BMJ Neurology Open

Altered amyloid plasma profile in patients with disabling headaches after SARS-CoV-2 infection and vaccination

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To cite: Aamodt AH, Ueland T, Boldingh M, *et al*. Altered amyloid plasma profile in patients with disabling headaches after SARS-CoV-2 infection and vaccination. *BMJ Neurology Open* 2025;**7**:e001013. doi:10.1136/bmjno-2024-001013

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/bmjno-2024-001013).

Received 21 December 2024
Accepted 10 August 2025

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ABSTRACT

Background and objectives Persistent headache has emerged as a symptom following acute COVID-19 and, to a lesser extent, after SARS-CoV-2 vaccination. However, the underlying mechanisms remain poorly understood. This study aimed to evaluate plasma levels of amyloid-related biomarkers in patients experiencing persistent headaches after COVID-19 or SARS-CoV-2 vaccination.

Methods In this prospective observational cohort, patients presenting with severe headache as the dominating symptom after COVID-19 (n=29) or SARS-CoV-2 vaccination (n=31) had neurological assessments with reassessments after 6 months. Plasma levels of amyloid precursor protein (APP), pregnancy zone protein (PZP), cathepsin L1 (CTSL) and serum Amyloid A (SAA1) were measured using ELISA and compared with levels in healthy controls (n=16).

Results We found a strong and persistent upregulation of APP in patients with headache after COVID-19 as compared with the two other groups. Notably, APP levels remained elevated at both inclusion and after 6 months in individuals with accompanying cognitive symptoms. In contrast, PZP levels were increased in patients with headache after SARS-CoV-2 vaccination at both time points relative to healthy controls. CTSL was only elevated in the post-COVID-19 at baseline, whereas SAA1 showed levels comparable across all groups.

Conclusion Altered plasma levels of soluble markers, potentially reflecting changes in amyloid processing, were found in patients with persistent headache following SARS-CoV-2 vaccine, particularly in those with persistent headache after COVID-19. In the latter group, we also found some association with cognitive symptoms.

Trial registration numbers NCT04576351 and NCT05235776.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Persistent new-onset headache has been reported in a subset of individuals following COVID-19 and, to a lesser extent, after SARS-CoV-2 vaccination. However, the underlying pathophysiological mechanisms remain largely unclear.

WHAT THIS STUDY ADDS

⇒ This study identified altered plasma levels of soluble markers potentially indicative of disrupted amyloid processing in patients with persistent headache following SARS-CoV-2 vaccine and particularly in those with post-COVID-19 headache accompanied by cognitive symptoms.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These findings highlight the need for long-term monitoring of individuals with new-onset headache after COVID-19 or SARS-CoV-2 vaccination. Such follow-up might also include blood tests for amyloid processing and neuroinflammation. This study identified altered plasma levels of soluble biomarkers potentially related to amyloid processing in patients with persistent headache following SARS-CoV-2 vaccination and, more prominently, in those with post-COVID-19 headache—particularly in individuals reporting concurrent cognitive symptoms.

INTRODUCTION

During the COVID-19 pandemic, headache emerged as a significant health concern, contributing to the overall burden of headache disorders.[1 2] Both infection with SARS-CoV-2 and, to a lesser extent, SARS-CoV-2 vaccination, have been associated with secondary headache disorders and exacerbation of pre-existing primary headache conditions.[1 3] Individuals with primary headache disorders are more likely to experience headache during the acute phase of COVID-19

or following vaccination, compared with the general population.[1] Approximately 20% of patients who develop headache during acute COVID-19 go on to experience persistent headache, often accompanied by cognitive symptoms and fatigue.[1] Similarly, a subset of individual reports chronic headache following SARS-CoV-2 vaccinations.[1 3] Moreover, in those with pre-existing primary headache disorders, both SARS-CoV-2 infection and, to a lesser degree, vaccination have been associated with an increased overall headache burden.[3]

The mechanisms underlying persistent headaches after SARS-CoV-2 infection or vaccines remain poorly understood. One proposed mechanism involves sustained immune activation and inflammation triggered by SARS-CoV-2 or vaccination[4 5] potentially targeting the trigeminovascular system.[1] Additionally, the inflammatory responses during acute COVID-19—characterised by the release of prostaglandins, interleukin-6 (IL-6) and other inflammatory cytokines—may increase blood–brain barrier permeability, leading to neuroinflammation with accompanying headache.[6 7]

