UNMASKING COVID-19

CEREBRAL CONSEQUENCES FROM A NEUROIMAGING PERSPECTIVE

RESA VAN LITH

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Unmasking COVID-19

Cerebral consequences from a neuroimaging perspective

Resa van Lith

The studies in chapter 2-6 in this thesis were carried out at the Department of Neurology, Donders Center for Medical Neurosciences, Neuroscience, Radboud University Medical Center, Nijmegen, The Netherlands. The study in chapter 7 was carried out at the Department of Medical Imaging, Radboud University Medical Center, Nijmegen.

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Cerebral consequences from a neuroimaging perspective

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Ik draag dit proefschrift op aan mijn ouders

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Introduction

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General introduction, aims and outline

General introduction

The clinical course of coronavirus disease 2019 (COVID-19)

The arrival of the severe acute respiratory syndrome coronavirus (SARS-CoV-2) in 2020 and the subsequent worldwide spread has led to socio-economic disruption, impacting daily life and health care in particular. COVID-19, characterized by several clinical manifestations, has opposed a great array of health challenges. Besides the initial characterization as a respiratory illness, neurological sequelae and cardiovascular complications have emerged as prominent elements of the disease. (1, 2) The urgency to understand the dynamics of viral infections in the brain has become more apparent in the evolution of the COVID-19 pandemic.

COVID-19 is known to induce increased inflammation, trigger autoimmune responses and cause oxidative stress. These effects have primarily been observed in the (cardio)pulmonary system, but may also affect other organs, including the brain. During the pandemic in 2020, it became clear that infection with COVID-19 could be complicated by thrombo-embolic events, including pulmonary embolism and deep venous thrombosis, but also ischemic stroke, particularly in patients admitted to the intensive care unit (ICU). (3, 4) These complications, specifically ischemic stroke, are associated with adverse clinical outcomes and increased mortality rates. (3, 5) In addition to overt ischemic strokes, due to the proinflammatory and procoagulant response of COVID-19, there may also occur small ischemic lesions that initially appear clinically silent. These lesions can impair long-term recovery, eventually leading to for example long COVID. Therefore, there is an urgent need to investigate these potential ''clinically silent'' ischemic brain lesions arising from this procoagulant or proinflammatory response to COVID-19.

Conventional cerebrovascular MRI markers

By providing non-invasive insights into the (changes of the) structure of the brain, MRI offers a unique advantage to explore these possible changes and the attendant neurological symptoms associated with the virus. Not only ischemic lesions, but also other cerebrovascular lesions such as microbleeds, intracerebral hemorrhage and white matter hyperintensities (WMH) are known to occur in COVID-19 patients. One recent systematic review (2023) investigating COVID-19 patients with neurological symptoms reported (sub)acute infarcts as the most common MRI finding in up to a quarter of the patients. (6) It is known that (sub)acute infarcts are markers for cerebral small vessel disease (SVD), an important contributor to stroke, dementia but also impairment of functional and cognitive outcomes among elderly individuals. (7)

Existing studies, however, have predominantly examined the effect of COVID-19 on the brain of symptomatic (hospitalized) patients, with clinical symptoms as the reason for scanning. Therefore, there remains a gap in knowledge on the presence of cerebrovascular (MRI) markers of unselected, hospitalized COVID-19 patients, regardless of (neurological) symptoms. Subsequently, little is known about the evolution of these cerebrovascular (MRI) markers over time in these patients. Since these abnormalities may influence long-term recovery, this information is crucial for better understanding of the disease, optimization of treatment strategies and long-term prognosis of the ''general'' hospitalized patient with COVID-19.

In addition to conventional MRI markers, MRI can also be utilized to perform vessel wall imaging to examine the brain's vasculature. Currently, there is a gap in understanding the frequency and characteristics of vascular involvement in COVID-19, particularly intracranial vasculopathy. The application of intracranial vessel wall imaging, often conducted in the context of ischemic stroke, presents a unique opportunity to investigate active inflammation and shed light on the pathophysiology underlying these vascular abnormalities.

