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No Evidence of CNS Inflammatory Activity in Individuals with post-COVID-19 Condition Experiencing Neurological Symptoms

Insights from TSPO-PET Imaging

Human Neuroscience/Faculty of Medicine

Master's thesis

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Background: Following recovery from COVID-19, some individuals experience long-term symptoms, referred to as post-COVID-19 condition (PCC). PCC often includes symptoms potentially of neurological or neuropsychiatric origin such as fatigue, brain fog and sleep disturbances. The underlying mechanisms of PCC symptoms are still largely unknown. Neuroinflammation has been suggested as a potential cause for the neurological symptoms of PCC; however, the research in PCC patients is limited. In current literature, increased translocator protein (TSPO) expression indicates increased microglial activation. Positron emission tomography (PET) imaging with TSPO-binding radioligands, such as [^{11}C]PK11195, enables studying neuroinflammation *in vivo*.

Methods: PET-imaging with [^{11}C]PK11195 was performed to evaluate neuroinflammation in 20 participants with PCC compared to 13 healthy controls. [^{11}C]PK11195 binding was assessed as distribution volume ratio (DVR). Blood biomarkers neurofilament light chain and glial fibrillary acidic protein were measured to assess neuroaxonal damage and evidence of astrocyte activation. Additionally, clinical assessments including neurological examinations and mental health questionnaires were conducted in the PCC group.

Results: PCC participants did not exhibit increased [^{11}C]PK11195 DVRs in the brain compared to healthy controls ($p = 0.84$), nor signs of neuroaxonal damage or astrocyte activation in PCC participants based on soluble biomarker analysis. However, [^{11}C]PK11195 binding correlated with various variables in PCC participants. Higher quality of life was associated with decreased DVRs in hippocampus ($\rho = -0.87$, $p < 0.001$) and amygdala ($\rho = -0.77$, $p = 0.0091$), while increased depression and anxiety were associated with increased DVRs in these regions of interest.

Conclusion: Other than brain inflammatory or neuroaxonal damage-related mechanisms are likely to contribute to the neurological symptoms experienced by the individuals with PCC.

Key words: Post-COVID-19 condition, TSPO-PET, microglial activation, PET/MR, NfL, GFAP.

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List of Abbreviations

ACE-2	Angiotensin converting enzyme 2
BBB	Blood-brain barrier
CoV	Coronavirus
COVID-19	Coronavirus disease 19
CRS	Cytokine release syndrome
DAMP	Damage associated molecular pattern
DVR	Distribution volume ratio
GFAP	Glial fibrillary acidic protein
ME/CFS	Myalgic encephalomyelitis/chronic fatigue syndrome
MERS	Middle East respiratory syndrome
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NfL	Neurofilament
NO	Nitric oxide
NOS	Nitric oxide synthase
PAMP	Pathogen associated molecular pattern
PCC	Post-COVID-19 condition
PET	Positron emission tomography
PHOX	Phagocyte NAPDH oxidase
SARS	Severe acute respiratory syndrome
TNF	Tumour necrosis factor
TSPO	18kDA translocator protein

1 Introduction

1.1 COVID-19 Pandemic and Previous Coronavirus Outbreaks

Coronaviruses (CoVs) cause respiratory, enteric and systemic diseases in humans and other animals (Woo et al., 2009). CoVs are a group of viruses that belong to the order *Nidovirales*, family *Coronaviridae* and subfamily *Coronavirinae* (Chan et al., 2012; Zumla et al., 2016). They are enveloped, positive sense, single-stranded RNA viruses that are subdivided into four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus,* and *Deltacoronavirus* (Chan et al., 2012; Llanes et al., 2020). The seven human infecting CoVs belong to the *Alphacoronavirus* and *Betacoronavirus* genera (Llanes et al., 2020). Four of these, CoV-229E, CoV-NL63, CoV-OC43, and CoVHKU1, cause mild upper respiratory tract infections (He et al., 2020; Nickbakhsh et al., 2020). However, three of the human infecting CoVs are associated with severe respiratory infections: severe acute respiratory syndrome CoV (SARS-CoV), Middle East respiratory syndrome CoV (MERS-CoV), and SARS-CoV-2 (Channappanavar et al., 2014; He et al., 2020; Nickbakhsh et al., 2020). These three viruses have caused disease outbreaks ranging from local outbreaks to a global pandemic (LeDuc & Barry, 2004; Martellucci et al., 2020; Memish et al., 2020).

According to the World Health Organization (WHO) SARS-CoV first emerged in 2002, causing a yearlong epidemic in which over 8000 individuals were infected (WHO, 2003b). The first cases of SARS were in Guangdong Province, China from where it spread to 29 countries (WHO, 2003a). It is assumed that the virus was first transmitted to humans from masked palm civets in animal markets, but horseshoe bats may be the natural reservoir (Shi & Hu, 2008). Despite spreading to several countries, 87% of all cases and 84% of all deaths occurred in mainland China and Hong Kong (Lam et al., 2003). The clinical symptoms of SARS-CoV infection are those typical of lower respiratory tract disease, and Centers for Disease Control and Prevention describes that the symptoms typically begin with fever and progress to a dry cough and dyspnoea with possible diarrhoea (CDC, 2017; Peiris et al., 2004). The SARS-CoV epidemic had a global case-fatality ratio of approximately 11%, ranging from 0% to over 50% with the ratio increasing with age (Chan-Yeung & Xu, 2003; WHO, 2003a).

The first documented case of MERS-CoV occurred in Saudi Arabia in June of 2012 (Cunha & Opal, 2014). The virus is thought to have transmitted from dromedary camels to humans, and

camels may be the primary reservoir of the virus (Nowotny & Kolodziejek, 2014). MERS cases are still detected sporadically throughout the year, but the number of cases has decreased significantly from the peaks of spring 2013 and 2014 (Zumla et al., 2015). Between 2012 and 2019, 2499 laboratory confirmed cases from 27 different countries were reported (Memish et al., 2020). Common symptoms of MERS-CoV infection are shortness of breath, cough, fever, diarrhoea, and pneumonia (WHO, 2022a). MERS-CoV has a higher mortality rate than SARS-CoV, of around 35%, but it is transmitted relatively inefficiently to humans (Cunha & Opal, 2014). One aspect that may cause the high mortality rate is that comorbidities seem to have a significant effect on the disease prognosis (Cunha & Opal, 2014). MERS-CoV causes an influenza-like illness that typically progresses rapidly to pneumonia approximately a week after the beginning of the infection (Cunha & Opal, 2014).

In December 2019 a novel CoV, later named SARS-CoV-2, emerged in Wuhan, China leading to the declaration of the coronavirus disease 2019 (COVID-19) pandemic by the WHO in March 2020 (Lu et al., 2020; WHO, 2023a). The pandemic is still ongoing, though not as a public health emergency of international concern anymore, as stated by the WHO in May of 2023 (WHO, 2023a). The origin of SARS-CoV-2 has been largely debated in public. The two predominant ideas are that the virus escaped from a laboratory, or that the virus has a zoonotic origin. In a global study on SARS-CoV-2 origins, WHO (2021) rated the likelihood of introduction through an intermediary host as likely to very likely, direct zoonotic transmission as possible to likely, and the likelihood of introduction through a laboratory incident as extremely unlikely. The animal host of SARS-CoV-2 has not yet been identified, but the virus may have spread from horseshoe bats to an intermediary host and from there to humans (WHO, 2021). By the end of 2023 there have been over 700 million confirmed cases of COVID-19 and over 7 million reported deaths since the beginning of the pandemic (WHO, 2023b). Compared to SARS and MERS the mortality of COVID-19 is low, ranging from 0.1% in South Korea to 4.9% in Peru (Johns Hopkins University, 2023); however, the number of infected is considerably higher. The symptoms of COVID-19 resemble symptoms of other viral respiratory infections. According to the WHO (2023a), the most common symptoms are fever, chills, and sore throat. In addition, a variety of neurological symptoms and complications have been reported in COVID-19 patients. These include more common symptoms like hyposmia and hypogeusia, but also neurological disorders such as Guillain-Barré syndrome, and neurovascular and thromboembolic disease (Ren et al., 2021).

The severity of the disease varies between different human-infecting CoVs, and while the mortality of COVID-19 is lower than that of SARS and MERS, the impact of COVID-19 on the world has been significant, solely based on the number of people infected. It seems however, that the obstacles of COVID-19 are not limited to the initial infection, but that a growing number of people experience residual or emerging symptoms after recovering from the acute infection.

1.2 Post-COVID-19 Condition

1.2.1 Terminology

An increasing number of studies show that some people experience persistent symptoms after recovering from COVID-19 (Fernández-de-las-Peñas et al., 2021; Han et al., 2022; Michelen et al., 2021). These post-acute symptoms have been called multiple names such as long COVID, long-haul COVID, post-acute sequelae of COVID-19, and chronic COVID. Since September 2020 the condition has been listed in the ICD-10 as post-COVID-19 condition (PCC), which is how it is referred to in this thesis as well (Soriano et al., 2022). According to the WHO, PCC is defined as symptoms continuing or developing 3 months after the initial COVID-19, with these symptoms lasting for at least 2 months (WHO, 2022b).

1.2.2 Epidemiology

In recent years, the epidemiology of PCC has been studied extensively. The prevalence estimates of PCC vary widely from study to study due to many factors such as the study population, region, what symptoms were included, and follow-up time. In the largest epidemiology meta-analysis to date including a total of 1 680 000 COVID-19 patients worldwide, C. Chen et al. (2022) reported a global pooled prevalence of PCC of 43%. Estimates of the global pooled prevalence ranged from 9% to 81% (C. Chen et al., 2022). The pooled prevalence was higher in hospitalised patients (54%) compared to non-hospitalised patients (34%), and in females (49%) compared to males (37%) (C. Chen et al., 2022). Regionally the pooled prevalence was highest in Asia (51%), followed by Europe (44%), and the US (31%) (C. Chen et al., 2022). These estimates are high compared to the estimate by the WHO, which suggests that 10–20% of people infected with SARS-CoV-2 may develop PCC symptoms (WHO, 2022b). A nationwide study conducted on Scottish adults revealed a PCC prevalence ranging from 6.6 to 10.3% (Hastie et al., 2023), while a similar study in the Netherlands estimated a prevalence of 12.7% (Ballering et al., 2022).

Recognising the risk factors of PCC is important for identifying high-risk individuals, for offering follow-up care, and for planning public health measures. In a meta-analysis of 41 studies that included 860 783 PCC patients, hospitalisation or admission to the ICU for COVID-19 and the presence of comorbidities were associated with a high risk of PCC (OR = 2.37 and OR = 2.48, respectively) (Tsampasian et al., 2023). In more detail, the comorbidities included immunosuppression (OR = 1.5), chronic obstructive pulmonary disease (OR = 1.38), ischemic heart disease (OR = 1.28), asthma (OR = 1.24), anxiety and/or depression (OR = 1.19), chronic kidney disease (OR = 1.12), and diabetes (OR = 1.06) (Tsampasian et al., 2023). Additionally, female sex (OR = 1.56), older age (OR = 1.21), high BMI (OR = 1.15), and smoking (OR = 1.1) were associated with a higher risk of PCC (Tsampasian et al., 2023).

In an analysis of 384 137 non-hospitalised COVID-19 patients from the UK, Subramanian et al. (2022), reported that female sex; belonging to a certain ethnic minority group, including black Afro-Caribbean, mixed ethnicity, native American, Middle Eastern, and Polynesian ethnicities; socioeconomic deprivation; smoking; BMI greater than 30kg/m^2; and comorbidities such as chronic obstructive pulmonary disease, fibromyalgia, anxiety, depression, and multiple sclerosis (MS) were risk factors for PCC symptoms (Subramanian et al., 2022). Interestingly, older age was associated with a lower risk of PCC symptoms (Subramanian et al., 2022).

Notarte et al. (2022) reported in their meta-analysis of 37 articles and one preprint, that the most important risk factor for PCC was female sex (OR = 1.48). Comorbidities such as pulmonary disease, obesity, and organ transplantation were also recognised as potential risk factors (Notarte et al., 2022). Old age was not found to have significant association with PCC (OR = 0.86) (Notarte et al., 2022).

In summary, PCC is a health condition affecting a significant number of people around the world. It seems that female sex increases the risk for experiencing PCC symptoms and there may be other minority groups that are overrepresented. High BMI and comorbidities such as anxiety, depression, chronic obstructive pulmonary disease, and immunosuppression may also be risk factors for the condition, while research on whether older age increases the risk for PCC is conflicting.

1.2.3 Clinical Symptoms

PCC is a condition that affects multiple organ systems, and includes pulmonary, hematologic, cardiovascular, endocrine, gastrointestinal, hepatobiliary, dermatological, neuropsychiatric, and neurological manifestations (Nalbandian et al., 2021). Common symptoms of PCC include fatigue, shortness of breath, and cognitive dysfunction (Nalbandian et al., 2021; Soriano et al., 2022). Other symptoms like headache, heart palpitations, joint pain, physical limitations, depression and insomnia have also been reported (The Lancet, 2020).

In a meta-analysis of 63 articles, Alkodaymi et al. (2022) reported the most common PCC symptoms experienced during three different follow up periods at least 12 weeks after acute COVID-19 infection. In the first follow up period between 3 and 6 months the most common symptoms were fatigue (32%), shortness of breath (25%), and difficulty when concentrating (22%). At the second follow up period from 6 to 9 months the most common symptoms reported were intolerance to effort (45%), fatigue (36%), sleep disorder (29%), and shortness of breath (25%). Finally, in the last follow up period from 9 to 12 months, the most prevalent symptoms were fatigue (37%) and shortness of breath (21%) (Alkodaymi et al., 2022).

Many of the PCC symptoms seem to be of neurological or neuropsychiatric origin. As described by Stefanou et al. (2022), the neurological symptoms can involve both the central nervous system (CNS) and the peripheral nervous system (PNS). CNS manifestations of PCC include symptoms such as fatigue, “brain fog”, headache, sleep disorders, and cognitive impairment. Symptoms involving the PNS include muscle weakness, muscle pain, reduced ability to taste (hypogeusia) or smell (hyposmia), tinnitus or hearing loss, and sensorimotor deficits such as reduced sense of touch sensation (hypoesthesia), unpleasant and abnormal sense of touch (dysesthesia) and tremor (Stefanou et al., 2022).

In a retrospective cohort study Taquet et al. (2021) reported that as many as 33–66 % of 236 379 patients diagnosed with COVID-19 were diagnosed with a neurological or psychiatric disorder 6 months after the infection. The risk was increased for patients admitted to intensive care, suggesting that severe infection may worsen the outcome (Taquet et al., 2021).

Premraj et al. (2022) reported the prevalence of neurological and neuropsychiatric symptoms of PCC patients in their meta-analysis of 18 studies including 10 530 patients. The reported neurological findings included fatigue (37%), “brain fog” (32%), memory problems (28%), attention deficits (22%), muscle pain (17%), headache (15%), anosmia (12%), and dysgeusia

(10%). Neuropsychiatric symptoms included sleep disorder (31%), anxiety (23%), and depression (17%). Notably, hospitalisation did not increase the risk for neurological or neuropsychiatric symptoms (Premraj et al., 2022).

These studies indicate that of the neurological symptoms, fatigue, cognitive symptoms, and various PNS symptoms are the most common manifestations of PCC. Additionally, it seems that psychiatric manifestations such as anxiety and depression are common. Although the persistence of PCC symptoms has not been studied extensively, according to Y. Kim et al. (2023), the symptoms of PCC can last for years. Although time seems to improve symptoms, fatigue, cognitive symptoms and neuropsychiatric symptoms may persist for up to 24 months (Y. Kim et al., 2023). As for now, there is no specific treatment for PCC. These symptoms can decrease the quality of life of PCC patients, preventing them from returning to normal life.

1.2.4 Pathophysiology of Neurological Symptoms

The pathophysiology of PCC is still largely unknown. There are multiple hypothesized mechanisms that could underlie the neurological symptoms such as neuronal injury caused by SARS-CoV-2 neuroinvasion, damage to blood vessels caused by coagulopathy or endothelial dysfunction, systemic inflammation, and neuroglial dysfunction (Davis et al., 2023; Leng et al., 2023).

SARS-CoV-2 virion is made up of four structural proteins – nucleocapsid, membrane, envelope, and spike proteins (Harrison et al., 2020; Jackson et al., 2022). The entry to host cell – including binding to host cell membrane and fusion – is orchestrated by the spike protein. The spike protein binds to angiotensin-converting enzyme 2 (ACE2) on the host cell surface starting the viral entry. According to Jackson et al. (2022) two spike protein cleavage events are typically necessary for the viral entry, S1/S2 cleavage and S2’ cleavage. First the S1/S2 site is cleaved by furin, which exposes the S2’ site. The exposed S2’ site is proteolytically cleaved by transmembrane protease serine 2 or by intracellular cathepsin L (Jackson et al., 2022). This is followed by membrane fusion and uncoating of viral RNA to the cytosol (Jackson et al., 2022). The host cell entry is followed by a typical replication process of positive-strand RNA viruses (Harrison et al., 2020).

The possibility of SARS-CoV-2 infecting neurons remains highly debated (Leng et al., 2023). As mentioned above, SARS-CoV-2 enters host cells by binding to ACE2 receptor. ACE2

receptors are expressed in multiple organs and especially in the small intestine, testis, kidneys, heart, thyroid, and adipose tissue (Li et al., 2020). However, the expression of ACE2 in the brain is still debated. Low levels of ACE2 mRNA expression in the human brain have been demonstrated through quantitative real-time PCR (Harmer et al., 2002), but in another study immunohistochemical staining localised the expression in the brain to endothelium and vascular smooth muscle cells (Hamming et al., 2004). Still, studies show that SARS-CoV-2 can infect and replicate in human brain organoids derived from human induced pluripotent stem cells, and that infection in these brain organoids can be blocked with ACE2 antibodies (Song et al., 2021; Zhang et al., 2020). In an extensive study on cellular tropism, quantification and persistence of SARS-CoV-2 across the human body, S. R. Stein et al. (2022) demonstrated that the virus is capable to infect and replicate in the human brain. SARS-CoV-2 was detected in the CNS tissue in 10 out of 11 cases examined, and replication-competent SARS-CoV-2 was recovered from the thalamus (S. R. Stein et al., 2022). While direct brain infection has also been reported in other post-mortem studies (Matschke et al., 2020; Song et al., 2021), several studies in hospitalised COVID-19 patients with neurological symptoms have failed to detect SARS-CoV-2 in the brain (Thakur et al., 2021; A. C. Yang et al., 2021) or cerebrospinal fluid (Alexopoulos et al., 2020; Bellon et al., 2021; Neumann et al., 2020). According to a systematic review, it also seems that the presence of SARS-CoV-2 RNA or proteins in the brain is not associated with the presence of neurological symptoms (Cosentino et al., 2021).

An increased risk of cerebrovascular events, including ischemic stroke and intracranial haemorrhage, has been reported in patients with COVID-19 (Klok et al., 2020b; Rothstein et al., 2020). Rothstein et al. (2020) reported in their retrospective study, that out of 844 hospitalised COVID-19 patients, 20 (2.4%) had confirmed ischemic stroke. In a study of 184 COVID-19 patients admitted to the ICU a 31% incidence in thrombotic complications was reported, of which 3.7% were arterial thrombotic events (Klok et al., 2020b), and the results were repeated later with similar results (Klok et al., 2020a). In a neuropathological study of COVID-19 patients, Thakur et al. (2020) reported that out of 41 patients examined, 43.9% had brain infarcts. These infarcts included chronic infarcts, acute or subacute infarcts, and microscopic acute or subacute infarcts (Thakur et al., 2021). There are several mechanisms by which SARS-CoV-2 could cause thrombotic events, though the exact mechanism remains unknown. Possible mechanisms include infection of endothelial cells, hypercoagulability caused by infection, viral cardiomyopathy, and systemic hyperinflammatory state (Rothstein

et al., 2020). While it is acknowledged that vascular changes and thrombotic events worsen the prognosis during acute COVID-19, they may also be behind the long-term symptoms of PCC (Pretorius et al., 2021). Pretorius et al. (2021) propose that microclots formed during the infection may obstruct small capillaries, leading to inhibition of oxygen exchange in the brain. This CNS hypoxia could be a contributing factor to the persistent PCC symptoms (Pretorius et al., 2021).