Serum amyloid A (SAA1), an acute phase protein and systemic marker of inflammation, was initially identified as a biomarker of poor prognosis in COVID-19.[8] More recently, amyloid precursor protein (APP), a membrane protein considered to play a main role in Alzheimer's disease pathology,[9] was described as a novel receptor for SARS-CoV-2 offering new mechanistic insight into the neuropathological sequelae of COVID-19.[10] Moreover, pregnancy zone protein (PZP), originally recognised for its broad-spectrum immune-suppressive function in pregnancy by inhibiting T-cell activity to prevent fetal rejection, has also been proposed as a biomarker for various inflammatory disorders,[11 12] including COVID-19 prognosis.[13] Notably, elevated serum PZP levels have been observed in women with pre-symptomatic Alzheimer's disease, suggesting relevance to central nervous system involvement.[14]

Furthermore, cathepsin L1 (CTSL), a lysosomal cysteine protease central to intracellular protein degradation, has been shown in preclinical models to facilitate SARS-CoV-2 virus entry into host cells.[15] Intriguingly, CTSL has also been implicated in the degradation of certain amyloid fibrils.[16] Despite these connections, circulating levels of these biomarkers—SAA1, APP, PZP and CTSL—have not yet been characterised in patients experiencing persistent headaches after SARS-CoV-2 infection or vaccination, regardless of whether headache occurred during the acute phase of COVID-19 or shortly after vaccination.

In this study, we aimed to provide novel insight into the pathological mechanisms underlying persistent headache following COVID-19 and SARS-CoV-2 vaccination. We hypothesised that plasma levels of soluble markers—potentially indicative of altered amyloid processing—are related to persistent headache in these contexts, particularly among individuals experiencing accompanying cognitive symptoms. To test this hypothesis, we measured relevant biomarkers in plasma samples from patients with new-onset persistent headache, identified through clinical assessments and validated questionnaires. These levels were compared with those in healthy controls who had experienced COVID-19 or received SARS-CoV-2 vaccination but did not develop headaches. Additionally, biomarker levels were assessed in relation to co-occurring symptoms such as fatigue and cognitive symptoms, and in the COVID-19 group, the requirement for hospitalisation during the acute phase of infection.

METHODS

In this prospective observational cohort study performed at Norwegian neurological departments and led by Oslo University Hospital, Oslo, Norway, clinical characteristics and blood biomarker profiles were assessed in patients with severe headaches for a minimum of 6 months after COVID-19 or SARS-CoV-2 vaccination and compared with healthy controls. The inclusion period lasted from September 2020 to June 2023. We hypothesised that plasma levels of soluble markers that potentially could reflect changes in amyloid processing such as APP, PZP, CTSL and SAA are linked to persistent headaches after SARS-CoV-2 vaccination and COVID-19 in patients with accompanying cognitive symptoms. This hypothesis was tested by longitudinally measuring relevant plasma markers in patients with new-onset persistent headaches, complemented by clinical assessments and validated questionnaires. Differences in plasma levels were compared with healthy controls, and their relationship to cognitive symptoms was a major outcome.

Study population and clinical assessments of the participants

The subgroup with persistent headache following COVID-19 (n=29) was recruited from a cohort of 149 individuals aged ≥18 years who reported ongoing neurological symptoms 6 months after COVID-19. These participants were enrolled in the Norwegian observational multicentre study of nervous system manifestations and sequelae after COVID infection (NNC), a substudy of the ENERGY study led by European Academy of Neurology.[17] The subgroup with persistent headache after SARS-CoV-2 vaccine (n=31) was drawn from the Norwegian observational multicentre study CovaxHEAD: New-onset severe headache after COVID-19 vaccine (NCT05235776). This parallel study led by the same team as the NNC study, collecting comparable clinical and demographic variables.

For both subgroups, inclusion criteria were age ≥18 years and new-onset severe headache, or a marked worsening of pre-existing headache occurring within 1 week after SARS-CoV-2 infection or vaccination. Headache severity had to be sufficiently pronounced that the headaches were daily or almost daily, resulting in significant disability or sick leave and remain the dominant symptom at 6-month follow-up. Exclusion criteria included short-lasting headaches, lack of follow-up assessments or presence of alternative diagnoses more likely to explain the

symptoms (eg, other infections including COVID-19, head trauma or somatoform disorders). Participants in the COVID-19 subgroup who reported headache onset after SARS-CoV-2 vaccine were excluded. None of the individuals in the COVID-19 subgroup experienced vaccine-induced headache, although 24 participants (82.8%) had received a SARS-CoV-2 vaccine. The types of vaccines administered were recorded and included BNT162b2 (BioNTech/Pfizer), mRNA-1273 (Moderna) and ChAdOx1 nCoV-19 (AstraZeneca).

To be included in the study, participants in both headache groups were required to have available plasma samples for biomarker analysis. The CovaxHEAD study included 62 participants across seven Norwegian centres. However, since blood samples for amyloid profiling were only collected at Oslo University Hospital, only participants included from that centre were included. Similarly, the NNC included 149 patients, of whom 46 (30%) presented with headache as the dominant symptom. For the present study, only participants with available plasma samples were eligible, which limited inclusion to patients recruited at Oslo University Hospital.