White matter (WM) integrity and its relation with clinical outcomes and blood pressure variability (BPV)

The conventional MRI markers (such as WMH) may fall short in representing more subtle, pathological changes in the microstructure of the WM. This can be visualized through advanced neuroimaging techniques such as diffusion tensor imaging (DTI). Diffusion MRI measures the movement of water in brain tissue in vivo, with DTI metrics like fractional anisotropy (FA), mean diffusivity (MD) and peak width of skeletonized mean diffusivity (PSMD) referring to the microstructural integrity or condition of WM tracts. Additionally, neurite orientation dispersion and density imaging (NODDI) can further distinguish between neuronal compartments within the voxel, displaying the structure of dendrites and axons. (8) Current insights into microstructural integrity in COVID-19 patients are limited or inconsistent, yet the loss of WM integrity has been often linked to cognitive decline in several brain diseases. (9, 10) Assessing the WM integrity in COVID-19 patients is therefore important to identify possible causes of long-term or even persistent (cognitive) symptoms patients are currently experiencing after infection.

Given the importance of WM integrity in understanding potential long-term neurological effects, examining factors that could influence these microstructural changes is crucial. Blood pressure variability (BPV), characterized by fluctuations in blood pressure (BP) levels, has been associated with poorer outcomes, including

increased in-hospital mortality and ICU admissions in COVID-19 patients (11, 12). Since BPV is a known cardiovascular risk factor, we explored its relationship with WM integrity, as elevated BPV has been linked to adverse neurological outcomes in other conditions. Investigating BPV during hospitalization in relation to WM microstructural changes may help clarify potential long-term neurological sequelae in COVID-19 survivors.

PET/CT imaging for assessing inflammation

Whilst MRI imaging is able to visualize structural abnormalities in the brain, PET has the advantage of showing cellular metabolism, with the anatomical detail provided by CT. It is highly sensitive in detecting areas of inflammation, for example in blood vessels, associated with COVID-19. There is a research gap between in vivo localization and quantification of endothelial activation (leading to inflammation) in COVID-19 patients, in which PET/CT imaging could provide an understanding. αvβ3 integrins can be upregulated on endothelial cells due to SARS-CoV-2 infection. Therefore, by measuring the uptake of the PET/CT tracer to these integrins, this can be used as a proxy for endothelial activation in patients. We can enhance our understanding of the underlying pathogenesis of COVID-19 and its systemic impact on multiple organs.

Research during a pandemic

Our objectives encompass not only filling the existing gaps in knowledge regarding COVID-19 and the brain, but also identify implications of this pandemic on scientific research. Beyond these acute health implications, the pandemic has led to a reevaluation of infectious diseases and their consequences on a worldwide scale, especially in terms of research. The challenges encountered during the pandemic revealed how conducting rapid research—within the constraints of current legislation and regulatory policies—proved difficult. With limited initial knowledge about the virus, it became clear that research methodologies had to be continually adapted. While urgent efforts yielded some notable successes (such as the implementation of therapies like dexamethasone) many other studies, including our own, experienced significant delays due to complex approval processes, ethical reviews, and logistical barriers. These obstacles underscore the need for more streamlined procedures and enhanced international collaboration, particularly in the context of future pandemics. To improve future responses, integrating the lessons learned from COVID-19 with increased governmental investment in pandemic preparedness will be essential to ensure research can proceed more efficiently during future health crises.

Aim of this thesis

This thesis has three major objectives. Our first aim was to investigate the prevalence and incidence of cerebrovascular MRI markers and vessel wall abnormalities in hospitalized COVID-19 patients. Second, we aimed to provide insights in the structural WM integrity of COVID-19 patients and their association with long-term clinical outcomes and BPV. Third, we aimed to explore PET/CT tracer uptake by αvβ3 integrins in the carotid arteries as a proxy for carotid endothelial activation (and subsequently inflammation) in COVID-19.