In some cases, COVID-19 patients develop a systemic inflammatory condition called cytokine release syndrome (CRS), also known as cytokine storm. CRS can be triggered by several factors, including certain medications and infections such as influenza and COVID-19 (Fajgenbaum & June, 2020; Fara et al., 2020; Shimabukuro-Vornhagen et al., 2018). In COVID-19, CRS has been associated with lung injury, multi-organ failure and poor disease prognosis (L. Yang et al., 2021). Higher plasma levels of proinflammatory cytokines such as interferon gamma (IFNγ), interleukin-6 (IL-6), interleukin-1B (IL-1B), and tumour necrosis factor alpha (TNF-α) have been reported in COVID-19 patients (Huang et al., 2020; Qin et al., 2020; Zhu et al., 2021). In addition, lower levels of type I IFN or delayed type I IFN responses have been reported (Arunachalam et al., 2020; Blanco-Melo et al., 2020). In a study comparing neurological symptoms in COVID-19 patients with and without CRS, the patients with CRS had significantly more often altered level of consciousness (69.4%) compared to patients without CRS (25.3%) (Tutal Gursoy et al., 2023). It has also been shown that the immunological dysfunction can persist for up to 8 months following COVID-19 (Phetsouphanh et al., 2022). If cytokine release is prolonged after COVID-19, it could possibly initiate neuroinflammation through dysregulation of the blood-brain barrier (BBB) and activation of glial cells, leading to neurological symptoms experienced by individuals with PCC (Gonçalves De Andrade et al., 2021; Hanisch & Kettenmann, 2007).

Neuroaxonal damage has been suggested as one possible mechanism causing cognitive symptoms in PCC. Neurofilament light chain (NfL) is a protein expressed exclusively in neurons (Khalil et al., 2018). Increased levels of NfL in the cerebrospinal fluid and serum is a biomarker of axonal damage in neurodegenerative, inflammatory, vascular and traumatic diseases of the brain (Khalil et al., 2018). Increased plasma and serum levels of NfL in COVID-19 patients compared to healthy controls have been reported (Ameres et al., 2020; Havdal et al., 2022; Kanberg et al., 2020; Verde et al., 2022). In two of these studies, NfL levels were higher in patients with only minor neurological symptoms (Ameres et al., 2020; Verde et al., 2022). These studies indicate that SARS-CoV-2 may cause neuroaxonal damage

even in patients with mild-to-moderate symptoms. This damage could contribute to the neurological symptoms in patients with PCC.

Another well-established biomarker for brain injury is glial fibrillary acidic protein (GFAP) (Olsson et al., 2011). GFAP is a protein expressed by astrocytes in the CNS, and increased levels of this protein are a sign of CNS injury and the following astrogliosis (Lee et al., 2000). Increased GFAP concentrations have been reported in COVID-19 patients compared to healthy controls (Havdal et al., 2022; Kanberg et al., 2020). Havdal et al. (2022) did not find association with serum NfL or GFAP and fatigue or cognitive symptoms. However, NfL and GFAP levels correlated strongly with female sex and older age (Havdal et al., 2022). Kanberg et al. (2020) found that in COVID-19 patients with severe disease NfL levels increased with the disease duration, while GFAP levels decreased (Kanberg et al., 2020). As astrogliosis is a response to CNS damage and disease (Sofroniew, 2015), these studies indicate that astrocytes may participate in a neuroinflammatory response during COVID-19.

Kanberg et al. (2021) conducted a long-term follow-up study, where they measured NfL and GFAP in acute phase of COVID-19 and six months after the infection. Notably, six months after the infection, the NfL and GFAP concentrations had normalised, with no significant group differences. However, some of the patients still reported neurological symptoms. The NfL and GFAP levels during the acute infection did not correlate with neurological symptoms (Kanberg et al., 2021). Peluso et. al (2022) measured NfL and GFAP concentrations in patients with self-reported neurological PCC symptoms. In early recovery (<90 days) from COVID-19, patients who later reported neurological PCC symptoms, had higher concentration of GFAP, but not NfL compared to patients who did not report neurological PCC symptoms (Peluso et al., 2022). However, in late recovery (>90 days) neither biomarker concentration was elevated (Peluso et al., 2022). These results suggest that axonal damage and astrogliosis may occur during COVID-19 and in early recovery, but ongoing neuroaxonal injury or astrocyte activation may not be associated with the neurological symptoms of PCC.

Finally, many researchers have noted a potential correlation between PCC and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). ME/CFS is an umbrella term that refers to the two conditions characterized by severe fatigue and autonomic and neurocognitive symptoms (Institute of Medicine, 2015). T. L. Wong and Weitzer (2021) conducted a systemic review of 21 articles comparing ME/CFS and PCC. The results showed a high degree of similarities between ME/CFS and PCC symptoms (T. L. Wong & Weitzer, 2021).

Notably, the major criteria symptoms for ME/CFS diagnosis, fatigue, reduced daily activity, and post-exertional malaise, were reported in multiple studies as symptoms of PCC (T. L. Wong & Weitzer, 2021). However, the diagnostic criteria of ME/CFS requires that the symptoms have lasted for at least six months (Institute of Medicine, 2015), and this criterion was not met for most of the patients with PCC in the examined articles (T. L. Wong & Weitzer, 2021). PCC did also have three symptoms unique from ME/CFS, including olfactory dysfunction, gustatory dysfunction, and rash (T. L. Wong & Weitzer, 2021). Viral infections have long been considered as the main triggers of ME/CFS, but the exact pathogenesis is still unknown (Deumer et al., 2021). Given the overlap in symptoms between PCC and ME/CFS, it is conceivable that they may share underlying mechanisms contributing to their symptomatology.

1.3 Microglia and Neuroinflammation

The CNS parenchyma is considered a relatively immune privileged site, meaning that the afferent arm of immune response is deficient, because antigen presentation is limited, and the efferent arm is inhibited, because immune cell migration is restricted by the BBB (Barker & Billingham, 1973; Engelhardt et al., 2017; Galea et al., 2007). This relative immune privilege is essential for limiting damage in the CNS during inflammation due to its poor regenerative capacity (Galea et al., 2007). Considering that infiltration of peripheral immune cells is tightly regulated by the BBB, the CNS necessitates its own resident immune cells. Microglia are one of the three glial cells in the CNS, alongside astrocytes and oligodendrocytes. Microglia are yolk sac originated immune cells, accounting for about 10% of all CNS cells (Prinz et al., 2021). Del Rio Hortega (1927, as cited in S. U. Kim & De Vellis, 2005) was the first to identify microglia as a separate cell type from other glial cells. He theorised that the cells could transform from the ramified resting state into active ameboid macrophages (S. U. Kim & De Vellis, 2005). To this day, the morphological features of microglia that Del Rio Hortega described using a silver staining technique, are accurate (Nayak et al., 2014).

In a healthy CNS, microglia are important homeostatic regulators through promoting neuronal survival, initiating programmed cell death, cleaning the resulting cellular debris by phagocytosis, and maintaining synaptic homeostasis via synaptic pruning (Nayak et al., 2014). The homeostatic role of microglia has been studied extensively; however, microglia also have a very important role in initiating neuroinflammation in various pathological conditions. Neuroinflammation is a fundamental response that protects the brain from

pathologies, yet if prolonged or uncontrolled, neuroinflammation can be harmful and lead to neuronal damage (Cherry et al., 2014). Frank-Cannon et al. (2009) describe chronic neuroinflammation as a state where activation of microglia, sustained release of proinflammatory mediators, and increased oxidative stress persist after the initial damage on the CNS has long been resolved. The sustained release of proinflammatory mediators creates a positive feedback loop where further microglia are activated that in turn release more proinflammatory mediators. The outcomes of neuroinflammation – whether beneficial or harmful – may depend on the duration of the inflammatory response (Frank-Cannon et al., 2009).

1.3.1 Microglial Activation

In their review article titled "Physiology of Microglia", Kettenmann et al. (2011) describe extensively the intricate process of microglial activation. Under normal conditions in a healthy CNS, microglia are in a so called "resting" state, which is characterised by a ramified morphology – a small soma with fine cellular processes. These "resting" microglia constantly survey the CNS environment for signals indicating a threat to the brain's homeostasis. Microglia recognise numerous homeostasis threatening molecules and conditions, including pathogens, complement, antibodies, and cytokines, among others (Hanisch & Kettenmann, 2007). When the homeostasis is disturbed indicating danger to the CNS, a process called "microglial activation" occurs. This process evokes changes in the microglial cell shape, gene expression and functional behaviour. One of the major phenotypical changes is the reduction of complex cellular processes, resulting in an amoeboid appearance. Activated microglia gain mobility and migrate to the site of disturbance. Local populations of microglia can proliferate, providing more cells for the defence against invading pathogens and to protect and restore the CNS homeostasis. Additionally, release of immunoregulatory and proinflammatory mediators are important functions of microglia during the activation process. Activated microglia can also recruit immune cells through releasing chemoattractive factors and present antigens to recruited T cells. Finally, activated microglia are phagocytotic and important in clearing tissue debris, damaged cells, and pathogens in the CNS (Kettenmann et al., 2011).

Microglial activation is one of the four hallmarks of neuroinflammation, alongside with elevated levels of proinflammatory cytokines, infiltration of peripheral leukocytes, and nervous tissue damage (Estes & McAllister, 2014). Activated microglia have both neuroprotective and neurotoxic functions. Traditionally the view has been that during the

activation process, microglia can either adapt a proinflammatory M1 phenotype via classical activation, or an immunosuppressive M2 phenotype via alternative activation or acquired deactivation (Tang & Le, 2016). This dichotomous classification may be misleading, given the evolving understanding that microglia seem to exhibit multidimensional activation states (Ransohoff, 2016). The pathogenesis of numerous neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease (Hickman et al., 2018), and psychiatric disorders such as depression and schizophrenia (Najjar et al., 2013) is associated to chronic neuroinflammation. Additionally, viral infections can trigger chronic neuroinflammation (Streit et al., 2004). In diseases such as Alzheimer's disease, Parkinson's disease, and MS activated microglia may act as a double-edged sword, having first and foremost a neuroprotective role; however, in many of the diseases microglial activation may be dysregulated or excessive, leading to neuronal damage and loss (Hickman et al., 2018).

Activated microglia have various mechanisms that can damage or kill neurons (Brown & Vilalta, 2015; Hickman et al., 2018) (Figure 1). One of these mechanisms is reactive oxygen species production by phagocyte NAPDH oxidase (PHOX). PHOX produces reactive oxygen species to kill pathogens, but they may also cause damage to neurons and increase further activation of microglia (Brown & Vilalta, 2015). The increased activation of microglia leads to production of proinflammatory cytokines such as TNFα and IL-1β which further amplify the inflammatory response (Brown & Vilalta, 2015). Inducible nitric oxide synthase (iNOS) expression is mediated by inflammatory mediators and PHOX (Brown & Vilalta, 2015). High levels of nitric oxide produced by iNOS can cause neuronal death by inhibiting mitochondrial function (Brown & Vilalta, 2015). Together, superoxide produced by PHOX and nitric oxide produced by iNOS can react to give peroxynitrite which can cause neuronal apoptosis (Brown & Vilalta, 2015). Proteases released by activated microglia, like cathepsin B and matrix metalloproteases may also be neurotoxic. Cathepsin B is a protease responsible for degradation of intracellular proteins. It has been shown to be neurotoxic in neurodegenerative disorders *via* promotion of neuronal apoptosis (Brown & Vilalta, 2015). Matrix metalloproteases can be neurotoxic in hypoxic states caused by ischemia (Hickman et al., 2018). Microglia can release glutamate and glutaminase, an enzyme that converts glutamine to glutamate. Excessive and sustained levels of glutamate can be toxic to neurons expressing glutamate receptors by causing sustained activation of NMDA receptor leading to excitotoxic death (Brown & Vilalta, 2015). Neuronal death by glutamate or glutaminase release by microglia has been shown in Japanese encephalitis virus and HIV (Brown & Vilalta, 2015).

Furthermore, stressed but viable neurons may express eat-me signals that can trigger microglial phagocytosis (Brown & Vilalta, 2015).

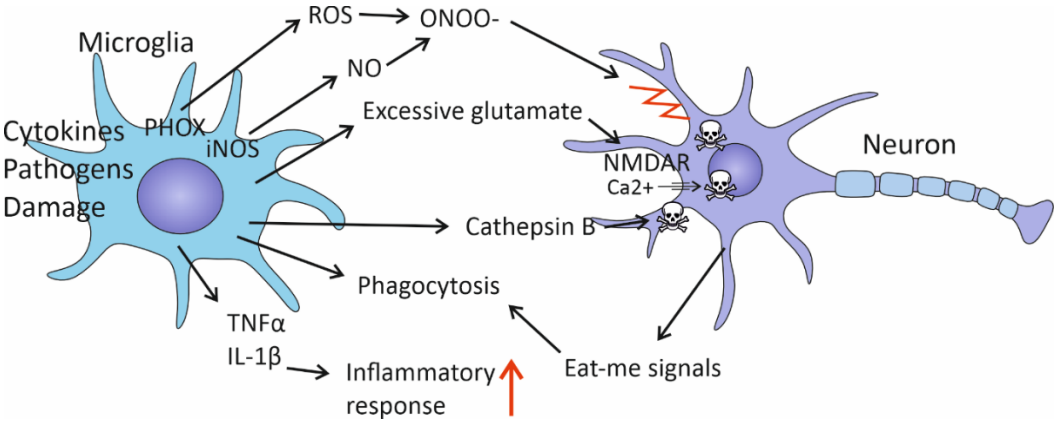

Figure 1. Mechanisms how activated microglia can injure or kill neurons. Peripheral cytokines, pathogens or neuronal damage may initiate microglial activation. Activated microglia can form reactive oxygen species (ROS) and nitric oxide (NO) that can react to form peroxynitrite ($ONOO^-$) which can cause neuronal apoptosis. Excessive glutamate or glutaminase released by microglia can cause sustained activation of NMDA receptors (NMDAR) which may lead to excitotoxic death. Cathepsin B released by activated microglia can promote neuronal apoptosis. Stressed, but viable neurons can release eat-me signals which may lead to microglial phagocytosis. Inflammatory cytokines (TNFα and IL-1β) increase the inflammatory response, activating more microglia. Adapted from Brown & Vilalta (2015).

1.3.2 Post-COVID-19 Condition and Microglia

Multiple postmortem studies have shown that COVID-19 patients exhibit microglial activation (Matschke et al., 2020; Schurink et al., 2020; J. A. Stein et al., 2023; Thakur et al., 2021). In their postmortem study of 43 COVID-19 patients, Matschke et al. (2020) found a variable degree of astrogliosis in all patients. Prominent diffuse activation of microglia with occasional microglial nodules was found in the brainstem and cerebellum (Matschke et al., 2020). In their autopsy study on 21 COVID-19 patients, Schurink et al. (2020) observed microglial activation in the olfactory bulb, medulla, cervical spinal cord, cerebellum, and deep grey matter of the cerebellum. Formation of microglial nodules was observed in the medulla and cerebellum (Schurink et al., 2020). In their neuropathological study on 41 patients, Thakur et al. (2021) found microglial activation in 34 (81%) of the patients. Microglial nodules were found in 26 (63%) of the brains, most prevalently in the brainstem and cerebellum (Thakur et al., 2021). Less frequently microglial nodules were found in the hippocampus (20%), isocortex (5%), and olfactory bulb (5%) (Thakur et al., 2021). J. A. Stein et al. (2023) reported in their neuropathological study that all 17 patients had diffuse

parenchymal microglial activity. This activity was most pronounced in the cerebellar nuclei, white matter areas of the cerebrum, and brainstem areas (J. A. Stein et al., 2023). There are also two positron emission tomography (PET) studies on microglial activation in study subjects with persisting symptoms after COVID-19, that are discussed in more detail later.

There are multiple mechanisms how COVID-19 could initiate microglial activation (Gonçalves De Andrade et al., 2021). In their review article Gonçalves de Andrade et al. (2021) present four possible mechanisms: (1) hypoxic injuries in the brain; (2) viral infection of brain endothelial cells, leukocytes migrating to the brain, or neurons; (3) CRS caused by excessive inflammatory response; and (4) psychological stress. The mechanisms are summarised in Figure 2. Notably, these mechanisms are parallel to some of the potential causes of neurological symptoms in PCC.

COVID-19 is associated with symptoms that can lead to systemic hypoxia such as shortness of breath, pneumonia, and even acute respiratory distress syndrome (N. Chen et al., 2020). In addition, as mentioned previously, patients with COVID-19 have an increased risk for cerebrovascular events (Klok et al., 2020b; Rothstein et al., 2020). Both systemic hypoxia and limited circulation to the brain can cause CNS hypoxia. In a functional near-infrared spectroscopy study on 34 PCC patients, 24% of PCC patients had reduced arterial oxygen saturation compared to healthy controls (Adingupu et al., 2023). Hypoxia, while also damaging neurons, may initiate microglial activation (Yenari et al., 2010). Activated microglia may cause further injury to neurons through cytotoxic effects, while also having beneficial effects on neuronal survival in hypoxia (Yenari et al., 2010).

Referring to the earlier chapter on PCC pathophysiology, SARS-CoV-2 could possibly infect neurons directly *via* neuronal retrograde route. Another way the virus could infiltrate the CNS is through the hematogenous route by infecting peripheral immune cells or endothelial cells (Desforges et al., 2019). Cells infected by the virus express damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) which are recognised by pattern recognition receptors such as Toll-like receptors. Toll-like receptors are expressed in the CNS by neurons and all glial cells, but particularly by microglia (Kumar, 2019). Once DAMPs and PAMPs bind to a toll-like receptor on the surface of microglia, microglial activation is initiated. In viral infections activated microglia try to recover the CNS homeostasis; however, if microglial activation is prolonged, it may contribute to astrocyte-

mediated neurotoxicity and excessive synapse elimination mediated by complement (Klein et al., 2019).

As discussed previously, COVID-19 is associated with CRS. Increased proinflammatory cytokines such as TNF-α and IL-1 may increase BBB permeability (Tsao et al., 2001; R. Wong et al., 2019). This BBB disruption and hyperinflammatory state may lead to increased leakage of cytokines to the brain parenchyma. The surveying microglia of the CNS are sensitive to changes in their environment, and leakage of proinflammatory cytokines can initiate microglial activation (Hanisch & Kettenmann, 2007). Long-term overexpression of proinflammatory cytokines in the brain may be an important factor in neurotoxic and neurodegenerative disorders (Szelényi, 2001).

In addition to the pathological changes caused by COVID-19, the pandemic is associated with psychological stress. In China, close to 35% of participants (N = 52 730) had experienced psychological distress during the pandemic according to a COVID-19 peritraumatic distress index that measured anxiety, depression, phobias, cognitive change, avoidance, and compulsive behaviour (Qiu et al., 2020). In a study on adults from the United States, using Kessler 6 Psychological Distress Scale, 13.6% of participants reported symptoms of serious psychological distress in 2020 compared to 3.9% in 2018 (McGinty et al., 2020). Stress triggers sympathetic nervous system and hypothalamus-pituitary-adrenal gland axis activity to release catecholamines and glucocorticoids. Additionally, psychological stress increases the levels of circulating cytokines and may increase cytokine levels in the amygdala and medial prefrontal cortex (Vecchiarelli et al., 2016). Increased levels of cytokines, glucocorticoids, and catecholamines released during persistent stress can disturb the balance between microglia–neuron interaction, and lead to neuronal damage by activated microglia (Tian et al., 2017).

In summary, several mechanisms could underlie microglial activation in COVID-19. While activation of microglia can have beneficial effects on the CNS, if dysfunctional or excessive, it may be harmful. Whether the microglial activation observed in COVID-19 patients is associated with the long-term neurological symptoms of individuals with PCC, remains to be explored.