Cognitive symptoms were assessed using questionnaires from the ENERGY study, conducted by the European Academy of Neurology.[17] In addition, all participants in the group with persistent headache following COVID-19 (n=29) underwent evaluation with the Montreal Cognitive Assessment (MoCA), administered by a trained neurologist. A MoCA score above 26 was considered within the normal range. Patients with chronic post-COVID-19 headaches who reported cognitive symptoms were also evaluated by a psychologist, neuropsychologist or psychiatrist as part of the multidisciplinary headache treatment programme.

Clinical data, including information on headache characteristics, were reviewed by a senior neurologist with expertise in headache disorders (AHA). Headaches were classified according to their clinical phenotype. Clinical visits and first set of blood samples were obtained at the time of inclusion, on average 181.0 days after COVID-19 (SD 145.8) in the group with post-COVID-19 headache and 290.3 days after SARS-CoV-2 vaccine (SD 145.8) in the postvaccine headache group. Both groups were followed up at 6 months, which included clinical assessments and blood sampling.

Controls: Healthy individuals aged over 18 years with no history of migraine or other disabling headache disorders, and no neurological symptoms following either SARS-CoV-2 vaccine or COVID-19. Additional exclusion criteria included presence of immunological disorders or the use of immunosuppressive medication. A total of 16 plasma samples were collected in the period between April and July 2023 from 8 men and 8 women (mean age 40.6 years, SD 11.2). The control group did not undergo detailed questionnaires or neurological, neuropsychiatric or neuropsychological examinations. However, serum antibody assessments revealed high antibody levels in all participants (median 15 831 BAU/mL, minimum 4309, maximum 64 676 BAU/mL) indicating a strong or very strong immune response consistent with prior COVID-19 or SARS-CoV-2 vaccination.

Blood sampling protocol

Peripheral venous blood was collected in pyrogen-free blood collection tubes containing EDTA as an anticoagulant. Samples were immediately placed on melting ice and centrifuged at 4°C at 2500×*g* for 20 min within 60 min to obtain platelet-poor plasma. The plasma samples were stored in multiple aliquots and thawed only once.

Biomarker assessments

Plasma markers were assessed in all three groups—patients with persistent headache after COVID-19 infection (n=29), patients with persistent headache after the COVID-19 vaccine (n=31), and healthy controls (n=16). The participants had blood samples drawn when they were included in the study. Additionally, 6-month follow-up blood sampling was done in the headache groups. Plasma levels of APP (Cat# DY850), PZP (Cat# DY8280-05), CTSL (Cat# DY952) and SAA1 (Cat# DY3019-05) were measured by ELISA using commercially available antibodies (R&D Systems, Minneapolis, Minnesota, USA) in a 384-format using a combination of a SELMA pipetting robot (Analytik Jena AG, Jena, Germany) and a BioTek dispenser/washer (BioTek Instruments, Winooski, VT). Absorption was read at 450 nm by using an EIA plate reader (BioTek Instruments) with wavelength correction set to 540 nm. Intra-assay and interassay coefficients of variation for these analyses were all <10%.

Statistics

Statistical Package for Social Science (IBM SPSS, V.29 for Windows) was used for statistical analysis. Differences in patient characteristics were tested using the χ^2 test or Fisher's exact test for categorical variables, and the Mann-Whitney U test for continuous variables. Prevalence of characteristics (frequency and percentages) was calculated for each group. Plasma marker levels were compared between groups using a multivariate general linear model using markers as dependent, group as fixed and age as covariate. A two-sided p <0.05 was deemed statistically significant.

Patient and public involvement

The user panel at the Department of Neurology, Oslo University Hospital, along with the user organisation 'Hodepine Norge' contributed actively to the project. Hodepine Norge also facilitated public information meetings to raise awareness about the study.

RESULTS

Characteristics and headache phenotypes in the study group

Demographic data including age, gender and comorbidities are presented in table 1. The two headache groups—those with persistent headache following COVID-19 and those with persistent headache after SARS-CoV-2

