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ORIGINAL ARTICLE

^{18}F-FDG brain PET hypometabolism in patients with long COVID

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Abstract

Purpose In the context of the worldwide outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), some patients report functional complaints after apparent recovery from COVID-19. This clinical presentation has been referred as "long COVID." We here present a retrospective analysis of ^{18}F-FDG brain PET of long COVID patients from the same center with a biologically confirmed diagnosis of SARS-CoV-2 infection and persistent functional complaints at least 3 weeks after the initial infection.

Methods PET scans of 35 patients with long COVID were compared using whole-brain voxel-based analysis to a local database of 44 healthy subjects controlled for age and sex to characterize cerebral hypometabolism. The individual relevance of this metabolic profile was evaluated to classify patients and healthy subjects. Finally, the PET abnormalities were exploratory compared with the patients' characteristics and functional complaints.

Results In comparison to healthy subjects, patients with long COVID exhibited bilateral hypometabolism in the bilateral rectal/orbital gyrus, including the olfactory gyrus; the right temporal lobe, including the amygdala and the hippocampus, extending to the right thalamus; the bilateral pons/medulla brainstem; the bilateral cerebellum (p-voxel < 0.001 uncorrected, p-cluster < 0.05 FWE-corrected). These metabolic clusters were highly discriminant to distinguish patients and healthy subjects (100% correct classification). These clusters of hypometabolism were significantly associated with more numerous functional complaints (brainstem and cerebellar clusters), and all associated with the occurrence of certain symptoms (hyposmia/anosmia, memory/cognitive impairment, pain and insomnia) ($p < 0.05$). In a more preliminary analysis, the metabolism of the frontal cluster which included the olfactory gyrus was worse in the 7 patients treated by ACE drugs for high blood pressure ($p = 0.032$), and better in the 3 patients that had used nasal decongestant spray at the infectious stage ($p < 0.001$).

Conclusion This study demonstrates a profile of brain PET hypometabolism in long COVID patients with biologically confirmed SARS-CoV-2 and persistent functional complaints more than 3 weeks after the initial infection symptoms, involving the olfactory gyrus and connected limbic/paralimbic regions, extended to the brainstem and the cerebellum. These hypometabolisms are associated with patients' symptoms, with a biomarker value to identify and potentially follow these patients. The hypometabolism of the frontal cluster, which included the olfactory gyrus, seems to be linked to ACE

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drugs in patients with high blood pressure, with also a better metabolism of this olfactory region in patients using nasal decongestant spray, suggesting a possible role of ACE receptors as an olfactory gateway for this neurotropism.

Keywords ^{18}F-FDG · PET · SARS-CoV-2 · COVID-19 · Anosmia · Stress · Complaints · Memory · Fatigue · Angiotensin-converting enzyme · Long COVID · Dysautonomia

Introduction

In the context of the worldwide outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, some patients have reported functional complaints after apparent recovery from COVID-19, including fatigue, dyspnea, hyposmia/anosmia, dysgeusia/ageusia, memory/cognitive impairment, sleeping alterations, and painful syndromes [1–3]. This clinical presentation has been referred as "long COVID," with urgent need of research works [4, 5]. It has been hypothesized that this disorder could be related to the brain inflammation associated with the neurotropism of SARS-CoV-2 from the olfactory bulb [6, 7]. In this line, we previously reported a clinical case of two patients with a confirmed diagnosis of SARS-CoV-2 infection presenting with brain ^{18}F-FDG PET hypometabolism in the post-infection context involving the olfactory bulb, and in one of them, an extension of this metabolic impairment to other limbic structures as well as to the pre-/post-central gyrus, the thalamus/hypothalamus, the cerebellum, and the brainstem [8]. This preliminary report thus suggested that ^{18}F-FDG PET hypometabolism could constitute a cerebral quantitative biomarker of long COVID [8–10].

We here present a retrospective analysis of ^{18}F-FDG brain PET of long COVID patients from the same center with a biologically confirmed diagnosis of SARS-CoV-2 infection and persistent functional complaints at least 3 weeks after the initial infection. We hypothesize that persistent functional complaints in long COVID is related to functional brain involvement, and that this involvement can be identified using ^{18}F-FDG PET. In this line, PET scans were compared using whole-brain voxel-based analysis to a local database of healthy subjects controlled for age and sex to characterize cerebral hypometabolism. The individual relevance of this metabolic profile was evaluated to classify patients and healthy subjects. Finally, on a more preliminary way, the PET abnormalities were exploratory correlated with the patients' characteristics and functional complaints.

Methods

Subjects

Forty-five consecutive patients with long COVID were retrospectively included in our center from May 18, 2020, to September 30, 2020, using the following criteria: adult over 18 years old with a history of confirmed SARS-CoV-2 infection, having a medical post-COVID evaluation for persistent fatigue, and an ^{18}F-FDG brain PET for functional complaints of possible neurological origin (dyspnea, hyposmia/anosmia, dysgeusia/ageusia, memory/cognitive impairment, insomnia, pain) at least 3 weeks after the initial infection (W3+). This W3+ definition of long COVID is concordant with those of the French association of patients (https://www.apresj20.fr/). Patients with brain lesions on CT or MRI were excluded (2 patients with encephalopathy/encephalitis concomitant with the SARS-CoV-2 infection, and 1 patient with a posterior fossa malformation). Patients without a confirmed diagnosis of SARS-CoV-2 infection, patients without follow-up in our center, and patients with neurologic/psychiatric antecedents or symptoms before SARS-CoV-2 were also excluded. The definition of a confirmed case encompassed clinical features (one or several of the following items: fever, cough, headache, rhinitis, pharyngitis, lumbar pain, muscular pain, arthralgia, diarrhea, hyposmia, dysgeusia) and a positive biological test established by rt-PCR at the time of symptoms or by serology technics (ELISA or immune-assay) from the same microbiology department. Seven patients were consequently excluded in addition to the 3 patients with brain lesions (10 in total), with a final group of 35 patients for the analyses.

