

Varying levels of inflammatory activity in brain and body of patients with persistent fatigue and difficulty concentrating after COVID-19: a TSPO PET study

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Clinical trial registration number: 2021-000781-15 (European Union Drug Regulating Authorities Clinical Trials Database (EudraCT)) and NCT05371522 (ClinicalTrials.gov)

Financial disclosure: This study was funded by a ZonMw grant (number: 10430302110003)

Conflicts of interest: RB received a research grant from Siemens Healthineers. FB has several interests (reported in the supplement), but none relevant to the work described in this article. No other potential conflict of interest relevant to this article was reported.

Data sharing statement: Data will be made available upon reasonable request.

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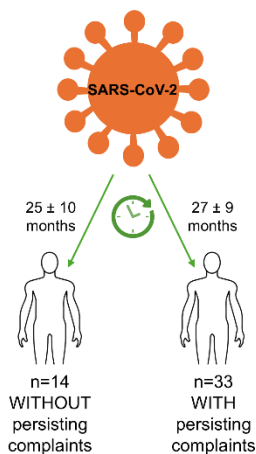
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Keywords: post-COVID, TSPO, PET, Imaging, fatigue

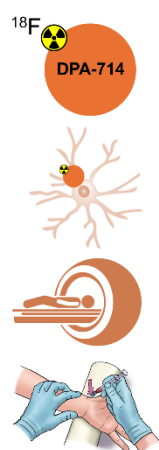
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Post-COVID neuroinflammation: a TSPO PET study

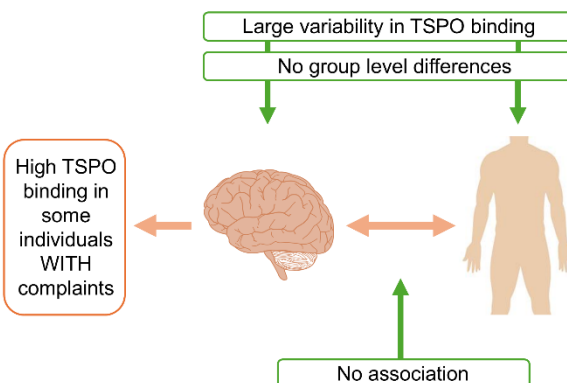
Study population
Individuals with and without persisting fatigue and difficulty concentrating after COVID-19



Main measurement
60-minute total-body dynamic TSPO PET scan with arterial sampling



Main results



Conclusions

Increased specific TSPO binding was found in some individuals with post-COVID syndrome, suggesting presence of an inflammatory subtype and further consolidating the role of neuroinflammation in subtypes of post-COVID syndrome.

ABSTRACT

Purpose: A significant number of patients report persisting complaints after infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), known as post-corona virus disease (COVID) syndrome. Underlying mechanism for these complaints remain poorly understood. Dysregulated immune- and neurological systems may play a role in the pathophysiology of post-COVID syndrome. A target providing direct information on immune-activation is the 18-kDa translocator protein (TSPO), which is upregulated in activated microglia. We aimed to assess whole body inflammatory activity with TSPO PET in individuals with and without persisting severe fatigue and difficulty concentrating two years after SARS-CoV-2 infection and relate it to complaint severity.

Methods: In this cross-sectional cohort study we included 47 post-COVID individuals of whom 33 with persisting complaints (50 ± 8 years, 61% female, 27 ± 9 months after initial infection) and 14 without (47 ± 9 years, 50% female, 25 ± 10 months after initial infection). All individuals were high affinity binder according to their TSPO genotype, and underwent total-body 60-minute dynamic [^{18}F]DPA-714 PET with arterial sampling, MRI, genotyping, and questionnaires. Tracer binding was quantified using binding potential (k_3/k_4) for cerebral regions and K_i or V_T for extracerebral regions. Parameters were compared between 33 individuals with persistent complaints (severe fatigue and difficulty concentrating) and 14 without, and associations between parameters were assessed.

Results: We found globally increased cerebral [^{18}F]DPA-714 binding in some individuals reporting persisting complaints when compared to individuals without. No group-level differences were found in extracerebral binding. Large variability in cerebral and extracerebral binding was observed between individuals. Cerebral and extracerebral binding levels did not associate with each other, nor with complaint severity.

Conclusion: Increased specific TSPO binding was found in some individuals with post-COVID syndrome, indicating presence of an inflammatory subtype and further consolidating the role of neuroinflammation in subtypes of post-COVID syndrome.

INTRODUCTION

A substantial proportion of individuals infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) experience persistent symptoms months or years after initial infection, known as ‘post-COVID-19 syndrome’(1). The underlying mechanisms and long-term effects remain poorly understood. Dysregulated immune and neurological systems likely contribute to these symptoms(2). Post-mortem studies show extensive brain inflammation, including microglia activation and astrogliosis, while *in vivo* imaging reveals similar changes in brain regions associated with cognitive dysfunction and blood inflammatory markers(3-11). However, direct *in vivo* evidence of microglia activation is limited(10,11).

The 18-kDa translocator protein (TSPO) serves as a biomarker for neuroinflammation, as it is overexpressed in activated microglia and macrophages(12). TSPO PET imaging with ligands like [¹⁸F]-FEPPA and [¹¹C]PBR28 has demonstrated increased neuroinflammation across various brain regions in post-COVID patients(10,11). These findings may help link regional brain inflammation to specific symptoms, aiding understanding of symptom heterogeneity. Nonetheless, small sample sizes(11) and methodological factors(10,11) restrict the generalizability of current studies. Besides neuroinflammation, TSPO PET imaging is also used to assess peripheral inflammation(13), which is relevant since post-COVID-19 syndrome affects multiple organ systems(2), including the lungs, causing symptoms like dyspnea and chest pain(1).

Our primary aim was to investigate differences in cerebral inflammatory activity using total body TSPO PET between post-COVID-19 individuals with and without persistent severe fatigue and cognitive complaints, and investigate its associations with severity of complaints and cognitive functioning. We used [¹⁸F]DPA-714, as this is a second generation TSPO PET tracer which shows high affinity, selectivity and reversible binding(14,15). We hypothesized that post-COVID individuals with persistent complaints would show higher levels of cerebral inflammatory activity compared to post-COVID-19 individuals without these persistent complaints. Our secondary aim was to explore differences in extracerebral inflammatory activity, where we hypothesized that post-COVID individuals with persistent complaints would show higher inflammatory activity in the lungs.