Table 1 Demographic and clinical characteristics at inclusion

Characteristics	Headaches after COVID-19 n=29	Headaches SARS-CoV-2 vaccine n=31	Control group n=16	P value*
Mean age at inclusion, (SD)	43.5 (11.2)	43.8 (12.4)	40.6 (11.2)	0.836
Female, n (%)	22 (75.9)	24 (77.4)	8 (50.0)	0.862
Education				
Secondary school n (%)	2 (6.8)	7 (22.6)		0.193
College/university ≤4 years, n (%)	14 (48.3)	12 (38.7)		
College/university >4 years, n (%)	13 (44.8)	12 (38.7)		
Hospitalisation during COVID-19, n (%)	9 (31.0)	3 (9.7)	0	0.357
Smoking, n (%)	1 (4.4)	2 (6.5)		0.610
Pre-existing migraine, n (%)	11 (31.0)	12 (38.7)	0	0.871
Comorbidities				
Allergy, n (%)	16 (55.1)	13 (41.9)		0.240
Asthma, n (%)	2 (6.9)	6 (19.4)		0.150
Depression, n (%)	8 (27.6)	10 (32.3)		0.759
Anxiety, n (%)	7 (24.1)	13 (41.9)		0.170
Diabetes, n (%)	0	1 (3.3)	0	0.330
Cardiovascular disease, n (%)	0	1 (3.3)	0	0.330
Stroke, n (%)	0	0	0	
Associated symptoms				
Fatigue, n (%)	22 (75.9)	20 (64.5)		0.204
Subjective cognitive symptoms, n (%)	22 (75.9)	17 (54.8)		0.119
Sick leave at first visit, n (%)	21 (72.4)	18 (58.1)	.	0.244
Sick leave at 6-month follow-up, n (%)	19 (65.5)	14 (45.2)		0.207

*Comparison of the two headache groups.

vaccination—were similar in terms of age and sex distribution, with three out of four participants being female. Common comorbidities in both groups included pre-existing migraine, allergies, anxiety and depression. Cardiovascular and metabolic disorders were infrequent. Among patients with persistent headache after COVID-19, one-third had been hospitalised during the acute phase of infection. In the postvaccination headache group, 17 participants (54.8%) reported a prior COVID-19 at baseline; however, none had experienced persistent headache following the infection (table 1).

Chronic migrainous headache was the most prevalent headache phenotype in both headache groups, followed by tension-type headache and episodic migrainous headache (figure 1). In the post-COVID-19 group, cranial neuralgia—often accompanied by painful paraesthesia in the extremities—was the dominant phenotype in five participants (17%).

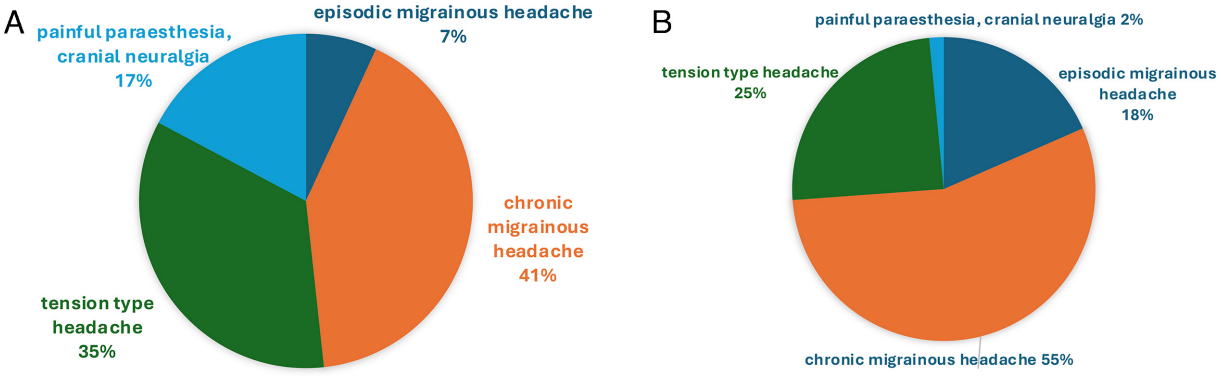

Figure 1 Phenotype of persistent headache reported after COVID-19 (A) and SARS-CoV-2 vaccine (B).

At inclusion, more than two-thirds of the participants with headache post-COVID-19 headache and over half of those with postvaccination headache were on sick leave due to the current symptoms. There was only a modest reduction in sick leave rates at the 6-month follow-up: from 72.4% to 65.5% in the post-COVID-19 group and from 58.1% to 45.2% in the postvaccination group (table 1). Subjective cognitive symptoms and fatigue were common, though not dominating features (see Methods), in the headache groups (table 1). All the patients in the post-COVID-19 group scored within the normal range of MoCA, except one patient who had experienced critical illness myopathy during the acute COVID-19 and scored 24 at follow-up. Patients with chronic headaches after COVID-19 vaccine were assessed by a psychologist, neuropsychologist or psychiatrist as part of the multidisciplinary headache treatment. Among them, three were diagnosed with depression, one with functional neurological disorder in the context of worsening chronic migraine, while two had no identifiable psychiatric disorders.

