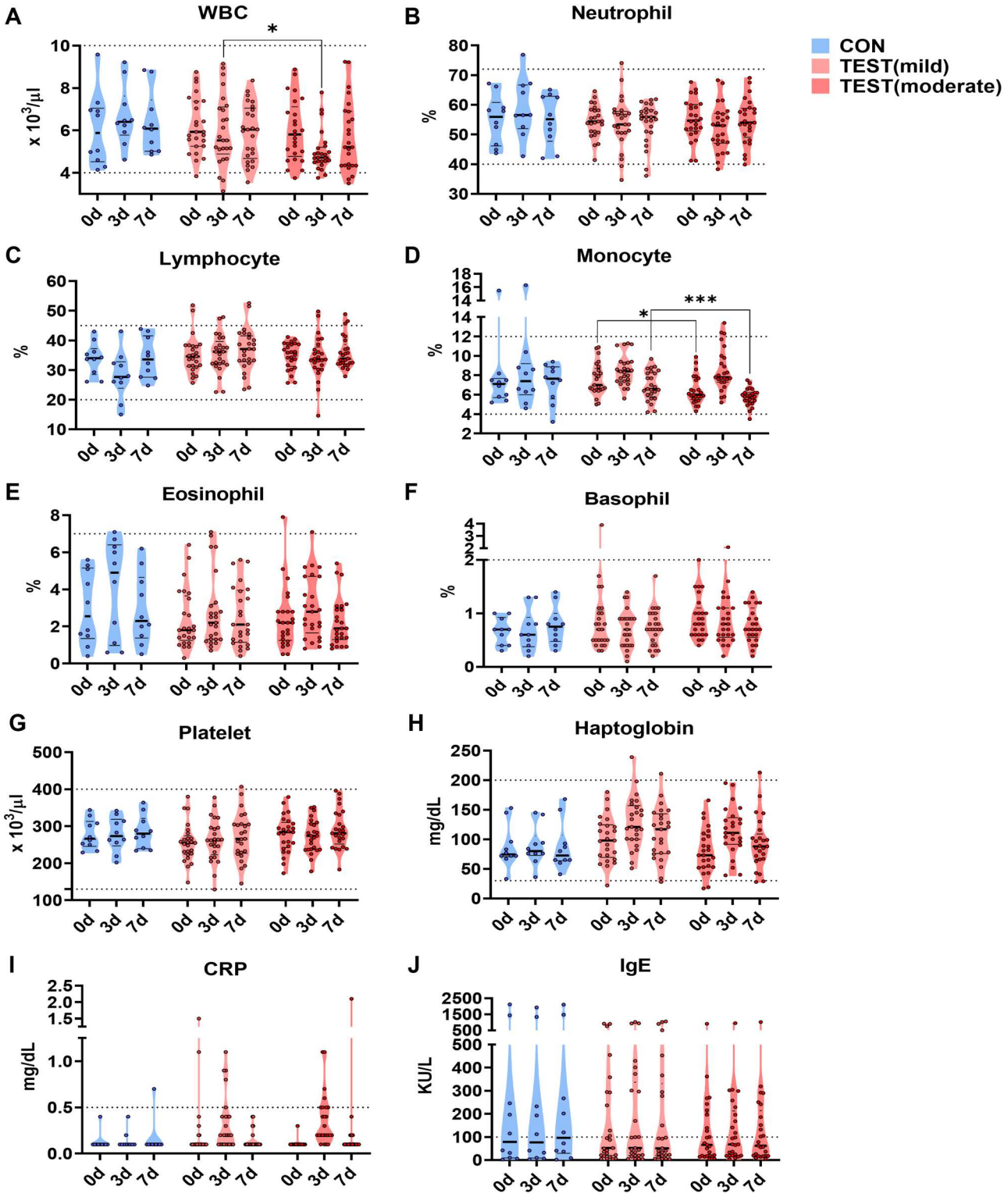


Figure 3. Hematological and serological parameter distributions and median values based on reported symptoms of mRNA and conventional vaccine recipients. The analysis used EDTA blood and serum samples from 60 healthy participants before vaccination and 3 and 7 days after vaccination. The 60 participants were divided into test (n = 50) and control groups (n = 10), and the test group (n = 50) was divided into Grade 1 (n = 25) and Grade 2 (n = 25) according to the severity of AEs. TEST (Grade 1), COVID-19 mRNA vaccine (BNT162b2 or mRNA-1273) immunized group with mild or no AEs; TEST (Grade 2), COVID-19 mRNA vaccine (BNT162b2 or mRNA-1273) immunized group with moderate AEs; CON, Meningococcal protein conjugate vaccine (Menveo) immunized groups. COVID-19, coronavirus disease 2019; mRNA, messenger ribonucleic acid; AEs, adverse events. Data are shown as the mean ± SEM. Statistical significance was analyzed using the Wilcoxon non-parametric test for comparisons between groups (*$p < 0.05$, ***$p < 0.001$ vs Grade 1). Analyses were performed using SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, NC, USA).

IgM antibodies. The levels of anti-PEG IgG and IgM antibodies increased on Days 3 and 7 after mRNA vaccine administration, respectively (Figure 4A and B). Additionally, the levels of anti-PEG IgG and IgM antibodies were slightly increased in the Grade 2 group compared with those in the Grade 1 group (Figure 4C and D).