The following variables were collected: age, sex, day 0 of SARS-CoV-2 infection as defined by initial symptoms, day of PET exam (concomitant with the clinical evaluation for subjective complaints obtained during the medical interview before the radiopharmaceutical administration by a same physician), body mass index (BMI), diabetes status (y/n), high blood pressure (y/n), current smoking status (y/n), hospitalization in intensive care unit (y/n), mechanical ventilation (y/n), initial level of C-reactive protein, initial clinical general status (NEWS score, *see below*), initial CT lung severity score (using 2 grades: normal/minimal vs intermediate/severe involvement), treatment by hydroxychloroquine (y/n), treatment by antibiotics (y/n), treatment targeting the angiotensin-converting enzyme (ACE drugs, including ACE inhibitors and angiotensin II receptor blockers), initial treatment by nasal decongestant spray (y/n), subjective complaint (y/n at W3+ for each of the following symptoms: dyspnea, hyposmia/anosmia, dysgeusia/ageusia, memory/cognitive complaint, insomnia, pain), and post-traumatic stress disorder (PTSD) checklist scale (PCLS) score at W3+. Data were missing for 7.3% of these items. In more detail, the clinical severity of general status was assessed using the National Early

Warning Score (NEWS) adapted to COVID-19 patients [11], including derived scores from age, respiration rate, oxygen saturation, supplemental oxygen, systolic blood pressure, heart rate, consciousness, and temperature. The total score provides a risk grading as low (score of 1 to 4), medium (5–6), or high (7 or higher).

This data collection was retrospective with no ethical approval requirement other than informed consent according to the French and European regulations. This study respects the GPDR (General Data Protection Regulation) requirements with a registration on the following reference: PADS20-296.

Forty-four healthy subjects, with no neurological/psychiatric symptoms or antecedents and a normal brain MRI, who underwent an ^{18}F-FDG brain PET in our own department, were also selected from a previous project including 60 subjects to match them for age and sex with the SARS-CoV-2 patients (55.06 years ± 11.22 for patients vs 55.11 years ± 14.07 for healthy subjects; 57.1% of women for patients vs 56.8% of women for healthy subjects; p value > 0.9). This local PET database of healthy controls was collected before the SARS-CoV-2 outbreak.

^{18}F-FDG PET imaging and processing

^{18}F-FDG brain PET scans were acquired in the same center using an acquisition protocol conforming to European guidelines, at resting-state, in fasting subjects for at least 4 h with a controlled, normal glycemic level, using an integrated PET/CT General Electric camera (Waukesha, WI), after an intravenous administration of 150 MBq per 15-min acquisition at 30 min post-injection. Images were reconstructed on a 192 × 192 matrix using the ordered subsets expectation maximization algorithm and corrected for attenuation using a CT transmission scan.

Whole-brain statistical analysis was performed at the voxel-level using SPM8 software (Wellcome Department of Cognitive Neurology, University College, London, UK) first to compare patients to healthy subjects considering their age and sex as covariables, identifying clusters of hypometabolism in the patients. We secondarily specified the classification relevance of this metabolic profile (between long COVID patients and healthy subjects) and, on a more exploratory and preliminary way, searched for mean differences or correlations between metabolic values of clusters and demographic, clinical, biological, and radiological data, including functional complaints and treatments (*see below*). In detail, the PET images were spatially normalized onto the Montreal Neurological Institute (MNI) atlas. The dimensions of the resulting voxels were 2 × 2 × 2 mm. The images were then smoothed with a Gaussian filter (8 mm full-width at half-maximum) to blur individual variations in the gyral anatomy and to increase the signal-to-noise ratio. The analysis was limited by a mask from PickAtlas (https://www.nitrc.org/projects/wfu_pickatlas/), including the whole cortex, the olfactory bulb, thalamus and basal ganglia, brainstem, and cerebellum, excluding the white matter and the cerebrospinal fluid. Proportional scaling was performed to give the same global metabolic value to each PET examination.

Statistical analyses

Categorical variables are presented as numbers and percentages, and continuous variables are presented as the means ±SD (standard deviation). The normality of the quantitative value was determined by the Shapiro non-normality test and visual normality verification. All values followed a normal distribution. The significance of the differences was determined by Student's t test between continuous and categorical variables. Correlations were determined by Pearson's test between continuous variables. Chi-squared tests were performed between categorical variables in cases of subgroup samples strictly higher than 5 and by a Fisher exact test otherwise. These various tests were two-sided or one-sided according to the hypothesis (*see below*). The statistical analyses were performed using SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp and R version 4.0.2.