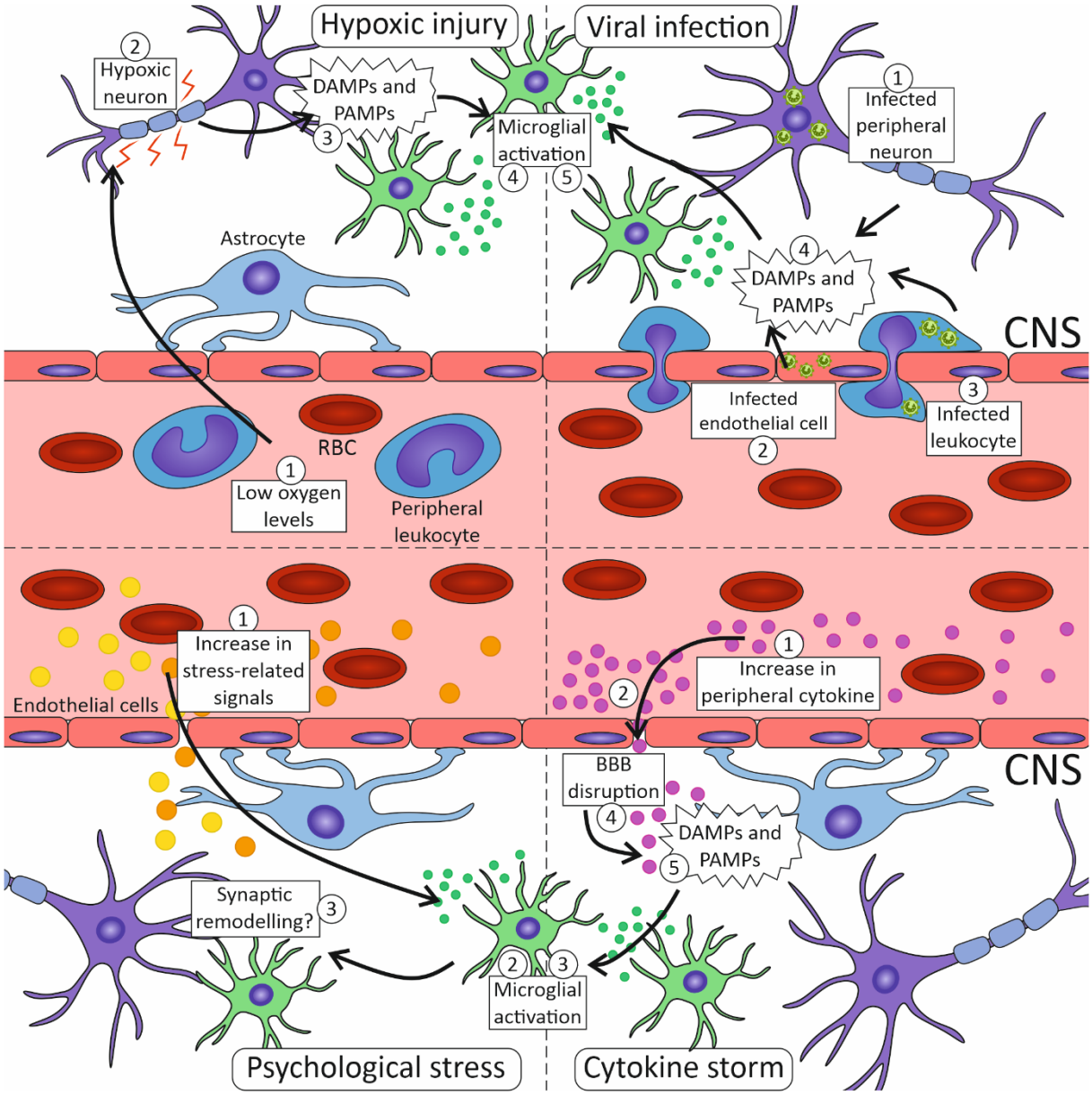

Figure 2. Possible mechanisms of microglial activation in COVID-19. (Upper left) (1) Low oxygen levels caused by COVID-19 may (2) damage neurons. Hypoxic neurons release (3) damage associated molecular patterns (DAMPs) and pathogen associated molecular patterns (PAMPs) that are sensed by (4) microglia, causing microglial activation. **(Upper right)** SARS-CoV-2 could enter the CNS via (1) infected peripheral neurons, (2) infected endothelial cells, or (3) infected peripheral leukocytes. The infected cells release (4) DAMPs and PAMPs that when sensed by microglia, (5) initiate microglial activation. **(Lower left)** Psychological stress caused by COVID-19 (1) increases cortisol, catecholamine, and cytokine release into circulation. This can cause (2) microglial activation. **(Lower right)** Excessive inflammatory response caused by COVID-19 results in (1) increased cytokine in circulation. Cytokines that pass (2) the blood-brain barrier (BBB) can (3) activate microglia. Furthermore, cytokines can drive (4) BBB disruption, increasing cytokine and (5) DAMP and PAMP levels. Edited from Gonçalves de Andrade et al. (2021)

1.4 TSPO-PET Imaging of Neuroinflammation

1.4.1 PET Imaging

PET is a functional imaging method where ligands labelled with positron emitting radioisotopes, radioligands, are used as molecular probes to visualise and measure metabolic changes and physiological processes. It is a relatively non-invasive method that enables studying molecular changes of the body *in vivo*. The radioligands can be designed to bind to certain receptors, or they can be structural analogs to compounds. The radioligands are labelled with radioactive labels such as carbon-11 or fluorine-18 (Pike et al., 1993).

During PET imaging, a small amount of chosen radioligand is injected into the patient's bloodstream. Once the radioligand has accumulated into tissues and organs it undergoes β^+ decay, also called positron emission. In this process a proton in the nucleus is converted into a neutron, and a positron and a neutrino are emitted out of the nucleus. In the tissue, the positron collides with an electron in a process called annihilation. During annihilation the positron and the electron are annihilated resulting in the creation of two photons with an energy of 511 keV emitted at an angle of 180° to each other (Bailey et al., 2005). These photons are then detected by scintillation detectors on opposite sides of the PET device. A line of response can be calculated between the two detectors which can be used to localise the site of annihilation. The data collected from multiple scintillation events is used to reconstruct a three-dimensional image showing the spread of the radioligand throughout the patient's body. As a common practice, anatomical imaging with magnetic resonance imaging (MRI) or computer tomography is performed in addition to the PET scan to provide anatomically detailed images that can be combined with the PET images.

PET imaging has made it possible to quantify the concentration of accumulated radioligand *in vivo*. To relate this concentration to the underlying physiological processes, mathematical modelling of the radioligand kinetics within a chosen region of interest is necessary (Bertoldo et al., 2014). There are multiple alternatives for semi-quantitative and quantitative PET analysis methods. Semi-quantitative methods include standardised uptake value and tissue-to-plasma ratio. In dynamic imaging, compartmental models, spectral analysis, and graphical methods are used (Bertoldo et al., 2014). Distribution volume (DV) is a linear function of free receptor concentration, and proportional to the ratio of transport constants, which is a function of plasma protein binding. Because of this, the calculation of DV requires blood sampling (Logan et al., 1996). Distribution volume ratio (DVR) is the ratio of DV in a receptor region

to the DV in a non-receptor containing region. Logan et al. (1996) described a graphical method by which DVR can be calculated without blood sampling by approximating the plasma integral using a non-receptor containing region of interest.

1.4.2 The 18kDa Translocator Protein

The 18 kDa translocator protein (TSPO), previously known as the peripheral benzodiazepine receptor, is a transmembrane protein located on the outer mitochondrial membrane (Papadopoulos et al., 2014). First recognised as a binding site for diazepam, it was later thought to function as a cholesterol transporter in steroid biosynthesis (Papadopoulos et al., 2014). This role has been challenged by studies done in TSPO knockout mice showing that TSPO function may not be essential for steroid biosynthesis (Guilarte, 2019). TSPO has multiple proposed functions including steroid biosynthesis, immunomodulation, regulation of mitochondrial metabolism and functions, and apoptosis (Liu et al., 2014; Papadopoulos et al., 2014).

The protein has also been implicated in pathological conditions such as brain injury and neurodegenerative diseases (Papadopoulos et al., 2014). In normal conditions TSPO is expressed in low levels in the brain, but its expression is increased in pathological conditions (Guilarte, 2019). The source of this increased expression is a controversial topic. Early on, the TSPO response was attributed to microglial activation and macrophage infiltration with no association to astrocytes (Conway et al., 1997; Myers et al., 1991). Later studies have shown that activated astrocytes contribute to the TSPO response together with microglia (Cosenza-Nashat et al., 2009; Kuhlmann & Guilarte, 2000), and in a study on neuroinflammation in schizophrenia, TSPO response was found in endothelial cells in addition to microglia and astrocytes (Notter et al., 2018). Furthermore, in recent years, there has been a dispute on whether the increase in TSPO expression represents microglial activation or rather the microglia and macrophage density. While activated microglia do proliferate to increase local cell density, studies have shown that proinflammatory stimulation of human microglia does not increase TSPO expression but does so in rodent-derived microglia (Nutma et al., 2023; Owen et al., 2017).

Nevertheless, numerous studies have shown that TSPO is a sensitive biomarker for neuroinflammation in conditions such as MS (Airas et al., 2015; Banati et al., 2000), Alzheimer's disease (Cagnin et al., 2001), HIV infection (Coughlin et al., 2014), and sports-related brain injury (Coughlin et al., 2017). Previous studies on rodents have shown that

because of its low levels in the brain parenchyma that increase regionally following brain injury or inflammation, it is a sensitive biomarker for changes in the injury region (M. K. Chen & Guilarte, 2008). Development of TSPO selective ligands such as [^{11}C]PK11195 has made it possible to visualise TSPO distribution *in vitro* with receptor autoradiography, as well as *in vivo* with PET imaging.

1.4.3 TSPO-PET Imaging With [^{11}C]PK11195 Radioligand

The first discovered TSPO binding radioligand among several is [^{11}C]PK11195. PK11195, also known as 1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinolinecar-boxamide, was first found to bind to TSPO in rat tissues with high affinity, displacing [^{3}H]R05-4864, diazepam, and clonazepam (Le Fur et al., 1983). Later, the distribution of TSPO in human brain was studied using [^{3}H]PK11195 autoradiography (Doble et al., 1987). In 1984 PK11195 was first labelled with carbon-11 (Camsonne et al., 1984), and in 1986 [^{11}C]PK11195 was used in a PET study to characterise TSPO binding sites in human and dog hearts (Charbonneau et al., 1986). Quickly, [^{11}C]PK11195 imaging was utilised for imaging human gliomas and glioblastomas (Junck et al., 1989; Pappata et al., 1991). First the radioligand was used as a racemate, but later it was observed that the R-enantiomer has a higher binding to TSPO than the S-enantiomer (Shah et al., 1994). Today, (R)-[^{11}C]PK11195 is the most widely used PET radioligand for imaging neuroinflammation in various brain pathologies (Chauveau et al., 2021).

The quantification of (R)-[^{11}C]PK11195 data has proved challenging for several reasons. Firstly, PK11195 has numerous binding sites in the blood, including platelets, monocytes and plasma proteins. Arterial radioactivity can be corrected by separating blood cells from plasma, if the bound fraction is small compared to the free fraction in plasma, or if the variation of binding is small across subjects (Turkheimer et al., 2015). However, PK11195 binds to plasma proteins, which can be upregulated in inflammatory states, and therefore plasma concentrations may be unreliable (Lockhart et al., 2003). Secondly, the localisation of microglial activity is unknown, so a reference region cannot be selected *a priori* (Turkheimer et al., 2015). Thirdly, TSPO is widespread in the normal brain and BBB. In normal conditions, the binding of PK11195 to the BBB generates a low intensity background signal which doesn't affect quantification in reference region approaches (Turkheimer et al., 2015). However, neuropathological conditions disrupt the density of TSPO at the BBB necessitating correction of the signal. For these reasons, a supervised cluster algorithm (SVCA) was

developed to extract a grey matter reference region with no specific binding of [^{11}C]PK11195, allowing for quantification of the radioligand without arterial sampling (Turkheimer et al., 2007).

While (R)-[^{11}C]PK11195 is still, after 30 years, the most used TSPO radioligand, it has several limitations, including low BBB permeability, relatively high nonspecific binding, poor signal-to-noise ratio, and the short half-life of carbon-11, that limits its wide clinical use (Chauveau et al., 2008). Therefore, researchers have actively searched for better ligands to quantify TSPO expression. These second-generation TSPO radioligands include [^{11}C]PBR28, [^{18}F]FEPPA, and [^{18}F]DPA-713 among others (Chauveau et al., 2021). Of these radioligands [^{11}C]PBR28 has a higher binding potential than (R)-[^{11}C]PK11195, and [^{18}F]FEPPA and [^{18}F]DPA-713 have higher specific binding (Cumming et al., 2018). However, all these second-generation radioligands are limited in their applications because genetic polymorphism (rs6971) affects their binding affinity, which necessitates genotyping study subjects (Mizrahi et al., 2012; Owen et al., 2011, 2012). In addition, the high affinity of these radioligands increases the TSPO signal in BBB compared to that of the target tissue. Because of this high background signal, an appropriate kinetic model is required to quantify the signal originating from the brain tissue (Rizzo et al., 2014). Because the TSPO bound at the BBB masks various brain tissues, identification of an appropriate reference region is very difficult or impossible, and therefore obtaining accurate estimates of free plasma concentrations is crucial (Turkheimer et al., 2015). For these reasons, further research is needed to develop a TSPO radioligand that would improve on the image quality of TSPO-PET with (R)-[^{11}C]PK11195, while also being as straightforward to use in practice. While researchers are eager to develop radioligands that address the limitations of (R)-[^{11}C]PK11195, it remains a valuable tool in neuroimaging research. Being the first developed TSPO radioligand, it has been studied extensively and validated in preclinical and clinical settings. Additionally, binding of (R)-[^{11}C]PK11195 is not affected by the TSPO polymorphism like the second-generation radioligands, making its use more straightforward (Chauveau et al., 2021).

Though (R)-[^{11}C]PK11195 is more commonly used to study neuroinflammation in neurodegenerative diseases, MS, and psychiatric disorders (Chauveau et al., 2021), it has been utilised in studying viral infections (Hammoud et al., 2005; Pflugrad et al., 2016), and even ME/CFS (Nakatomi et al., 2014; Raijmakers et al., 2022). Studies on ME/CFS have shown conflicting results, as Nakatomi et al. (2014) reported widespread neuroinflammation and association with neuropsychiatric symptoms, but Raijmakers et al. (2022) found no significant

differences between patients with ME/CFS and healthy subjects, and negative association between BP_{ND} of [^{11}C]-PK11195 and symptom severity scores. There are no previous studies using (R)-[^{11}C]PK11195 to study neuroinflammation in patients with PCC, but there are two studies using different, second-generation TSPO binding radioligands (Braga et al., 2023; Visser et al., 2022). Visser et al. (2022) showed a widespread increase in [^{18}F]DPA-714 binding in two patients with PCC. These patients had typical symptoms associated with PCC including fatigue, cognitive symptoms, anosmia, and headaches (Visser et al., 2022). Instead of PCC, the study conducted by Braga et al. (2023) focused on patients with persistent depressive and cognitive symptoms after COVID-19, referred to as COVID-DC in the article. In their study, Braga et al. (2023) showed increased [^{18}F]FEPPA V_T across all regions of interest in subjects with COVID-DC (n = 20) compared to healthy controls (n = 20). To my knowledge there are no other studies utilising TSPO-PET to quantify neuroinflammation in patients with PCC.

1.5 Aims and Hypotheses

The primary aim of this master's thesis is to evaluate whether microglial activation is increased in patients with PCC with neurological symptoms *via* TSPO-PET imaging with [^{11}C]PK11195 radioligand. Microglial activation is evaluated as the specific binding of the radioligand, calculated as DVRs. The study entails comparing [^{11}C]PK11195 DVRs across several regions of interest between healthy volunteers and participants with PCC. Secondary aims are to describe the pattern of neurological symptoms experienced by participants with PCC by collecting patient history, conducting a clinical neurological examination, conducting a six-minute walking test, and assessing questionnaire scores. Within the PCC group, the effects of hospitalisation during COVID-19 and results of the neurological examination on [^{11}C]PK11195 DVRs are assessed. Biomarker measurements, including GFAP and NfL, and MRI volumes are also assessed to complement the information acquired from PET results. Finally, correlations between DVRs and clinical characteristics, questionnaire scores, and biomarker results are assessed.

The hypothesis is that microglial activation is increased in patients with PCC compared to the healthy control group, demonstrated by increased binding of [^{11}C]PK11195. Increased microglial activation has been shown in two PET studies on patients with persisting neurological symptoms after COVID-19 (Braga et al., 2023; Visser et al., 2022). Increased microglial activation indicates that neuroinflammation may contribute to the development of neurological symptoms typical to PCC. Currently, the pathophysiology of PCC is unknown; however, studying the mechanisms contributing to the condition could lead to preventing its onset or improving the outcomes for affected individuals.

2 Materials and Methods

2.1 Study Subject Recruiting and Selection

PCC patients were recruited from neurology outpatient clinics in the hospital district of southwest Finland and from long COVID outpatient clinic at Helsinki University Hospital. Healthy participants were recruited through advertising in the Turku University Hospital (TYKS) intranet. The PCC group consisted of 20 participants and the healthy control group consisted of 13 healthy individuals. Healthy participants were compensated 120 euros for participation in the study.

The inclusion criteria for the PCC group were age of 18 years or older, having neurological symptoms such as fatigue, various cognitive symptoms, and sleep disturbances that have lasted for over four weeks after a PCR or antibody test confirmed COVID-19 infection. The exclusion criteria for post COVID-19 patients were another condition causing similar symptoms associated with PCC, pregnancy or breast feeding, corticosteroid treatment within past four weeks before PET/MRI, claustrophobia or history of severe anxiety or panic attacks, exposure to experimental radiation within the past 12 months, and intolerance to PET or MR scans. The inclusion criteria for the healthy control group were age of 18 years or older, being reportedly healthy, and matching the age and sex demographics of the PCC group. The exclusion criteria for the healthy control group were the same as for participants with PCC with an additional criterion of CNS disease or major or malignant underlying disease of other organ systems.

This study was approved by Ethics Committee of the Hospital District of Southwest Finland. Participants included in the study signed written informed consent according to the principles of the Declaration of Helsinki.

2.2 Clinical Assessment

Individuals with PCC who were interested in the study were invited to the Neurology Outpatient Clinic in Turku to a clinical assessment conducted by the research neurologist. The physician performed a clinical neurological examination and collected information on the current symptoms related to PCC, as well as those experienced during the COVID-19 infection through an interview. Healthy controls were not invited to a clinical assessment.

During the clinical assessment, participants with PCC performed a six-minute walk test. During the test, participants walked back and forth a 30-meter walkway continuously for six minutes. The test consisted of four stages, during which heart rate and breathing frequency were measured and the participants assessed their level of exertion using the Borg scale. The first stage was after a 10-minute seated break before starting the walk, the second stage was at a standing position directly before starting the walk, the third stage was directly after walking, and the final stage was after a three-minute standing break.

2.3 Questionnaires

Participants were given the EuroHIS-8, Fatigue Severity scale, General Anxiety Disorder-7, Modified Fatigue Impact Scale**,** Insomnia Severity Index, Patient Health Questionnaire, RAND 36-item health survey 1.0, and WHO disability assessment schedule questionnaires to fill in.

2.3.1 EuroHIS-QoL 8-item index

EuroHIS quality-of-life 8-item index (EuroHIS-8) (see Appendix 1 for the Finnish version of the questionnaire) is a shortened version of the WHOQOL-BREF 26-item questionnaire, developed for quick and easy assessment of quality of life (QoL) (Power, 2003). The questionnaire consists of eight items concerning overall QoL, health, energy, finances, daily life activities, self-esteem, social relationships, and home. The participants were asked to answer each statement by choosing one of five response options that best described their experience during the past two weeks. The response options were individualised for each question and scored from one to five, indicating discontent and contentment respectively. EuroHIS-8 scores were calculated as an average of the total questionnaire score.

2.3.2 Fatigue Severity Scale

Fatigue Severity Scale (FSS) (see Appendix 2 for the Finnish version of the questionnaire) is a 9-item fatigue questionnaire for assessing fatigue in neurological diseases (Krupp et al., 1989). The participants were asked to answer each statement by choosing a number from one to seven that best described their degree of agreement to each statement, one indicating “strongly disagree” and seven “strongly agree”. FSS scores were calculated according to the scoring guidelines as an average of the total questionnaire score. This study employed a scoring method where an average score of less than four was interpreted as no fatigue, while a score greater than or equal to four indicated fatigue.

2.3.3 Generalised Anxiety Disorder 7-item questionnaire

Generalised Anxiety Disorder 7-item questionnaire (GAD-7) (see Appendix 3 for the Finnish version of the questionnaire) is developed for identifying probable cases of generalized anxiety disorder (Spitzer et al., 2006). The participants were asked to answer each statement by choosing the response option that best described their experience during the past two weeks. The response options were “not at all”, “several days”, “more than half the days” and “nearly every day”, scored as zero, one, two, and three, respectively. The scores for GAD-7 questionnaire were calculated as a sum of all statements, according to the guidelines. GAD-7 employs a threshold of 10 or higher to indicate possible generalised anxiety disorder.

2.3.4 Modified Fatigue Impact Scale

Modified Fatigue Impact Scale (MFIS) (see Appendix 4 for the Finnish version of the questionnaire) is a 21-item modified version of the Fatigue Impact Scale (Fisk et al., 1994). The questionnaire is commonly used to assess the effects fatigue has on physical, cognitive, and psychosocial functioning in patients with MS. The participants were asked to answer the statements by choosing the response option that best described their experience during the past four weeks. The five response options were, “no/never”, “rarely”, “sometimes”, “often”, and “almost always”, scored as zero, one, two, three, and four, respectively. The final scores were calculated as a sum of all statements according to the guidelines. A sum score of 38 has been used as a threshold for fatigue in patients with MS and this threshold was chosen for this study as well (Flachenecker et al., 2002).

2.3.5 Insomnia Severity Index

Insomnia Severity Index (ISI) (see Appendix 5 for the Finnish version of the questionnaire) is a 5-item questionnaire developed for assessing the severity of both nighttime and daytime components of insomnia (Morin et al., 2011). The participants were asked to choose the response option from zero to four, that best described their situation in the past month. The scores for ISI were calculated as a sum of all statements according to the guidelines. For ISI, the scoring guidelines were following: a sum score between zero and seven indicates no clinically significant insomnia, a score between eight and 14 indicates mild insomnia, a score between 15 and 21 indicates moderate insomnia, and a score between 22 and 28 indicates severe insomnia.