A slight yet consistent rise in PEG-specific IgG (Day 3) and IgM (Day 7) was observed after mRNA vaccination, with mean fold changes of 2.00 (range, 0.04–33.54) and 1.34 (range, 0.50–2.40), respectively (Figure 4E and F). In the test group, four outliers exhibited anti-PEG IgG levels with a >3-fold or <0.1-fold change after mRNA vaccination, whereas the anti-PEG IgM antibody levels were within the normal range. Three outliers experienced AEs with severity levels exceeding 60 points after mRNA vaccination. Excluding these outliers, 26% (13 participants) showed an increase in anti-PEG IgG levels by >1.3-fold, and 22% (11 participants) showed an increase in anti-PEG IgM levels by >1.3-fold, concurrently with moderate AEs (>10 points) after mRNA vaccination (indicated in the blue-colored portion of Figure 4E and F). Notably, among these participants, 69% (9 of 13) and 72% (8 of 11) were in their 20s. This suggests that a significant proportion of younger individuals in their 20s exhibited both higher anti-PEG antibody levels and moderate AEs after mRNA vaccination.

In contrast, the control group did not show a similar increase in anti-PEG antibodies, and the severity of AEs in this group was not associated with the blue-colored portion in the figures. Collectively, these findings imply a potential role of anti-PEG antibodies in modulating the vaccine response and AE profiles following mRNA vaccination, particularly in younger individuals. The observed correlation highlights the need for further investigation into individual immune responses and vaccine safety.

Proteomic analysis of blood samples following mRNA or conventional vaccination

Hematological and serological analyses confirmed that COVID-19 mRNA vaccination significantly reduced WBC count. Moreover, it led to an elevation in haptoglobin and CRP proteins, indicating a distinct inflammatory response compared to meningococcal protein conjugate vaccination. To further elucidate this response, plasma samples were subjected to proteomic analysis to identify specific mediators of inflammation. Participants under 35 were selected from the control (n=10) and test (n=25) groups for robust data analysis. Bioinformatics analysis revealed a significant increase in several proteins on Day 3 in the mRNA vaccine group (FDR < 0.05). Notably, among the 267 analyzed proteins, nine were predominantly complement or acute-phase proteins, recognized factors associated with inflammation (Figure 5A-I). These findings highlight the intricate immune response triggered by mRNA vaccination, emphasizing the role of inflammatory mediators in vaccine-induced immune reactions.

Correlation analysis of the nine proteins selected from proteomic analysis

A STRING network analysis was conducted to explore potential biological functions correlated among the nine identified proteins including complement C1r subcomponent (C1R), complement factor B (CFB), complement component C9 (C9), haptoglobin (HP), lipopolysaccharide-binding protein (LBP), leucine-rich alpha-2-glycoprotein (LRG1), alpha-1-acid glycoprotein (ORM1), serum amyloid A-1 protein (SAA1), and serum amyloid A-2 protein (SAA2). These proteins have diverse functions. C1R, CFB, and C9 are involved in the complement system, which plays a crucial role in immune defense and inflammation. HP and ORM1 are acute-phase proteins involved in modulating immune responses. LBP is important for pathogen recognition, and LRG1 is associated with inflammation and immune regulation. SAA1 and SAA2 are involved in the inflammatory response and lipid metabolism (Supplementary Table 4). The results unveiled numerous correlations, indicating the likely co-expression of these proteins. Particularly noteworthy was the interaction of C9 with HP and ORM1, both acute-phase proteins, underscoring their pivotal role in linking complement components to the inflammatory response. Analyzing the edges connecting these proteins revealed multiple co-expressing partners, supported by text-mining data derived from gene/protein name co-occurrences in abstracts, highlighting significant correlations. Moreover, experimentally determined edges between C9, C1R, CFB, HP, and LRG1 further validated these interactions (Figure 6A). K-means clustering with a value of 3 identified three clusters: complement components, serum amyloid A proteins, and other proteins. HP and ORM1 appeared as intermediary proteins with multiple interactions (Figure 6B). Applying the Markov Cluster Algorithm (MCL) with an inflation parameter revealed cluster 2 comprising C1R and CFB, cluster 3 comprising C9, and cluster 1 comprising other proteins. Notably, cluster 1, excluding complement proteins, exhibited a statistically significant average local clustering coefficient of 0.9 and a PPI enrichment *p*-value of 1.0e-16. HP and ORM1 showed a higher combined co-expression score than other proteins (Figure 6C). Furthermore, an increase in complement proteins beyond the nine