The SPM(T) PET maps were obtained at an uncorrected height threshold (voxel-level significance) of $p < 0.001$, with a correction for multiple comparisons at the level of the cluster using the family-wise error (FWE) rate for a corrected p value < 0.05. Significant findings at a more constraint threshold of p value < 0.05 corrected for the voxel using FWE method were secondarily specified. Values of the metabolic clusters were found by comparing patients with long COVID to healthy subjects, and then were extracted at the individual level using the mean of the region with MARSBAR (http://marsbar.sourceforge.net/). One-sided Student's test of metabolic values of each cluster was applied to compare patients and healthy subjects, and then they were illustrated by Z-scores. Based on these metabolic clusters, a multivariate variance analysis (Wilks lambda) was also performed to individually classify patients and healthy subjects. Finally, the metabolic clusters obtained from the patients were exploratory compared to the other collected data (without correction for multiple comparisons). In detail, all continuous variables (age, BMI, CRP, NEWS score, PCLS score, and the delay between first symptoms and the ^{18}F-FDG PET scan in days) were correlated with the metabolic values of the clusters using a Pearson test. The mean difference of the metabolic values of each cluster was tested with Student's t test according to sex, the presence of W3+ complaints (separately for dyspnea, hyposmia/anosmia, dysgeusia/ageusia, memory/cognitive impairment, pain, and insomnia), the diabetes status, the high blood pressure status/ACE drugs (*same value in our group since all patients with high blood pressure were treated by ACE drugs*), the hospitalization in intensive care unit, the need of mechanical

ventilation, the CT lung severity score, the initial treatment (nasal decongestant spray, hydroxychloroquine, antibiotic). The statistical threshold of these analyses was one-sided at $p < 0.05$ on the hypothesis linking hypometabolism to clinical, biological, and radiological abnormalities, and two-sided in the absence of a hypothesis about the direction of the relationship (age, sex, delay, the use of nasal decongestant spray, and current smoking status).

Results

Clinical characteristics of the patients with long COVID

The clinical characteristics of the 35 included patients with long COVID are detailed in Table 1. The mean age of the patients was 55.06 years ± 11.22, with 57% of women (20/35). Notably, 29% of the patients had diabetes (10/34), 21% had high blood pressure (7/33), and only 6% of them were active smokers (2/34). At infection diagnosis, their median NEWS clinical score was 4, corresponding to a low-risk grading; 39% of patients required hospitalization in intensive care unit (12/31), and 16% a mechanical ventilation (5/31). At this step, intermediate or severe lung involvement was found in 16 of 30 patients who had a CT scan (53%), and the CRP level was higher than 5 mmol/L in 71% of the patients (20/28). Among the 35 patients (no missing data), 86% were treated with antibiotics (30/35) and 74% with hydroxychloroquine (26/35). All patients with high blood pressure (7/33) were treated with drugs targeting ACE (*same value for these 2 variables in our sample*). Finally, 3 of 28 patients (11%) used nasal decongestant spray during the infection phase. No patient had received systemic corticoids.

At W3+, among the 35 patients (no missing data), dyspnea was reported in 28 patients (80%), pain in 23 patients (66%, with chest pain, in addition to headache or more peripheral articular/muscular pain in 8/23 patients), memory/cognitive

Table 1 Characteristics of patients with long COVID

Variable	N[1]	Mean (SD) or n (%)
Age	35	55.06 (11.22)
Sex	35	
Women		20 (57)
Men		15 (43)
BMI	35	28.25 (4.82)
High blood pressure	33	7 (21)
Diabetes	34	10 (29)
Current smoking status	34	2 (6)
Hospitalization in intensive care unit	31	12 (39)
Mechanical ventilation	31	5 (16)
NEWS clinical score	26	4.04 (3.40)
CT lung severity score	30	
Normal/minimal		14 (47)
Intermediate/severe		16 (53)
CRP	28	61.2 (74.67)
ACE drugs	33	7 (21)
Hydroxychloroquine	35	26 (74)
Antibiotic	35	30 (86)
Nasal decongestant spray	28	3 (11)
Hyposmia/anosmia W3+	35	10 (29)
Dysgeusia/ageusia W3+	35	9 (26)
Memory/cognitive impairment W3+	35	17 (49)
Pain W3+	35	23 (66)
Dyspnea W3+	35	28 (80)
Insomnia W3+	35	16 (46)
PCLS score	21	35.81 (14.87)
Delay between initial symptoms and PET (in days)	35	95.57 (30.65)

[1] N is the number of patients with the available information

complaint in 17 patients (49%), insomnia in 16 patients (46%), hyposmia/anosmia in 10 patients (29%), and dysgeusia/ageusia in 9 patients (26%). The PCLS total score was impaired in 33% of patients (7/21, with a score higher than 44). The median duration between this evaluation, including the PET scan, and the first infectious symptoms was 97 days (26 to 155 days, with no missing data).

An increased number of functional complaints at W3+ was correlated with a younger age ($r = -0.443$, $p = 0.008$; bilateral Pearson's test) and with a longer duration from the initial infection symptoms ($r = 0.375$, $p = 0.026$; bilateral Pearson's test).