2.3.6 Patient Health Questionnaire 9

Patient Health Questionnaire 9 (PHQ-9) (see Appendix 6 for the Finnish version of the questionnaire) is the depression module of the patient health questionnaire (PHQ) that encompasses each of the 9 DSM-IV criteria for depression (Kroenke et al., 2001). Each statement was scored as “not at all”, “several days”, “more than half the days” or “nearly every day”, from zero to four respectively. The questionnaire scores were calculated as a sum of each statement according to guidelines. For PHQ-9 following scoring guidelines were used: a sum score between zero and four indicates no depression, a score between five and nine indicates mild depression, a score between 10 and 14 indicates moderate depression, a score between 15 and 19 indicates moderately severe depression, and a score equal or above 20 indicates severe depression.

2.3.7 RAND 36-item Health Survey

RAND 36-item health survey (RAND-36) (see Appendix 7 for the Finnish version of the questionnaire) is a set of health-related QoL measures (Hays & Morales, 2001). The questionnaire consists of 36-items from eight health concepts: general health perceptions, limitations in physical functioning, psychological distress and well-being, limitations in social functioning, energy and fatigue, bodily pain, physical limitations in usual role activities, and emotional limitations in usual role activities. The questionnaire is divided into segments where response options are either on a scale from one to five, one to three or binary choices of yes or no. The final scores were calculated according to the guidelines (RAND healthcare, n.d.).

2.3.8 WHO Disability Assessment Schedule 2.0

WHO Disability Assessment Schedule 2.0 (WHODAS 2.0) (see Appendix 8 for the Finnish version of the questionnaire) is an assessment instrument for measuring health and disability at population level or in clinical practice (Rehm et al., 1999). It captures the level of functioning in six domains of life: cognition, understanding and communication; mobility; self-care; social interactions; everyday life activities; and participating in community activities and society. The questionnaire has five response options, “not at all difficult”, “slightly difficult”, “moderately difficult”, “considerably difficult”, and “very difficult or unable to”, scored from zero to four respectively. The participants were asked to choose the response option that best described their experience in the last 30 days. The final scores were

calculated as a sum of all statements according to the guidelines. WHODAS 2.0 questionnaire scores were converted to percentages to enable the use of the International Classification of Functioning (ICF) in assessing the results. The ICF scale defines impairment levels for WHODAS as follows: no impairment (0–4%), mild impairment (5–24%), moderate impairment (25–49%), severe impairment (50–95%), and complete impairment (96–100%) (Üstün et al., 2010).

2.4 PET/MR Imaging

The PET/MR imaging was performed at the Turku PET Centre with GE SIGNA^{TM} PET/MR scanner (GE HealthCare, Chicago, IL, U.S.).

A 60-minute 3.0 T brain MRI was performed. The following MRI sequences were used for image acquisition: axial T2, 3D fluid attenuated inversion recovery (FLAIR), 3D T1, and 3D T1 with gadolinium enhancement.

After the MRI a dynamic 60-minute whole brain [^{11}C]PK11195 PET imaging was performed by the study physician. The [^{11}C]PK11195 radioligand radiochemical synthesis was performed as described previously (Rissanen et al., 2018). The mean (SD) injected dose of radioligand was 436.4 (55.1) in total, 444.9 (60.7) for healthy controls and 430.4 (52.0) for participants with PCC, with no significant differences between groups.

2.5 Biomarker Analysis

Blood samples were collected before the PET/MRI in 10ml Vacuette(R) serum clot-activator tubes (product number 455092, Greiner Bio-One, Kremsmünster, Austria). Blood was allowed to clot for 30 minutes at room temperature and serum was stored in aliquots at -80°C in the Auria Biobank (Turku, Finland) within 2 hours of sampling. Frozen samples were shipped packed with dry ice to Basel, Switzerland, where serum NfL and GFAP concentrations were measured by a single molecule array assay (Simoa® Technology, Quanterix, Billerica, MA, U.S.).

2.6 Data Analysis

2.6.1 PET/MR Imaging

MR image and PET image co-registration was performed using statistical parametric mapping (SPM8, version 8; Wellcome Trust Center for Neuroimaging) running on Matlab 2017a (The MathWorks, Natick, MA, U.S.).

PET images were reconstructed with Q.Clear using 17 time frames. Mutual information realignment in SPM8 was used to correct possible displacements between frames. All images were resliced to match an MR voxel size of 1 mm x 1mm x 1mm.

T1 MR images were used for auto segmentation of regions of interest (ROIsF) with FreeSurfer image analysis suite v7.2.0., which is documented and freely available (http://surfer.nmr.mgh.harvard.edu/). ROIs chosen for this study were the whole brain, white matter, cortical grey matter, brainstem, cerebellum, cingulate cortex, thalamus, hippocampus, putamen, pallidum, amygdala, and caudate.

MRI volumes for white matter, whole brain, and cortical grey matter were calculated based on respective segments created in FreeSurfer as described previously (Rissanen et al., 2018). Intracranial volumes (ICVs) were calculated using SPM8.

Microglial activation was evaluated as specific binding of [^{11}C]PK11195 radioligand as DVRs using the logan method within a time interval of 20–60 minutes. A supervised clustering algorithm with four predefined kinetic tissue classes was used with Matlab SuperPK software package to acquire the time activity curve corresponding to a reference region devoid of specific radioligand binding (Turkheimer et al., 2007).

2.6.2 Statistical Analysis

All statistical analyses were performed using R (version 4.3.2) (R Core Team, 2023) and RStudio (Posit Team, 2023).

Shapiro-Wilk normality test was used to test the normality of the data. When normally distributed, an unpaired t-test with Welch's correction was used to compare two groups, otherwise a Wilcoxon rank-sum test was applied. Effect sizes were calculated as rank-biserial correlation coefficient (r_{rb}), where values range from -1 meaning the dominance of the second sample, to +1 meaning the dominance of the first sample (Cureton, 1956). The magnitude of

effect size was defined as $|r_{rb}| \leq 0.1$ trivial, $|r_{rb}| < 0.3$ small, $0.3 \leq |r_{rb}| \leq 0.5$ moderate, and $|r_{rb}| > 0.5$ large. For multiple comparisons, Kruskal-Wallis test and pairwise Wilcoxon rank-sum test was applied. Multiple comparison correction was not used because the number of compared groups was three at most. Spearman correlation was used for correlation analysis. For all tests, $p \leq 0.05$ was considered significant.

3 Results

3.1 Demographics

The study sample consisted of a total of 46 participants of which 13 were healthy controls (HC) and 20 were participants with PCC experiencing neurological symptoms. Additionally, 13 participants with MS were included to serve as a positive control group for biomarker analysis and MRI volumetrics. The demographic information of all participants is presented in Table 1. The HC and PCC groups were matched based on age and sex, and there were no significant differences between groups in either variable (Table 1). The age and sex of the MS group did not differ significantly from either the HC or the PCC group (Table 1). There were no significant differences between groups in BMI (Table 1). However, the mean BMI for HC group was above the threshold for overweight (>25 kg/m^2), and for the PCC group and MS group it was above the threshold for obese (>30 kg/m^2).

Table 1. Demographic information.

HC = healthy control, PCC = post-COVID-19 condition, MS = multiple sclerosis

Variable	Total (*N* = 46)	HC (*n* = 13)	PCC (*n* = 20)	MS (*n* = 13)	*p* (HC vs. PCC)	*p* (HC vs. MS)	*p* (PCC vs. MS)
Age, years							
Mean (SD)	45 (8.9)	44 (12)	45 (7.8)	47 (6.6)	0.58	0.3	0.59
Min-Max	26-67	26 - 67	32 - 62	38-61			
Sex					1	0.227	0.146
Male, *n* (%)	21 (46)	7 (54)	11 (55)	3 (23)			
Female, *n* (%)	25 (54)	6 (46)	9 (45)	10 (77)			
BMI (kg/m^2)							
Mean (SD)	29.6 (6.9)	27 (3.6)	31 (7.3)	30 (8.3)	0.16	0.42	0.79

3.2 Clinical Characteristics

3.2.1 Clinical Assessment

Of the participants with PCC, 19 out of 20 individuals participated in the clinical assessment. Of these participants, two had had their first COVID-19 infection in 2020, nine in 2021, and eight in 2022. Most participants had had PCR or antibody test proven COVID-19 only once, but three participants had had it twice and one thrice. Seven of the participants were hospitalised during COVID-19 infection, with none requiring ICU admission. Most participants reported that the post-COVID-19 symptoms started immediately after recovering from the infection, while two of the participants reported that the symptoms started around three months after the infection. At the time of clinical examination and PET-imaging, the median (Q1–Q3) duration of PCC symptoms was 365 (258.5–746) days. The shortest disease duration was 8 months while the longest was 2 years and 9 months.

Participants described a wide range of PCC symptoms, of which any symptom experienced by at least two participants were included in analysis, resulting in a total of 14 symptoms. The PCC symptoms and their frequency are shown in Table 2. The most common symptom was post-exertional malaise i.e., the worsening of symptoms after physical or mental exertion, with 16 out of 19 participants. Fatigue and brain fog were also common, with 12 and 11 incidents respectively.

Table 2. Localisation and frequency of neurological symptoms in PCC patients.

Localisation in the NS	Neurological symptoms	Freq, n (%)
Central nervous system	Fatigue	12 (60)
	Brain fog	11 (55)
	Headache	7 (35)
	Memory problems	7 (35)
	Nausea	2 (10)
Peripheral nervous system	Anosmia	6 (30)
	Muscle or joint ache	6 (30)
	Weakness or numbness of limbs	5 (25)
	Sensory issues	4 (20)
	Ageusia	3 (15)
	Heart palpitations	3 (15)
	Shortness of breath	2 (10)
	Sweating	2 (10)
Not categorised	Post exertional malaise	16 (80)

3.2.2 Neurological Examination

All 20 participants presenting with PCC underwent neurological examinations. Of these, 11 participants did not have a fully normal neurological profile. Specific impairments were identified in five domains: higher cognitive functions in five participants, cranial nerves in four participants, motor functions in one participant, coordination in one participant, and sensory functions in two participants (Figure 3).

Within the subset of participants with abnormal higher cognitive functions, three out of five participants reported concentration problems and brain fog, one out of five experienced memory issues, and one out of five exhibited acalculia. Concerning cranial nerves, all four participants had impaired olfaction. Motor function abnormalities were observed in one participant, who had difficulty in toe walking, heel walking, squat sitting, rising from squat sitting, and jumping on one leg. In terms of coordination, dysmetria in the finger-nose test and heel-knee test were identified in one participant. Sensory examination revealed abnormalities in two participants: impaired vibration sense in both feet for one participant, and impaired sense of touch on the back of left foot and impaired sense of pain in the back of right palm, for another participant.

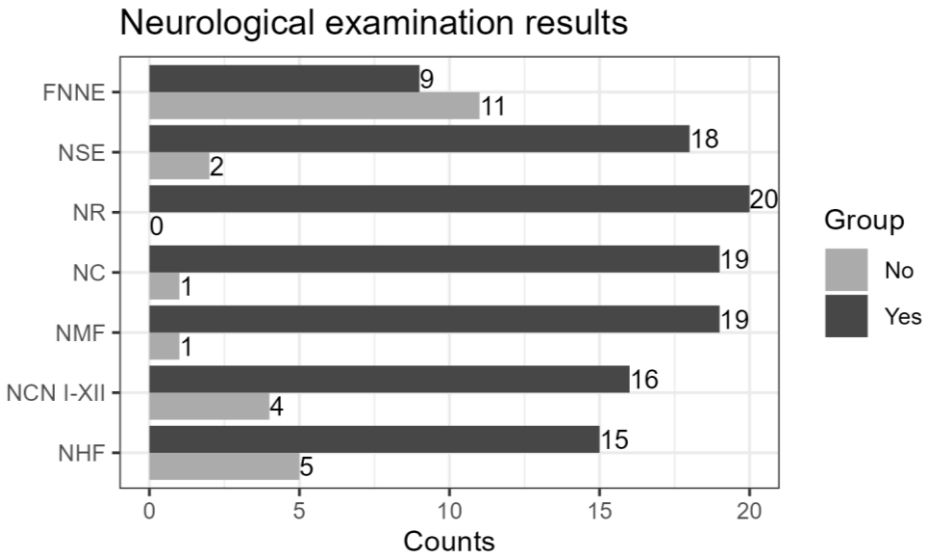

Figure 3. Results of neurological examination. FNNE = fully normal neurological exam, NSE = normal sensory exam, NR = normal reflexes, NC = normal coordination, NMF = normal motor functioning, NCN I-XII = normal cranial nerves 1-12, NHF = normal higher functions.

3.2.3 Six-Minute Walk Test

Out of 20 participants in the PCC group, 19 participated in a six-minute walk test (6MWT) to assess endurance and perceived exertion. Reference values for men and women were calculated according to Enright and Sherrill (2012). The calculated reference values included lower limit of normal (LLN) and predicted 6-minute walking distance (6MWD) and were calculated separately for men and women (Enright & Sherrill, 2012). The average LLN, predicted 6MWD, and measured 6MWD are presented in Table 3. Unpaired t-test with Welch's correction was used to compare the predicted 6MWD and the measured 6MWD for men and women, revealing a significant difference for both groups, $p = 0.001$ and $p = 0.033$ respectively (Table 3). As 6MWT was not performed on the healthy control group, comparing results between HC and PCC groups was not done.

Borg scale for ratings of perceived exertion was used to assess effort and exertion, breathlessness, and fatigue during the 6MWT. In the first two stages, Borg scores for men ranged from "no exertion" (6) to "extremely light exertion" (8), and for women, from "no exertion" (6) to "light exertion" (10). Following the 6-minute walk, Borg scores varied from "very light exertion" (6) to "hard exertion" (15) for men and from "no exertion" (6) to "hard exertion" (16) for women. After a standing break, Borg scores ranged from "extremely light exertion" (7) to "light exertion" (12) in men and from "no exertion" (6) to "somewhat hard exertion" (14) in women. Baseline values and peak values of median heartrate, breathing frequency, and Borg scores are presented in Table 4.

Table 3. Six-minute walking test results.

Mean (SD) of lowest limit of normal (LLN), predicted six-minute walking distance, and measured six-minute walking distance.

Group	LLN, m	Predicted 6MWD, m	Measured 6MWD, m	*p* (pred. vs. meas. 6MWD)
Men (*n* = 10)	496 (44)	649 (44)	531 (79)	0.001
Women (*n* = 9)	433 (79)	572 (79)	485 (80)	0.033

Table 4. Baseline and peak values for heart rate, breathing frequency (BF) and Borg scale.

	Men (*n* = 10)		Women (*n* = 9)	
Variable, median (Q1 – Q3)	**Baseline**	**Peak value**	**Baseline**	**Peak value**
Heart rate, bpm	68 (61 – 85)	96 (87 – 113)	74 (60 – 93)	95 (93 – 116)
BF, breaths/min	12 (10 – 14)	20 (17 – 20)	13 (12 – 14)	18 (16 – 20)
Borg scale	6.5 (6 – 7)	13 (12 – 14)	7 (6 – 9)	13 (12 – 14)

3.3 Questionnaire Scores

Of the 20 participants with PCC, four participants were excluded from questionnaire analyses due to incomplete questionnaire submissions. Of the participants included, 15 completed all questionnaires and one participant completed three of the questionnaires (FSS, MFIS, and RAND-36).

The means (SD), minimum and maximum values, and Cronbach's α values for EuroHIS-8, FSS, GAD-7, MFIS, ISI, PHQ-9, and WHODAS 2.0 questionnaires are shown in Table 5. All questionnaires had a Cronbach's α higher than the threshold of 0.7, which is commonly set as a threshold for adequate internal consistency.

There are no official guidelines on interpreting the results of EuroHIS-8 questionnaire. However, the Finnish institute for health and welfare (Terveyden ja hyvinvoinnin laitos, THL) has conducted a national EuroHIS-8 survey in 2022, revealing that 51% of Finns felt that their QoL was "good". The individuals who felt their QoL was "good" were defined as having an average score of at least four out of five (THL, 2022). In our study, participants with PCC had an average EuroHIS-8 score of 3.1. Out of 15 participants that completed this questionnaire, 13 had a score lower than four.

The average FSS score for participants with PCC was 5.8. Out of 16 participants that completed the questionnaire, 15 participants had a score higher than 4, indicating fatigue.

The average GAD-7 score was 12.1. Out of 15 participants that completed this questionnaire, eight had a score higher than 10, indicating anxiety.

The mean MFIS score for participants with PCC was 48.3. Out of 16 participants that completed the questionnaire, 12 participants had a score higher than 38, indicating fatigue.

The mean ISI score for participants with PCC was 11.9. Out of 15 participants who answered this questionnaire, five had no insomnia, five had mild insomnia, four had moderate insomnia, and one had severe insomnia according to the questionnaire scores.

The mean PHQ-9 score for participants with PCC was 8.1. Out of 15 participants who answered this questionnaire, four had no depression, seven had mild depression, two had moderate depression, and two had moderately severe depression according to the questionnaire scores.

The mean WHODAS 2.0 score was 42.1. Out of 15 participants who answered this questionnaire, five had mild impairment, nine had moderate impairment, and one had severe impairment according to the ICF classification.

RAND-36 scoring was done according to the guidelines (RAND healthcare, n.d.). Reference values based on Finnish population were published in 1999 (Aalto et al., 1999). Each of the eight health concepts had a Cronbach's α exceeding 0.7 signifying adequate internal consistency. Participants with PCC exhibited a lower mean score in all eight health concepts in comparisons to the reference values representative of the average Finnish population. The median (Q1 – Q3), mean (SD), and Cronbach's α values of RAND-36 for participants with PCC and refence mean (SD) values are presented in Table 6.

Table 5. EuroHIS-8, FSS, GAD-7, MFIS, ISI, PHQ-9, and WHODAS 2.0 questionnaire results.

Variable	EuroHIS-8	FSS	GAD-7	MFIS	ISI	PHQ-9	WHODAS
Mean (SD)	3.1 (0.7)	5.8 (1.2)	12.1 (3.9)	48.3 (18)	11.9 (6.4)	8.1 (4.8)	42.1 (21.3)
Min – Max	2.1 – 4.25	2 – 7	7 – 20	3 – 75	2 – 23	2 – 17	12 – 91
Cronbach's α	0.85	0.95	0.84	0.95	0.86	0.85	0.94

Table 6. RAND-36 questionnaire results.

	PCC (*n* = 16)			Reference (*n* = 2060)
	Median (Q1 – Q3)	**Mean (SD)**	**Cronbach's α**	**Mean (SD)**
General	25 (25 – 50)	38.4 (31.6)	0.75	65.0 (19.8)
Physical functioning	50 (50 – 100)	62.5 (40.0)	0.88	84.9 (20.1)
Emotional	60 (40 – 80)	59.8 (26.1)	0.93	73.7 (19.7)
Social functioning	50 (25 – 50)	46.1 (27.0)	0.89	82.1 (23.2)
Energy	20 (0 – 40)	30.6 (30.9)	0.82	64.0 (22.4)
Pain	60 (40 – 75)	56.1 (31.3)	0.9	76.2 (24.0)
Physical role lim.	0 (0 – 0)	12.5 (33.3)	0.84	74.8 (35.5)
Emotional role lim.	50 (0 – 100)	50 (50.5)	0.88	75.0 (36.4)

3.4 DVR Group Comparisons in Different Brain Regions

Out of the 20 participants with PCC, and 13 healthy controls that enrolled to this study, successful PET imaging was performed to 15 and 11 participants respectively. Groupwise median (Q1–Q3) DVRs and statistics for all chosen ROIs are presented in Tables 7, 8, and 9.

To investigate the effects of PCC on microglial activation, specific binding of [^{11}C]PK11195 was compared between HC subjects and PCC subjects. Comparisons of DVRs in chosen ROIs between HC group and PCC group are presented in Figure 4 and Table 7. There were no significant differences between the HC group and patients with PCC in any of the examined ROIs (Table 7). Still, the PCC group exhibited lower median DVRs in brainstem and cingulate cortex with a moderate effect size; in pallidum, thalamus, amygdala, putamen, and caudate with a small effect size; and higher median DVR in cortical grey matter with a small effect size, while in other regions the DVRs remained similar (Table 7).

The PCC group was further divided into two subgroups based on hospitalisation status during the acute phase of COVID-19 to study the impact of infection severity on microglial activation. There were differences in DVRs between groups, though not statistically significant (Table 8). Subjects who had been hospitalised demonstrated higher DVRs in cortical grey matter with a large effect size; in brain, cingulate cortex, putamen, and amygdala with a moderate effect size; and in caudate with a small effect size (Table 8, Figure 5).