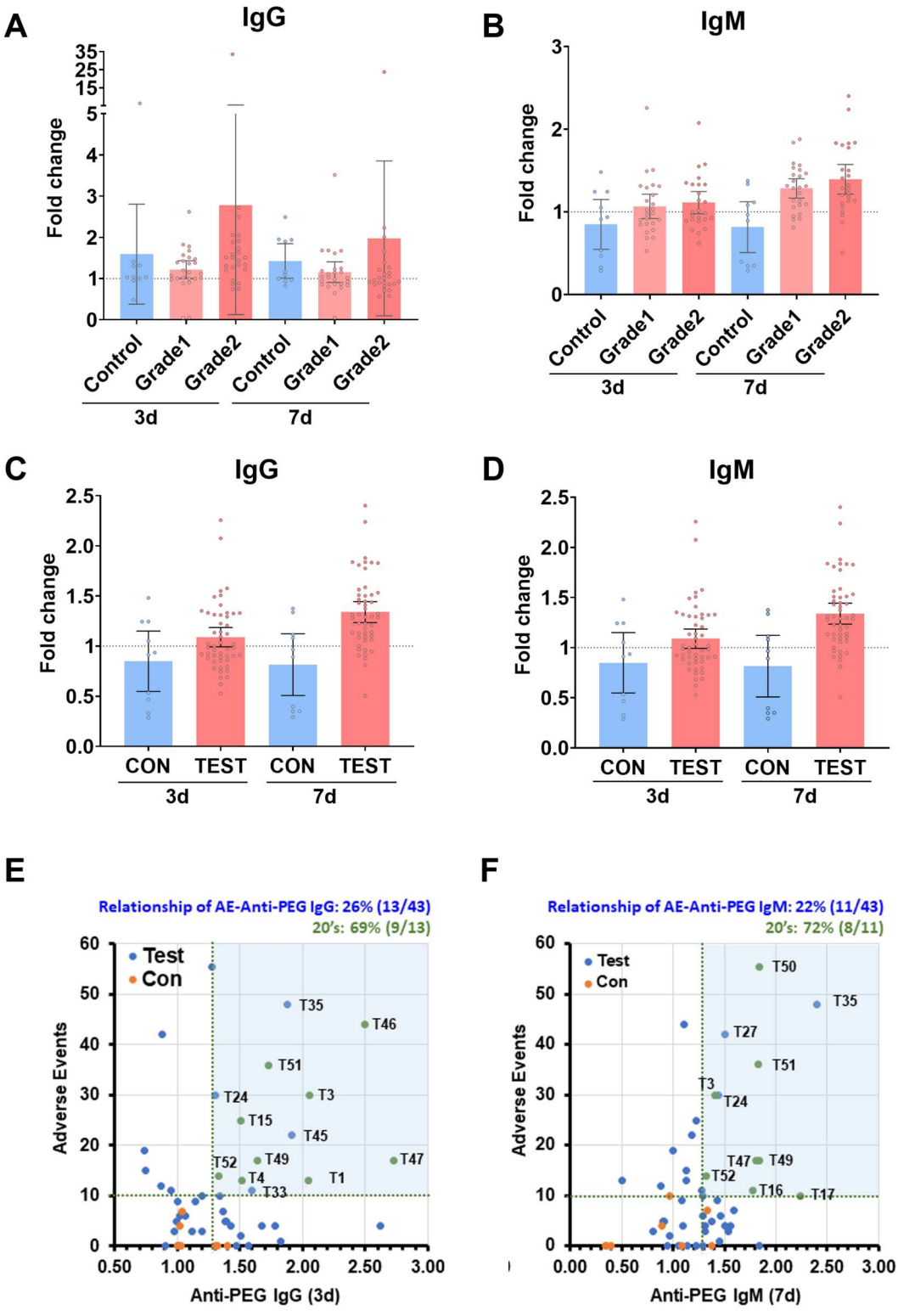


Figure 4. Relationship between adverse events and anti-PEG antibodies. (A and B) Comparison of plasma anti-PEG IgG and IgM levels before (0 d) and after (3 and 7 d) administering mRNA vaccines (COVID-19) and the conventional protein conjugate vaccine (meningococcal vaccine). The anti-PEG levels at 3 d and 7 d are expressed as the fold normalized to the level at 0 d. Sixty participants were divided into the test (n = 50) and control groups (n = 10). (C and D) Relationship between the fold change of anti-PEG IgG detected at 3 d and the severity score of AEs. (E and F) Relationship between the fold change of anti-PEG IgM detected at 7 d and the severity score of AEs. Con, Meningococcal protein conjugate vaccine (Menveo) immunized group; COVID-19, coronavirus disease 2019; Ig, immunoglobulin; mRNA, messenger ribonucleic acid; PEG, polyethylene glycol; Test, COVID-19 mRNA vaccine (BNT162b2 or mRNA-1273) immunized group; AEs, adverse events.