Study design

Part I, II and III are based on the CORONavirus and Ischemic Stroke (CORONIS) study, a multicenter observational cohort study on the prevalence, incidence and risk factors of silent cerebral ischemia and other cerebrovascular MRI markers and long-term clinical outcomes after hospitalization (Figure 1). Patients included were hospitalized with COVID-19 (≥18 years) and healthy controls with proven absence of SARS-CoV-2 infection, recruited in the Radboudumc, LUMC and UMCU hospital. At baseline (2011-2022), a total of 202 participants enrolled and follow-up was performed 3 and 12 months after baseline study procedures.

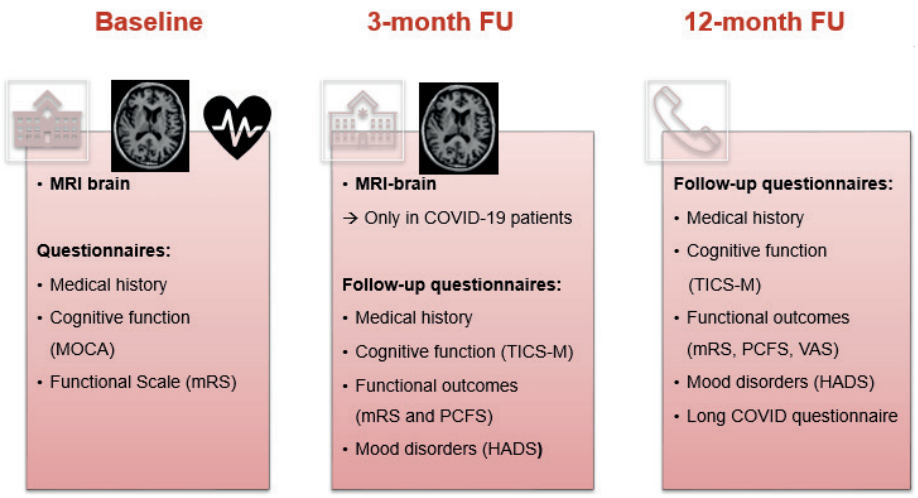

Figure 1. CORONIS study. Prospective observational cohort study investigating the prevalence, incidence and long-term clinical outcomes of hospitalized COVID-19 patients, compared to controls. Baseline study procedures included brain MRI and collection of medical history and cognitive function (MOCA = Montreal Cognitive Assessment). During follow-up, MRI was repeated in patients during first follow-up and questionnaires were carried out by telephone and online including cognitive function (TICS-M = Modified Telephone Interview for Cognitive Status), mood and depression (HADS = Hospital Anxiety and Depression Scale) and functionals outcomes (mRS = modified Rankin Scale, PCFS = Post-COVID-19 Functional Status scale, VAS = Visual Analogue Scale) during both follow-up points.

In another sample of COVID-19 patients we conducted PET/CT imaging (Part IV).

Outline of this thesis

In **Part I (chapter 2)**, the rationale and design of the CORONIS study is described. This study was designed to gain insight the prevalence, risk factors and long-term effects of (silent) cerebral ischemia and other cerebrovascular MRI markers in hospitalized patients with COVID-19 (≥18 years).

In **Part II (chapter 3)**, we present the main results of the CORONIS study, specifically the prevalence and 3-month incidence of cerebrovascular MRI markers in hospitalized COVID-19 patients scanned shortly after discharge, compared to healthy controls. In **Part II (chapter 4)**, we provide more insight into the presence of vasculopathy and intracranial vessel wall enhancements (VWE) in these patients at baseline and during follow-up.

In **Part III**, we further explored the WM integrity after hospitalization in patients of the CORONIS study. In **chapter 5**, we investigated WM integrity in COVID-19 patients compared to controls, changes over time and their association with clinical outcomes during follow-up. In **chapter 6,** we investigated the association between loss of WM integrity and increased BPV in patients during hospitalization.