The PCC group was also divided into two subgroups to investigate whether outcomes of neurological examination affected microglial activation. There were no significant differences between the subgroups across all examined ROIs (Table 9, Figure 6). Those who exhibited abnormalities in the neurological examination had slightly elevated DVRs in all examined ROIs compared to the subgroup with fully normal neurological exam results (Table 9). DVRs were higher in cingulate cortex with a large effect size; in white matter, brainstem, cerebellum, thalamus, hippocampus, pallidum, and caudate with a moderate effect size; and in brain, cortical grey matter, and putamen with a small effect size (Table 9).

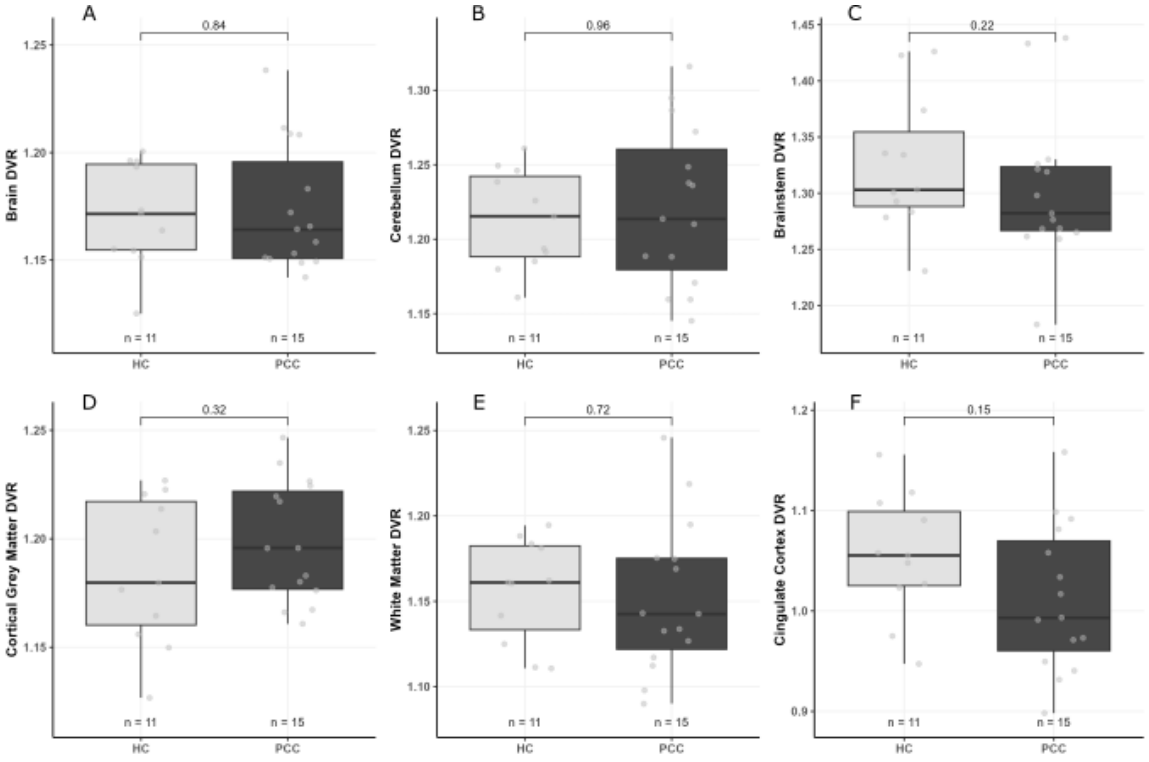

Figure 4 Comparison of DVRs between HC group and PCC group in chosen ROIs. A) Whole brain DVRs were very similar between HC and PCC group, with no significant differences. B) No significant differences between HC and PCC group in cerebellum DVRs. C) PCC patients had lower DVRs in the brainstem compared to HC group, with no significant differences. D) PCC patient had slightly higher DVRs in cortical grey matter with no significant differences between groups. E) In the white matter, there were no significant differences between groups. F) In cingulate cortex, PCC patients had lower DVRs compared to HC group with no significant differences.

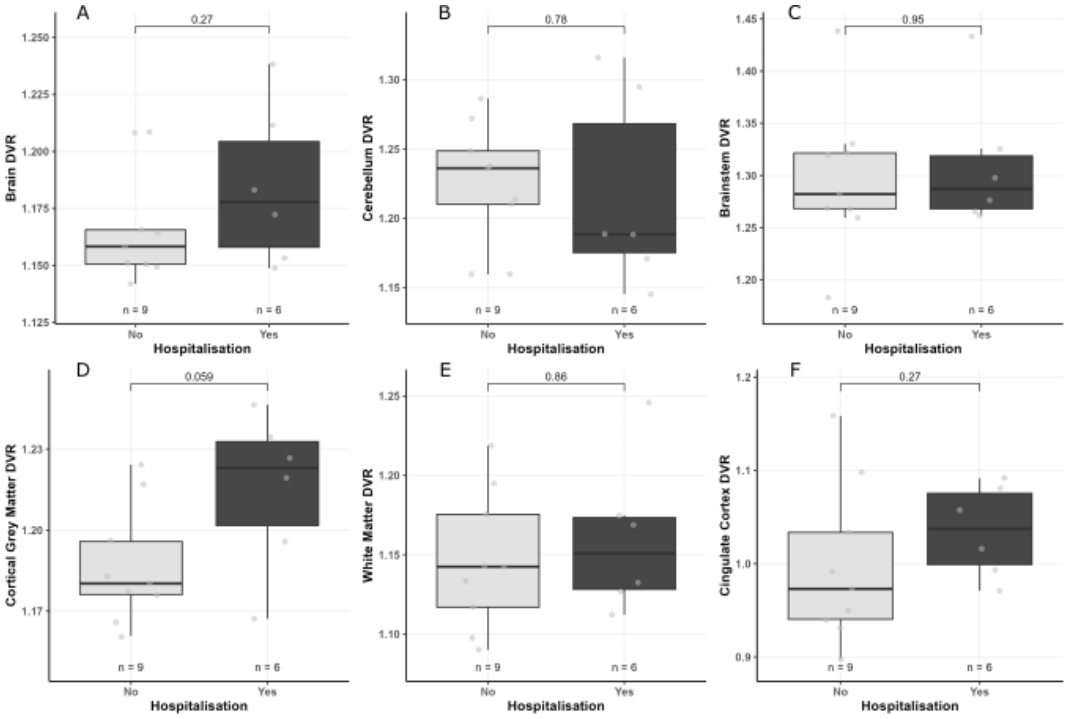

Figure 5 Comparison of DVRs between hospitalised and non-hospitalised PCC patients in chosen ROIs. There were no significant differences between groups in any ROIs. Median DVRs were higher in hospitalised patients in A) the brain, D) the cortical grey matter, and F) the cingulate cortex.

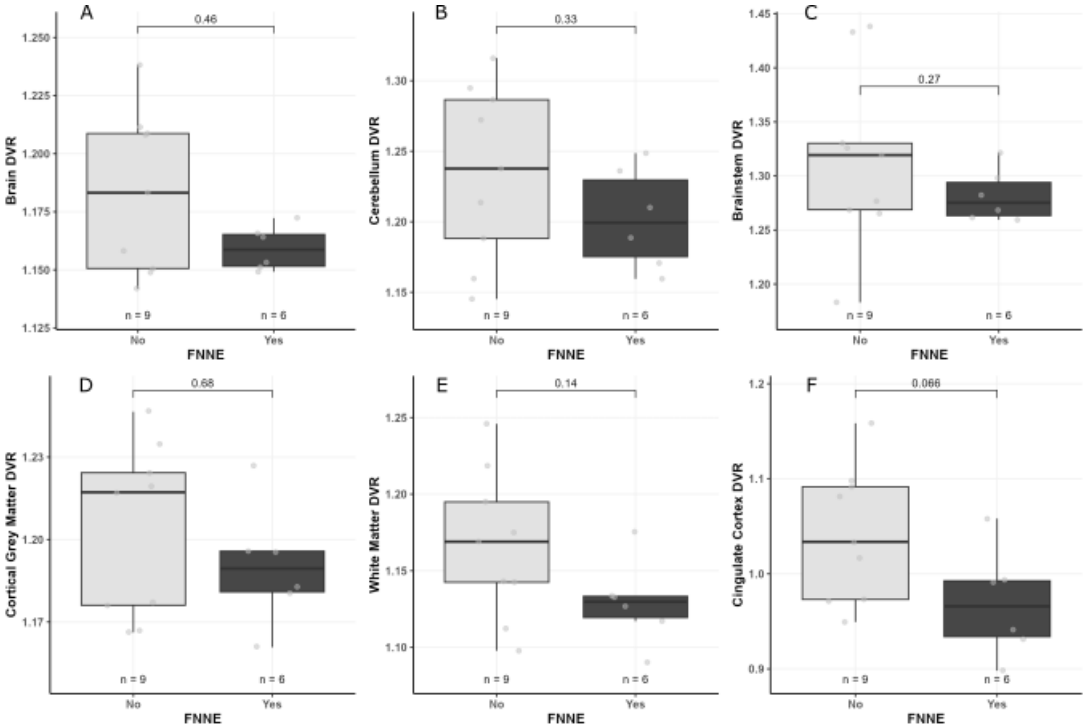

Figure 6 Comparison of DVRs between PCC patients with fully normal neurological exam (FNNE) and patients with abnormal findings in the neurological exam in chosen ROIs. There were no significant differences between groups. Median DVRs were lower in participants with FNNE in all chosen ROIs.

Table 7 Comparison of DVRs between HC group and PCC group in ROIs.

WM = white matter, GM = grey matter, r_{rb} = rank-biserial correlation coefficient.

DVR, median (Q1–Q3)	HC (*n* = 11)	PCC (*n* = 15)	*p*	r_{rb}
Brain	1.17 (1.15–1.19)	1.16 (1.15–1.2)	0.84	0.05
WM	1.16 (1.13–1.18)	1.14 (1.12–1.18)	0.72	0.09
Cortical GM	1.18 (1.16–1.22)	1.2 (1.18–1.22)	0.32	-0.24
Brainstem	1.3 (1.29–1.35)	1.28 (1.27–1.32)	0.22	0.30
Cerebellum	1.22 (1.19–1.24)	1.21 (1.18–1.26)	0.96	-0.02
Cingulate cortex	1.06 (1.03–1.1)	0.99 (0.96–1.07)	0.15	0.35
Thalamus	1.29 (1.21–1.31)	1.26 (1.22–1.28)	0.44	0.19
Hippocampus	1.12 (1.09–1.13)	1.11 (1.06–1.15)	0.76	0.08
Putamen	1.22 (1.19–1.24)	1.19 (1.17–1.23)	0.45	0.18
Pallidum	1.23 (1.19–1.24)	1.18 (1.15–1.23)	0.31	0.25
Amygdala	1.13 (1.11–1.15)	1.12 (1.07–1.16)	0.65	0.12
Caudate	1.01 (1–1.07)	1.02 (0.97– 1.07)	0.47	0.18

Table 8 Comparison of DVRs between hospitalised and non-hospitalised PCC patients in ROIs.

WM = white matter, GM = grey matter, r_{rb} = rank-biserial correlation coefficient.

	Hospitalisation			
DVR, median (Q1-Q3)	Yes (*n* = 6)	No (*n* = 9)	*P*	r_{rb}
Brain	1.18 (1.16–1.2)	1.16 (1.15–1.17)	0.27	0.37
WM	1.15 (1.13–1.17)	1.14 (1.12–1.18)	0.86	0.07
Cortical GM	1.22 (1.2–1.23)	1.18 (1.18–1.2)	0.06	0.61
Brainstem	1.29 (1.27–1.32)	1.28 (1.27–1.32)	0.95	0.04
Cerebellum	1.19 (1.18–1.27)	1.24 (1.21–1.25)	0.78	-0.11
Cingulate cortex	1.04 (0.99–1.08)	0.97 (0.94–1.03)	0.27	0.37
Thalamus	1.25 (1.21–1.3)	1.26 (1.23–1.27)	1	0.00
Hippocampus	1.1 (1.07–1.12)	1.13 (1.05–1.16)	0.78	-0.11
Putamen	1.22 (1.19–1.24)	1.19 (1.16–1.21)	0.33	0.33
Pallidum	1.18 (1.15–1.22)	1.18 (1.17–1.24)	0.95	-0.04
Amygdala	1.15 (1.1–1.16)	1.07 (1.07–1.13)	0.33	0.33
Caudate	1.03 (1–1.07)	0.99 (0.96–1.06)	0.46	0.26

Table 9 Comparison of DVRs between PCC patients with fully normal neurological exam and patients with abnormalities in neurological exam.

FNNE = Fully normal neurological exam, WM = white matter, GM = grey matter, r_{rb} = rank-biserial correlation coefficient.

	FNNE			
DVR, median (Q1-Q3)	**Yes (*n* = 6)**	**No (*n* = 9)**	***p***	***r_{rb}***
Brain	1.16 (1.15–1.17)	1.18 (1.15–1.21)	0.46	-0.26
WM	1.13 (1.12–1.13)	1.17 (1.14–1.19)	0.14	-0.48
Cortical GM	1.19 (1.18–1.2)	1.22 (1.18–1.22)	0.68	-0.15
Brainstem	1.28 (1.26–1.29)	1.32 (1.27–1.33)	0.27	-0.37
Cerebellum	1.2 (1.18–1.23)	1.24 (1.19–1.29)	0.33	-0.33
Cingulate cortex	0.97 (0.93–0.99)	1.03 (0.97–1.09)	0.07	-0.59
Thalamus	1.22 (1.2–1.26)	1.27 (1.23–1.28)	0.39	-0.33
Hippocampus	1.06 (1.04–1.13)	1.12 (1.09–1.15)	0.33	-0.33
Putamen	1.18 (1.16–1.2)	1.22 (1.18–1.24)	0.11	-0.22
Pallidum	1.17 (1.16–1.19)	1.21 (1.15–1.27)	0.53	-0.30
Amygdala	1.09 (1.07–1.13)	1.13 (1.07–1.16)	0.78	-0.11
Caudate	0.99 (0.96–1.03)	1.04 (0.99–1.08)	0.18	-0.44

3.5 MRI Volumetrics

MR images were acquired from 12 healthy participants and 18 PCC participants. In addition, 13 subjects with MS were included as a positive control for MRI volume loss. Groupwise comparisons are presented in Figure 7 and Table 10.

Brain volumes were significantly lower in MS subjects compared to HC subjects (p = 0.0012), and PCC subjects (p = 0.0018) (Figure 7A). There were no significant differences between HC subjects and PCC subjects. There were no significant differences in ICVs between groups (Figure 7B). Cortical grey matter volumes were significantly lower in MS subjects compared to HC group (p = 0.015) and PCC group (p = 0.02) (Figure 7C). There were no significant differences between HC group and PCC group in cortical grey matter volumes. White matter volumes were significantly lower in MS subjects compared to HC subjects (p = 0.0039) and PCC subjects (p = 0.0078) (Figure 7D). There were no significant differences between HC and PCC groups in white matter volumes.

Table 10 Comparison of MRI brain volumes between HC group, PCC group, and MS group.

ICV = Intracranial volume, GM = grey matter, WM = white matter.

Variable, median (Q1-Q3)	HC (*n* = 12)	PCC (*n* = 18)	MS (*n* = 13)	*p* (HC vs. PCC)	*p* (HC vs. MS)	*p* (PCC vs. MS)
Brain	1233 (1183–1259)	1194 (1105–1305)	1089 (1065–1159)	0.69	0.0012	0.018
ICV	1475 (1358–1518)	1399 (1283–1526)	1356 (1276–1405)	0.63	0.087	0.465
Cortical GM	469 (451–485)	467 (439–497)	413 (383–464)	0.79	0.015	0.02
WM	496 (478–518)	512 (441–543)	433 (412–465)	0.61	0.0039	0.0076

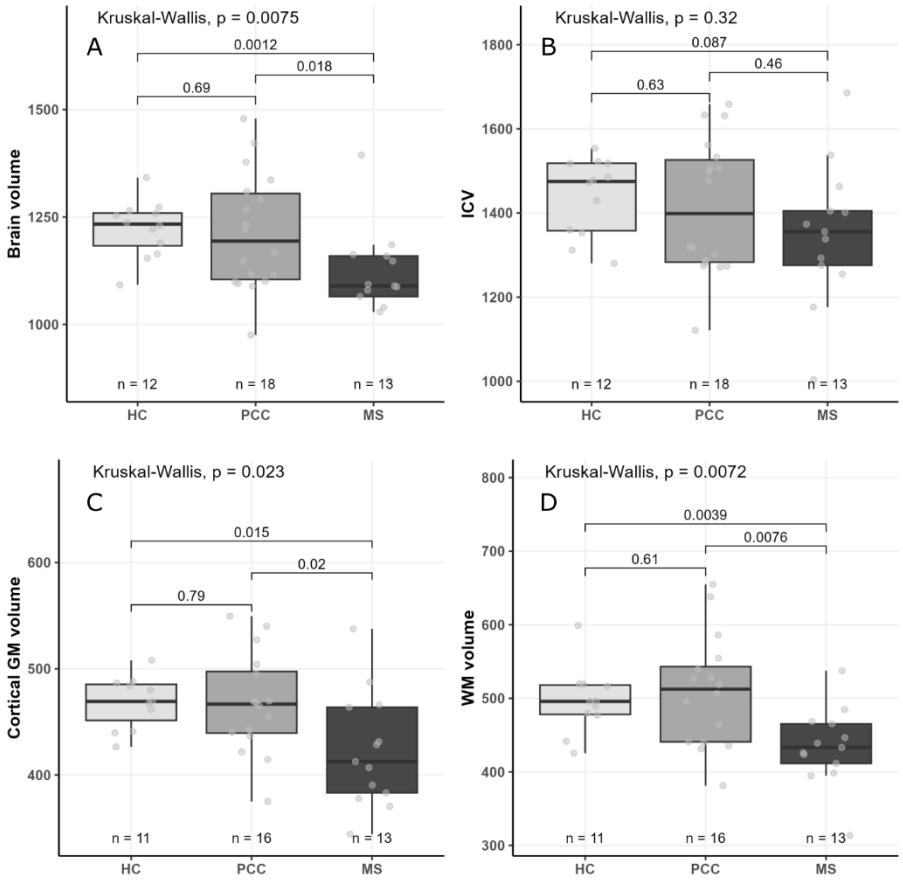

Figure 7 Comparison of MRI brain volumes between HC group, PCC group, and MS group. A) MRI brain volumes were significantly lower between HC group and MS group and PCC group and MS group. B) There were no significant differences between groups in intracranial volumes. C) MRI volumes were significantly lower in cortical grey matter between HC and MS groups and PCC and MS groups. D) White matter MRI volumes were significantly lower in MS groups compared to HC and PCC groups.

3.6 Biomarkers

GFAP and NfL measurements were performed in 12 healthy participants and 10 PCC participants. In addition, 13 subjects with MS were included as a positive control for increased biomarker concentration. Results of biomarker assessment are presented in Table 11 and Figure 8. There were no significant differences between HC group and PCC group in NfL concentration (Figure 8A). PCC group had significantly lower NfL concentration compared to the MS group ($p = 0.021$), but there were no significant differences between HC and MS groups (Figure 8A). No significant differences were found in GFAP concentration between HC group and PCC group (Figure 8B). MS group had significantly higher GFAP concentration compared to the HC group ($p = 0.035$) and PCC group ($p = 0.021$) (Figure 8B).

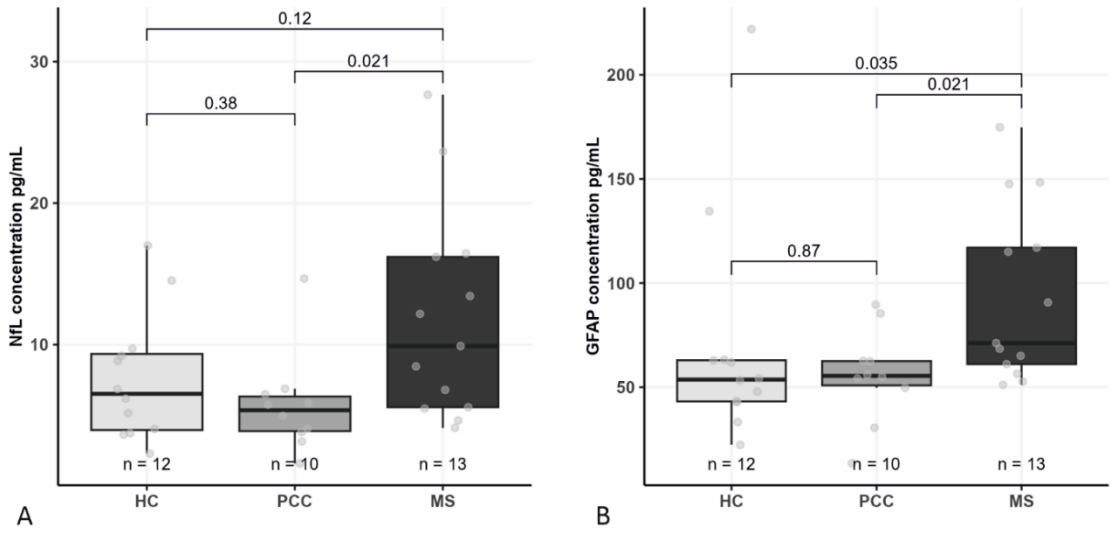

Figure 8 Biomarker concentrations. A) Group comparisons of NfL concentrations. B) Group comparisons of GFAP concentrations.