METHODS

Participants

This study is part of the VeCosCO study; a case-control study in participants with a previous SARS-CoV-2 infection of which a detailed study protocol was published previously(16). Briefly, individuals between 30 and 65 years who were high affinity binders according to their TSPO genotype were included (see sTable 1). Presence of persistent complaints was determined using the Checklist Individual Strength (CIS), where validated cut-off scores of ≥ 35 on the CIS-fatigue subscale and/or a score ≥ 18 on the CIS-concentration subscale indicated persisting complaints, while scores of < 35 on the CIS-fatigue subscale and a score < 18 on the CIS-concentration subscale indicated absence of complaints (see supplemental section 1.1 for details)(16,17).

This study was approved by the Medical Ethics Review Committee of the Amsterdam UMC, location VU Medical Center, and registered under 2021-000781-15 in the European Union Drug Regulating Authorities Clinical Trials Database (EudraCT) and under NCT05371522 in ClinicalTrials.gov. All participants provided written informed consent prior to study participation.

Questionnaires and neuropsychological assessment

A variety of questionnaires were used to assess different complaints and functioning. Specifically, the CIS-fatigue and -concentration subscales to evaluate fatigue and concentration complaints(17,18), the Work and Social Adjustment Scale (WSAS) to measure functional impairment(19), the Research and Development-36 (Rand-36) physical functioning subscale to assess physical limitations(20), the Beck Depression Inventory for Primary Care (BDI-PC) and Hospital Anxiety and Depression Scale (HADS) to evaluate depressive symptoms(21,22), and the Cognitive Failure Questionnaire (CFQ) for subjective cognitive performance(23). The Stroop Color and Word Test (SCWT) was used for objective cognitive functioning, since we previously reported this to be the most sensitive cognitive test in our larger cohort(24).

PET

[¹⁸F]DPA-714 radiosynthesis was performed as described previously(25). Simultaneous with injection of 258±20 MBq (individuals with complaints) or 269±12 MBq (individuals without complaints) [¹⁸F]DPA-714, 60-minute dynamic PET scans with continuous online and manual arterial blood sampling were acquired between March 2022 and March 2024 on the Ingenuity TF PET-CT scanner (n=10; Philips Medical Systems) as described previously(26), or on the long-axial-field-of-view (LAFOV) Biograph Vision Quadra (n=37; Siemens Healthineers; reconstruction settings in supplemental section 1.2).

Preprocessing of cerebral PET images has been described elsewhere and more details on the arterial sampling and quantification of cerebral tracer binding are described in supplemental section 1.3 and 1.4(26). Continuous whole blood time activity curves (TACs) were corrected for plasma to whole blood ratios, metabolites and time delay to obtain metabolite-corrected arterial plasma input functions for analyses of cerebral data. A plasma input two-tissue-compartment model with blood volume parameter (2T4k_V_B) with delay correction was used (as it was found previously to be the optimal model to quantify cerebral [¹⁸F]DPA-714 binding(26)) to estimate binding potential (BP_{ND}) in global gray matter, cerebellum gray matter, brainstem and thalamus. Details on quantification of extracerebral tracer binding are described in supplemental section 1.5 and elsewhere(27). In short, extracerebral data was quantified by using the manual arterial blood samples to establish calibrated metabolite-corrected plasma input function extracted from ascending aorta image derived input function (IDIF)(28). Quantitative parameters in nine organs (adrenal gland, bone, kidney, lung, myocardium, pancreas, skeletal muscle, spleen, and thyroid) were obtained using several kinetic models best describing tracer kinetics in the different organs (see supplemental section 1.5).

MRI

Participants underwent magnetic resonance imaging (MRI) at the Amsterdam UMC, location VUmc, on a 3.0T whole-body scanner (GE Signa, Discovery MR750). The scanning protocol included a high

resolution 3DT1-weighted magnetization prepared rapid acquisition gradients-echo (MP-RAGE) image. Details are described elsewhere(26).

Data analysis

Differences in demographics and clinical characteristics were analyzed using unpaired t-tests (continuous variables) and χ^2 tests (categorical variables). Questionnaire and neuropsychological scores were compared with ANCOVA, adjusting for age, sex, time after infection, and education (for neuropsychological tests). Spearman correlations assessed relationships between [^{18}F]DPA-714 binding and age, sex, BMI, time after infection, and PET scanner type (cerebral data only); significantly correlated variables were included as nuisance variables in subsequent analyses.

Differences in [^{18}F]DPA-714 binding levels across four cerebral and nine extracerebral regions between individuals with and without persistent complaints were evaluated using ANCOVA, adjusting for relevant covariates. Non-parametric Mann-Whitney U tests were also performed for global gray matter, lung, thyroid and pancreas binding levels due to variance differences. Linear regression models, adjusted for covariates, examined associations between binding levels and symptom severity or cognitive scores (SCWT), with separate analyses for groups with and without complaints. Due to small sample size ($n=5$), correlations with depression and subjective cognitive impairment were not calculated in individuals without complaints.

Associations between cerebral and extracerebral binding levels were assessed via linear regression and for global cerebral binding levels with binding levels in skeletal muscle, thyroid, and pancreas via Spearman correlations. All analyses were conducted in R 4.2.1, with multiple comparison correction using Benjamini-Hochberg FDR; $p<0.05$ was considered significant.

RESULTS

Sample

In total we included 47 individuals who suffered a SARS-CoV-2 infection. Data from all individuals were used for cerebral analyses. Thirty-three post-COVID individuals reported persistent complaints

(age 50 ± 8 years, 61% female, 27 ± 9 months after initial infection) and 14 did not (age 47 ± 9 years, 50% female, 25 ± 10 months after initial infection). Sample characteristics are presented in **Table 1**. All post-COVID individuals with complaints reported both severe fatigue and difficulty concentrating, except for 3 reporting only severe fatigue and 3 only difficulty concentrating. BMI was higher in individuals with persistent complaints compared to individuals without (29 ± 6 versus 25 ± 3 , $p_{FDR}=0.021$). CIS-fatigue, CIS-concentration, WSAS (functional impairment), and BDI-PC were higher (=worse functioning/more severe complaints) in post-COVID individuals with persistent complaints (all $p_{FDR}<0.05$). Rand-36 physical impairment was lower (=more impaired) in post-COVID individuals with persistent complaints ($p_{FDR}<0.001$). All other demographic characteristics were similar between groups ($p_{FDR}>0.05$). For extracerebral analyses, only those participants who were scanned on the LAFOV PETCT system were included. One total body scan from an individual without complaints was excluded due to motion artefacts, leaving a sample of $n=25$ with and $n=11$ without persistent complaints for extracerebral analyses. Sample characteristics did not significantly differ between the cerebral and extracerebral sample.