Patients with W3+ hyposmia/anosmia and patients with W3+ memory/cognitive complaints were younger (respectively, $p = 0.018$ and $p = 0.007$; bilateral Student's t tests). A longer delay from initial infection was found in patients with W3+ pain and in patients with W3+ memory/cognitive complaints ($p = 0.048$ and $p = 0.010$, respectively; bilateral Student's t tests).

A more pejorative PCLS score for PTSD was found for patients with W3+ memory/cognitive complaints and for patients with W3+ dysgeusia/ageusia ($p = 0.002$ and $p = 0.012$; bilateral Student's t tests).

PET metabolic profile of patients with long COVID in comparison to healthy subjects

In comparison to the 44 healthy subjects, the patients with long COVID presented with significant hypometabolism (p-voxel < 0.001 uncorrected, p-cluster < 0.05 FWE corrected) involving 4 clusters (Figs. 1 and 2): the bilateral rectal/orbital gyrus, including the olfactory gyrus (BA11; T-max = 9.80; $k = 767$); the right temporal lobe, including the amygdala and the hippocampus, extending to the right thalamus (BA20, BA21, BA22, BA28; T-max = 5.16; $k = 3353$); the bilateral pons/medulla brainstem (T-max = 6.84; $k = 1485$); the bilateral cerebellum (T-max = 5.18; $k = 1969$). As detailed in Table 2, significant results were also obtained using a FWE correction at voxel-level for these four regions but with a lesser cluster size. No significant hypermetabolism was found at this statistical threshold.

Wilks lambda model was performed with the metabolic PET cluster values to classify patients with long COVID and healthy subjects. Among the 4 PET clusters, the right temporal and frontal metabolisms were finally retained in the model. Wilks lambdas were 0.147 for the right temporal cluster and 0.123 for the frontal cluster, and the Fisher exact test proved that the class center of these variables was not confounded ($p < 0.001$). This model was able to correctly classify 100% of the individuals.

Relationships between brain PET hypometabolisms and clinical characteristics

The exploratory relationships between brain PET hypometabolisms and clinical characteristics are presented in Figs. 3 and 4. An increased number of functional complaints at W3+ was correlated with decreased metabolism of the brainstem and cerebellum clusters ($r = -0.440$ and -0.581, $p = 0.004$ and $p < 0.001$, respectively; unilateral Pearson's tests).

Lower metabolic values of the frontal cluster were associated with W3+ pain and with high blood pressure ($p = 0.040$ and $p = 0.032$, respectively, using unilateral Student's t tests). Higher metabolic values of the frontal cluster were found in the only 3 patients who had used nasal decongestant spray ($p < 0.001$, using bilateral Student's t test).

Lower metabolic values of the right temporal cluster were associated with W3+ pain and W3+ insomnia (respectively $p = 0.037$ and $p = 0.045$; unilateral Student's t tests). A trend was found between hypometabolism of the right temporal cluster and a longer duration from the initial infection symptoms ($r = -0.323$, $p = 0.058$; bilateral Pearson's test).

Lower metabolic values of the brainstem cluster were associated with W3+ pain and W3+ insomnia (respectively $p = 0.024$ and $p = 0.042$; unilateral Student's t tests).

Lower metabolic values of the cerebellum cluster were associated with W3+ hyposmia/anosmia, W3+ memory/

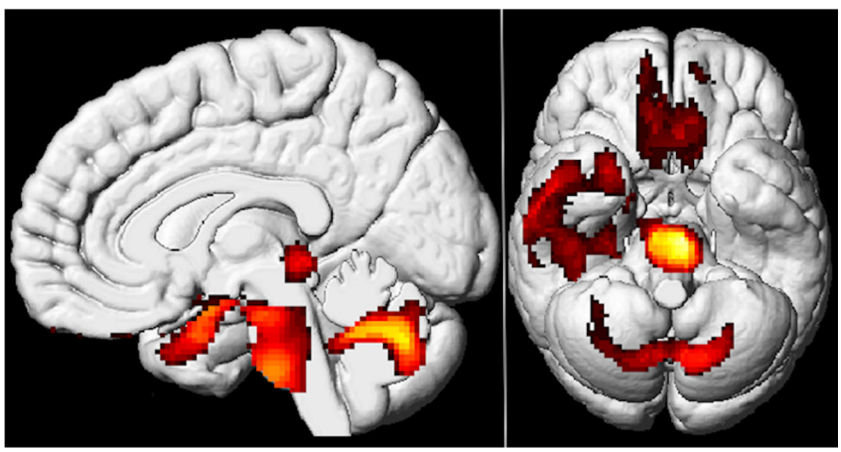

Fig. 1 Brain ^{18}F-FDG PET hypometabolism in patients with long COVID. In comparison to healthy subjects, the patients exhibit hypometabolism in the bilateral rectal/orbital gyrus, including the olfactory gyrus; the right temporal lobe, including the amygdala and the hippocampus, extending to the right thalamus; the bilateral pons/medulla brainstem; the bilateral cerebellum (p-voxel < 0.001 uncorrected, p-cluster < 0.05 FWE-corrected; SPM8 3D rendering)