Table 11 Biomarker concentrations.

Variable, median (Q1–Q3)	HC (*n* = 12)	PCC (*n* = 10)	MS (*n* = 13)	*p* (HC vs. PCC)	*p* (HC vs. MS)	*p* (PCC vs. MS)
NfL (pg/mL)	6.5 (4–9.3)	5.4 (3.9–6.3)	9.9 (5.6–16)	0.38	0.12	0.021
GFAP (pg/mL)	54 (43–63)	55 (51–63)	71 (61–117)	0.87	0.035	0.021

3.7 DVR Correlation with Other Variables in Patients with PCC

Correlation analyses with Spearman correlation were performed to analyse associations of various variables with the [^{11}C]PK11195 DVRs across ROIs. The variables included the reported number of PCC symptoms, GFAP concentration (pg/mL), NfL concentration (pg/mL), six-minute walking distance (6MWD) (m), questionnaire scores, BMI (kg/m^2), and age. The correlation coefficient, and statistical significance of correlation for each analysed variable and ROI is presented in Figure 9.

The number of PCC symptoms exhibited statistically significant moderate negative correlations with [^{11}C]PK11195 DVRs in cortical grey matter ($\rho = -0.64$, $p = 0.013$) and brainstem ($\rho = -0.64$, $p = 0.013$), and a strong negative correlation with cerebellum DVRs ($\rho = -0.71$, $p = 0.0046$) (Figure 10).

Among the biomarkers studied, NfL concentration did not exhibit statistically significant correlation with any DVR measurements across all ROIs. GFAP concentration had strong negative correlation with cerebellum DVRs ($\rho = -0.79$, $p = 0.028$) (Figure 11C). Additionally, the six-minute walking distance showed a moderate positive correlation with globus pallidum DVRs ($\rho = 0.54$, $p = 0.047$) and cortical grey matter DVRs ($\rho = 0.59$, $p = 0.026$) (Figure 11A, B).

Of the questionnaires, ISI, WHODAS 2.0, and FSS showed no significant correlation with any DVR measurements across all ROIs (Figure 12). EuroHIS-8 scores exhibited strong negative correlation with hippocampus ($\rho = -0.87$, $p < 0.001$) and amygdala DVRs ($\rho = -0.77$, $p = 0.009$) (Figure 12C, F). PHQ-9 scores showed strong positive correlation with hippocampus ($\rho = 0.78$, $p = 0.0073$) and amygdala DVRs ($\rho = 0.74$, $p = 0.014$) (Figure 12B, E). MFIS scores showed strong positive correlation with hippocampus DVR ($\rho = 0.79$, $p = 0.0061$) (Figure 12G). GAD-7 scores exhibited strong positive correlation with hippocampus ($\rho = 0.8$, $p = 0.0052$) and amygdala DVRs ($\rho = 0.83$, $p = 0.0028$) (Figure 12A, D).

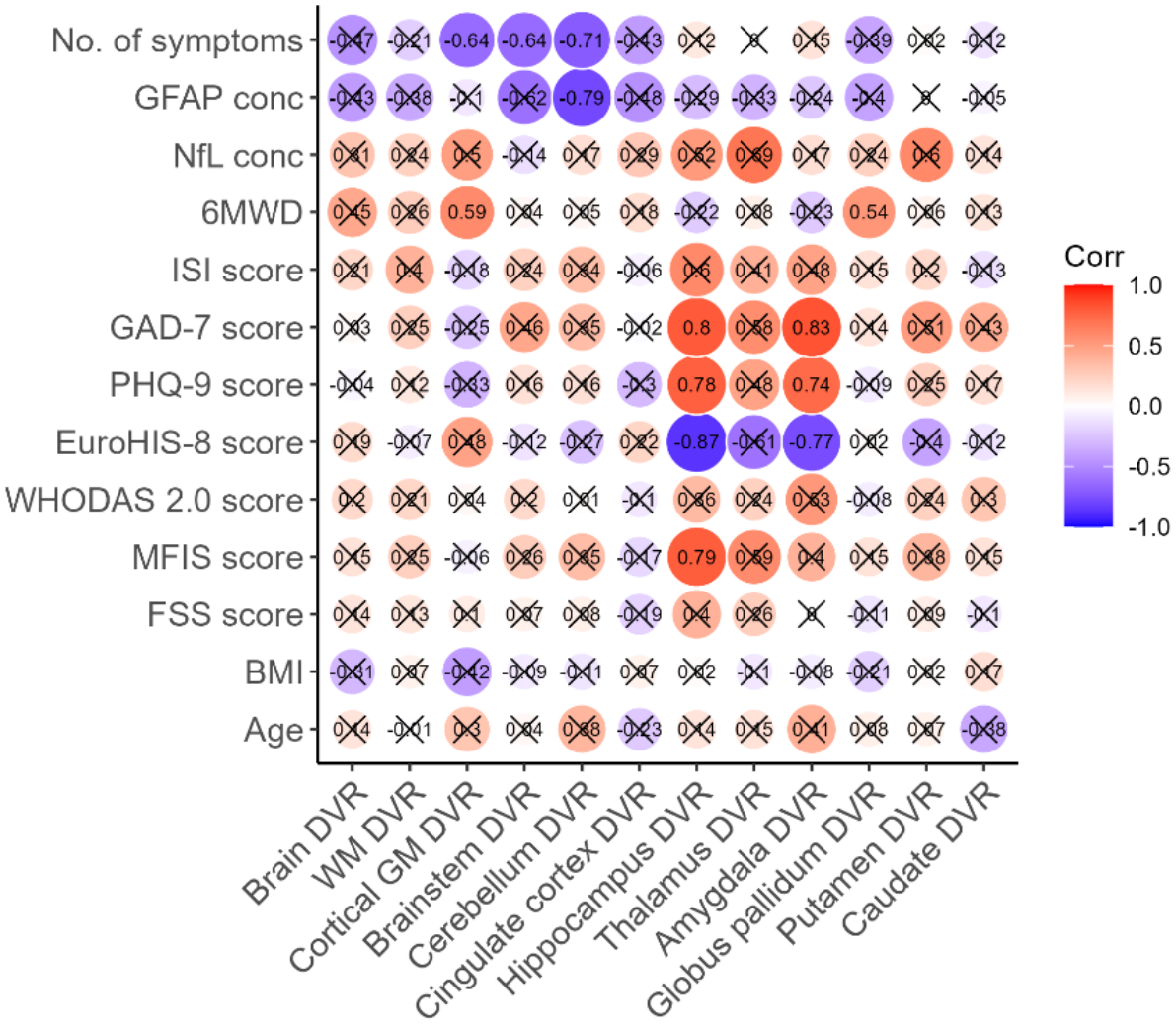

Figure 9. **Correlation heatmap showing correlation between [^{11}C]PK11195 DVRs in chosen ROIs and several variables.** Variables included the number of post-COVID-19 symptoms (No. of symptoms), concentration of GFAP (pg/mL), concentration of NfL (pg/mL), six-minute walking distance (6MWD), questionnaire scores, BMI, and age. Most variables showed no significant correlation between any of the DVR measurements in different ROIs. Nonsignificant correlations are crossed out.

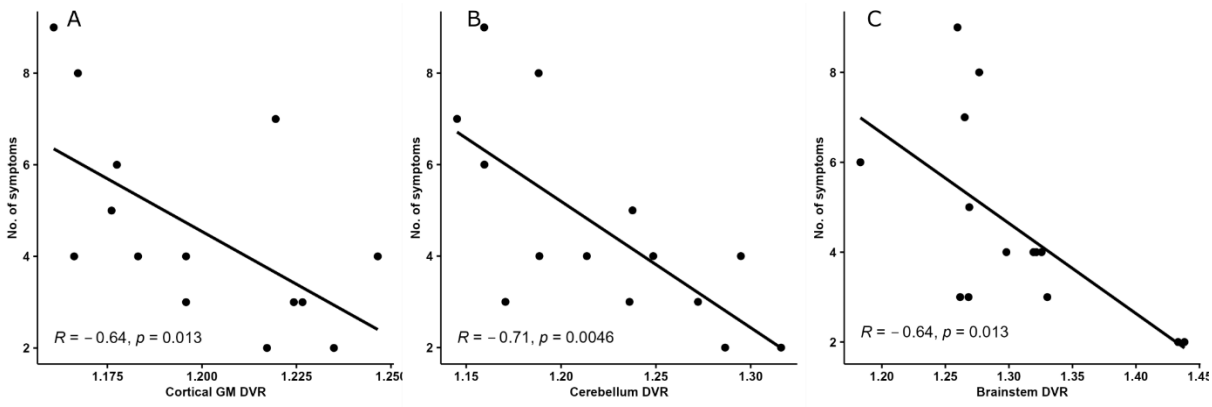

Figure 10 Statistically significant correlations between number of PCC symptoms and cortical grey matter, cerebellum and brainstem DVRs. A) Moderate negative correlation between number of PCC symptoms and cortical grey matter DVRs. B) Strong negative correlation between number of PCC symptoms and cerebellum DVRs. C) Moderate negative correlation between number of PCC symptoms and brainstem DVRs.

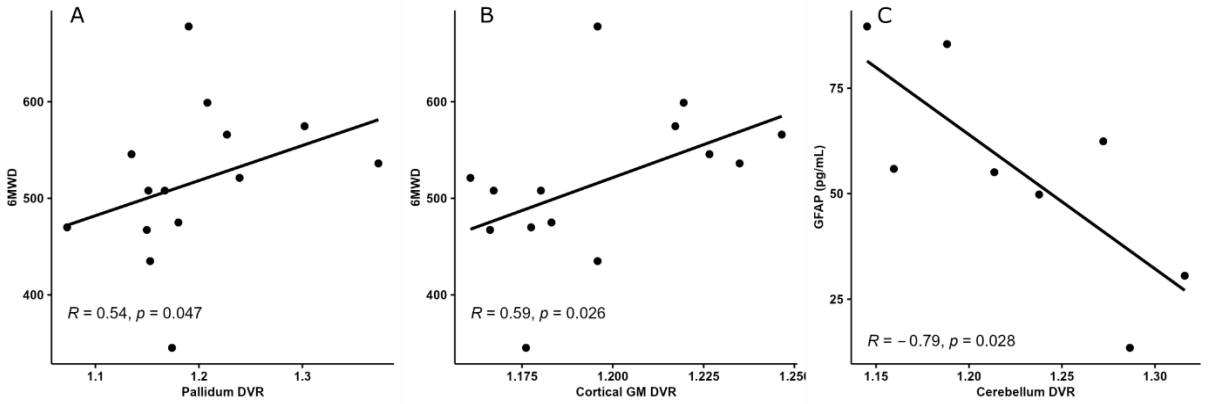

Figure 11 Statistically significant correlations between six-minute walking distance (6MWD) and pallidum and cortical grey matter DVR, and GFAP concentration and cerebellum DVR. A) 6MWD had a moderate positive correlation with pallidum and B) cortical grey matter DVRs. C) GFAP concentration had a strong negative correlation with cerebellum DVRs.

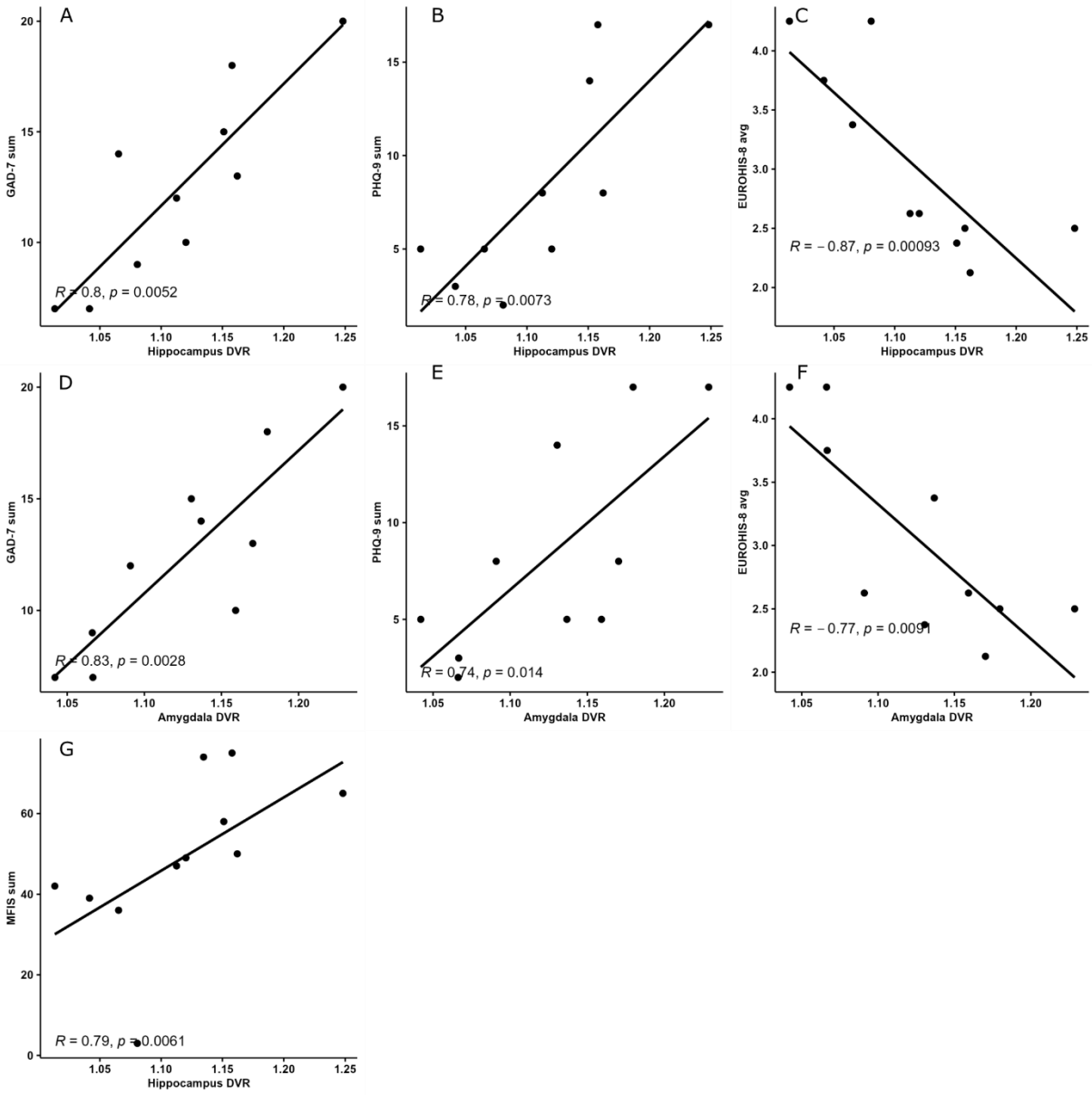

Figure 12 Statistically significant correlations between questionnaire scores and hippocampus and amygdala DVRs in PCC patients. A) GAD-7 score and hippocampus DVRs showed strong positive correlation. B) PHQ-9 score and hippocampus DVRs had strong positive correlation. C) EuroHIS-8 score and hippocampus DVRs had strong negative correlation. D) GAD-7 sum and amygdala DVRs showed strong positive correlation. E) PHQ-9 sum and amygdala DVRs showed strong positive correlation. F) EuroHIS-8 score and amygdala DVRs had strong negative correlation G) MFIS sum and hippocampus DVRs had strong positive correlation.

4 Discussion

Post-COVID-19 is a condition that affects many organ systems, with neurological manifestations being commonly reported. Although the COVID-19 pandemic is no longer a public health emergency of international concern, it is still ongoing with daily incidences of new infections. While many countries have ceased reporting the frequency of COVID-19 cases, tens of thousands of cases are still reported weekly around the world (WHO, 2023b). WHO estimates that 10–20% of infected individuals may develop PCC symptoms (WHO, 2022b). With so many people affected globally, it is increasingly important to study the mechanisms of the condition. The aims of this thesis were to assess whether PCC is associated with signs of central inflammation or neuroaxonal damage, to describe the neurological symptoms experienced by participants with PCC, and to assess whether these symptoms are associated with the objective biomarker findings of neuroaxonal damage or central inflammation.

4.1.1 Clinical Characteristics and Questionnaire Results

One of the aims of this thesis was to describe the clinical characteristics of the participants with PCC to assess the impact of the condition on general health, QoL, and possible neurological causes of the symptoms experienced by participants. Clinical characteristics were collected by collecting patient history, conducting neurological examination, performing 6MWT, and giving questionnaires to fill in.

Participants with PCC reported a wide range of symptoms, the most common being fatigue, brain fog, headache, and memory problems. Post-exertional malaise was reported by 80% of the participants, emphasising how the condition affects the day-to-day life of people with PCC. The symptoms reported by the participants represent the symptoms of PCC described in previous literature (Nalbandian et al., 2021). Many of the symptoms reported are similar to those in ME/CFS, except for hyposmia and hypogeusia (T. L. Wong & Weitzer, 2021).

In the neurological examination, more than half of the participants with PCC presented with abnormalities from a fully normal neurological profile. Participants had impairments in five domains of the examination: higher cognitive functions, cranial nerves, motor functions, coordination, and sensory functions. It is reasonable to assume that not all observed impairments can be attributed to PCC. However, certain impairments, including impaired olfactory function, brain fog, and attentional deficits, exhibit a higher likelihood of direct

association with PCC. Previously reported neurological abnormalities in PCC patients reflect our findings and include symptoms such as hyposmia, hypogeusia, cognitive deficits, and motor or sensory deficits (Bungenberg et al., 2022; Pilotto et al., 2021).

The six-minute walk test was originally used as a measure of function in patients with heart and lung disease (Guyatt et al., 1985), but today it is also used for individuals with compromised ability, such as patients with MS, Parkinson's disease, and stroke (Canning et al., 2006; Chetta et al., 2004; Eng et al., 2002). We conducted 6MWT to assess whether PCC affects physical functioning. Results of the 6MWT show that participants with PCC performed worse than predicted in the measured walking distance. These results are supported by previous literature (Kersten et al., 2022; Peroy-Badal et al., 2024). It must be acknowledged that participants with PCC had an average BMI exceeding the threshold for obese, and that the distance walked in 6MWT correlated with BMI in other studied cohorts (Capodaglio et al., 2013). However, in our study 6MWD did not associate with BMI (data not shown), supporting that the observed shortened walking distance could be associated with PCC.

The participants with PCC were given several questionnaires concerning psychiatric symptoms, quality of life, general health, and fatigue. The purpose of these questionnaires was twofold: to assess the neuropsychiatric symptoms in more detail, and to gauge the impact of PCC on patients' QoL.

The questionnaires on psychiatric symptoms showed that several participants with PCC experienced anxiety, depression, and insomnia, but that there was considerable variation among participants, especially in the ISI and PHQ-9 questionnaires. In the GAD-7 questionnaire, more than half of the participants exceeded the threshold for GAD. Studies have shown that the COVID-19 pandemic increased the rate of mental health problems (Penninx et al., 2028). The increase in mental health problems peaked during the beginning of the pandemic but was still observed, though to a lesser extent, later in pandemic with reduced restrictions and declining infection rates (Penninx et al., 2028). While the symptoms could be caused by the pandemic itself, PCC has been associated with mental health symptoms including sleep disturbances, depression, and anxiety (Penninx et al., 2028). Whether the observed mental health symptoms are caused by the pandemic or by some underlying mechanism of PCC is difficult to ascertain.

WHODAS 2.0 is a standardised measure developed by the WHO for measuring health and disability. It consists of six domains for assessing individual's level of functioning: cognition, mobility, self-care, interaction with other people, life activities, and participation in society. Participants with PCC had an average score indicating moderate impairment according to ICF scale. The scores varied considerably among participants, ranging from mild impairment to severe impairment indicating that the limitations to functioning are heterogeneous.

MFIS questionnaire is a modified version of fatigue impact scale, which was originally developed to assess how fatigue impacts the QoL of individuals with chronic disease (Fisk et al., 1994). The questionnaire is commonly used in MS patients and is included in MS quality of life inventory, a battery of scales for measuring the QoL in MS patients (National MS society, n.d.). FSS is a questionnaire created for individuals with chronic illness for self-reporting the severity of their fatigue symptoms. It has been shown to distinguish between patients with MS or systemic lupus erythematosus and healthy individuals with high accuracy (Krupp et al., 1989). In both MFIS and FSS, most participants with PCC scored higher than the threshold for fatigue. Naik et al. (2022) found that patients recovering from COVID-19 referred for post-COVID-19 assessment have increased fatigue based on FSS scores. The patients had had their first COVID-19 infection approximately a month before (Naik et al., 2022). Our results show that this fatigue may continue for years after recovering from COVID-19.