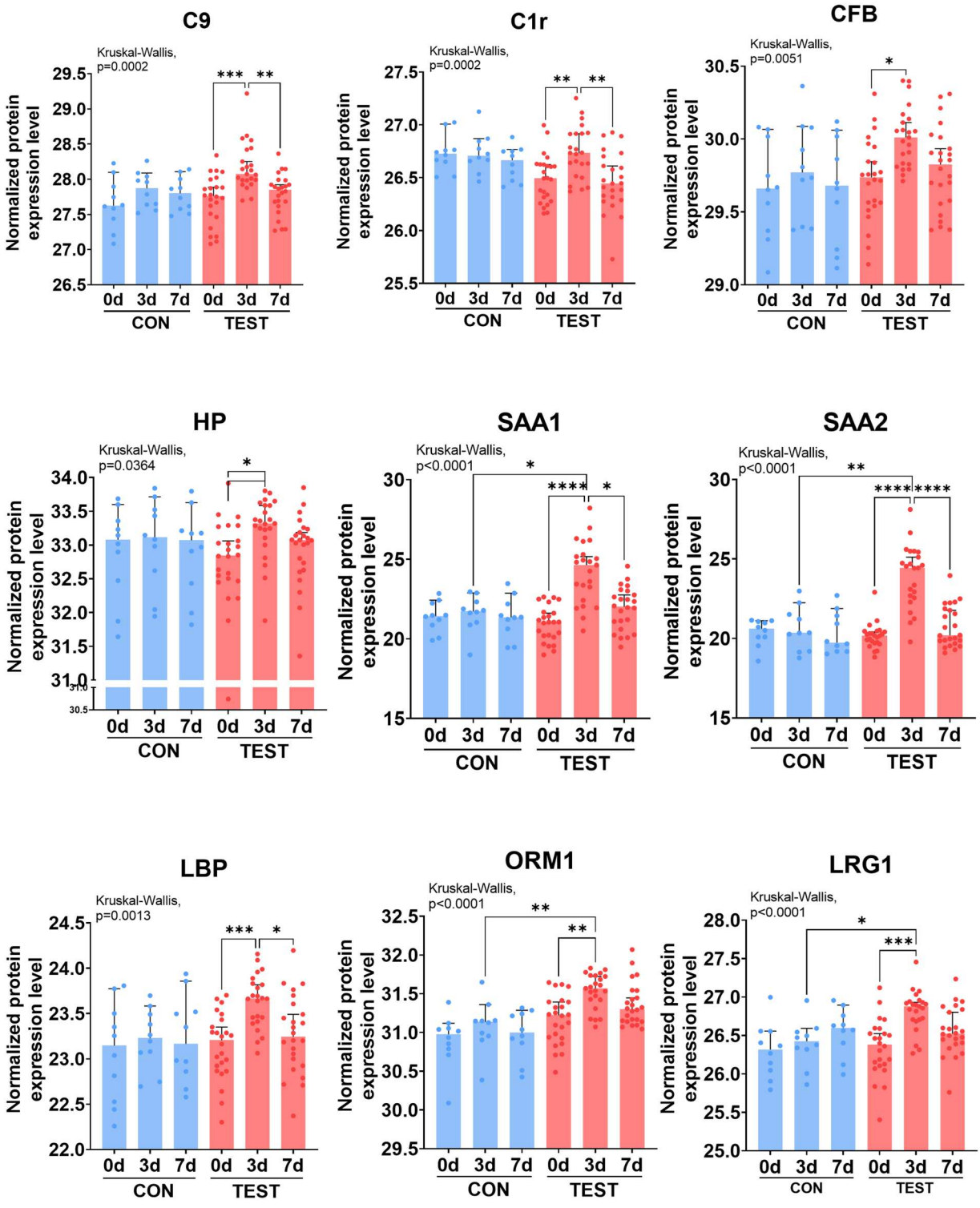
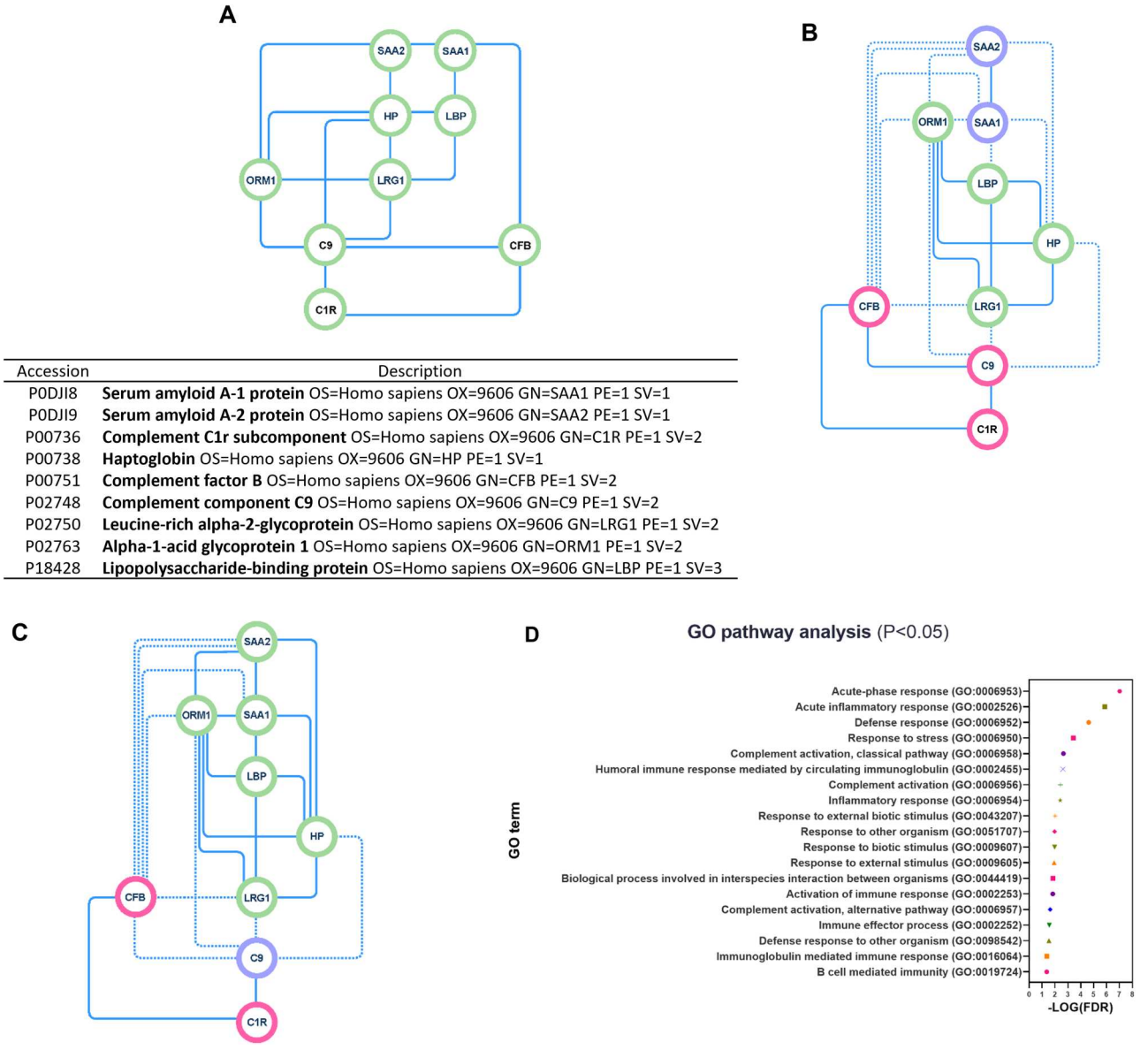