In the group with new-onset headache following SARS-CoV-2 vaccine, 34.5% reported the headache starting after the first dose, 41.4% after the second dose and 24.1% reported the headache to start after the third dose. Although BNT162b2 (Pfizer-BioNTech) was the most widely used vaccine overall, 89.7% of participants reported headache onset following RNA-1273 (Moderna), 10.3% after BNT162b2 and none after ChAdOx1 nCoV-19 (AstraZeneca).

Biomarkers in post-COVID-19 headache and headache following SARS-CoV-2 vaccination

At both inclusion and 6-month follow-up, we found a strong and persistent upregulation of APP in patients with headache after COVID-19 as compared with individuals with vaccine-induced headache and healthy controls (figure 2A). In contrast, CTSL was upregulated in patients with headaches following vaccine compared with those with post-COVID-19 headache at baseline; however, this difference was not observed at the 6 months follow-up (figure 2B). Plasma levels of PZP were elevated in patients with headache following SARS-CoV-2 vaccination at both inclusion and at follow-up, compared with healthy controls. In the post-COVID-19 headache group, a more modest increase in PZP was observed, limited to baseline measurement (figure 2C). For SAA1, there were no differences between the two headache groups or in comparison to healthy controls either, either at baseline or during follow-up (figure 2D). Although post-COVID-19 symptoms have previously been associated with severity during acute illness,[18] we found no differences in biomarker levels between patients who had been hospitalised and those who had not during the acute phase of COVID-19 (online supplemental table 1).

Biomarker levels in relation to fatigue and cognitive symptoms

In the group with persistent headache following COVID-19, participants reporting cognitive symptoms showed a

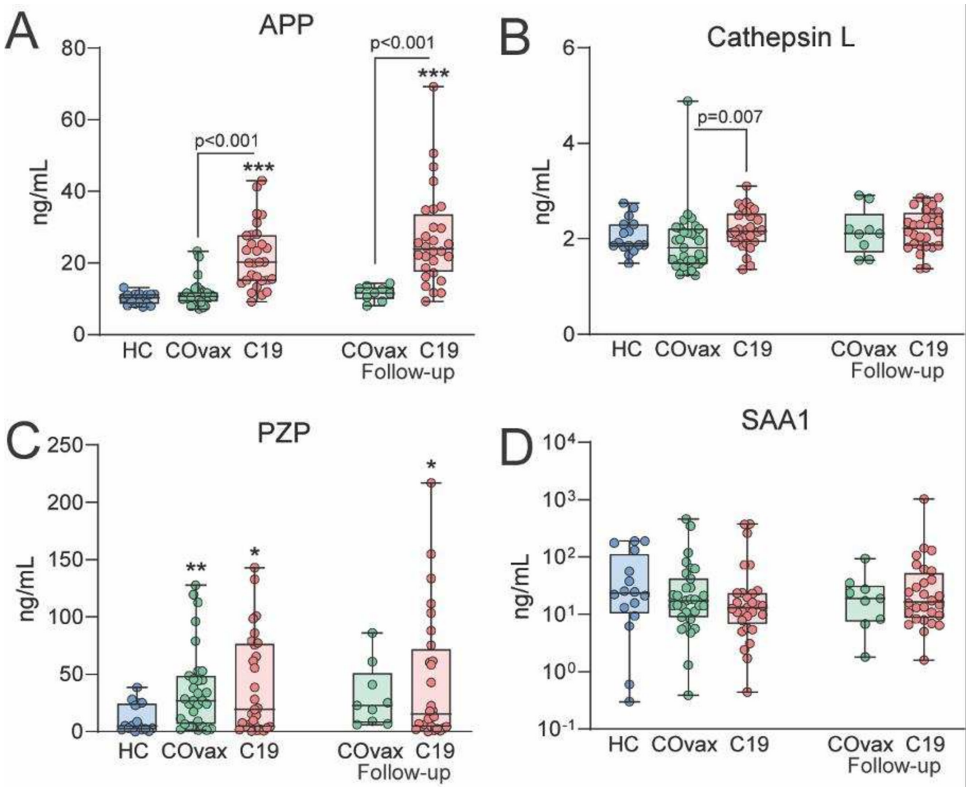

Figure 2 Plasma levels of (A) amyloid precursor protein (APP), (B) cathepsin L, (C) pregnancy zone protein (PZP) and (D) serum amyloid A (SAA1) in healthy controls (HC) (n=16), participants with persistent headache after SARS-CoV-2 vaccine (COvax) (n=31) and participants with persistent headache after COVID-19 (C19) (n=29).