The results of the EuroHIS-8 questionnaire demonstrate that on average, participants with PCC did not describe their QoL as "good", suggesting that PCC has a negative impact on overall health, functioning, and perceived QoL. The RAND-36 questionnaire is a widely used survey that measures health related QoL in eight health concepts. Participants with PCC scored lower on average in all eight health concepts compared to the reference values of the general Finnish population. These results emphasise the negative effects PCC has on coping in day-to-day life.

The questionnaire results show that on average the participants with PCC experience mental health problems including depression, anxiety, and sleep problems; lowered levels of functioning; increased fatigue; and reduced QoL. Reduced functionality, lower levels of physical activity, and increased fatigue have been associated with poorer quality of life in PCC patients (Vélez-Santamaría et al., 2023). In this study, depression (PHQ-9), fatigue

(MFIS), and anxiety (GAD-7) were associated with poorer quality of life (EuroHIS-8) in PCC participants (data not shown).

4.1.2 Microglial Activation and Neuronal Injury

The primary aim of this thesis was to evaluate whether participants with PCC exhibit elevated specific binding of [^{11}C]PK11195, interpretated as microglial activation, compared to healthy participants. Utilising TSPO-PET, a widely used method for studying neuroinflammation in various neuropathologies, allowed for minimally invasive *in vivo* assessment of neuroinflammation. In addition to PET imaging, serum concentrations of NfL and GFAP were assessed to evaluate the presence of neuroaxonal injury and astrocyte activation in participants with PCC. Finally, volumetric analysis of MRI was performed to evaluate whether participants with PCC exhibited signs of brain atrophy.

Multiple post-mortem studies on COVID-19 patients have reported microglial activation especially in the brainstem and cerebellum (Matschke et al., 2020; Schurink et al., 2020; J. A. Stein et al., 2023; Thakur et al., 2021). These studies included individuals that had deceased due to COVID-19 infection, indicating a severe disease and high degree of inflammation. These factors likely contributed to the central inflammation observed in these studies. No post-mortem brain data analysis has been performed on patients with PCC. The *in vivo* research on microglial activation in PCC patients is so far limited, but as previously noted a few TSPO-PET studies have reported that patients with neuropsychiatric symptoms after recovering from COVID-19 have increased TSPO expression in the brain (Braga et al., 2023; Visser et al., 2022). Visser et al. (2022) reported extensive increase in [^{18}F]DPA-714 binding in two patients with PCC symptoms that had lasted for over a year. The patients in this study experienced symptoms including verbal memory deficits, visuo-constructive deficits, fatigue, concentration problems, functional impairment, and attentional deficits (Visser et al., 2022). These patients had widespread neuroinflammation in all brain regions (Visser et al., 2022). The study conducted by Braga et al. (2023) focused more on the persistent depressive symptoms occurring after COVID-19. The study included 20 participants who had experienced a major depressive episode within three months of COVID-19 (COVID-DC) and 20 healthy controls. Braga et al. (2023) reported increased TSPO binding in participants with COVID-DC across regions of interest, most prominently in the ventral striatum and dorsal putamen.

In our study no such increase in TSPO expression was observed. The binding of [^{11}C]PK11195 was similar between healthy controls and participants with PCC across all ROIs. Additionally, subgroup analyses of PCC participants based on hospitalisation status and neurological examination results did not reveal any significant differences between subgroups. It is worth noting that these results may be affected by the small sample sizes, and a larger study could reveal significant differences between groups. The PCC subjects included in this study were heterogeneous, with symptom durations ranging from less than a year to close to three years. It is plausible that microglial activation is more pronounced shortly after the initial infection and decreases over time, as findings in post-mortem studies show that microglial activation is observed in severe cases of COVID-19 (Matschke et al., 2020; Schurink et al., 2020; J. A. Stein et al., 2023; Thakur et al., 2021). However, these post-mortem studies do not specify whether the patients had any neurological symptoms. Yet, both previous TSPO-PET studies included participants with symptom durations of over a year or even two years after the initial COVID-19 infection (Braga et al., 2023; Visser et al., 2022). It should be noted that the study conducted by Visser et al. (2022) included only two participants with PCC, increasing the possibility that the neuroinflammation observed in the study may be coincidental or influenced by other factors. On the other hand, the study conducted by Braga et al. (2023) was focused on depression after COVID-19, and the neuroinflammation they observed may be related to the depressive episodes rather than PCC. TSPO-PET studies have shown that depressed individuals exhibit increased neuroinflammation compared to healthy controls (Troubat et al., 2021). Our PET findings are further reinforced by biomarker assays as NfL, shown to be associated with neuroinflammation in MS, showed no increase in concentration in PCC participants (Saraste et al., 2023). Additionally, the group comparisons of GFAP concentrations and MRI volumes revealed no signs of axonal injury or neuronal atrophy, further reinforcing the absence of neurodegenerative changes in PCC subjects. The biomarker results observed in our study are supported by previous studies showing that elevated GFAP or NfL concentrations are not observed in late recovery from COVID-19 (Kanberg et al., 2021; Peluso et al., 2022).

4.1.3 Correlations

While the [^{11}C]PK11195 binding showed no increase in PCC patients, correlation analysis indicated negative and positive associations between various factors and DVRs in certain ROIs. The strongest negative association was between EuroHIS-8 scores and hippocampus DVRs, while the strongest positive association was between GAD-7 scores and amygdala

DVRs. These results indicate that higher EuroHIS-8 scores, signifying better QoL, are associated with lower hippocampus and amygdala TSPO expression and therefore low microglial activity, while higher GAD-7 scores, signifying increased anxiety, are associated with increased TSPO expression in the same ROIs. PHQ-9 questionnaire scores were also positively associated with DVRs in the same ROIs, indicating that participants with increased depression have elevated microglial activity in these areas. Nakatomi et al. (2014) observed that depression scores and specific binding of [^{11}C]PK11195 are correlated in the hippocampus of patients with ME/CFS.

Surprisingly, the number of PCC symptoms was negatively associated with DVRs in the cortical grey matter, brainstem, and cerebellum, indicating that participants with higher number of symptoms exhibited lower DVRs in these ROIs. The association was most pronounced in the cerebellum. These results could indicate that the number of PCC symptoms may not adequately reflect the severity of PCC considering that the symptomatology of the condition is still relatively unknown. However, it is worth noting that activated microglia can also exert beneficial effects (Hickman et al., 2018) This association could indicate that increased activation of microglia, even in the relatively low levels observed in this study, could alleviate symptoms experienced by individuals with PCC.

4.1.4 Limitations

There may be methodological reasons that explain why no differences in [^{11}C]PK11195 binding were observed. Firstly, as discussed previously, because [^{11}C]PK11195 has a relatively high nonspecific binding, it is good practice to extract the reference tissue for PET quantification using SVCA if arterial blood sampling is not performed (Turkheimer et al., 2007). SVCA extracts a grey matter tissue reference with no specific binding using four defined kinetic classes. However, these kinetic classes should be defined separately for each scanner (Schubert et al., 2021). In this study, these kinetic classes were defined based on the High Resolution Research Tomograph (HRRT, CTI/Siemens), rather than the scanner used in this study, Signa PET/MRI. Until the results are reanalysed with kinetic classes based on images taken on the PET/MRI scanner, the findings of this thesis should be taken with a grain of salt, since it is unclear how this methodological difference affects the DVRs. The reanalysis of the results was not conducted for this thesis due to time limitations. Secondly, while [^{11}C]PK11195 is a popular radioligand in neuroinflammation studies, the second-generation ligands, such as [^{18}F]DPA-714 or [^{11}C]PBR28, may have lower non-specific

binding when genetic polymorphism is taken into consideration (Chauveau et al., 2008). When comparing our results to the previous PET studies on individuals with persisting symptoms after COVID-19 by Visser et al. (2022) and Braga et al. (2023), it must be noted that both studies used a second-generation radioligand.

Concerning the clinical characteristics, it needs to be acknowledged that clinical assessment and neurological examination were not performed on healthy controls, and that the questionnaires were filled in only by participants with PCC, so the symptoms, questionnaire scores, and neurological impairments of PCC patients could not be compared to those of healthy individuals. Gottlieb et al. (2023) found that approximately the same number of participants who tested COVID-negative and participants who tested COVID-positive experienced persistent symptoms at six months follow-up. The study conducted by Kantele et al. (2024) revealed that of symptoms commonly associated with PCC, only impaired olfaction and taste had a higher prevalence among infected individuals. These results suggest that some of the symptoms associated with PCC may be caused by other factors, such as the distress caused by the pandemic or other infectious diseases (Gottlieb et al., 2023; Kantele et al., 2024). As PCC is a relatively new condition, the exact definition of it is still changing as more research is carried out. Studies that include symptom comparisons between individuals with PCC and healthy controls may shed more light on the symptomatology of PCC.

5 Conclusion

In conclusion, this study shows that based on TSPO-PET with the [^{11}C]PK11195 radioligand, microglial activation/central inflammation is not increased in individuals with PCC experiencing persisting neurological symptoms when compared to healthy participants. While the results of this thesis contribute to our understanding on PCC pathophysiology, they should be interpreted with caution until the results are reanalysed with appropriate methodology when defining the reference tissue. The results of this thesis show that participants with PCC experience various neurological and psychiatric symptoms and that these symptoms have a negative effect on the QoL of these individuals. Still, if PCC is not associated with increased central inflammation, the question remains: what mechanisms cause the neurological symptoms experienced by so many patients? While neuroinflammation remains a possible mechanism in PCC pathophysiology, other mechanisms such as injury to blood vessels and multiorgan injury should also be considered (Davis et al., 2023; Leng et al., 2023). Additionally, the similarities between PCC and ME/CFS should not be overlooked, and while the conditions do have their differences, they may share disease mechanisms (Davis et al., 2023). Further research on PCC pathophysiology could lead to a better understanding of the condition and possibly development of specific treatments that could help alleviate the symptoms experienced by patients. Finally, more research is needed on the exact definition and symptomatology of PCC, to define which symptoms result from COVID-19, and whether some of the symptoms are more related to the mental health effects of the pandemic or other infectious diseases.

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Appendices

Appendix 1 EuroHIS-8 questionnaire

EuroHIS-8-elämänlaatumittari

Ohjeet

Tällä lomakkeella pyydämme Teitä arvioimaan elämänlaatuanne, terveyttänne ja muita arkielämänne asioita. **Vastatkaa kaikkiin kysymyksiin.** Jos olette epävarma siitä, minkä vastausvaihtoehdon valitsisitte, **valitkaa se, joka vaikuttaa sopivimmalta**. Usein sopivin vastaus on se, joka ensimmäiseksi tulee mieleen.

Kun vastaatte, ajatelkaa tärkeinä pitämiänne asioita, toiveitanne sekä mielihyvän ja huolenaiheitanne. Muistelkaa elämäänne kahden viimeisen viikon aikana. Kahta viimeistä viikkoa koskeva kysymys voi kuulua esimerkiksi seuraavasti:

	En lainkaan	Vähän	Kohtuul-lisesti	Paljon	Täysin riittävästi
Oletteko saanut muilta tarvitsemaanne tukea?	1	2	3	4	5

Ympyröikää numero, joka parhaiten vastaa sitä, kuinka paljon olette saanut tukea muilta viimeisten kahden viikon aikana. Jos olette saanut paljon tukea, ympyröikää numero 4. Jos ette ole lainkaan saanut tarvitsemaanne tukea muilta viimeisen kahden viikon aikana, ympyröikää numero 1.

Vastaaja, etunimi ja sukunimi:

EuroHIS-8-elämänlaatumittari

Tällä lomakkeella pyydämme Teitä arvioimaan elämänlaatuanne, terveyttänne ja muita arkielämänne asioita. Pyydämme Teitä miettimään elämäänne kahden viime viikon aikana.

		Erittäin huonoksi	Huonoksi	Ei hyväksi eikä huonoksi	Hyväksi	Erittäin hyväksi
1	Millaiseksi arvioitte elämänlaatunne?	1	2	3	4	5

		Erittäin tyytymätön	Melko tyytymätön	Ei tyytyväinen eikä tyytymätön	Melko tyyty-väinen	Erittäin tyyty-väinen
2	Kuinka tyytyväinen olette terveyteenne?	1	2	3	4	5

Seuraavat kysymykset koskevat sitä, missä määrin olette viimeisten kahden viikon aikana kokenut seuraavia asioita.

		Ei lainkaan	Vähän	Kohtuulli-sesti	Lähes riittävästi	Täysin riittävästi
3	Onko Teillä riittävästi tarmoa arkipäivän elämäänne varten?	1	2	3	4	5
4	Onko Teillä tarpeeksi rahaa tarpeisiinne nähden?	1	2	3	4	5

Seuraavissa kysymyksissä Teitä pyydetään kertomaan, kuinka tyytyväinen olette ollut viimeisten kahden viikon aikana erilaisiin asioihin elämässänne.

		Erittäin tyytymätön	Melko tyytymätön	Ei tyytyväinen eikä tyytymätön	Melko tyyty-väinen	Erittäin tyyty-väinen
5	Kuinka tyytyväinen olette kykyynne selviytyä päivittäisistä toiminnoistanne?	1	2	3	4	5
6	Kuinka tyytyväinen olette itseenne?	1	2	3	4	5
7	Kuinka tyytyväinen olette ihmis-suhteisiinne?	1	2	3	4	5
8	Kuinka tyytyväinen olette asuin-alueenne olosuhteisiin?	1	2	3	4	5

Haluatteko sanoa jotain tästä kyselystä?

_____

_____

_____

_____

_____

KIITOS AVUSTANNE!

Lähde: Power M. Development of a common instrument for quality of life. Teoksessa Nosikov A, Gudex C. (toim.). EUROHIS: Developing Common Instruments for Health Surveys. IOS Press, Amsterdam, Hollanti. 2003. Ss. 145–159.
Saatavilla Internetistä: http://www.euro.who.int/__data/assets/pdf_file/0015/101193/WA9502003EU.pdf

Appendix 2 FSS questionnaire

Nimi _____ Syntymäaika _____

Päivämäärä _____

FATIGUE SEVERITY SCALE (FSS) - UUPUMUSASTEIKKO

Ohessa on yhdeksän neurologisille sairauksille ominaiseen uupumukseen liittyvää väittämää. Jokaista väittämää varten on numeroasteikko yhdestä seitsemään. **Ympyröi** se numero (vain yksi numero), joka vastaa mielestäsi parhaiten yläpuolella esitettyä väittämää. *Yksi* tarkoittaa, että olet *vahvasti eri mieltä* väittämästä. *Seitsemän* tarkoittaa, että olet *vahvasti samaa mieltä* väittämästä.

Väittämät:

1. **Olen haluttomampi mihinkään, kun olen uupunut.**

Olen vahvasti eri mieltä 1 2 3 4 5 6 7 Olen vahvasti samaa mieltä

2. **Liikunta uuvuttaa minua.**

Olen vahvasti eri mieltä 1 2 3 4 5 6 7 Olen vahvasti samaa mieltä

3. **Uuvun helposti**

Olen vahvasti eri mieltä 1 2 3 4 5 6 7 Olen vahvasti samaa mieltä

4. **Uupumus haittaa fyysisiä toimintojani.**

Olen vahvasti eri mieltä 1 2 3 4 5 6 7 Olen vahvasti samaa mieltä

Käännä lomake !

5. **Uupumus aiheuttaa usein minulle ongelmia.**

Olen vahvasti eri mieltä 1 2 3 4 5 6 7 Olen vahvasti samaa mieltä

6. **Uupumiseni estää pitempiaikaisen fyysisen toiminnan.**

Olen vahvasti eri mieltä 1 2 3 4 5 6 7 Olen vahvasti samaa mieltä

7. **Uupumus haittaa minua tiettyjä tehtäviä hoitaessani.**

Olen vahvasti eri mieltä 1 2 3 4 5 6 7 Olen vahvasti samaa mieltä

8. **Uupumus kuuluu kolmen eniten toimintakykyäni estävän oireen joukkoon**

Olen vahvasti eri mieltä 1 2 3 4 5 6 7 Olen vahvasti samaa mieltä

9. **Uupumus haittaa työ- ja perhe-elämääni tai ihmissuhteitteni hoitoa.**

Olen vahvasti eri mieltä 1 2 3 4 5 6 7 Olen vahvasti samaa mieltä

Ole hyvä ja tarkista, että olet vastannut kaikkiin kysymyksiin (vain yhtä vaihtoehtoa käyttäen).

Kiitos!

Appendix 3 GAD-7 questionnaire

Ahdistuneisuuskysely (GAD-7)

1. Hermostuneisuuden, ahdistuneisuuden tai kireyden tunne

- ◯ Ei lainkaan (0 p)
- ◯ Useana päivänä (1 p)
- ◯ Suurimpana osana päivistä (2 p)
- ◯ Lähes joka päivä (3 p)

2. Kyvyttömyys lopettaa huolehtiminen tai pitää se kurissa

- ◯ Ei lainkaan (0 p)
- ◯ Useana päivänä (1 p)
- ◯ Suurimpana osana päivistä (2 p)
- ◯ Lähes joka päivä (3 p)

3. Liiallinen huolestuneisuus erilaisista asioista

- ◯ Ei lainkaan (0 p)
- ◯ Useana päivänä (1 p)
- ◯ Suurimpana osana päivistä (2 p)
- ◯ Lähes joka päivä (3 p)

4. Vaikeus rentoutua

- ◯ Ei lainkaan (0 p)
- ◯ Useana päivänä (1 p)
- ◯ Suurimpana osana päivistä (2 p)
- ◯ Lähes joka päivä (3 p)

5. Niin levoton olo, että on vaikea pysyä aloillaan

- ◯ Ei lainkaan (0 p)
- ◯ Useana päivänä (1 p)
- ◯ Suurimpana osana päivistä (2 p)
- ◯ Lähes joka päivä (3 p)

6. Taipumus harmistua tai ärsyyntyä helposti

- ◯ Ei lainkaan (0 p)
- ◯ Useana päivänä (1 p)
- ◯ Suurimpana osana päivistä (2 p)
- ◯ Lähes joka päivä (3 p)

www.thl.fi/toimia/tietokanta/mittariversio/109/

Ahdistuneisuuskysely (GAD-7)

7. Pelko siitä, että jotakin kauheaa saattaisi tapahtua

- ◯ Ei lainkaan (0 p)
- ◯ Useana päivänä (1 p)
- ◯ Suurimpana osana päivistä (2 p)
- ◯ Lähes joka päivä (3 p)

Pisteet yhteensä: ☐

Tulkinta

0-4 pistettä: Vähäinen ahdistuneisuus
5-9 pistettä: Lievä ahdistuneisuus
10-15 pistettä: Kohtalainen ahdistuneisuus
16-21 pistettä: Vaikea ahdistuneisuus

GAD-7 -mittarille ≥ 10 pistettä on asetettu raja-arvoksi mahdolliselle GAD-diagnoosille. Tällöin sensitiivisyys GAD-diagnoosille on 89 % ja spesifisyys 82 %.

Appendix 4 MFIS questionnaire

TOIMIA

MUOKATTU ASTEIKKO UUPUMUKSEN VAIKUTUSTEN ARVIOINTIIN (MFIS)

Alla on luettelo väittämistä, jotka kuvaavat uupumuksen vaikutuksia ihmiseen. Uupumuksella (fatiikki) tarkoitetaan epänormaalia väsymystä fyysisen tai psyykkisen ponnistelun yhteydessä, jota monet ihmiset kokevat silloin tällöin. Tietyissä sairauksissa, kuten MS-taudissa, uupumuksen tunnetta saattaa ilmetä useammin ja sen vaikutukset saattavat olla voimakkaampia kuin tavallisesti.

Ole hyvä ja lue jokainen väittämä huolellisesti. Ympyröi tämän jälkeen se numero, joka parhaiten vastaa sitä, kuinka usein (ei koskaan, harvoin, joskus, usein, lähes aina) uupumus on vaikuttanut sinuun tällä tavalla viimeksi kuluneiden neljän viikon aikana. (Mikäli tarvitset apua vastausten merkitsemisessä, kerro haastattelijalle parhaiten sopivan vastauksen numero.) Vastaa kaikkiin kysymyksiin. Mikäli olet epävarma vastauksen suhteen, valitse se vastausvaihtoehto, joka parhaiten kuvaa tilannettasi. Jos et ymmärrä joitakin sanoja tai lauseita, haastattelija voi selittää ne sinulle.

Nimi:_____ **Päiväys:** _____/_____/_____
päivä kuukausi vuosi

Sotu:_____ **Testinumero:** 1 2 3 4

Uupumukseni vuoksi, viimeksi kuluneiden neljän viikon aikana...