Figure 5. Comparison of differentially expressed proteins between the mRNA vaccine and protein-conjugate vaccine groups. Proteome analysis was performed using LC-MS, and the proteomic profile was statistically analyzed using bioinformatics. Plasma samples were obtained prior to vaccination and on Days 3 and 7 following the vaccination. For statistical significance, study participants from the test group were chosen based on their age, specifically those under 35 years of age. To identify the significantly different proteins between the mRNA and protein vaccine groups, the Kruskall – Wallis test was employed using R, and the *p*-value was adjusted using BH FDR. C9, Complement component C9; CFB, Complement factor B; CON, Meningococcal protein conjugate vaccine (Menveo) immunized group (n = 10); COVID-19, coronavirus disease 2019; FDR, false discovery rate; HP, Haptoglobin; LBP, Lipopolysaccharide-binding protein; LC-MS, liquid chromatography-mass spectrometry; LRG1, Leucine-rich alpha-2-glycoprotein; mRNA, messenger ribonucleic acid. C1R, Complement C1r subcomponent; ORM1, Alpha-1-acid glycoprotein; SAA1, Serum amyloid A-1 protein; SAA2, Serum amyloid A-2 protein; TEST, COVID-19 mRNA vaccine (BNT162b2 or mRNA-1273) immunized participants under the age of 35 ($n = 25$).



Accession	Description
P0DJI8	**Serum amyloid A-1 protein** OS=Homo sapiens OX=9606 GN=SAA1 PE=1 SV=1
P0DJI9	**Serum amyloid A-2 protein** OS=Homo sapiens OX=9606 GN=SAA2 PE=1 SV=1
P00736	**Complement C1r subcomponent** OS=Homo sapiens OX=9606 GN=C1R PE=1 SV=2
P00738	**Haptoglobin** OS=Homo sapiens OX=9606 GN=HP PE=1 SV=1
P00751	**Complement factor B** OS=Homo sapiens OX=9606 GN=CFB PE=1 SV=2
P02748	**Complement component C9** OS=Homo sapiens OX=9606 GN=C9 PE=1 SV=2
P02750	**Leucine-rich alpha-2-glycoprotein** OS=Homo sapiens OX=9606 GN=LRG1 PE=1 SV=2
P02763	**Alpha-1-acid glycoprotein 1** OS=Homo sapiens OX=9606 GN=ORM1 PE=1 SV=2
P18428	**Lipopolysaccharide-binding protein** OS=Homo sapiens OX=9606 GN=LBP PE=1 SV=3

Figure 6. Interaction network between differentially expressed proteins using STRING. STRING analysis indicates potential interactions between these nine proteins, including three complement component proteins and four acute phase proteins. Predicted interactions between differentially expressed proteins are denoted by colored lines. (A) Interaction network between differentially expressed proteins by STRING analysis. (B) Analysis using a K-means value of 3 by STRING analysis. (C) Analysis using an MCL value of 7 by STRING analysis. C1R, Complement C1r subcomponent; CFB, Complement factor B; C9, Complement component C9; HP, Haptoglobin; LBP, Lipopolysaccharide-binding protein; LRG1, Leucine-rich alpha-2-glycoprotein; ORM1, Alpha-1-acid glycoprotein; SAA1, Serum amyloid A-1 protein; SAA2, Serum amyloid A-2 protein. (D) Gene Ontology (GO) biological process analysis of differentially expressed proteins. Pathway analysis was conducted by using Protein ANalysis THrough Evolutionary Relationships (PANTHER) database v 6.1 (www.pantherdb.org) in the COVID-19 mRNA vaccine group on Day 3 after immunization. The pathway presented satisfied FDR < 0.05. COVID-19, coronavirus disease 2019; FDR, false discovery rate; mRNA, messenger ribonucleic acid.

pro-inflammatory proteins was observed, although not statistically significant, in the mRNA vaccination group compared to that in the conventional vaccination group (Supplementary Figure 3). To elucidate the association of the nine significantly increased proteins with biological processes, a Gene Ontology (GO) analysis was performed, revealing significant involvement in the acute-phase response, innate immune response (complement activation process), and humoral immune response mediated by circulating immunoglobulins (Figure 6D). Collectively, these findings underscore the intricate network of interactions among pro-inflammatory proteins following mRNA vaccination, emphasizing their role in modulating immune responses and highlighting potential targets for therapeutic interventions.

Discussion

mRNA vaccines are currently one of the most common vaccine platforms used in public health. The side effects of the mRNA vaccines that have already been or are currently being developed are being closely monitored. Such studies are key to advancing the development of safe and efficient mRNA vaccines and the effective management of recipients. Nevertheless, the underlying causes and mechanisms of the side effects remain unclear. In this study, various analyses were performed, encompassing hematological, serological, and proteomic evaluations to select biomarkers and propose mechanisms underlying the AEs of mRNA vaccines.

The mRNA vaccine recipients exhibited a significant decline in WBC count compared with the protein vaccine recipients. Decreased WBC count after vaccination is not a common or expected outcome (Tefferi et al. 2005; Al-Saadi and Abdulnabi 2022; Sing et al. 2022); thus, it could be a sign of an unusual reaction or underlying health issue, even though the count did not reach pathological levels (Liu et al. 2021). These results suggested that mRNA vaccines may influence the bone marrow, thereby highlighting the need for more detailed toxicity studies. Furthermore, the transient increase of CRP and haptoglobin levels in the serological analysis was observed exclusively in the mRNA vaccine recipients. These vital biomarkers, CRP and haptoglobin, are associated with inflammation and the acute phase response, respectively. Although the delineation of pathological serum concentrations remains undefined (Beimdiek et al. 2022), and our results indicated that their levels were within normal ranges, they may potentially be affected by immunogenic responses. These proteins are mainly secreted by the liver and are influenced by LNPs, which serve as vehicles for mRNA delivery. Carrier molecules migrate to the liver immediately after vaccination (Hou et al. 2021; Johnson et al. 2022). Consistent with our results, CRP levels were abnormally elevated in BNT162b1 mRNA vaccine recipients (Li and Chen 2020; Li et al. 2021). Moreover, CRP was a risk factor for cardiovascular diseases, such as myocarditis, in a healthy population (Anderson et al. 1998).