Higher cerebral [^{18}F]DPA-714 binding in the global gray matter correlated with higher age (Spearman's $\rho=0.32$, $p=0.029$), as was previously found for cerebral TSPO binding(29), higher BMI (Spearman's $\rho=0.32$, $p=0.027$), and PET scanner type (Spearman's $\rho=0.61$, $p<0.001$). No correlations with sex or time after infection (both $p>0.05$) were found (**Figure 1**). We included all three variables (age, BMI and PET scanner type) as nuisance variables in our following analyses. Extracerebral data showed associations with age (bone), sex (skeletal muscle and thyroid), BMI (lung), and time after infection (adrenal gland) and we included all as nuisance variables in following analyses for all extracerebral regions.

Cerebral [^{18}F]DPA-714 PET

Overall, a large variety in [^{18}F]DPA-714 BP_{ND} levels was observed (**Figure 2&3, Table 2**). Using parametric testing while adjusting for age, BMI and PET scanner type, we found significant higher binding in individuals with versus without complaints in the global gray matter region, which did not

survive FDR-correction (BP_{ND} 0.80 ± 0.34 versus 0.65 ± 0.17 , $p=0.036$, $p_{FDR}=0.144$) (**Figure 3, Table 2**), and was driven by some individuals with high binding. Non-parametric testing without adjustment for nuisance variables revealed no difference in global gray matter binding between groups ($p=0.216$). No significant differences between groups were observed in the other regions (**Figure 3, Table 2**). Visual inspection of the data, however, showed similar patterns of high binding in some post-COVID individuals with persistent complaints compared to post-COVID individuals without complaints in the cerebellum as well (**Figure 3**). Binding levels in gray and white matter separately in the global and cerebellum region are shown in **sFigure 1**. Information on global gray matter binding in relation to approximated virus variants is provided in **sFigure 2**.

Association between cerebral [^{18}F]DPA-714 PET with complaint severity and SCWT

In individuals with complaints, no significant associations were found between [^{18}F]DPA-714 binding levels (in any of the regions) and any of the questionnaire scores (CIS-concentration and -fatigue subscales, WSAS, Rand-36, BDI-PC, HADS, and CFQ) (**sFigure 3**). In individuals without complaints, we found associations between higher [^{18}F]DPA-714 binding levels in the brainstem and more functional impairment on the WSAS ($\beta=2.59$ SE=0.76, $p=0.009$), and more physical impairment on the Rand-36 ($\beta=-14.60$ SE=3.55, $p=0.003$). No associations between binding levels and CIS-concentration subscale scores were found.

We found no associations between cerebral [^{18}F]DPA-714 binding levels (in any of the regions) and any of the three SCWT scores, both in individuals with and without complaints (all $p>0.05$).

Extracerebral [^{18}F]DPA-714 PET

As for the cerebral data, a large variety in extracerebral [^{18}F]DPA-714 binding levels was observed (**Figure 4, sTable 2**). On group level, no significant differences in extracerebral binding were found between individuals with and without persistent severe fatigue and difficulty concentrating in any of the organs. Visual inspection of the data, however, showed diverse subsets of individuals showing (very) high binding in specific tissues, such as skeletal muscle, lung or thyroid (**Figure 4&5**). These subsets

consisted of different individuals, meaning that individuals often showed isolated high binding in one extracerebral organ (instead of multiple).

No associations between extracerebral [^{18}F]DPA-714 binding with any of the questionnaire scores or SCWT were found (all $p>0.05$).

Cerebral versus extracerebral [^{18}F]DPA-714 PET

Cerebral binding levels were not associated with binding levels in any of the extracerebral organs investigated. Individuals with high binding in a certain extracerebral organ did generally not demonstrate high cerebral binding (**Figure 5**).

DISCUSSION

In this study we investigated the presence of cerebral inflammatory activity in individuals with and without persistent severe fatigue and difficulty concentrating two years following a SARS-CoV-2 infection by using fully quantitative TSPO PET imaging with an arterial input function. We found widespread (globally) elevated cerebral inflammatory activity in some individuals reporting persistent complaints when compared to individuals without. Exploratory analysis of total body data revealed variable levels of extracerebral inflammatory activity with increased binding in multiple (isolated) organs. Large variability in both cerebral and extracerebral binding levels was observed between individuals. No clear associations between TSPO PET binding and measures of persistent severe fatigue and difficulty concentrating, self-reported depressive symptoms and cognitive test performance on the SCWT were found.

Our findings are partially in keeping with two previous studies showing (globally) increased TSPO signal in the brain of post-COVID individuals(10,11) and SARS-CoV-2 infected rhesus macaques(30), albeit in different populations and with different methods, further consolidating the role of neuroinflammation in post-COVID syndrome. Studies into potentially related (post-infectious) syndromes such as myalgic encephalomyelitis/chronic fatigue syndrome and Q-fever fatigue syndrome using the first-generation TSPO ligand [^{11}C](R)-PK11195 report conflicting results(31), which might be the result of the high degree of heterogeneity within these syndromes and the use of different tracers accompanied by methodological differences.

TSPO binding in extracerebral organs has been relatively unexplored. Although presence of inflammation in extracerebral organs must be interpreted with caution, since other factors like diet, injury or certain drugs may impact organ-inflammation(32), our study further supports that multisystemic inflammatory activity might play a role in post-COVID complaints. The high extracerebral binding present in some of the individuals was most often limited to one organ, and organ type also differed between individuals. Furthermore, high binding in (one of the) extracerebral organs was in general not accompanied by high cerebral inflammatory activity. This might point to either a

different temporal evolution for cerebral and extracerebral inflammatory activity post-COVID, as was also suggested by others based on [^{18}F]FDG PET(33) or to differential subtypes (cerebral, extracerebral or even organs-specific) within the inflammatory subtype of post-COVID syndrome. This may be relevant for patient stratification and (personalized) treatment.

To our knowledge, this study is one of the first and largest fully quantitative studies evaluating neuroinflammation using TSPO PET in individuals with post-COVID syndrome more than two years after their initial infection. We explored extracerebral inflammatory activity, as total body data was obtained. There are some limitations to consider. Although patients were carefully selected using validated neurocognitive questionnaires, the lack of a biomarker means our cohort likely included different post-COVID subtypes. In addition, no diagnostic instrument was used for the assessment of psychiatric illness prior to COVID-19. Also, as we selected individuals based on severe fatigue and difficulty concentrating, total body data of these individuals may not represent the general post-COVID population and exploration of association between extracerebral binding levels and (severity of) complaints were hampered by the lack of non-neurocognitive questionnaires. Furthermore, although a sample size of 47 individuals is considered extensive for highly complex studies like ours, statistically the sample suffered from limited power. In addition, two PET scanners were used in our study. Although we minimized the impact of PET scanner type on the data by harmonizing the two scanners using phantom-scans according to the EARL brain standard, we cannot rule out that (small) differences might have been induced due to remaining differences in noise, scatter-correction and time-of-flight performance. Furthermore, PET itself has a certain detection threshold, potentially preventing us from detecting low/subthreshold inflammatory activity.