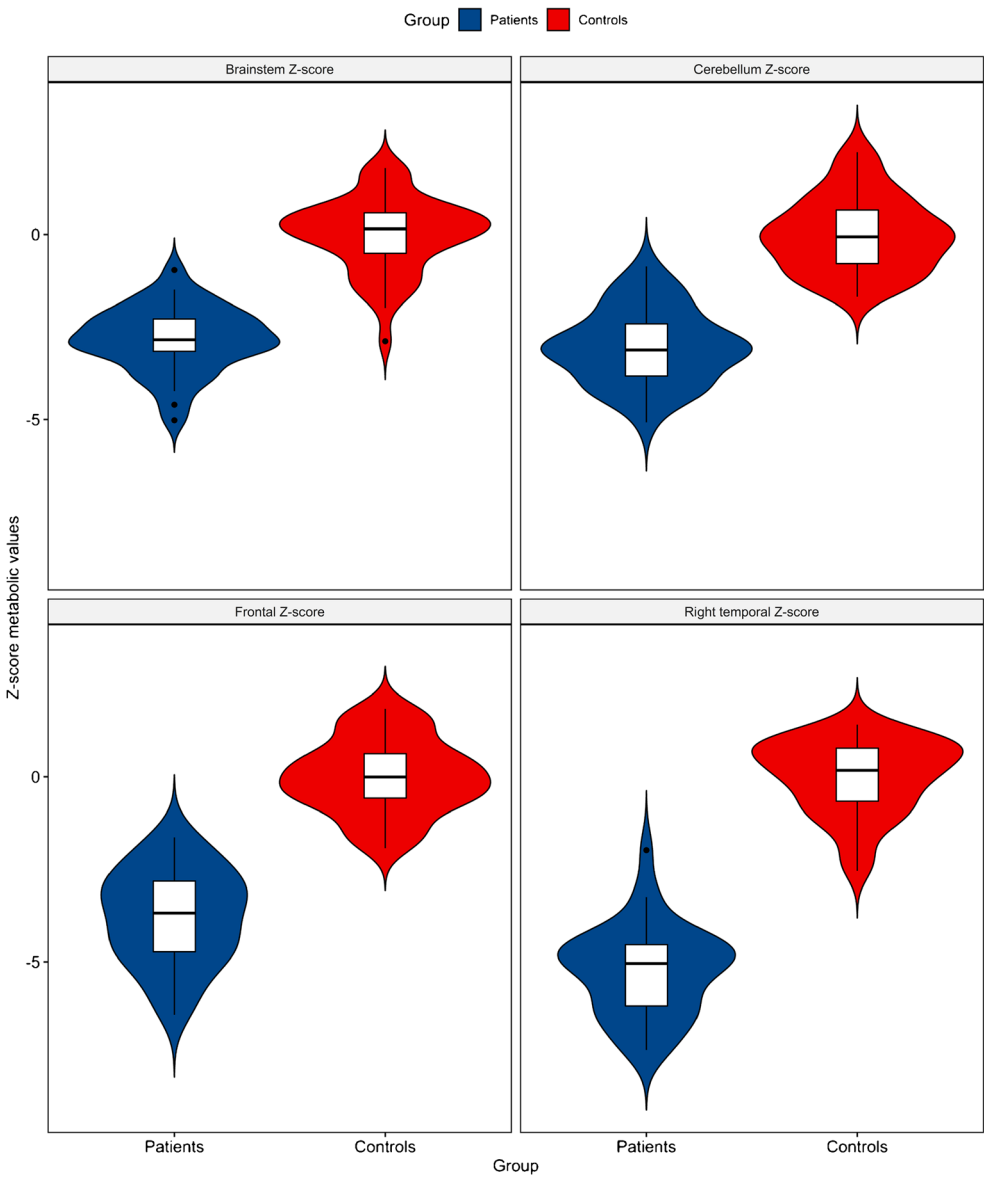

Fig. 2 Mean metabolic PET values of clusters expressed as Z-scores, found by comparing patients with long COVID to healthy subjects in SPM8. These metabolic values were significantly different between patients and healthy subjects ($p < 0.001$; one-sided Student's t test)

cognitive complaint, W3+ pain, and W3+ insomnia ($p = 0.007$, $p = 0.004$, $p = 0.014$, and $p = 0.006$, respectively; unilateral Student's t tests).

Discussion

This whole-brain voxel-based PET study demonstrates brain hypometabolism in long COVID patients with biologically confirmed SARS-CoV-2 infection and functional complaints of a possible central origin, 26 to 155 days after the initial symptoms of infection, in comparison to healthy subjects without antecedents of SARS-CoV-2 infection matched for age and sex. In detail, these areas of hypometabolism involved the bilateral rectal/orbital gyrus (including the olfactory gyrus), the right temporal lobe (including the amygdala and the hippocampus extending to the right thalamus), the bilateral pons/medulla brainstem, and the bilateral cerebellum. These metabolic clusters were highly discriminant to distinguish patients and healthy subjects. Moreover, they were significantly associated with more numerous functional complaints and, with a trend, with a longer duration of functional complaints post-recovery. On a more exploratory and preliminary way, the metabolism of the frontal cluster which included the olfactory gyrus was worse in the 7 patients treated by ACE drugs for high blood pressure, and better in the only 3 patients that had used nasal decongestant spray.

