In this investigation, researchers at the Yale University School of Medicine, United States, examined the immunological profiles of individuals with persistent symptoms of post-COVID-19 vaccination syndrome (PVS), 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 PVS, although it significantly limits patient care and support.

Since the molecular mechanisms of PVS remain largely unknown, the authors hypothesized about multiple mechanisms that may contribute to long-term symptoms in susceptible individuals. Vaccine components, such as mRNA, lipid nanoparticles, and adenoviral vectors, trigger the activation of pattern recognition receptors. The 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 circulates in the plasma as early as one day after vaccination. The full-length S protein, its subunits (S1, S2), and/or peptide fragments may interact with host molecules. Biodistribution studies in animal models indicate that the S protein can cross the blood-brain barrier. And thirdly, vaccine-induced immune responses may trigger autoreactive lymphocytes.

About the study

The study included participants with PVS and no pre-existing comorbidities, as well as

healthy controls who did not develop PVS after receiving the COVID-19 vaccination. All blood samples were collected between December 2022 and November 2023 as part of the LISTEN study. 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.

To evaluate the possibility that PVS might result from an undiagnosed, asymptomatic SARS-CoV-2 infection that coincided with the vaccination period and not directly caused by COVID vaccine administration, researchers compared the immunophenotypic profiles of participants with PVS, with or without a history of SARS-CoV-2 infection. They also compared the immunophenotypic profiles of vaccinated individuals who developed PVS with those of vaccinated individuals who did not report PVS.

The authors analyzed circulating immune cells, antibody responses, and immune modulators. They also analyzed 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.

Results

The cohort with PVS included 42 participants, 29 females and 13 males, with no pre-existing comorbidities. The control cohort consisted of 22 participants, 11 from each biological sex.

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.

Information on vaccine type was available for 39 of 42 participants: 14 participants had received Comirnaty (Pfizer), 21 had received Spikevax (Moderna), and 4 had received Jcovden (Johnson & Johnson) vaccines. Participants with PVS received significantly fewer COVID-19 vaccine doses than controls (median vaccine number was 2 in PVS *versus* 4 in

controls).

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%). Following COVID vaccination, the median number of days for any symptom onset was four, while the median number of days for the severe symptom onset was ten. Most participants with PVS (70%) developed symptoms within ten days of vaccination.

The general health status, based on the GHVAS scores, of the PVS participants was far below the average for the general US population. Median GHVAS scores were lower in both PVS subgroups than in the controls. The patient-reported scores on 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.

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 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). The infection-positive PVS subgroup had lower proportions of both cDC1 and cDC2 cells than the infection-positive control subgroup.

There were no differences in the percentages of eosinophils between the subgroups. 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.

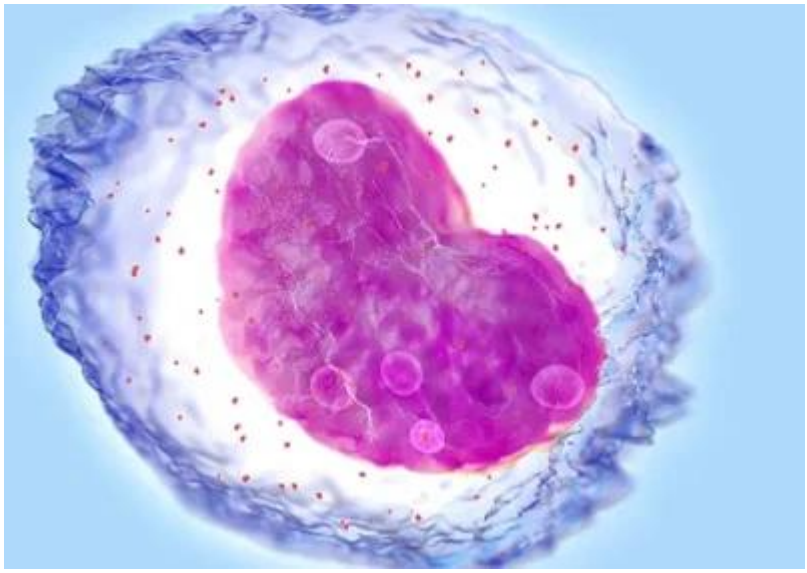
Among T-cell lineages, the PVS cohort had lower proportions of effector memory (CD4+ Tem) and resting natural (CD4+ Treg) CD4+ T-cell subsets and higher proportions of exhausted CD8+ T-cells (CD8+ Tex) than controls. CD4+ central memory T-cell population

(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 expression of CXCR3 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 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 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 the circulating S1 protein in individuals with post-COVID-19 vaccination syndrome

In the infection-positive PVS subgroup, the highest levels of detectable S1 were observed after the longest time interval from the last known exposure (ranging between more than 600-700 days). This suggested that prolonged antigen persistence might be associated with post-COVID-19 vaccination syndrome in this subgroup.

Machine learning-based identification of PVS

Machine-learning models, built to establish a combined global immune signature for persistent symptoms following COVID-19 vaccination, demonstrated that several parameters were negatively associated with PVS, including circulating factors sIL-1R1, fetuin A36, granzyme A and B, FLT−3L, and HMGB1, as well as some 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 titer, MMP1 level, 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 a specific immunological profile with 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.

PVS participants also 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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