Concluding, we found widespread (globally) elevated neuroinflammatory activity as measured with TSPO PET in some of the post-COVID individuals with persistent severe fatigue and/or difficulty concentrating when compared to individuals without. Exploratory analyses showed high inflammatory activity in different and isolated extracerebral organs in some post-COVID individuals, which was not linked to neuroinflammatory activity. The clinical relevance of elevated neuroinflammatory activity in post-COVID is unclear, since no clear associations with level of complaints, depressive symptoms or

cognitive measures were found in our study. Furthermore, a common factor in the available data linking the individuals with elevated cerebral TSPO signal has not been found in amongst others age, sex or/and time after infection. In addition to hinting at the pathophysiology of post-COVID syndrome, diagnosing chronic neuroinflammatory activity in these patients is important as we know from other diseases, such as multiple sclerosis(34), Alzheimer's Disease (AD)(35) and Parkinson's Disease (PD)(36), it is associated with neurodegeneration and thus may be equally relevant for post-COVID syndrome. A recent study described cortical thinning and gray matter volume loss over time (as well as cognitive decline) in patients with post-COVID syndrome(7) which is an imaging hallmark for the previously mentioned neurodegenerative diseases. Assessing neuroinflammatory status with TSPO PET may therefore provide prognostic information, providing for example information on individuals' recovery potential/speed. Longitudinal imaging studies and clinical follow up of the individuals with but also without elevated TSPO binding are highly valuable, to evaluate the temporal relationship and clinical relevance of the present findings, especially in relation to neurodegenerative disorders. Visa versa, the COVID pandemic and post-COVID syndrome may provide unique information regarding the role of chronic neuroinflammation in the pathophysiology of neurodegenerative disorders like AD and PD.

ACKNOWLEDGEMENTS

We would like to thank all VeCosCO study participants, the VeCosCO Study Group, and Stichting Long COVID for their contribution. The Biograph Vision Quadra PET/CT system at Amsterdam UMC was made possible by many contributors (see supplemental section 4).

KEY POINTS

QUESTION: Are there differences in neuroinflammatory activity measured with [¹⁸F]DPA-714 PET between post-COVID individuals with and without severe fatigue and difficulty concentrating?

PERTINENT FINDINGS: In this cross-sectional cohort study with 33 post-COVID individuals with and 14 without persistent complaints we found globally increased cerebral [^{18}F]DPA-714 binding in some individuals reporting persisting complaints when compared to individuals without. No group-level differences were found in extracerebral binding, and cerebral and extracerebral binding levels did not associate with each other, nor with complaint severity.

MEANING: Increased inflammatory activity on PET was found in some individuals with post-COVID syndrome, indicating presence of an inflammatory subtype, and suggesting [^{18}F]DPA-714 PET can serve as a biomarker.

References

1. O'Mahoney LL, Routen A, Gillies C, et al. The prevalence and long-term health effects of Long Covid among hospitalised and non-hospitalised populations: A systematic review and meta-analysis. *EClinicalMedicine*. 2023;55.
2. Davis HE, McCorkell L, Vogel JM, Topol EJ. Long COVID: major findings, mechanisms and recommendations. *Nature Reviews Microbiology*. 2023;21:133-146.
3. Schurink B, Roos E, Radonic T, et al. Viral presence and immunopathology in patients with lethal COVID-19: a prospective autopsy cohort study. *The Lancet Microbe*. 2020;1:e290-e299.
4. Lou JJ, Movassaghi M, Gordy D, et al. Neuropathology of COVID-19 (neuro-COVID): clinicopathological update. *Free neuropathology*. 2021;2.
5. Matschke J, Lütgehetmann M, Hagel C, et al. Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. *The Lancet Neurology*. 2020;19:919-929.
6. Guedj E, Campion J, Dudouet P, et al. 18F-FDG brain PET hypometabolism in patients with long COVID. *European journal of nuclear medicine and molecular imaging*. 2021;48:2823-2833.
7. Douaud G, Lee S, Alfaro-Almagro F, et al. SARS-CoV-2 is associated with changes in brain structure in UK Biobank. *Nature*. 2022;604:697-707.
8. Braga J, Kuik EJ, Lepra M, et al. Astroglisis Marker [11C] SL25. 1188 After COVID-19 With Ongoing Depressive and Cognitive Symptoms. *Biological Psychiatry*. 2025.
9. Besteher B, Rocktäschel T, Garza AP, et al. Cortical thickness alterations and systemic inflammation define long-COVID patients with cognitive impairment. *Brain, Behavior, and Immunity*. 2024;116:175-184.
10. Braga J, Lepra M, Kish SJ, et al. Neuroinflammation after COVID-19 with persistent depressive and cognitive symptoms. *JAMA psychiatry*. 2023;80:787-795.
11. VanElzakker MB, Bues HF, Brusafferri L, et al. Neuroinflammation in post-acute sequelae of COVID-19 (PASC) as assessed by [11C] PBR28 PET correlates with vascular disease measures. *Brain, Behavior, and Immunity*. 2024.
12. Nakajima K, Kohsaka S. Microglia: activation and their significance in the central nervous system. *The journal of biochemistry*. 2001;130:169-175.