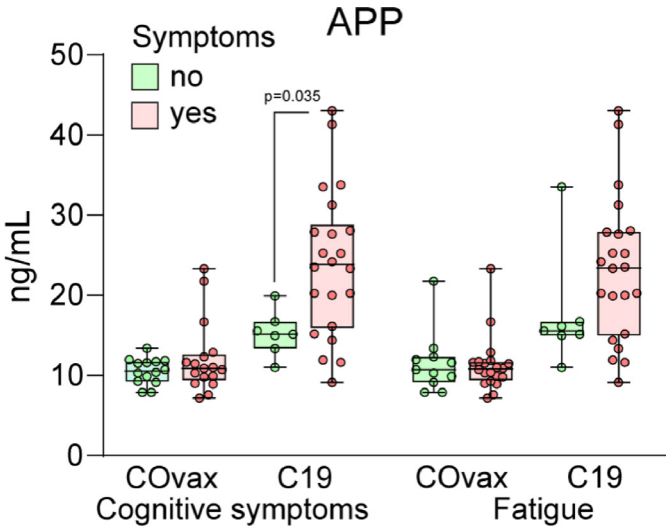

Figure 3 Plasma levels of amyloid precursor protein (AAP) in relation to cognitive symptoms and fatigue in those with persistent headache after SARS-CoV-2 vaccine (COvax) or COVID-19 (C19).

significant upregulation of APP. This association was not observed in those with headache following SARS-CoV-2 vaccination (figure 3). Although APP levels were also higher among participants with fatigue compared with those without, the difference did not reach statistical significance (figure 3). For PZP and CTSL, no differences were observed between participants with or without associated cognitive symptoms or fatigue, in either the post-COVID-19 or postvaccination headache groups (table 2).

DISCUSSION

Persistent headache has been reported following acute COVID-19 and to some extent also after SARS-CoV-2 vaccination.[1 3] In this study, we observed higher plasma levels of APP in patients with persistent headache after COVID-19, but not in those with persistent headache following SARS-CoV-2 vaccination, when compared with healthy controls. Notably, APP levels remained elevated at 6-month follow-up and were associated with subjective cognitive symptoms. In contrast, plasma levels of CTSL were higher in patients with headaches following SARS-CoV-2 vaccination than in those with post-COVID-19 headache. Furthermore, PZP levels were persistently elevated in patients with headache after SARS-CoV-2 vaccination, while only a modest, transient increase was observed in those with post-COVID-19 headache. To our knowledge, this is the first report linking proteins involved in amyloid formation to persistent headache following COVID-19, and CTSL and PZP to persistent headache after SARS-CoV-2 vaccination.

As depicted in figure 4, our findings suggest a plausible mechanism for the serum APP and PZP proteins in the pathophysiology of COVID-19 and SARS-CoV-2 vaccine-induced headache. In brief, APP is primarily produced by neurons and undergoes proteolytic processing by various secretases, resulting in generation of a soluble fragment (sAPP) and release of the neurotoxic amyloid-β (Aβ) peptide. SARS-CoV-2 infection may upregulate APP expression via interactions with the ACE2 receptor[19 20] and APP itself has been shown to facilitate virus entry into cells, thereby enhancing Aβ-associated pathology.[10]

Table 2 Median biomarker levels (ng/mL) with IQR of cathepsin L, pregnancy zone protein (PZP) and serum amyloid A (SAA1) in relation to (A) subjective cognitive symptoms and (B) fatigue in participants with persistent headache following SARS-CoV-2 vaccine (COvax) (n=31) and participants with persistent headache following COVID-19 (C19) (n=29)

Biomarker levels	Headache group	No subjective cognitive symptoms (IQR)	Subjective cognitive symptoms (IQR)	P value
A				
Cathepsin L	COvax	1.6 (1.5, 2.1)	2.0 (1.5, 2.2)	0.451
	C19	2.2 (1.9, 2.7)	2.2 (1.9, 2.5)	0.760
PZP	COvax	36.5 (4.7, 52.9)	24.2 (9.1, 37.9)	0.889
	C19	19.3 (4.3, 79.1)	17.2 (4.2, 75.8)	0.308
SAA1	COvax	17.7 (10.8, 42.0)	17.3 (8.2, 29.1)	0.165
	C19	23.3 (9.2, 72.4)	12.0 (5.7, 19.7)	0.575
Biomarker levels	**Headache group**	**Fatigue (IQR)**	**No fatigue reported (IQR)**	**P value**
B				
PZP	COvax	10.7 (9.2, 12.4)	10.8 (9.5, 11.7)	0.397
	C19	15.6 (15, 16.7)	23.5 (15.2, 27.9)	0.683
Cathepsin L	COvax	26.8 (4.7, 48.7)	27.7 (7.8, 49.3)	0.967
	C19	15.4 (4.2, 77.4)	24.3 (5.5, 75.8)	0.646
SAA1	COvax	2.9 (2.49, 3.46)	2.4 (1.97, 2.82)	0.088
	C19	2.4 (1.06, 3.25)	3.3 (1.96, 5.54)	0.248