In addition to MRI neuroimaging, in **Part IV (chapter 7)**, we investigated the uptake of the PET/CT tracer to αvβ3 integrins as an indication of endothelial activation (by COVID-19 in the carotid arteries of patients.

The final part of this thesis **(Part VI, chapter 8-10)** contains a general discussion of the thesis with methodological considerations, clinical interpretation of the results and future perspectives and the summary (English and Dutch).

Chapter 2

The CORONIS study: study protocol and rationale

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Prevalence, risk factors, and long-term outcomes of cerebral ischemia in hospitalized COVID-19 patients – study rationale and protocol of the CORONIS study: A multicentre prospective cohort study

European Stroke Journal; 2022;7(2):180–187

Abstract

Background

COVID-19 is often complicated by thrombo-embolic events including ischemic stroke. The underlying mechanisms of COVID-19-associated ischemic stroke, the incidence and risk factors of silent cerebral ischemia and the long-term functional outcome in these patients are currently unknown.

Patients and Methods

CORONavirus and Ischemic Stroke (CORONIS) is a multicentre prospective cohort study investigating the prevalence, risk factors and long-term incidence of (silent) cerebral ischemia and the long-term functional outcome among patients with COVID-19. We aim to include 200 adult patients hospitalized with COVID-19 without symptomatic ischemic stroke to investigate the prevalence of silent cerebral ischemia compared with 60 (matched) controls with MRI. In addition, we will identify potential risk factors and/or causes of cerebral ischemia in COVID-19 patients with (n=70) or without symptomatic stroke (n=200) by means of blood sampling, cardiac workup and brain MRI. We will measure functional outcome and cognitive function after 3 and 12 months with standardized questionnaires in all patients with COVID-19. Finally, the long-term incidence of (new) silent cerebral ischemia in patients with COVID-19 will be assessed with follow up MRI (n=120).

Summary

The CORONIS study is designed to add further insight into the prevalence, long-term incidence and risk factors of cerebral ischemia and the long-term functional outcome in hospitalized adult patients with COVID-19.

Keywords: COVID-19, SARS-CoV-2, silent cerebral ischemia, ischemic stroke

Introduction and rationale

The clinical course of coronavirus disease 2019 (COVID-19) is complicated by a high risk of thrombo-embolic complications, with a higher incidence in patients admitted to the intensive care unit (ICU) compared to the general ward. (4, 13, 14) The occurrence of these complications is associated with a poor clinical outcome and a higher risk of mortality. (15) The majority of these events consist of venous thrombosis and pulmonary embolism. (3, 16) However, arterial ischemic events, such as ischemic stroke have also been described. (17-21)

Possible mechanisms of COVID-19 -associated ischemic stroke include generalized coagulopathy, systemic embolism secondary to atrial fibrillation, paradoxical (venous) emboli due to a patent foramen ovale (PFO), arterial thrombosis and arterial wall inflammation of the cerebral or cervical arteries. (22, 23)

Previous studies have mainly investigated symptomatic ischemic stroke. However, it may very well be that 'clinically silent' ischemic brain lesions occur due to a procoagulant or proinflammatory COVID-19 response which can impair recovery. (19, 24) Insight into the magnitude, causes and long-term outcomes of cerebral ischemia in hospitalized patients with COVID-19 is crucial for patient care to provide optimal diagnostic strategies and prophylactic-and therapeutic treatment.

The CORONIS study was designed to investigate the prevalence, risk factors and the long-term effects of (silent) cerebral ischemia in hospitalized patients with COVID-19.

Methods

Design

The CORONavirus and Ischemic Stroke study (CORONIS) is a multicentre prospective observational cohort study. The study was approved by the medical ethics committee region Arnhem–Nijmegen and all patients will provide written informed consent.