13. Shah S, Sinharay S, Patel R, et al. PET imaging of TSPO expression in immune cells can assess organ-level pathophysiology in high-consequence viral infections. *Proceedings of the National Academy of Sciences*. 2022;119:e2110846119.
14. James ML, Fulton RR, Vercoullie J, et al. DPA-714, a new translocator protein–specific ligand: Synthesis, radiofluorination, and pharmacologic characterization. *Journal of Nuclear Medicine*. 2008;49:814-822.
15. Arlicot N, Vercouillie J, Ribeiro M-J, et al. Initial evaluation in healthy humans of [18F] DPA-714, a potential PET biomarker for neuroinflammation. *Nuclear medicine and biology*. 2012;39:570-578.
16. Verveen A, Verfaillie SC, Visser D, et al. Neurobiological basis and risk factors of persistent fatigue and concentration problems after COVID-19: study protocol for a prospective case–control study (VeCosCO). *BMJ open*. 2023;13:e072611.
17. Worm-Smeitink M, Gielissen M, Bloot L, et al. The assessment of fatigue: Psychometric qualities and norms for the Checklist individual strength. *Journal of psychosomatic research*. 2017;98:40-46.
18. Vercoulen JH, Swanink CM, Fennis JF, Galama JM, van der Meer JW, Bleijenberg G. Dimensional assessment of chronic fatigue syndrome. *Journal of psychosomatic research*. 1994;38:383-392.
19. Mundt JC, Marks IM, Shear MK, Greist JM. The Work and Social Adjustment Scale: a simple measure of impairment in functioning. *The British Journal of Psychiatry*. 2002;180:461-464.
20. Hays RD, Sherbourne CD, Mazel RM. The rand 36-item health survey 1.0. *Health economics*. 1993;2:217-227.
21. Beck AT, Guth D, Steer RA, Ball R. Screening for major depression disorders in medical inpatients with the Beck Depression Inventory for Primary Care. *Behaviour research and therapy*. 1997;35:785-791.
22. Spinhoven P, Ormel J, Sloekers P, Kempen G, Speckens AE, van Hemert AM. A validation study of the Hospital Anxiety and Depression Scale (HADS) in different groups of Dutch subjects. *Psychological medicine*. 1997;27:363-370.
23. Ponds R, Van Boxtel M, Jolles J. De Cognitive Failure Questionnaire als maat voor subjectief cognitief functioneren. *Tijdschrift voor neuropsychologie*. 2006;2:37-45.

24. Verveen A, Verfaillie SC, Visser D, et al. Neuropsychological functioning after COVID-19: minor differences between individuals with and without persistent complaints after SARS-CoV-2 infection. *The Clinical Neuropsychologist*. 2024;1-16.
25. Golla SS, Boellaard R, Oikonen V, et al. Quantification of [18F] DPA-714 binding in the human brain: initial studies in healthy controls and Alzheimer's disease patients. *Journal of Cerebral Blood Flow & Metabolism*. 2015;35:766-772.
26. Hagens MH, Golla SV, Wijburg MT, et al. In vivo assessment of neuroinflammation in progressive multiple sclerosis: a proof of concept study with [18 F] DPA714 PET. *Journal of neuroinflammation*. 2018;15:1-10.
27. Palard-Novello X, Visser D, et al. First Whole-Body [18F]DPA-714 Kinetics Assessment using Long Axial Field of View PET/CT scanner. *Journal of Nuclear Medicine*. 2025; Accepted.
28. Palard-Novello X, Visser D, Tolboom N, et al. Validation of image-derived input function using a long axial field of view PET/CT scanner for two different tracers. *EJNMMI physics*. 2024;11:25.
29. Schuitemaker A, van der Doef TF, Boellaard R, et al. Microglial activation in healthy aging. *Neurobiology of aging*. 2012;33:1067-1072.
30. Nieuwland JM, Nutma E, Philippens IH, et al. Longitudinal positron emission tomography and postmortem analysis reveals widespread neuroinflammation in SARS-CoV-2 infected rhesus macaques. *Journal of neuroinflammation*. 2023;20:179.
31. Raijmakers R, Roerink M, Keijmel S, et al. No signs of neuroinflammation in women with chronic fatigue syndrome or Q fever fatigue syndrome using the TSPO ligand [11C]-PK11195. *Neurology: Neuroimmunology & Neuroinflammation*. 2021;9:e1113.
32. Chen L, Deng H, Cui H, et al. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*. 2017;9:7204.
33. Sollini M, Morbelli S, Ciccarelli M, et al. Long COVID hallmarks on [18F] FDG-PET/CT: a case-control study. *European Journal of Nuclear Medicine and Molecular Imaging*. 2021;48:3187-3197.
34. Sucksdorff M, Matilainen M, Tuisku J, et al. Brain TSPO-PET predicts later disease progression independent of relapses in multiple sclerosis. *Brain*. 2020;143:3318-3330.
35. Scheltens P, De Strooper B, Kivipelto M, et al. Alzheimer's disease. *The Lancet*. 2021;397:1577-1590.

- 36.** Vivekanantham S, Shah S, Dewji R, Dewji A, Khatri C, Ologunde R. Neuroinflammation in Parkinson's disease: role in neurodegeneration and tissue repair. *International Journal of Neuroscience*. 2015;125:717-725.

Table 1. Cerebral sample characteristics

	Post-COVID WITH persistent complaints	post-COVID WITHOUT persistent complaints	P-value FDR corrected
Sample, n	33	14	
Age at PET, y	50±8	47±9	0.354
Female, n (%)	20 (61%)	7 (50%)	0.726
Time after infection, mo	27±9	25±10	0.471
Body Mass Index (BMI)	29*±6	25±3	0.021
PET/CT system			
Ingenuity TF	8 (24%)	2 (14%)	0.726
Biograph Vision Quadra	25 (76%)	12 (86%)	
<i>Questionnaires</i>			
CIS-fatigue	46*±8	15±7	<0.001
CIS-concentration	26*±6	11±5	<0.001
WSAS	21*±10	1±2	<0.001
Rand-36 physical impairment	55*±15	85±15	<0.001
BDI	2*±2	1±1	0.028
HADS [#]	6±4	3±2	0.084
CFQ [#]	48±15	30±11	0.062
<i>Cognitive functioning[†]</i>			
Stroop 1 (word), T-score	42±16	45±13	0.712
Stroop 2 (color), T-score	41±14	45±11	0.471
Stroop Color Word test, T-score	42±12	47±11	0.327

[#]only available in n=20 with and n=5 without complaints. [†]Stroop tests were not available in n=4 with and n=1 without complaints. *p_{FDR}<0.05

Table 2. Cerebral [¹⁸F]DPA-714 binding levels in gray matter for post-COVID individuals with and without persistent complaints.

	Post-COVID WITHOUT complaints (n=13)	Post-COVID WITH complaints (n=34)	% difference	P value
[¹⁸F]DPA714 BP_{ND} (k3/k4)				
Global	0.65±0.17	0.80*±0.34	20,7%	0.036
Cerebellum	0.66±0.19	0.74±0.29	11,4%	0.235
Brainstem	2.14±0.62	2.04±0.74	-4,8%	0.614
Thalamus	1.12±0.55	1.28±0.64	13.3%	0.395

*p<0.05, which did not survive FDR-correction (p=0.036, p_{FDR}=0.144) and was not significant when using a non-parametric test without adjustment for nuisance variables (p=0.216).

