In this decentralized, cross-sectional investigation, US researchers from the Yale University School of Medicine examined immunological features in individuals experiencing persistent symptoms of post-COVID-19 vaccination syndrome, a chronic debilitating condition that occurs after COVID-19 vaccination. The authors stressed that, in contrast to long COVID, health authorities do not officially recognize post-COVID vaccination syndrome. It has significantly limited patient care and support.

Since the molecular mechanisms of PVS remain largely unknown, the authors postulated multiple mechanisms that may contribute to long-term symptoms in susceptible individuals. Firstly, vaccine components, such as mRNA, lipid nanoparticles, and adenoviral vectors, trigger the activation of pattern recognition receptors. Thus, unregulated stimulation of innate immunity could lead to chronic inflammation. Secondly, it has been shown that the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein, expressed following BNT162b2 or mRNA-1273 vaccination, circulates in the plasma as early as one day after vaccination. Interaction with full-length S protein, its subunits (S1, S2), and/or peptide fragments with host molecules may result in prolonged symptoms. Also, biodistribution studies in animal models indicate that S protein can cross the blood-brain barrier. Third, vaccine-induced immune responses may trigger autoreactive lymphocytes.

About the study

The study included 64 participants, 42 with PVS with no pre-existing comorbidities and 22 healthy controls who did not report PVS after COVID-19 vaccination. To evaluate the possibility that PVS might result from an undiagnosed, asymptomatic SARS-CoV-2 infection coinciding with the vaccination period, instead of being directly caused by the COVID vaccine administration, researchers compared the immunophenotypic profiles of those with PVS and with or without a history of SARS-CoV-2 infection. They also compared the immunophenotypic profiles of individuals with PVS and vaccinated individuals who did not report PVS. The authors achieved this by profiling circulating immune cells, antibody responses, and immune modulators. They also assessed general health characteristics.

All blood samples were collected between December 2022 and November 2023 from the Listen to Immune, Symptom, and Treatment Experiences Now (LISTEN) study. The authors analyzed peripheral blood mononuclear cells (PBMC), the seropositivity rates for cytomegalovirus (CMV), Epstein-Barr Virus (EBV), Herpes Simplex Virus Type 1 (HSV-1), and Herpes Simplex Virus Type 2 (HSV-2), and whether the SARS-CoV-2 S protein and its S1 subunit could be detected in the plasma.

All participants fulfilled the self-reported General Health Visual Analogue Scale (GHVAS) and the PROMIS-29 scales for physical function, fatigue, pain interference, depression, anxiety, sleep disturbance, and pain.

Results

The cohort with post-COVID-19 vaccination syndrome (PVS) included 42 participants, 29 females and 13 males, with no preexisting comorbidities. The control cohort consisted of 22 participants, 11 from each biological sex.

The information on vaccine type was available for 39 of 42 participants: Comirnaty (Pfizer) received 14 participants, Spikevax (Moderna) 21, and Jcovden (J&J) 4. The most frequent symptoms in individuals with post-COVID-19 vaccination syndrome were excessive fatigue (85%), tingling and numbness (80%), exercise intolerance (80%), brain fog (77.5%), difficulty concentrating or focusing (72.5%), trouble falling or staying asleep (70%), neuropathy (70%), muscle aches (70%), anxiety (65%), tinnitus (60%), and burning sensations (58%).

36% (n=15) participants with PVS and 46% (n=10) control participants reported a history of

one or more previous SARS-CoV-2 infections. Asymptomatic SARS-CoV-2 infections accounted for up to 40% of cases. After serological analysis of high-affinity IgM, IgA, and IgG antibodies to SARS-CoV-2 nucleocapsid (N), the two cohorts were further classified into four subgroups: PVS with no history of infection (n=15), PVS with a history of infection (n=27), controls with no history of infection (n=11) and controls with a history of infection (n=11).