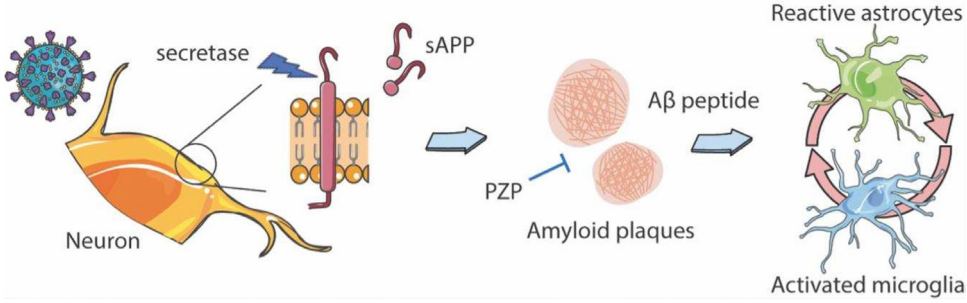

Figure 4 Possible mechanisms of soluble amyloid precursor protein (sAPP) and pregnancy zone protein (PZP) in relation to COVID-19 and SARS-CoV-2 vaccination associated headache. The authors wish to acknowledge SERVIER Medical Art (WWW.Servier.fr) for use of their medical art kits when making the illustration in the article.

The accumulation of Aβ peptides can lead to amyloid plaque formation,[21] which in turn activates astrocytes and microglia, triggering neuroinflammation through multiple mechanisms.[22] This neuroinflammatory response may potentially contribute to the development or persistence of headache symptoms.[23]

Increased levels of APP have been reported as a biomarker for preclinical Alzheimer disease; however, the biological functions of APP extend beyond amyloid formation[24] and elevated levels have been found in various metabolic and cardiovascular disorders.[25] Like in some other viruses, amyloids are identified in the SARS-CoV-2 proteins, that is, the structural spike, the nucleocapsid and the accessory ORF6/ORF10 proteins.[26] Several studies have reported worsening of pre-existing dementia including Alzheimer's Disease and the onset of cognitive impairment following SARS-CoV-2 infection. However, the underlying mechanisms remain incompletely understood.[26–28] Notably, a recent study investigating the relationship between COVID-19 and Alzheimer's disease demonstrated that changes in blood biomarkers were linked to brain structural imaging patterns associated with Alzheimer's disease, lower cognitive test scores and poorer overall health evaluations.[26–29]

In the present study, few or none of the participants had clinically verified comorbidities such as dementia or major neurodegenerative conditions. Nevertheless, we observed significant upregulation of APP among participants with accompanying cognitive symptoms in the group with persistent headache following COVID-19, compared with those without such symptoms. Supporting this, Camacho *et al* reported increased APP expression in brain microvascular endothelial cells in network analyses of SARS-CoV-2 infection.[19] Additionally, a previous study investigating patients with COVID-19 associated neurological syndromes found evidence of impaired amyloid processing with significantly reduced levels of sAPP in cerebrospinal fluid (CSF) from patients compared with controls.[30] However, the study primarily included patients with severe neurological diseases such as Guillain-Barré syndrome and encephalopathy and only one patient was described with central pain syndrome.[30] Furthermore, cognitive symptoms were not reported. At present, however, the reason for this as well as the consequence of the raised APP in patients with persistent headache following COVID-19 is elusive, and follow-up studies are clearly needed.

Recent studies suggest that serum biomarkers traditionally associated with cognitive impairment when measured in CSF are also linked to cognitive impairment when measured in serum.[31] Thus, Urbano *et al* found a positive correlation between neurofilament light chain protein levels in serum and CSF in patients with neurocognitive disorders.[31] Moreover, a recent study revealed that the changes in blood biomarkers were linked to brain structural imaging patterns associated with Alzheimer's disease, as well as lower cognitive test scores. Several of these proteins such as Tau, are known to be associated with Alzheimer's disease when detected in CSF.[29] To the best of our knowledge, whether a similar pattern will be seen for APP and PZP has not been studied.

Although a modest increase in the COVID-19-related headache group at inclusion, plasma levels of PZP were persistently elevated only in those with prolonged headache following SARS-CoV-2 vaccination. The reason for this pattern remains unclear. PZP is known to promote a beneficial immunosuppression during pregnancy to prevent rejection of the fetus. More recently, increased PZP levels have been observed during airway infections[12] and have been suggested as a marker of certain cancers.[32] Moreover, previous studies with mouse models have indicated that APP may increase the entry of the SARS-CoV-2 virus into cells, while PZP has been described as inhibiting amyloid aggregation.[33] Since APP was lower in the vaccine group than the COVID-19 group, one intriguing hypothesis is that PZP may have inhibited amyloid aggregation to a higher extent in the vaccine group than in the COVID-19 group. However, PZP has also been reported to stabilise misfolded proteins like Aβ protein, and its role in amyloid formation remains unclear.[33] Based on this relatively small study, which is at least hypothesis generating, it is hard to draw any firm conclusion regarding primary and secondary effects. In our, opinion, data in the literature regarding APP are more convincing in relation to its role in cognitive impairment compared with PZP. Therefore, we currently consider elevated APP as the most important finding, with potentially some 'additive' effects from PZP.