PEG is a component of LNP for mRNA vaccines; however, it remains unclear whether vaccination enhances anti-PEG antibodies and their impact on blood biomarkers. One study reported that four anti-PEG IgE-positive patients had received a second dose of the mRNA COVID-19 vaccine, and all tolerated it without allergic reactions (Mouri et al. 2022). Zhou et al. also found no positive correlation between anaphylaxis and anti-PEG IgG positivity; however, anti-PEG IgM positivity was higher in anaphylaxis cases than in the controls for Pfizer-BioNTech vaccination (Zhou et al. 2023). In contrast, anti-PEG IgG was detected in 10 of 11 anaphylaxis cases and in none of the three controls (Warren et al. 2021). Consistently, in our study, anti-PEG IgG and IgM levels were increased in the Grade 2 group, which had moderate AEs. Similarly, Lim et al. reported higher levels of anti-PEG IgG or anti-PEG IgM (anti-PEG IgE was not evaluated) in two out of three patients with suspected anaphylaxis from the Pfizer-BioNTech vaccine (BNT162b2) compared with the control levels (Lim et al. 2021).

The proteomic plasma analysis revealed a considerable increase in complement proteins, suggesting a potential link to changes in blood composition. The inflammatory response triggered by external pathogens leads to elevated acute-phase proteins in the early stages. Notably, haptoglobin, CRP, and serum amyloid A-1 and A-2 levels showed significant increases on Day 3 after mRNA vaccination compared with those after protein vaccination. These findings align with previous studies, confirming the association between mRNA vaccination and increased acute phase proteins (Barmada et al. 2023). Another potential mechanism linking the PEG antigen–antibody reaction to inflammation involves complement activation (Verhoef et al. 2014). Complement is a protein produced in the liver and circulating in the blood that functions in the innate immune system. The complement cascade results in the binding of complement to antigen–antibody complexes (Zipfel 2009). The complement components C3a, C4a, C5a, and others produced during this process are called anaphylatoxins and activate mast cells and eosinophils, inducing the release of histamine and other vasodilator mediators and increasing vascular permeability. This leads to inflammation and, if excessive, can cause allergic reactions or even anaphylaxis (Reber et al. 2017). Consistent with these studies, the proteomic analysis identified increased levels of the complement components C9, CFB, and C1r in mRNA vaccine recipients, suggesting that complement activation contributes to the inflammatory response. Thus, our study suggests a novel contributor to AE mechanisms, whereas other studies have focused mostly on C3a and C5a (Zipfel 2009; Shah et al. 2024; Csuth et al. 2025). Furthermore, this study suggests that the interactions between complement C9, haptoglobin, and alpha-1-acid glycoprotein may play an essential role in the inflammatory response induced by mRNA vaccines. Further research is required to elucidate these potential interactions. Moreover, STRING network analysis revealed significant co-expression and associations between C9, HP, ORM1, C1R, CFB, LBP, LRG1, SAA1, and SAA2 and acute

inflammatory responses, complement activation, and humoral immune response. The observed increase in IgE levels may be attributed to an enhanced allergic response triggered by PEGylated LNPs, leading to immune system sensitization. These results highlight the proteomic changes induced by mRNA vaccines consisting of ribonucleic acids and PEG.

With advancements in diagnostics, these parameters will play a crucial role in tailoring treatment strategies. More specific analyses based on individual protein profiles may enable personalized anti-inflammatory treatments and potentially serve as predictive or prognostic markers for mRNA vaccination side effects, contributing to the safer use of mRNA platforms.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Ethical statement

This study was approved by the Institutional Review Board (IRB) of the Kangnam Sacred Heart Hospital, College of Medicine, Hallym University (IRB approval number, 2022-02-014). Informed consent was obtained from all participants after the nature and possible consequences of the study had been fully explained to them.

Author contribution

Gahyun Roh, Jisun Lee: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. Hyun-Mee Park, Woori Kwak: ¨ Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. Hyo-Jung Park, Ayoung Oh: Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Yewon Na: Software, Methodology, Investigation. Dahyeon Ha, Yu-Sun Lee, Seo-Hyeon Bae, Seonghyun Lee, Subin Yoon, Sowon Lee: Methodology, Investigation. Jaehun Jung: Writing – review & editing, Investigation. Jacob Lee and Jae-Hwan Nam: Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplemental data. Raw data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Jae-Hwan Nam http://orcid.org/0000-0002-0974-4343

References

Al-Saadi EA, Abdulnabi MA. 2022. Hematological changes associated with COVID-19 infection. J Clin Lab Anal. 36(1): e24064. doi:10.1002/jcla.24064.

Anderson JL, Carlquist JF, Muhlestein JB, Horne BD, Elmer SP. 1998. Evaluation of C-reactive protein, an inflammatory marker, and infectious serology as risk factors for coronary artery disease and myocardial infarction. J Am Coll Cardiol. 32(1):35–41. doi:10.1016/s0735-1097(98)00203-4.

Barmada A, Klein J, Ramaswamy A, Brodsky NN, Jaycox JR, Sheikha H, Jones KM, Habet V, Campbell M, Sumida TS, et al. 2023. Cytokinopathy with aberrant cytotoxic lymphocytes and profibrotic myeloid response in SARS-CoV-2 mRNA vaccine–associated myocarditis. Sci Immunol. 8(83): eadh3455. doi:10.1126/sciimmunol.adh3455.