There were no significant age differences between PVS cases and controls and among the four subgroups. The general health status of the PVS participants was far below the general US population average based on the GHVAS scores. The patient-reported outcome scores from PROMIS29 domains were also indicative of lower quality of life. Participants with PVS had lower PROMIS-29 scores for physical function and higher PROMIS-29 scores for anxiety, depression, fatigue, and pain than the control group, regardless of infection status. Median GHVAS scores were also lower in both PVS subgroups than in the control subgroups.

Participants with PVS received significantly fewer COVID-19 vaccine doses than controls (median vaccine number was 2 in PVS *versus* 4 in controls). Following COVID vaccination, the median number of days for the onset of any symptoms was four, while the median number of days for the onset of severe symptoms was ten. Most participants with PVS (70%) developed symptoms within ten days of vaccination.

Differences in circulating immune cell populations

Among cell populations of the myeloid lineage, proportions of non-classical monocytes (CD14lowCD16high) were higher in the PVS cohort than in the controls, without differences in the percentage of total monocytes. The PVS cohort also had a lower median percentage of conventional type 2 dendritic cells (cDC2) than the controls, with no differences in the proportions of conventional type 1 dendritic cells (cDC1). But, comparisons among the subgroups revealed lower proportions of both cDC1 and cDC2 cells in the infection-positive PVS subgroup than in the infection-positive control subgroup.

There were no differences in the percentages of eosinophils in the subgroups. However, the infection-positive PVS subgroup had more neutrophils than convalescent controls.

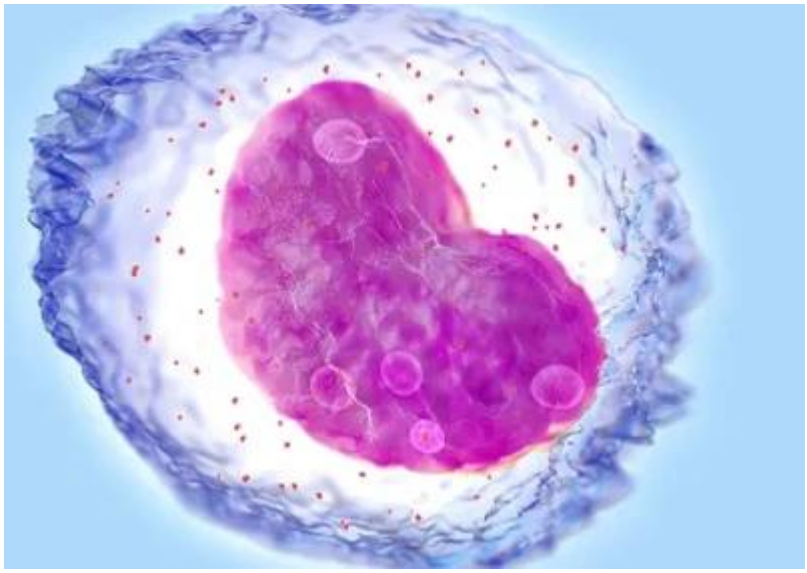
Among B-cell populations, the cohort with PVS had higher relative proportions of unswitched memory B cells (CD19+/CD27+/IgD+) and a lower proportion of double

negative B cells (DN B) than controls.

Among T-cell lineages, the cohort with PVS had lower proportions of effector memory (CD4+ Tem) and resting natural (CD4+ Treg) CD4+ T-cell subsets and higher proportions of exhausted CD8+ T-cells (CD8+ Tex) than controls. CD4+ central memory T-cell population (CD4+ Tcm) and CD4+ exhausted T-cell population (CD4+Tex) did not differ between the PVS cohort and controls. However, the infection-positive PVS subgroup had a higher proportion of CD8+ Tcm cells than the infection-positive controls.

After *in vitro* stimulation, CD4+ T-cells from the cohort with PVS and the infection-positive PVS subgroup had lower CXCR3 expression on the cell surface, lower intracellular IL-4 levels, and lower IL-4/IL-6 levels in combination than the controls.

There were no differences in IFNγ and TNFα levels, but TNFα levels increased in the stimulated CD8+ T-cells in the PVS cohort. Interestingly, there were no differences in immune cell populations between the infection-negative PVS cases and controls.