Beyond the link with the total number of symptoms, the rationale for the brain substrate of such functional complaints is more specially strengthened by the relationship found with PET hypometabolism. In detail, hyposmia/anosmia was associated with cerebellar hypometabolism. Previous reports have

Table 2 Main ^{18}F-FDG PET hypometabolism in patients with long COVID in comparison to healthy subjects (p-voxel < 0.001, uncorrected; p-cluster < 0.05, FWE corrected)

Cluster	Cluster FWE	Voxel	Voxel unc.	Talairach coordinates			Localization
k	*p* value	*T* score	*p* value	x	y	z	
767	0.010	9.80	<0.001*	−8	20	−30	Left rectal gyrus, BA11
		9.26	<0.001*	8	31	−32	Right orbital gyrus, BA11
		8.77	<0.001*	−10	29	−32	Left orbital gyrus, BA11
		7.61	<0.001*	6	24	−30	Right rectal gyrus, BA11
1485	<0.001	6.84	<0.001*	−6	−23	−36	Left medulla
		4.44	<0.001	6	−20	−19	Right pons
		4.08	<0.001	−4	−24	−21	Left pons
1969	<0.001	5.18	<0.001*	10	−71	−25	Right cerebellum, pyramis
		4.70	<0.001	−16	−72	−32	Left cerebellum, uvula
		3.79	<0.001	12	−48	−25	Right cerebellum, dentate
3353	<0.001	5.16	<0.001*	55	−3	−12	Right middle temporal gyrus, BA21
		4.99	<0.001*	61	−36	15	Right superior temporal gyrus
		4.75	<0.001	36	−14	−13	Right hippocampus
		4.61	<0.001	26	3	−27	Right uncus, BA28
		4.54	<0.001	26	1	−19	Right amygdala
		4.41	<0.001	57	−13	−18	Right inferior temporal gyrus, BA21
		4.36	<0.001	46	−22	−14	Right fusiform gyrus, BA20

The *k* value represents the number of voxels inside a particular cluster. *p* values for the voxel are all presented as uncorrected for multiple comparisons. *p* values still significant at $p < 0.05$ after correction for multiple comparisons for the voxel using the FWE (family-wise error) method are indicated with *. All *p* values for the cluster are corrected for multiple comparisons using the FWE method. Talairach coordinates are expressed in mm. *BA*, Brodmann area

linked anosmia and ageusia to lesions of the cerebellum [12, 13]. Cerebellar hypometabolism was also associated in our study with memory/cognitive impairment, which is concordant with the involvement of this region in executive functions and working memory [14, 15]. Pain was associated with the hypometabolism of the frontal cortex, brainstem, and cerebellum; these regions are known to be involved in the brain matrix of pain [16], especially in patients with fibromyalgia [17]. Finally, insomnia was associated with the hypometabolism of the brainstem and cerebellum, with again well-known substrates for dysautonomia [18]. Beyond these highlighted relationships, the retrospective qualitative evaluation of our study certainly hampered a more extended analysis linking functional complaints to PET hypometabolism.

Our findings reinforce the hypothesis of SARS-CoV-2 neurotropism through the olfactory bulb and the possible extension of this impairment to other limbic/paralimbic structures as well as to the thalamus, the cerebellum, and the brainstem within these highly connected regions [19]. The neurotropism of SARS-CoV-2 is first supported by cerebral complications already reported during the acute phase, such as strokes and encephalopathy/encephalitis [20, 21]. In the longer term, a delayed outbreak of psychiatric and neurological diseases of neuroinflammatory origin has also been anticipated [6]. This neurotropism is overall concordant with previous findings reported for other coronavirus infections, with demonstration of the presence of the virus in the brain and in the cerebrospinal fluid [22]. In this line, a recent meta-analysis reported psychiatric and neuropsychiatric presentations associated with severe CoV infection in more than 40% of patients in the early phase, and around 30% have post-traumatic stress disorder in the post-illness stage [23], with a chronic fatigue syndrome and fibromyalgia association [24].

From a pathophysiological perspective, these functional complaints could involve a systemic immune-inflammation disorder [1], with possible entanglement with other factors and particularly psychological factors. These presentations have been also related to the quarantine, possible adverse effects of treatments such as corticosteroids [2], or traumatic experience of media exposure or hospitalization with uncertainty regarding the prognosis [25]. Moreover, it has been suggested that women [3] and trauma survivors could be at risk for such complications [26]. In contrast, no relationship was found in our study with the initial clinical, biological, and radiological severity (NEWS score, hospitalization in intensive care unit, mechanical ventilation, CRP level, CT score). On the other hand, patients with memory/cognitive