Beimdiek J, Janciauskiene S, Wrenger S, Volland S, Rozy A, Fuge J, Olejnicka B, Pink I, Illig T, Popov A, et al. 2022. Plasma markers of COVID-19 severity: a pilot study. Respir Res. 23(1):343. doi:10.1186/s12931-022-02272-7.

Callaway E. 2023. The next generation of coronavirus vaccines: a graphical guide. Nature. 614(7946):22–25. doi:10.1038/d41586-023-00220-z.

Chaudhary N, Weissman D, Whitehead KA. 2021. mRNA vaccines for infectious diseases: principles, delivery and clinical translation. Nat Rev Drug Discov. 20(11):817–838. doi:10.1038/s41573-021-00283-5.

Chen G, Li X, Sun M, Zhou Y, Yin M, Zhao B, Li X. 2021. COVID-19 mRNA vaccines are generally safe in the short term: a vaccine vigilance real-world study says. Front Immunol. 12:669010. doi:10.3389/fimmu.2021.669010.

Csuth Á, Garvey LH, Jenmalm MC. 2025. COVID-19 mRNA vaccine allergy. J Allergy Clin Immunol. 155(4):1187–1189.

Dolgin E. 2021. How protein-based COVID vaccines could change the pandemic. Nature. 599(7885):359–360. doi:10.1038/d41586-021-03025-0.

Fabregat A, Jupe S, Matthews L, Sidiropoulos K, Gillespie M, Garapati P, Haw R, Jassal B, Korninger F, May B, et al. 2018. The reactome pathway knowledgebase. Nucleic Acids Res. 46(D1):D649–D655. doi:10.1093/nar/gkx1132.

Harris JM, Chess RB. 2003. Effect of pegylation on pharmaceuticals. Nat Rev Drug Discov. 2(3):214–221. doi:10.1038/nrd1033.

Hou X, Zaks T, Langer R, Dong Y. 2021. Lipid nanoparticles for mRNA delivery. Nat Rev Mater. 6(12):1078–1094. doi:10.1038/s41578-021-00358-0.

Johnson LT, Zhang D, Zhou K, Lee SM, Liu S, Dilliard SA, Farbiak L, Chatterjee S, Lin YH, Siegwart DJ. 2022. Lipid nanoparticle (LNP) chemistry can endow unique in vivo RNA delivery fates within the liver that alter therapeutic outcomes in a cancer model. Mol Pharm. 19(11):3973–3986. doi:10.1021/acs.molpharmaceut.2c00442.

Ju Y, Lee WS, Pilkington EH, Kelly HG, Li S, Selva KJ, Wragg KM, Subbarao K, Nguyen TH, Rowntree LC, et al. 2022. Anti-PEG antibodies boosted in humans by SARS-CoV-2 lipid nanoparticle mRNA vaccine. ACS Nano. 16(8):11769–11780. doi:10.1021/acsnano.2c04543.

Khurana A, Allawadhi P, Khurana I, Allwadhi S, Weiskirchen R, Banothu AK, Chhabra D, Joshi K, Bharani KK. 2021. Role of nanotechnology behind the success of mRNA vaccines for COVID-19. Nano Today. 38:101142. doi:10.1016/j.nantod.2021.101142.

Kim SH, Wi YM, Yun SY, Ryu JS, Shin JM, Lee EH, Seo KH, Lee SH, Peck KR. 2021. Adverse events in healthcare workers after the first dose of ChAdOx1 nCoV-19 or BNT162b2 mRNA COVID-19 vaccination: a single center experience. J Korean Med Sci. 36(14):e107. doi:10.3346/jkms.2021.36.e107.

Krammer F. 2020. SARS-CoV-2 vaccines in development. Nature. 586(7830):516–527. doi:10.1038/s41586-020-2798-3.

Lee J, Woodruff MC, Kim EH, Nam JH. 2023. Knife's edge: balancing immunogenicity and reactogenicity in mRNA vaccines. Exp Mol Med. 55(7):1305–1313. doi:10.1038/s12276-023-00999-x.

Li J, Hui A, Zhang X, Yang Y, Tang R, Ye H, Ji R, Lin M, Zhu Z, Türeci Ö, et al. 2021. Safety and immunogenicity of the SARS-CoV-2 BNT162b1 mRNA vaccine in younger and older Chinese adults: a randomized, placebo-controlled, double-blind phase 1 study. Nat Med. 27(6):1062–1070. doi:10.1038/s41591-021-01330-9.

Li L, Chen C. 2020. Contribution of acute-phase reaction proteins to the diagnosis and treatment of 2019 novel coronavirus disease (COVID-19). Epidemiol Infect. 148:e164. doi:10.1017/S095026882000165X.

Lim XR, Leung BP, Ng CY, Tan JW, Chan GY, Loh CM, Tan GL, Goh VH, Wong LT, Chua CR, et al. 2021. Pseudo-anaphylactic reactions to Pfizer BNT162b2 vaccine: report of 3 cases of anaphylaxis post pfizer BNT162b2 vaccination. Vaccines. 9(9):974. doi:10.3390/vaccines9090974.

Liu J, Wang J, Xu J, Xia H, Wang Y, Zhang C, Chen W, Zhang H, Liu Q, Zhu R, et al. 2021. Comprehensive investigations revealed consistent pathophysiological alterations after vaccination with COVID-19 vaccines. Cell Discov. 7(1):99. doi:10.1038/s41421-021-00329-3.