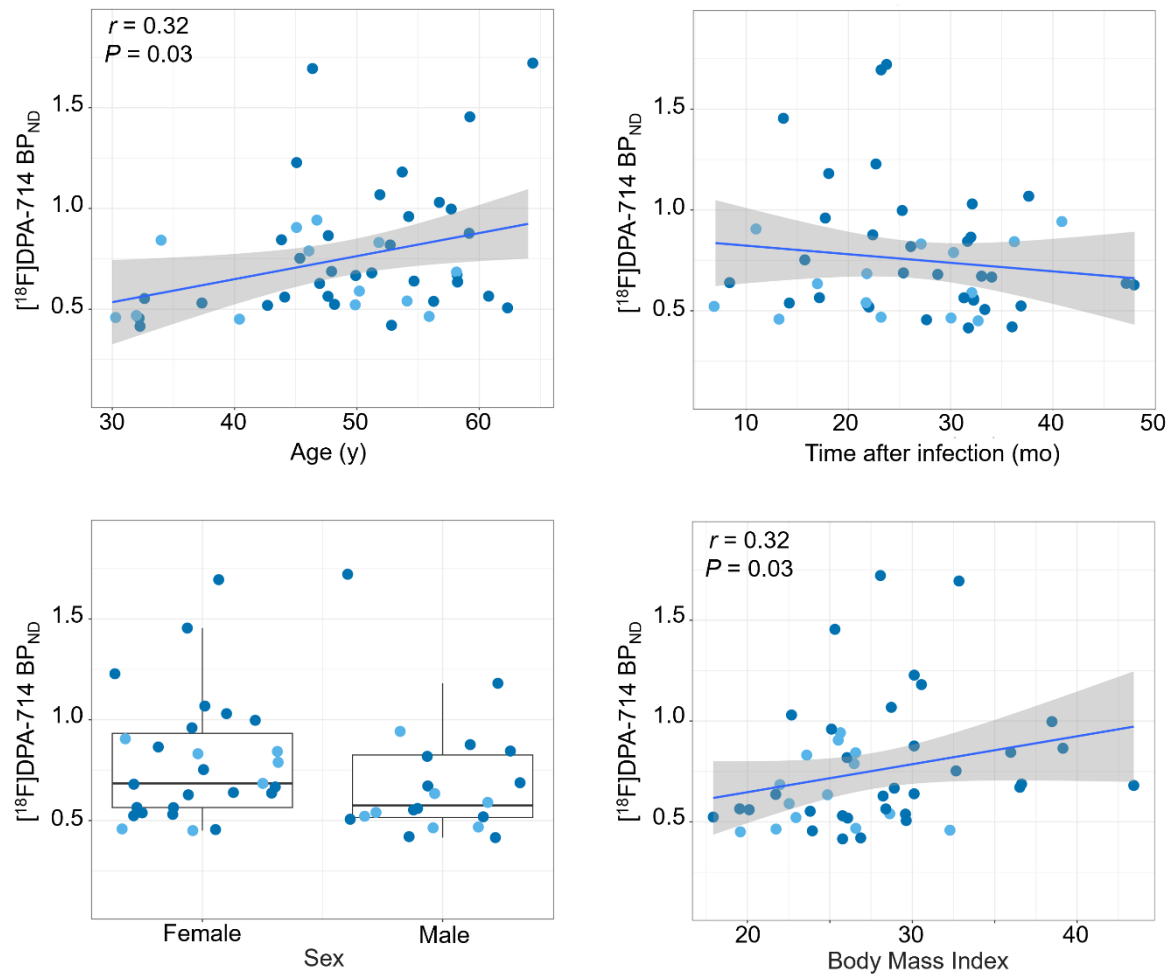


Figure 1. Correlation between global cerebral $[^{18}\text{F}]\text{DPA-714 BP}_{\text{ND}}$ with age, sex, time after infection and body mass index. Light blue = post-COVID without complaints, Dark blue = post-COVID with complaints.

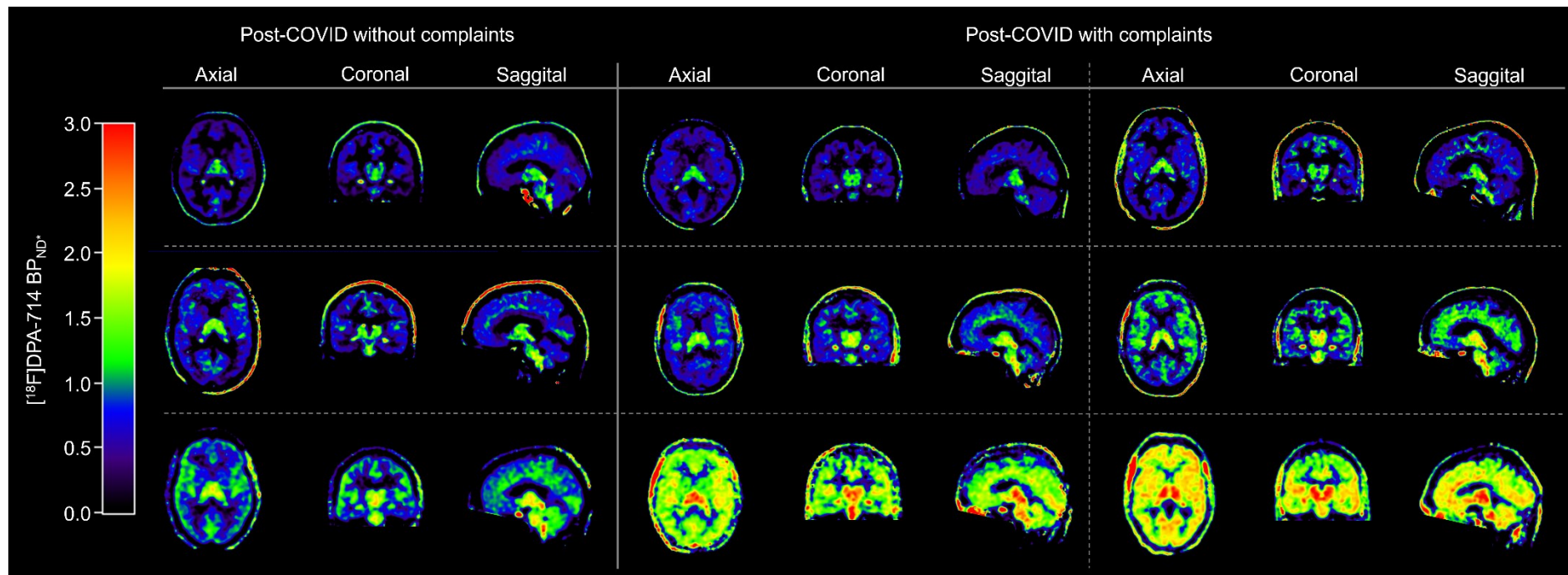


Figure 2. Examples of quantitative cerebral $[^{18}\text{F}]\text{DPA-714}$ images of the brain in post-COVID individuals. *Parametric Logan V_T images corrected for K_1/k_2 (global cerebral region - gray and white matter) and subtracted by one, creating indirect binding potential (BP_{ND}) images.