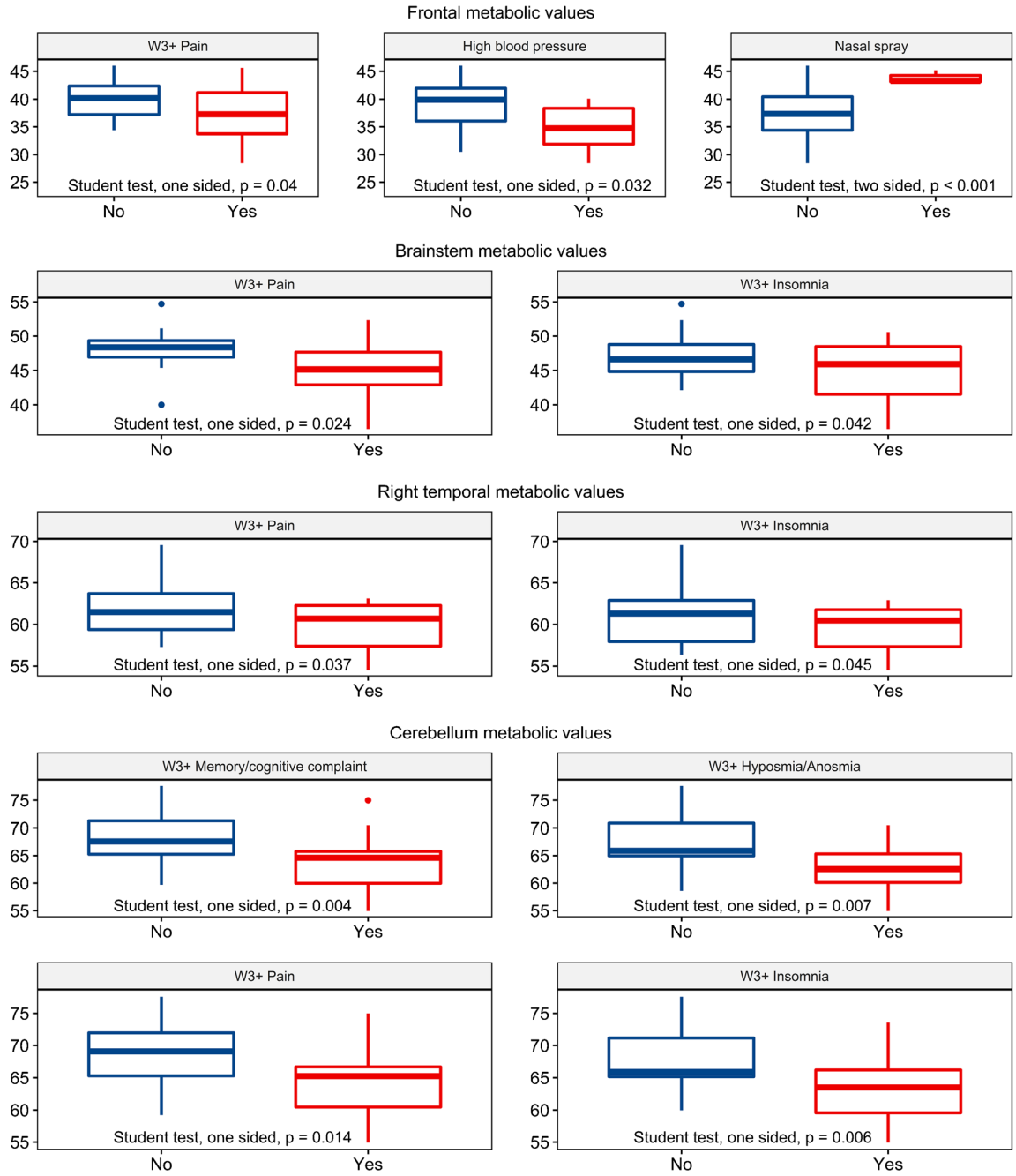

Fig. 3 Relationships between PET metabolic clusters and characteristics of patients with long COVID

impairment and more numerous complaints were younger. The relationship with age should be particularly noticed, especially because young people are supposed to develop a more benign form of the disease, which does not seem to be the case for these delayed/persistent functional complaints, even if we cannot exclude a bias of recruitment.

Concerning the brain PET hypometabolism, a significant association was found with high blood pressure status treated by ACE drugs (for the frontal cluster), with also a better metabolism of this region in patients using nasal decongestant spray (for the frontal cluster), and with a trend for a longer persistence of symptoms (for the temporal cluster). These preliminary results should be considered with caution given the small number of patients (3 patients for the use of nasal spray, and 7 for ACE drugs). The relationship with high blood pressure status could suggest the involvement of ACE2 receptors in the neurotropism of SARS-CoV-2, and especially for the possible gateway from the olfactory bulb. For other CoVs, a propagation mechanism has indeed been proposed across the lamina cribrosa of the ethmoid bone, from the nose to the olfactory bulb, where ACE2 receptors, which are targeted by the virus, are strongly expressed [7]. Beyond this, a trans-synaptic viral transfer has been previously