Mingot-Castellano ME, Butta N, Canaro M, Gómez Del Castillo Solano MD, Sánchez-González B, Jiménez-Bárcenas R, Pascual-Izquierdo C, Caballero-Navarro G, Entrena Urena L, Jose Gonzalez-Lopez T, et al. 2022. COVID-19 vaccines and autoimmune hematologic disorders. Vaccines. 10(6):961. doi:10.3390/vaccines10060961.

Moreira, Jr, ED, Kitchin N, Xu X, Dychter SS, Lockhart S, Gurtman A, Perez JL, Zerbini C, Dever ME, Jennings TW, et al. 2022. Safety and efficacy of a third dose of BNT162b2 Covid-19 vaccine. N Engl J Med. 386(20):1910–1921. doi:10.1056/NEJMoa2200674.

Mouri M, Imamura M, Suzuki S, Kawasaki T, Ishizaki Y, Sakurai K, Nagafuchi H, Matsumura N, Uchida M, Ando T, et al. 2022. Serum polyethylene glycol-specific IgE and IgG in patients with hypersensitivity to COVID-19 mRNA vaccines. Allergol Int. 71(4):512–519. doi:10.1016/j.alit.2022.05.007.

Pardi N, Hogan MJ, Porter FW, Weissman D. 2018. mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov. 17(4):261–279. doi:10.1038/nrd.2017.243.

Ranathunge C, Patel SS, Pinky L, Correll VL, Chen S, Semmes OJ, Armstrong RK, Combs CD, Nyalwidhe JO. 2023. Promor: a comprehensive R package for label-free proteomics data analysis and predictive modeling. Bioinform Adv. 3(1):vbad025. doi:10.1093/bioadv/vbad025.

Reber LL, Hernandez JD, Galli SJ. 2017. The pathophysiology of anaphylaxis. J Allergy Clin Immunol. 140(2):335–348. doi:10.1016/j.jaci.2017.06.003.

Sahin U, Karikó K, Türeci Ö. 2014. mRNA-based therapeutics–developing a new class of drugs. Nat Rev Drug Discov. 13(10):759–780. doi:10.1038/nrd4278.

Shah MM, Layhadi JA, Hourcade DE, Fulton WT, Tan TJ, Dunham D, Chang I, Vel MS, Fernandes A, Lee AS, Liu J. 2024. Elucidating allergic reaction mechanisms in response to SARS-CoV-2 mRNA vaccination in adults. Allergy. 79(9):2502–2523.

Sing CW, Tang CT, Chui CS, Fan M, Lai FT, Li X, Wan EY, Wong CK, Chan EW, Hung IF, et al. 2022. COVID-19 vaccines and risks of hematological abnormalities: nested case-control and self-controlled case series study. Am J Hematol. 97(4):470–480. doi:10.1002/ajh.26478.

Tefferi A, Hanson CA, Inwards DJ. 2005. How to interpret and pursue an abnormal complete blood cell count in adults. Mayo Clin Proc. 80(7):923–936. doi:10.4065/80.7.923.

Tenchov R, Sasso JM, Zhou QA. 2023. PEGylated lipid nanoparticle formulations: immunological safety and efficiency perspective. Bioconjug Chem. 34(6):941–960. doi:10.1021/acs.bioconjchem.3c00174.

Tregoning JS, Brown ES, Cheeseman HM, Flight KE, Higham SL, Lemm NM, Pierce BF, Stirling DC, Wang Z, Pollock KM. 2020. Vaccines for COVID-19. Clin Exp Immunol. 202(2):162–192. doi:10.1111/cei.13517.

Verhoef JJ, Carpenter JF, Anchordoquy TJ, Schellekens H. 2014. Potential induction of anti-PEG antibodies and complement activation toward pegylated therapeutics. Drug Discov Today. 19(12):1945–1952. doi:10.1016/j.drudis.2014.08.015.

Wang H, Wang Y, Yuan C, Xu X, Zhou W, Huang Y, Lu H, Zheng Y, Luo G, Shang J, Sui M. 2023. Polyethylene glycol (PEG)-associated immune responses triggered by clinically relevant lipid nanoparticles in rats. NPJ Vaccines. 8(1):169. doi:10.1038/s41541-023-00766-z.

Warren CM, Snow TT, Lee AS, Shah MM, Heider A, Blomkalns A, Betts B, Buzzanco AS, Gonzalez J, Chinthrajah RS, et al. 2021. Assessment of allergic and anaphylactic reactions to mRNA COVID-19 vaccines with confirmatory testing in a US regional health system. JAMA Netw Open. 4(9):e2125524. doi:10.1001/jamanetworkopen.2021.25524.

Watson OJ, Barnsley G, Toor J, Hogan AB, Winskill P, Ghani AC. 2022. Global impact of the first year of COVID-19 vaccination: a mathematical modelling study. Lancet Infect Dis. 22(9):1293–1302. doi:10.1016/S1473-3099(22)00320-6.

Wickham H. 2011. Ggplot2. WIRES Computational Stats. 3(2):180–185. doi:10.1002/wics.147.

Willforss J, Chawade A, Levander F. 2019. NormalyzerDE: online tool for improved normalization of omics expression data and high-sensitivity differential expression analysis. J Proteome Res. 18(2):732–740. doi:10.1021/acs.jproteome.8b00523.

Zhou ZH, Cortese MM, Fang JL, Wood R, Hummell DS, Risma KA, Norton AE, KuKuruga M, Kirshner S, Rabin RL, et al. 2023. Evaluation of association of anti-PEG antibodies with anaphylaxis after mRNA COVID-19 vaccination. Vaccine. 41(28):4183–4189. doi:10.1016/j.vaccine.2023.05.029.

Zipfel PF. 2009. Complement and immune defense: from innate immunity to human diseases. Immunol Lett. 126(1–2):1–7. doi:10.1016/j.imlet.2009.07.005.