Patient population

Two hundred hospitalized patients with laboratory-confirmed COVID-19 infection, without a symptomatic ischemic stroke, will be included. In addition, seventy patients with a symptomatic ischemic stroke or transient ischemic attack (TIA) will

be included. Ischemic stroke' must be confirmed with neuroimaging demonstrating either infarction in the corresponding vascular territory or absence of another apparent cause. 'TIA' must be diagnosed based on transient focal neurological symptoms lasting <24h presumed to be due to focal brain, spinal cord or retinal ischemia without evidence of acute infarction by neuroimaging or pathology (or in the absence of imaging). (25)

The study will be performed in three Dutch academical hospitals: Radboud University Medical Center, Leiden University Medical Center (LUMC) and the University Medical Center Utrecht (UMCU). Patients from other hospitals in the Netherlands can be referred to the participating hospitals for participation in the study. **Table 1** summarises the in- and exclusion criteria.

Table 1. Inclusion and exclusion criteria of the CORONIS study

Inclusion and exclusion criteria:
Inclusion criteria
• Age ≥18 years
• Admitted to the hospital because of COVID-19
Exclusion criteria
• MRI contraindication and/or post COVID-19 disability interfering with MRI acquisition (e.g. severe delirium)
• eGFR ≤ 30 ml/min
• Pregnancy
• Limited life expectancy (< 3 months)
• Major disease interfering with study participation or follow-up
• Not able to give informed consent

Abbreviations: COVID-19 = coronavirus disease 2019, MRI = magnetic resonance imaging, eGFR = estimated glomerular filtration rate

Controls

Age- and sex-matched adult (≥18 years) controls without previous COVID-19 infection from the general population will be recruited among the patients' next of kin or social environment.

Study objectives

The main study objectives are:

1. To determine the prevalence of asymptomatic (silent) cerebral ischemia on MRI in patients with COVID-19 compared to controls.
2. To assess causes of cerebral ischemia in patients with COVID-19
3. To measure functional outcome and cognitive function in patients with COVID-19 after 3 and 12 months

4. To determine the incidence of new cerebral ischemia on MRI after 3 months of follow-up in patients with COVID-19

Study procedures and follow-up

All eligible patients will be recruited during admission or shortly after discharge. Baseline measurements will be executed during admission or during a visit in the outpatient department (T0). Follow-up at three (T1) and twelve (T2) months after inclusion consists of a telephone interview using standardized questionnaires including cognitive assessment. A follow-up brain MRI will be performed three months after baseline MRI in a random sample of the patients with COVID-19 (n=120) (T1). In control subjects we will only perform baseline questionnaires and brain MRI. Study procedures are described in **Table 2.**

Table 2. Study assessments

	COVID-19 patients			**Controls**
Assessment	**Study phase:**			
	Baseline (T0)	*3 months follow-up (T1)*	*1 year follow-up (T2)*	*Baseline*
Medical history + vascular risk factors	x	x	x	x
Medication use	x	x	x	x
Recurrent events		x	x	
Demographics	x			x
Questionnaires (education, lifestyle)	x			x
Functional outcome: mRS	x	x	x	x
Functional outcome post-COVID: PCFS		x	x	-
Mood questionnaire: HADS		x	x	-
Cognitive assessment	x	x	x	-
Blood chemistry	x			-
Biobanking	x			-
Contrast transthoracic echocardiography	x			-
48h - 72h heart rhythm monitoring	x			-
Brain MRI	x	x		x

Abbreviations: mRS = Modified Rankin Scale, HADS = Hospital and Anxiety Depression Scale, PCFS = Post-COVID Functional Scale,, MRI = magnetic resonance imaging

Baseline questionnaires

Demographics, lifestyle and functional outcome

A structured questionnaire will be used at baseline to assess demographic data (age, sex, body mass index (BMI), ethnicity and education) and lifestyle behaviour. Education will be classified using seven categories: one being less than primary school and seven reflecting an academic degree. (26) Questions regarding lifestyle include current or past nicotine, alcohol and illicit drug use. Alcohol consumption is defined as units per day and the age alcohol consumption started (and if applicable stopped). Smoking behaviour is defined as the number of pack years, calculated as the number of packs of cigarettes smoked per day multiplied by the number of years a patient has smoked.