The presence of autoantibodies

The authors screened for IgM, IgG, and IgA reactivities to 120 autoantigens. The PVS cohort had increased IgM reactivities against 65 antigens, increased IgG reactivity against 1 antigen, and increased IgA reactivities against 39 antigens. The control participants had increased reactivities against 21 antigens, 18 of which were of the IgG isotype and five of

the IgA isotype.

The infection-positive PVS subgroup had higher anti-calprotectin/S100 IgM, anti-genomic DNA IgA, and anti-ssDNA IgA reactivities. The infection-positive controls had higher anti-histone H3 IgG, anti-MBP IgA, and anti-PR3 IgA reactivities.

Differences in circulating hormones and immune modulators

Analysis of circulating hormones and immune modulators revealed lower fetuin A26 and neurotensin levels in participants with PVS. The infection-positive PVS subgroup had lower circulating fetuin A36, neurotensin, and β endorphin levels than the infection-positive control subgroup. No differences were observed in the uninfected subgroups.

Serological evidence of recent EBV reactivation in PVS

The participants with PVS had a higher prevalence of *Epstein-Barr Virus (EBV)* and *Herpes Simplex Virus* coinfection and a lower prevalence of *Epstein-Barr Virus* and *Cytomegalovirus* coinfection than controls. PVS participants also had greater antibody reactivities to two peptides corresponding to two envelope glycoproteins, EBVgp42, and gp350, necessary for B cell infection. Study participants with greater antibody reactivity to gp42, as assessed by ELISA, also exhibited higher percentages of TNFα-producing CD8+ T-cells.

The presence of circulating S1 protein of post-COVID-19 vaccination syndrome

In the infection-positive PVS subgroup, the highest levels of detectable S1 were observed the furthest away from the last known exposure and ranging between greater than 600 to 700 days.

This suggested that prolonged antigen persistence might be associated with post-COVID-19 vaccination syndrome in this subgroup. Most participants in the infection-positive PVS subgroup experienced breakthrough SARS-CoV-2 infections, except for two cases, indicating that PVS symptoms started before infection.

Machine learning-based identification of PVS

To establish a combined global immune signature for persistent symptoms following COVID-19 vaccination, the authors built machine-learning models. Among the features selected, several were negatively associated with PVS, including circulating factors sIL-1R1, fetuin A36, granzyme A and B, FLT−3L and HMGB1, and subsets of circulating CD4 T cell populations (CXCR3+ CD4 T-cells, CD4+ TEMRA cells, and IL-4+/IL-6+ CD4+ T-cells). Multiple hormones and neuropeptides synthesized by the hypothalamus, pituitary glands, and the peripheral nerves involved in nociception and stress responses, such as oxytocin, neurotensin, endorphin, melanocyte-stimulating hormones (MSH), and substance P, were also negatively associated with PVS. The features that were positively associated with PVS were anti-EBV gp42 IgG titers, MMP1 levels, and TNFα+ CD8 T-cells. No single variable or small subset of variables had a particularly strong differentiating power.

Conclusion

This study showed that participants with post-COVID-19 vaccination syndrome had reduced CD4+ T-cell subsets in the circulation (both Th1 and Th2) and increased TNFα levels in CD8+ T-cells. Among the cell populations of myeloid origin, they had reduced cDC2 cells and elevated non-classical monocytes. According to the authors, the lower S protein-specific IgG levels observed in individuals with PVS were mainly due to the limited vaccine doses.

In addition, PVS participants had serological evidence for recent EBV reactivation. Importantly, elevated levels of spike protein (full-length S and S1 subunit) were detected in circulation up to 709 days after vaccination, even in individuals with no evidence of detectable SARS-CoV-2 infection.

The authors concluded that these results highlight potential immune differences in individuals with post-COVID-19 vaccination syndrome that merit further investigation to better understand this condition.

This study has been published on a preprint server and is currently being peer-reviewed.

Journal Reference

Bhattacharjee B, Lu P, Monteiro VS. Immunological and Antigenic Signatures Associated with Chronic Illnesses after COVID-19 Vaccination. medRxiv preprint, posted February 18, 2025. https://doi.org/10.1101/2025.02.18.25322379