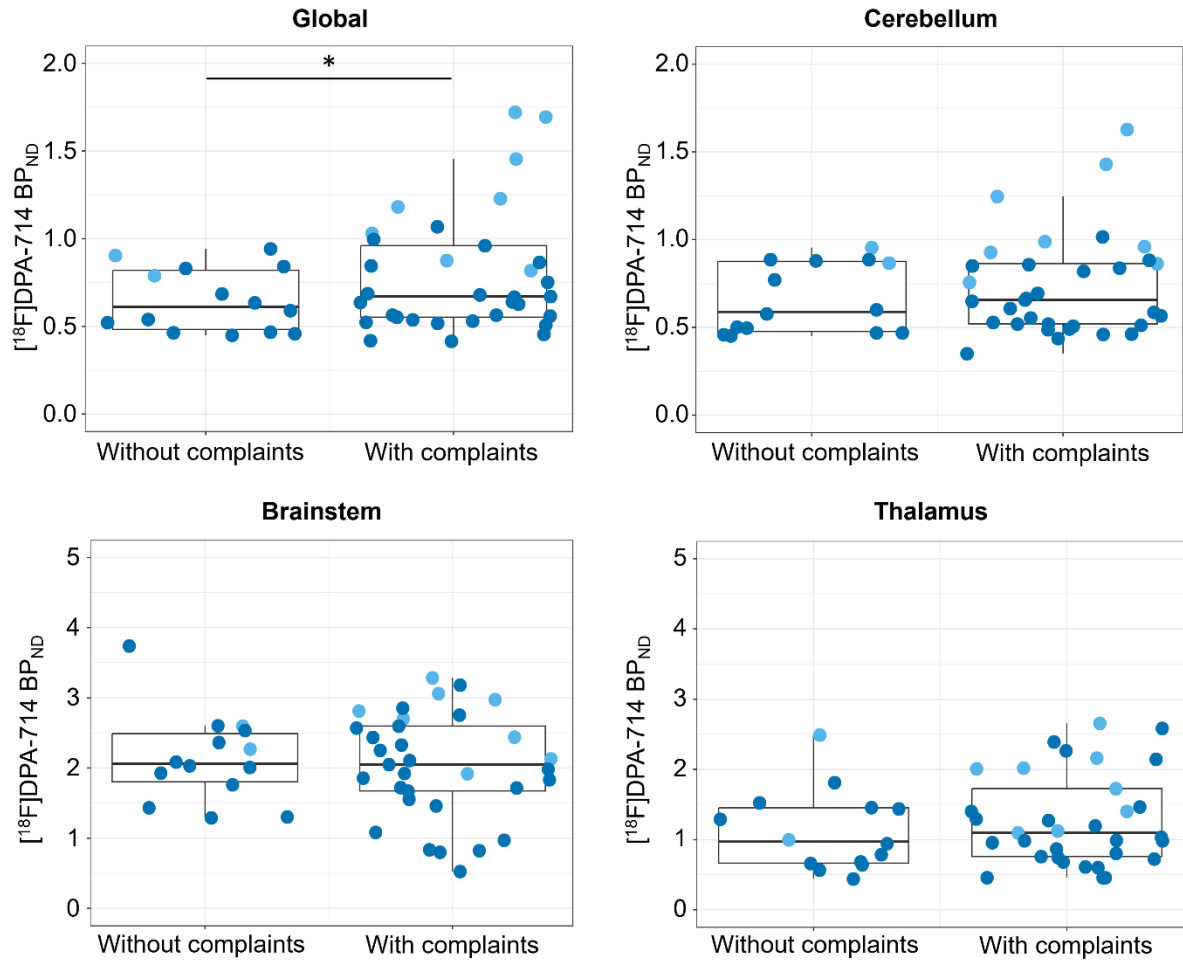


Figure 3. Boxplots of cerebral $[^{18}\text{F}]\text{DPA-714}$ binding in post-COVID individuals with and without complaints. Dark blue = Siemens Biograph Vision Quadra LAFOV PETCT scanner, Light blue = Philips Ingenuity PETCT scanner. * $p < 0.05$, which did not survive FDR-correction ($p = 0.036$, $p\text{FDR} = 0.144$) and was not significant when using a non-parametric test without adjustment for nuisance variables ($p = 0.216$).