Functional performance before hospital admission will be assessed by the modified Rankin Scale (mRS).

Medical history

For each patient a history of diabetes mellitus, hypertension, hypercholesterolemia, atrial fibrillation, TIA, ischemic and hemorrhagic stroke, myocardial infarction, peripheral arterial disease, venous thromboembolism, lung diseases, autoimmune diseases or malignancy will be collected. A past medical history of other neurological disease than the above will be recorded if applicable. The presence of a family history of all of the above, current medication use and vaccination status will be recorded.

Follow-up questionnaires

Through a telephone interview by one of the researchers, patients will undergo structured questionnaires at 3 and 12 months after baseline testing on the occurrence of new cardiovascular events, lung diseases, and persistence of COVID-19 symptoms such as fatigue and dyspnea. Presence of actual depressive or anxiety symptoms will be assessed using the HADS scale. (27) Patients will also be asked about current medication use. Functional outcome will be determined using the Post-COVID-19 Functional Status scale (PCFS) and the modified Rankin Scale (mRS). (28-30)

Brain MRI

Brain MRI scans will be rated qualitatively following a standardized, structured protocol by experienced neuroradiologists blinded to clinical data. The MRI protocol is designed to detect acute and chronic cerebral ischemia, markers of cerebral small vessel disease and vessel wall abnormalities. **Table 3** presents the MRI scanning protocol in each participating center.

Table 3. Scanning protocol MRI

		Participating hospital		
		Radboudumc Nijmegen	**LUMC**	**UMCU**
Type of scanner		Siemens 3T Prisma	Philips 3T Ingenia	Ingenia Elition 3T X
Contrast agent		15 ml Dotarem® (0.5 mmol/mL)	Clariscan (0.2 ml/kg)	0,1 ml Gadovist/kg
Duration (in min)		40	35	25
T1-weighted	Orientation	3D Space fatsat	3D T1	Axial
	Voxels	0.9 mm isotropic	1.15 mm isotropic	0.5 x 0.5 x 0.5 mm
FLAIR	Orientation	3D space flair fatsat	2D FLAIR	Axial
	Voxel + resolution	1 mm isotropic	0.7 x 0.7 x 5.00 mm	0.6 x 0.6 x 4 mm
Diffusion weighted imaging (DWI)	Orientation	Axial Resolve	Axial	Axial
	Target-slice thickness + resolution	5 mm	5 mm	4 mm
Susceptibility weighted imaging (SWI)	Orientation	Axial	3D	Axial
	Target-slice thickness + resolution	3 mm	2 mm	2 mm,
Intracranial vessel wall imaging with and without contrast	Orientation	3D space fatsat	3D	Axial
	Target-slice thickness + resolution	0.9 mm isotropic	0.6 x 0.6 x 1.0 mm	0.5 mm isotropic
Diffusion tensor image (DTI)		2mm B0, 1000, 2000 64 directions	-	-

Abbreviations: Radboudumc = Radboud University Medical Center, LUMC = Leiden University Medical Center; UMCU= University Medical Center Utrecht, FLAIR = Fluid-attenuated inversion recovery

MRI abnormalities

Brain MRIs will be evaluated for acute or previous ischemic lesions, markers for cerebral small vessel disease and intracranial vessel wall abnormalities. An acute ischemic lesion is defined by the presence of restricted diffusion on DWI. Markers of cerebral small vessel disease are defined as recent small (sub)cortical infarcts, white matter hyperintensities of presumed vascular origin and cerebral microbleeds. The markers of small vessel disease are assessed in concordance with the STandards for ReportIng Vascular changes on nEuroimaging (STRIVE) criteria for cerebral small vessel disease. (31)

Intracranial vessel wall abnormalities are defined as major vessel wall changes, such as dissections, occlusions and stenoses. In addition, images will be assessed for enhancing foci specified for the vessel segment. Vessel wall enhancement is classified as concentric or eccentric type enhancement. The MRI characteristics of interest are showed in **Table 4**.