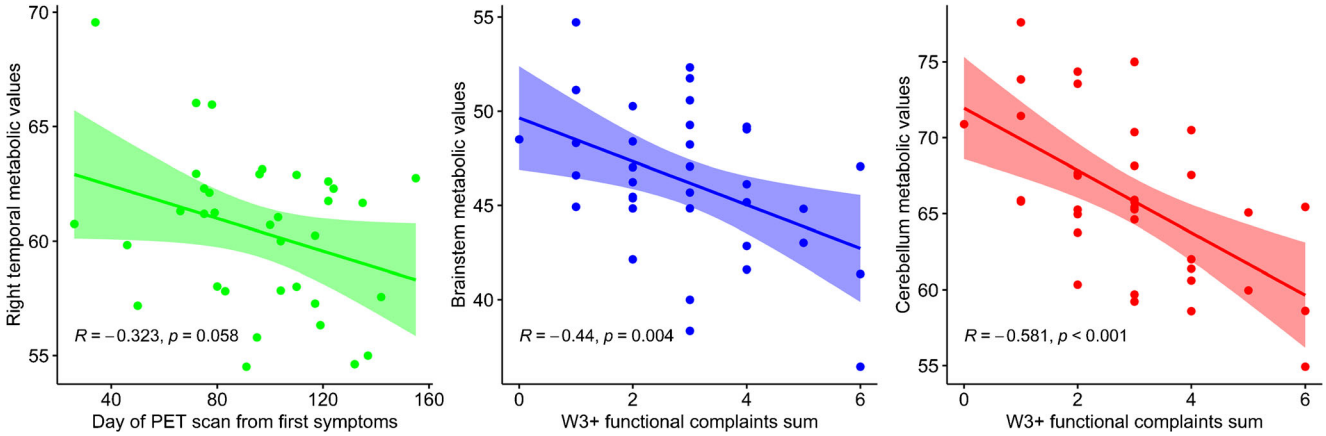

Fig. 4 Relationships between PET metabolic clusters and characteristics of patients with long COVID

demonstrated [7], with lesions possibly involving direct infection injury, hypoxia, and immune injuries. In this line, a post-mortem study confirmed the neurotropism of SARS-CoV-2, with moderate to intense microglial activation [27]. On the other hand, the better metabolism of the frontal cluster, including the olfactory bulb, found in patients who had used nasal decongestant spray could indeed argue for a direct process from the nose to the olfactory bulb, even if this result is obtained in few patients. Finally, the trend between PET hypometabolism and the duration after the initial infection symptoms could suggest a relationship with the clinical severity, linking a more severe clinical profile to more PET hypometabolism, and longer duration of symptoms in patients more severe hypometabolisms. This may alternatively suggest a pejorative dynamic with a worsening of the PET hypometabolism over time. We have also to notice the absence of significant brain hypermetabolism, suggesting the absence of brain inflammation at this step of the evaluation. Follow-up PET studies are required to specify the correct relationship between the PET abnormalities and the temporal sequence of functional complaints, and especially earlier PET examinations to investigate the hypothesis of brain hypometabolic dysfunction secondary to earlier hypermetabolic inflammation. We could also hypothesize that this brain hypometabolic dysfunction at least partly involves remote functional effects from focal olfactory lesion through diaschisis and deafferentation [28–30].

Classification analysis finally shows that the brain PET profile of hypometabolism could constitute a cerebral biomarker with high performance to discriminate affected patients with long COVID from healthy subjects. While a prolonged SARS-CoV-2 syndrome could have major social-economic and medical impacts, the possibility of implementing an effective biomarker could be very useful to confirm and follow-up functional brain involvement in these patients and to evaluate the efficiency of new therapeutic strategies such as medication or rehabilitation, to treat the possible inflammatory olfactive gateway and stimulate this hypofunctional brain network.

Limitations

The study is retrospective and monocentric, with a comprehensive clinical evaluation including few missing data, comparing patients to healthy subjects examined in the same PET department. This data collection is, however, mostly qualitative and without a dedicated quantitative scale for the functional complaints. In this line, the confrontation of PET abnormalities according to patients' characteristics and functional complaints is more exploratory, and has to be considered as a first preliminary report on a very emergent topic. Bias of recruitment cannot be excluded, especially to explain the relationships found with younger patients and those with longer delay, and those obtained in few subjects (3 patients for the use of nasal spray, and 7 for ACE drugs). Moreover, brain MRI was not systematically prescribed in the absence of encephalopathy/encephalitis or stroke (these patients were excluded from the study), and normal brain CT on hybrid PET. Finally, the impact of the drug treatment strategy including the use of systemic corticosteroids cannot be evaluated, since none of these patients was given such treatments during this first phase of the pandemic. Additional studies with a prospective multicentric design and larger samples are needed to confirm our results, especially the relevance of the PET biomarker at the individual level with distinct training and test samples, and to evaluate the correct impact of therapies such as local/systemic corticosteroids. These further studies could also involve brain MRI multimodal sequences to investigate the respective contribution of lesions and dysfunctions. The follow-up of these patients is finally required to confirm the functional nature and reversibility of these PET metabolic abnormalities.

Conclusion

On the whole, this study demonstrates a profile of brain PET hypometabolism in long COVID patients with biologically confirmed SARS-CoV-2 infection and persistent functional complaints more than 3 weeks after the initial infection

symptoms, involving the olfactory gyrus and connected limbic/paralimbic regions, extended to the brainstem and the cerebellum. This hypometabolic profile had an individual relevance to classify patients and healthy subjects, suggesting value as a biomarker to identify and follow these patients. The rationale for the affected brain substrate of these functional complaints is strengthened by the relationships found with the hypometabolic clusters. Finally, the hypometabolism of the frontal cluster, which included the olfactory gyrus, seems to be linked to ACE drugs in patients with high blood pressure, with also a better metabolism of this region in patients using nasal decongestant spray, suggesting a possible role of ACE receptors as an olfactory gateway for this neurotropism.

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Data availability The PET data that support the findings are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Declarations

Ethics approval and consent to participate The retrospective observations required no ethical approval requirement other than informed consent. The local PET database of healthy controls was acquired in accordance with the Declaration of Helsinki, with informed written consent from the patients and approval from the "CPP Sud Méditerranée V" ethics committee. Informed consent was obtained from all individual participants included in the study.

Consent for publication Not applicable.

Conflict of interest The authors declare no conflict of interest.

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