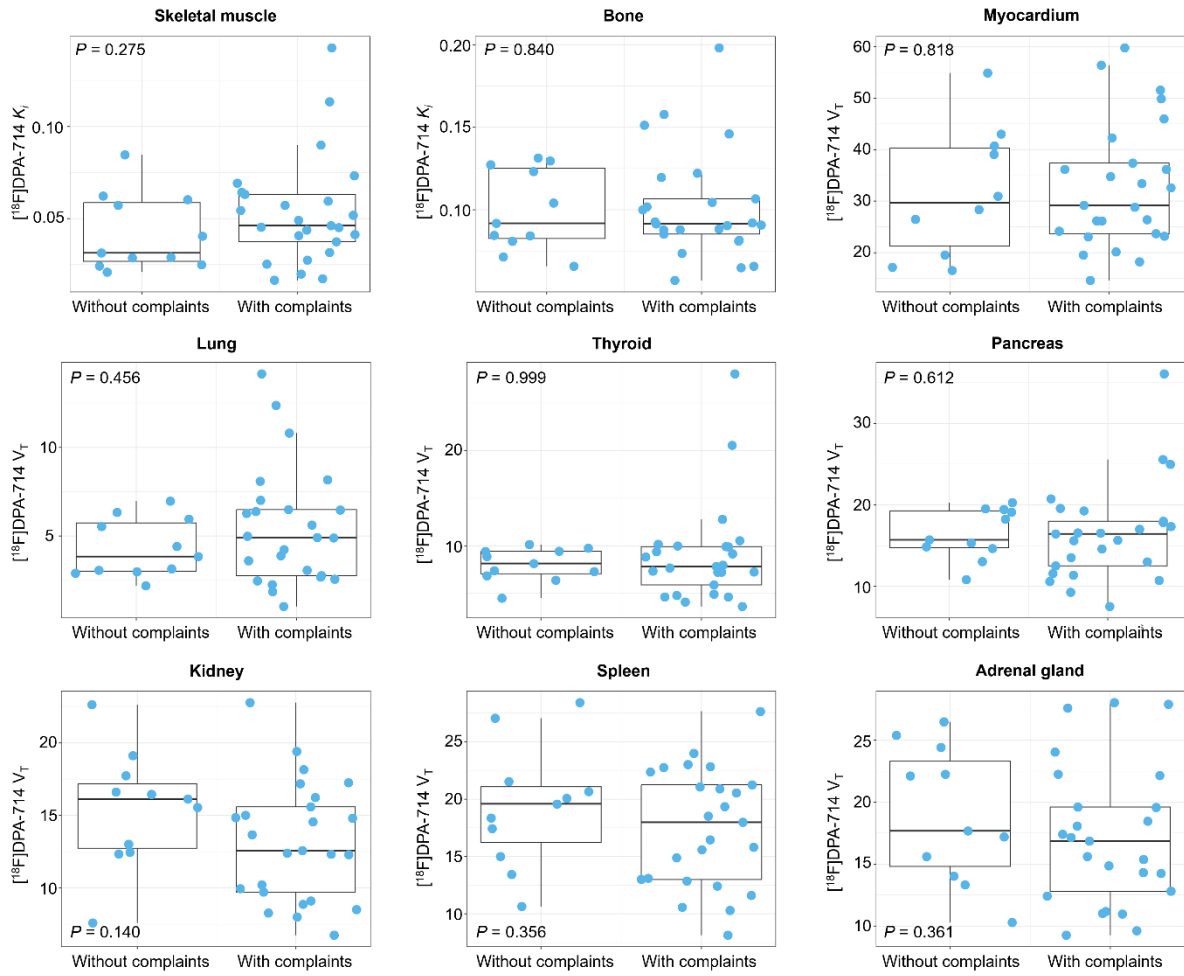


Figure 4. Boxplots of extracerebral $[^{18}\text{F}]$ DPA-714 binding in post-COVID individuals with and without complaints. Binding was quantified using an irreversible 1-tissue-compartment for bone and skeletal muscle (K_i); a reversible 2-tissue-compartment model for kidney and lungs (V_T) and a reversible 1-tissue-compartment model for the other organs (V_T).

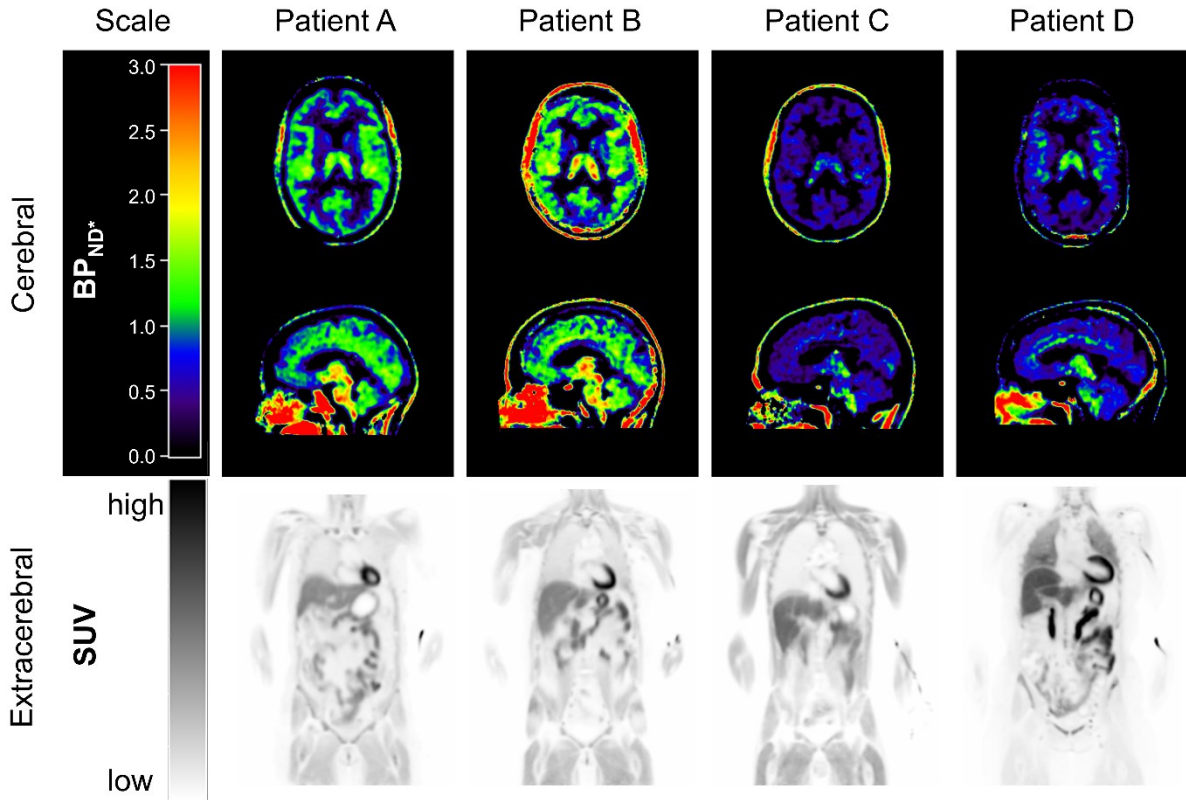


Figure 5. Cerebral and extracerebral [^{18}F]DPA-714 binding levels in four post-COVID patients with persistent fatigue and difficulty concentrating. Patient A: 54y old female, 18mo post-infection without apparent extracerebral binding and relatively high cerebral binding (global BP_{ND} 1.04). Patient B: 52y old female, 38mo post-infection with high binding in skeletal muscles (K_i 0.052) and relatively high cerebral binding (global BP_{ND} 1.07). Patient C: 44y old male, 32mo post-infection, with very high binding in skeletal muscles (K_i 0.076) and low cerebral binding (global BP_{ND} 0.51). Patient D: 56y old female, 14mo post-infection, with very high binding in the lungs (V_T 18.14). *Cerebral parametric Logan V_T images corrected for global (gray and white matter combined) K_1/k_2 and subtracted by one, creating indirect binding potential (BP_{ND}) images. For extracerebral data standardized uptake value (SUV) images, corrected for body weight and injected activity, were created.