	En/Ei koskaan	Harvoin	Joskus	Usein	Lähes aina
1. En ole ollut yhtä valpas kuin tavallisesti.	0	1	2	3	4
2. Minun on ollut vaikea keskittyä asioihin pitkäksi aikaa.	0	1	2	3	4
3. En ole pystynyt ajattelemaan selkeästi.	0	1	2	3	4
4. Olen ollut kömpelömpi ja liikkeeni eivät ole olleet hyvin hallittuja.	0	1	2	3	4
5. Olen ollut muistamaton.	0	1	2	3	4
6. Minun on täytynyt olla huolellinen sen suhteen, kuinka usein ja kuinka kauan liikun.	0	1	2	3	4
7. Olen ollut haluttomampi tekemään mitään, mikä vaatii ruumiillista ponnistelua.	0	1	2	3	4

TOIMIA

Uupumukseni vuoksi, viimeksi kuluneiden neljän viikon aikana...

	En/Ei koskaan	Harvoin	Joskus	Usein	Lähes aina
8. Olen ollut haluttomampi osallistumaan sosiaalisiin toimintoihin.	0	1	2	3	4
9. Kykyni tehdä asioita kodin ulkopuolella on ollut rajoittunut.	0	1	2	3	4
10. Minun on ollut vaikea jatkaa ruumiillisia ponnisteluja pitkää aikaa.	0	1	2	3	4
11. Minun on ollut vaikea tehdä päätöksiä.	0	1	2	3	4
12. Olen ollut haluttomampi tekemään mitään, mikä vaatii ajattelemista.	0	1	2	3	4
13. Lihakseni ovat tuntuneet heikommilta.	0	1	2	3	4
14. Minulla on ollut enemmän ruumiillisia vaivoja.	0	1	2	3	4
15. Minun on ollut vaikea saattaa loppuun asioita, jotka vaativat ajattelemista.	0	1	2	3	4
16. Minun on ollut vaikea pitää ajatukseni koossa tehdessäni asioita kotona tai työpaikalla.	0	1	2	3	4
17. Kykyni saattaa loppuun ruumiillista ponnistelua vaativia tehtäviä on ollut rajoittunut.	0	1	2	3	4
18. Ajatuksen juoksuni on tuntunut hitaammalta.	0	1	2	3	4
19. Minun on ollut vaikea olla tarkkaavainen.	0	1	2	3	4
20. Olen rajoittanut ruumiillisia toimintojani.	0	1	2	3	4
21. Minun on täytynyt pysähtyä lepäämään useammin tai pidempään.	0	1	2	3	4

Appendix 5 ISI questionnaire

UNETTOMUUDEN HAITTA-ASTEEN ARVIO (ISI; Insomnia Severity Index)

Vastaa alla oleviin kysymyksiin ympyröimällä se vaihtoehto, joka mielestäsi parhaiten vastaa tilannettasi viimeksi kuluneen kuukauden aikana*.

1. Arvioi univaikeuksiesi VAKAVUUTTA

	Ei lainkaan	Lievä	Kohtalainen	Vakava	Erittäin vakava
1) Nukahtamisvaikeus	0	1	2	3	4
2) Unessapysymisvaikeus	0	1	2	3	4
3) Liian aikainen herääminen aamulla	0	1	2	3	4

2. Kuinka TYYTYVÄINEN / tyytymätön olet tämänhetkiseen nukkumiseesi?

Erittäin tyytyväinen	0
Tyytyväinen	1
En osaa sanoa	2
Tyytymätön	3
Erittäin tyytymätön	4

3. Missä määrin arvioit nukkumisongelmasi HÄIRITSEVÄN päivittäistä toimintaasi (esim. aiheuttaa väsymystä päivällä, häiritsee suorituskykyäsi, aiheuttaa keskittymis- ja muistamis-vaikeuksia, vaikuttaa mielialaan)?

Ei häiritse yhtään	0
Vähän	1
Jonkin verran	2
Paljon	3
Häiritsee erittäin paljon	4

4. Kuinka helposti luulet muiden HUOMAAVAN nukkumisongelmasi heikentäneen elämänlaatuasi ?

Eivät huomaa lainkaan	0
Juuri ja juuri	1
Melko helposti	2
Helposti	3
Huomaavat erittäin helposti	4

5. Kuinka HUOLESTUNUT / ahdistunut olet tämänhetkisen nukkumisongelmasi vuoksi?

En ollenkaan	0
Vähän	1
Jonkin verran	2
Paljon	3
Erittäin paljon	4

Viite:
Morin CM, Belleville G, Bélanger L, Ivers H. The Insomnia Severity Index: psychometric indicators to detect insomnia cases and evaluate treatment response. Sleep 2011;34:601-8

1

* Kuukauden aikaraja perustuu Charles M. Morinilta 4.3.2014 saatuun suositukseen.

Pisteytysohje:

0–7 ei kliinisesti merkittävää unettomuutta
8–14 lievä unettomuus
15–21 keskivaikea unettomuus
22–28 vaikea unettomuus

Kokonaispistemäärä 0–28

Appendix 6 PHQ-9 questionnaire

PHQ-9-terveyskysely

Kuinka usein **viimeisen kahden viikon aikana** ovat seuraavanlaiset ongelmat vaivanneet sinua?

1. Vain vähäistä mielenkiintoa tai mielihyvää erilaisten asioiden tekemisestä

- ☐ Ei ollenkaan (0 p)
- ☐ Useina päivinä (1 p)
- ☐ Enemmän kuin puolet ajasta (2 p)
- ☐ Lähes joka päivä (3 p)

2. Alakuloisuutta, masentuneisuutta, toivottomuutta

- ☐ Ei ollenkaan (0 p)
- ☐ Useina päivinä (1 p)
- ☐ Enemmän kuin puolet ajasta (2 p)
- ☐ Lähes joka päivä (3 p)

3. Vaikeuksia nukahtaa, pysyä unessa tai liiallista nukkumista

- ☐ Ei ollenkaan (0 p)
- ☐ Useina päivinä (1 p)
- ☐ Enemmän kuin puolet ajasta (2 p)
- ☐ Lähes joka päivä (3 p)

4. Väsymystä tai voimattomuutta

- ☐ Ei ollenkaan (0 p)
- ☐ Useina päivinä (1 p)
- ☐ Enemmän kuin puolet ajasta (2 p)
- ☐ Lähes joka päivä (3 p)

5. Ruokahaluttomuutta tai liiallista syömistä

- ☐ Ei ollenkaan (0 p)
- ☐ Useina päivinä (1 p)
- ☐ Enemmän kuin puolet ajasta (2 p)
- ☐ Lähes joka päivä (3 p)

6. Huonommuuden tai epäonnistumisen tunteita tai tunne siitä, että olet tuottanut pettymyksen itsellesi tai perheellesi

- ☐ Ei ollenkaan (0 p)
- ☐ Useina päivinä (1 p)
- ☐ Enemmän kuin puolet ajasta (2 p)
- ☐ Lähes joka päivä (3 p)

PHQ-9-terveyskysely

7. Vaikeutta keskittyä asioihin kuten sanomalehden lukemiseen tai television katseluun

- ☐ Ei ollenkaan (0 p)
- ☐ Useina päivinä (1 p)
- ☐ Enemmän kuin puolet ajasta (2 p)
- ☐ Lähes joka päivä (3 p)

8. Puhumisen tai liikkumisen hitautta, jonka muutkin voisivat huomata tai vastakohtaisesti rauhattomuutta tai liikehtimistä paljon tavallista enemmän

- ☐ Ei ollenkaan (0 p)
- ☐ Useina päivinä (1 p)
- ☐ Enemmän kuin puolet ajasta (2 p)
- ☐ Lähes joka päivä (3 p)

9. Ajatuksia, että olisi parempi, jos olisit kuollut tai että haluaisit vahingoittaa itseäsi jotenkin

- ☐ Ei ollenkaan (0 p)
- ☐ Useina päivinä (1 p)
- ☐ Enemmän kuin puolet ajasta (2 p)
- ☐ Lähes joka päivä (3 p)

Pisteet yhteensä: ☐

0-4 pistettä: ei masennusta

5-9 pistettä: lievä masennus

10-14 pistettä: kohtalainen masennus

15-19 pistettä: kohtalaisen vakava masennus

20 pistettä tai yli: vakava masennus

Appendix 7 RAND-36 questionnaire

Liite 3

RAND 36-ITEM HEALTH SURVEY 1.0 (RAND-36)

Suomenkielinen versio

STAKES/KTL

Liite 3

1. Onko terveytenne yleisesti ottaen ...
(ympyröikää yksi numero)

1 erinomainen
2 varsin hyvä
3 hyvä
4 tyydyttävä
5 huono

2. Jos vertaatte nykyistä terveydentilaanne vuoden takaiseen, onko terveytenne yleisesti ottaen ...
(ympyröikää yksi numero)

1 tällä hetkellä paljon parempi kuin vuosi sitten
2 tällä hetkellä jonkin verran parempi kuin vuosi sitten
3 suunnilleen samanlainen
4 tällä hetkellä jonkin verran huonompi kuin vuosi sitten
5 tällä hetkellä paljon huonompi kuin vuosi sitten

Seuraavassa luetellaan erilaisia päivittäisiä toimintoja. Rajoittaako terveydentilanne nykyisin suoriutumistanne seuraavista päivittäisistä toiminnoista? Jos rajoittaa, kuinka paljon?
(ympyröikää yksi numero joka riviltä)

		kyllä, rajoittaa paljon	kyllä, rajoittaa hiukan	ei rajoita lainkaan
3.	huomattavia ponnistuksia vaativat toiminnat (esimerkiksi juokseminen, raskaiden tavaroiden nostelu, rasittava urheilu)	1	2	3
4.	kohtuullisia ponnistuksia vaativat toiminnat, kuten pöydän siirtäminen, imurointi, keilailu	1	2	3
5.	ruokakassien nostaminen tai kantaminen	1	2	3
6.	nouseminen portaita useita kerroksia	1	2	3
7.	nouseminen portaita yhden kerroksen	1	2	3
8.	vartalon taivuttaminen, polvistuminen, kumartuminen	1	2	3
9.	noin kahden kilometrin matkan kävely	1	2	3
10.	noin puolen kilometrin matkan kävely	1	2	3
11.	noin 100 metrin matkan kävely	1	2	3
12.	kylpeminen tai pukeutuminen	1	2	3

Liite 3

Onko teillä viimeisen 4 viikon aikana ollut RUUMIILLISEN TERVEYDENTILANNE TAKIA alla mainittuja ongelmia työssänne tai muissa tavanomaisissa päivittäisissä tehtävissänne?
(ympyröikää yksi numero joka riviltä)

		kyllä	ei
13.	Vähensitte työhön tai muihin tehtäviin käyttämäänne aikaa	1	2
14.	Saitte aikaiseksi vähemmän kuin halusitte	1	2
15.	Terveydentilanne asetti teille rajoituksia joissakin työ- tai muissa tehtävissä	1	2
16.	Töistänne tai tehtävistänne suoriutuminen tuotti vaikeuksia (olette joutunut esim. ponnistelemaan tavallista enemmän)	1	2

Onko teillä viimeisen 4 viikon aikana ollut TUNNE-ELÄMÄÄN LIITTYVIEN vaikeuksien (esim. masentuneisuus tai ahdistuneisuus) takia alla mainittuja ongelmia työssänne tai muissa tavanomaisissa päivittäisissä tehtävissänne?
(ympyröikää yksi numero joka riviltä)

		Kyllä	ei
17.	Vähensitte työhön tai muihin tehtäviin käyttämäänne aikaa	1	2
18.	Saitte aikaiseksi vähemmän kuin halusitte	1	2
19.	Ette suorittanut töitänne tai muita tehtäviänne yhtä huolellisesti kuin tavallisesti	1	2

20. **MISSÄ MÄÄRIN ruumiillinen terveydentilanne tai tunne-elämän vaikeudet ovat viimeisen 4 viikon aikana häirinneet tavanomaista (sosiaalista) toimintaanne perheen, ystävien, naapureiden tai muiden ihmisten parissa?**
(ympyröikää yksi numero)

1 ei lainkaan
2 hieman
3 kohtalaisesti
4 melko paljon
5 erittäin paljon

Liite 3

21. Kuinka voimakkaita ruumiillisia kipuja teillä on ollut viimeisen 4 viikon aikana?
(ympyröikää yksi numero)

1 ei lainkaan
2 hyvin lieviä
3 lieviä
4 kohtalaisia
5 voimakkaita
6 erittäin voimakkaita

22. Kuinka paljon kipu on häirinnyt tavanomaista työtänne (kotona tai kodin ulkopuolella) viimeisen 4 viikon aikana?
(ympyröikää yksi numero)

1 ei lainkaan
2 hieman
3 kohtalaisesti
4 melko paljon
5 erittäin paljon

Seuraavat kysymykset koskevat sitä, miltä teistä on tuntunut viimeisen 4 viikon aikana. Merkitkää kunkin kysymyksen kohdalla se numero, joka parhaiten kuvaa tuntemuksianne.
(ympyröikää yksi numero joka riviltä)

Kuinka suuren osan ajasta olette viimeisen 4 viikon aikana ...	koko ajan	suurim-man osan aikaa	huomat-tavan osan aikaa	jonkin aikaa	vähän aikaa	ei lain-kaan
23. tuntenut olevanne täynnä elinvoimaa	1	2	3	4	5	6
24. ollut hyvin hermostunut	1	2	3	4	5	6
25. tuntenut mielialanne niin matalaksi, ettei mikään ole voinut teitä piristää	1	2	3	4	5	6
26. tuntenut itsenne tyyneksi ja rauhalliseksi	1	2	3	4	5	6
27. ollut täynnä tarmoa	1	2	3	4	5	6
28. tuntenut itsenne alakuloiseksi ja apeaksi	1	2	3	4	5	6
29. tuntenut itsenne "loppuun-kuluneeksi"	1	2	3	4	5	6
30. ollut onnellinen	1	2	3	4	5	6
31. tuntenut itsenne väsyneeksi	1	2	3	4	5	6

Liite 3

32. **Kuinka suuren osan ajasta ruumiillinen terveydentilanne tai tunne-elämän vaikeudet ovat viimeisen 4 viikon aikana häirinneet tavanomaista sosiaalista toimintaanne (ystävien, sukulaisten, muiden ihmisten tapaaminen)?**
(ympyröikää yksi numero)

1 koko ajan
2 suurimman osan aikaa
3 jonkin aikaa
4 vähän aikaa
5 ei lainkaan

Kuinka hyvin seuraavat väittämät pitävät paikkansa teidän kohdallanne?
(ympyröikää yksi numero joka riviltä)

	pitää ehdotto-masti paikkansa	pitää enimmäk-seen paikkansa	en osaa sanoa	enimmäk-seen ei pidä paikkansa	ehdotto-masti ei pidä paikkansa
33. Minusta tuntuu, että sairastun jonkin verran helpommin kuin muut ihmiset	1	2	3	4	5
34. Olen vähintään yhtä terve kuin kaikki muutkin tuntemani ihmiset	1	2	3	4	5
35. Uskon, että terveyteni tulee heikkenemään	1	2	3	4	5
36. Terveyteni on erinomainen	1	2	3	4	5

Appendix 8 WHODAS 2.0 questionnaire

WHODAS 2.0
WORLD HEALTH ORGANIZATION
DISABILITY ASSESSMENT SCHEDULE 2.0

36	Itse täytettävä

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36 kysymyksen versio, itse täytettävä

Kysely selvittää terveydentilasta johtuvia vaikeuksia. Terveydentilalla tarkoitetaan sairauksia, tauteja ja muita lyhyt- tai pitkäaikaisia terveysongelmia, vammoja sekä mielenterveys-, tunne-elämän, alkoholi-, huume- tai lääkkeenkäytön ongelmia.

Vastatessasi mieti, kuinka isoja vaikeuksia sinulla oli kussakin suorituksessa tai toimessa viimeisten 30 päivän aikana. Ympyröi jokaisen kysymyksen kohdalta vain yksi vastausvaihtoehdoista.

Kuinka vaikeaa sinun oli viimeisten 30 päivän aikana:						
Ymmärtäminen ja yhteydenpito						
D1.1	Keskittyä johonkin tekemiseen 10 minuuttia?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D1.2	Muistaa tehdä tärkeät asiat?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D1.3	Ratkaista päivittäisiä ongelmia?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D1.4	Oppia uutta, esimerkiksi löytää reitti uuteen paikkaan?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D1.5	Ymmärtää yleensä toisten puhetta?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D1.6	Aloittaa ja jatkaa keskustelua?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
Liikkuminen						
D2.1	Seistä pidempään, esimerkiksi 30 minuuttia?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D2.2	Nousta istumasta seisomaan?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D2.3	Liikkua kotona?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D2.4	Lähteä kotoa?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D2.5	Kävellä pitkä matka, esimerkiksi kilometri?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt

Ole hyvä ja jatka seuraavalle sivulle ...

WHODAS 2.0
WORLD HEALTH ORGANIZATION
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Kuinka vaikeaa sinun oli viimeisten 30 päivän aikana:						
Itsestä huolehtiminen						
D3.1	Peseytyä?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D3.2	Pukeutua?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D3.3	Syödä?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D3.4	Olla yksin muutama päivä?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
Ihmissuhteet						
D4.1	Olla tekemisissä tuntemattomien ihmisten kanssa?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D4.2	Pitää yllä ystävyyssuhteita?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D4.3	Tulla toimeen läheisten ihmisten kanssa?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D4.4	Saada uusia ystäviä?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D4.5	Olla seksuaalisessa kanssakäymisessä?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
Arkitoimet						
D5.1	Hoitaa kotityöt?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D5.2	Hoitaa tärkeimmät kotityöt hyvin?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D5.3	Saada kaikki tarpeelliset kotityöt tehtyä?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D5.4	Tehdä kotityöt tarvittavan nopeasti?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt

Ole hyvä ja jatka seuraavalle sivulle ...

WHODAS 2.0
WORLD HEALTH ORGANIZATION
DISABILITY ASSESSMENT SCHEDULE 2.0

36	Itse täytettävä

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Jos työskentelet (palkkatyö, vapaaehtoistyö tai yrittäjyys) tai opiskelet, vastaa seuraaviin kysymyksiin D5.5–D5.8. Muuten siirry kysymykseen D6.1.

Kuinka vaikeaa sinun oli terveytesi takia viimeisten 30 päivän aikana:						
D5.5	Hoitaa päivittäinen työsi / opiskelusi?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D5.6	Tehdä tärkeimmät työ/opiskelutehtäväsi hyvin?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D5.7	Saada kaikki tarvittavat työsi tehtyä?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D5.8	Saada työt tehtyä tarvittavan nopeasti?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt

Yhteisöön osallistuminen						
Viimeisten 30 päivän aikana:						
D6.1	Kuinka vaikeaa sinun oli osallistua tapahtumiin (esim. juhliin tai muihin tilaisuuksiin) samaan tapaan kuin muut ihmiset?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt
D6.2	Kuinka paljon ympäristösi esteet rajoittivat osallistumistasi?	Ei lainkaan	Hieman	Kohtalaisesti	Huomattavasti	Erittäin paljon
D6.3	Kuinka paljon muiden asenteet tai teot vaikeuttivat ihmisarvoista elämääsi?	Ei lainkaan	Hieman	Kohtalaisesti	Huomattavasti	Erittäin paljon
D6.4	Kuinka paljon sinulla meni aikaa terveydentilan aiheuttamiin ongelmiin?	Ei lainkaan	Hieman	Kohtalaisesti	Huomattavasti	Erittäin paljon
D6.5	Kuinka paljon terveydentilasi on vaikuttanut tunteisiisi?	Ei lainkaan	Hieman	Kohtalaisesti	Huomattavasti	Erittäin paljon
D6.6	Kuinka paljon terveydentilasi rasitti sinun tai perheesi taloutta?	Ei lainkaan	Hieman	Kohtalaisesti	Huomattavasti	Erittäin paljon
D6.7	Kuinka paljon terveydentilasi aiheutti ongelmia perheellesi?	Ei lainkaan	Hieman	Kohtalaisesti	Huomattavasti	Erittäin paljon
D6.8	Kuinka vaikea sinun oli tehdä rentouttavia tai mielihyvää tuottavia asioita?	Ei lainkaan vaikeaa	Hieman vaikeaa	Kohtalaisen vaikeaa	Huomattavan vaikeaa	Erittäin vaikeaa tai en pystynyt

Ole hyvä ja jatka seuraavalle sivulle ...

WHODAS 2.0

WORLD HEALTH ORGANIZATION
DISABILITY ASSESSMENT SCHEDULE 2.0

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H1	Kuinka monena päivänä 30 päivän aikana näitä vaikeuksia kaikkiaan esiintyi?	***Päivien lukumäärä*** ____
H2	Kuinka monena päivänä 30 päivän aikana olit täysin kykenemätön tekemään tavallisia askareitasi tai työtäsi terveydentilan takia?	***Päivien lukumäärä*** ____
H3	Kuinka monena päivänä 30 päivän aikana, kun ei lasketa päiviä jolloin olit täysin kykenemätön, jouduit vähentämään tavallisia askareitasi tai työtäsi terveydentilan takia?	***Päivien lukumäärä*** ____

Kysely on valmis, kiitos vastaamisesta!