Given that one of the three vaccines was disproportionately associated with headache, it warrants a discussion of potential mechanisms. While the ChAdOx1 nCoV-19 vaccine has been associated with severe cerebral events,[34] none of the patients in the present study experienced new-onset persistent headaches after ChAdOx1 nCoV-19 vaccines. Moreover, although the BNT162b2 vaccine was the most commonly used vaccine, 89.7% of the participants reported that their headache started after RNA-1273 vaccine. The reason for this pattern is not clear, but interestingly, an online survey on self-reported adverse reactions in individuals who had received two doses of either the BNT162b2 or mRNA-1273 vaccine reported more side effects among those receiving the mRNA-1273 vaccine.[35] One could speculate that it was related to the higher mRNA content in mRNA-1273 as compared with the BNT162b2 vaccine.[36] Nonetheless, due to the low sample size in the present study, the results should be interpreted with caution.

The strengths of this study include the comprehensive examinations of the participants conducted by headache specialists, allowing for precise phenotyping of headache syndromes as well as the strong multidisciplinary collaboration involved. However, several limitations should be noted. First, CSF samples were not available from either headache group. Second, the overall sample size was small. Moreover, we lacked parallel samples from CSF and measurements of the actual protein in individuals who have experienced COVID-19 and SARS-CoV-2 vaccination without the development of persistent headache, which would have strengthened the comparison. The control group was particularly limited—not only small in size, but also suboptimally matched for age and did not undergo longitudinal assessments or detailed clinical interviews to the same extent as the patient groups.

In summary, altered plasma levels of soluble markers—potentially reflecting changes in amyloid processing—were observed in patients with persistent headache following SARS-CoV-2 vaccination, particularly among those with persistent headache after COVID-19 accompanied by cognitive symptoms. Larger, future studies that include CSF sampling are needed to further investigate and clarify the clinical significance of these findings.

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Acknowledgements We thank Marcus Boateng for registering data and Katrine Persgård Lund and Azita Rashidi for the sampling of blood biomarkers and Stephen J. Ryan for proofreading the manuscript.

Contributors AHA accepts full responsibility for the finished work and/or the conduct of the study, had access to the data and controlled the decision to publish. AHA has been the project manager, designed the project, recruited patients, performed analyses and drafted the manuscript. TU has done the analyses and contributed with the drafting of the manuscript. MB has recruited patients and contributed with the drafting of the manuscript. BEB has contributed with data management and with the drafting of the manuscript. MBA has recruited patients and contributed with the drafting of the manuscript. CAD has contributed with data management and contributed with the drafting of the manuscript. KO, TBD, IG, FL-J, VB and AEM has contributed with the biomarker analyses and contributed with the drafting of the manuscript. AH, ÅHM, KD, ACP, KBG, KS, THP, JS, SMM, EAH, MA-J have recruited patients and contributed with the drafting of the manuscript. THS has analysed images in CovaxHEAD study and contributed with the drafting of the manuscript. TBB has recruited patients, been responsible for the psychosomatic assessments and contributed with the drafting of the manuscript, HFH has contributed in the organisation of the NeuroCOVID project and contributed with the drafting of the manuscript. PA has contributed in the analyses, design and contributed with the drafting of the manuscript. ET has contributed to the design, the completion of the CovaxHEAD study and contributed with the drafting of the manuscript. BEH has contributed to the design, been leading the biomarker project and contributed with the drafting of the manuscript.

Funding Supported by Oslo University Hospital and the South-Eastern Norway Regional Health Authority (2021051).

Disclaimer The sponsor had no involvement in the study design; in the collection, analysis and interpretation of the data; in the writing of the report; or in the decision to submit the paper for publication.

Competing interests The authors report no disclosures relevant to this study. AHA has received honoraria for advice or lecturing from Pfizer Allergan, Teva, Novartis, Roche, Lundbeck and Teva and research grant from Boehringer Ingelheim. ACP: lecturing, advisory boards and/or clinical studies: AbbVie, Lundbeck, Pfizer, Novartis, Teva, Eli Lilly.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants and ethical approval for this study was obtained from The Norwegian South-Eastern Ethical Committee (152727 NNC, 351097 CovaxHEAD). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

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