Table 4. MRI characteristics of interest

MRI-sequence:	Outcome:	Additional information:
FLAIR	White matter hyperintensities (WMH)	Fazekas (0/1/2/3)
	Previous cerebral infarction	Location: • Local, multifocal • Cortical, lacunar
	Signs of delayed cerebral hypoxia	
DWI	Acute ischemic lesions, DWI+ lesion	Location: • Local, multifocal • Lacunar, territorial
SWI	Cerebral hemorrhage	Location
	Cerebral Microbleeds	Location: lobar, deep Number of lesions: <5, 5-10, >10
T1 intracranial vessel wall imaging	Vasculopathy	
	Mural hematoma (pre-contrast)	Location: • MCA, ACA, PCA, BA or VA • Concentric vs. eccentric
	Vessel wall abnormalities	Stenosis Dissections Occlusions Enhancement Location: • MCA, ACA, PCA, BA or VA • Concentric vs. eccentric
T1 Post-Contrast	Meningeal contrast enhancement	Location: • Leptomeningeal/ pachymeningeal
	Cranial nerve enhancement	Location
Coincidental findings	Presence / absence	

Abbreviations: MCA = middle cerebral artery, ACA = anterior cerebral artery, PCA = posterior cerebral artery, BA = basilar artery, VA = vertebral artery, DWI = Diffusion weighted imaging, SWI = susceptibility weighted imaging.

Cognitive assessment

At baseline, the patients with COVID-19 will undergo a short cognitive screening with the Montreal Cognitive Assessment (MoCA). This 10-min test covers various cognitive domains, including memory, visuoconstruction, attention, executive functioning and language. (32) During follow-up, patients will undergo the Telephone Interview for Cognitive Status (TICS), which covers verbal and working memory, orientation, language and attention. (33)

Contrast transthoracic echocardiography

To assess presence of PFO, patients will undergo agitated saline contrast transthoracic echocardiography. All echocardiography examinations will be performed by an experienced cardiologist. The interatrial septum will be assessed in multiple views. In addition shunting will be evaluated with color flow Doppler and first-generation contrast. The appearance of microbubbles in the left atrium within 3–6 cardiac beats after opacification of the right atrium is considered positive for the presence of an intracardiac shunt such as a PFO. Valsalva manoeuvre will be performed to promote right-to-left shunting of microbubbles to identify a PFO when no shunting is present without provocation. (34)

Heart rhythm monitoring

Patients will receive an ambulatory Holter to monitor heart rhythm for a period of 48 - 72 hours. Holter monitoring will be performed according to standard procedures. If patients have received rhythm monitoring during admission for standard medical practice (for example telemetry during ICU admission $\geq$48h), these data will be used for the current study and no additional Holter monitoring will take place to reduce the burden for the patients.

Blood sampling

Fifty-four ml blood (18 ml citrated plasma, 20 ml serum and 10-16 ml EDTA) will be sampled to assess biomarkers of inflammation and coagulation, including genetic variants of these factors. The samples will be stored locally according to the hospital's regulations or in the affiliated biobank.

Statistical analysis

Sample size calculation

To determine the prevalence of asymptomatic (silent) cerebral ischemia on MRI in patients with COVID-19 compared to controls, we based our sample size calculation on the currently available literature. We expect an incidence of

about 1-3% of symptomatic ischemic stroke in hospitalized patients with COVID-19 (15, 17, 19, 21). Extrapolating from existing literature we expect a 6-9 fold increased prevalence of asymptomatic (silent) cerebral ischemia (i.e. 18-25% assuming a 3% prevalence of symptomatic events) as compared to symptomatic ischemic stroke. (35) In 200 patients undergoing MRI scanning this would lead to identification of about 40 cases with asymptomatic (silent) cerebral ischemia and at least 160 controls without asymptomatic cerebral ischemia. The observed prevalence will be compared with that in controls. Assuming a prevalence of silent cerebral ischemia in these subjects of max 1% (36) and of 20% in the patients with COVID-19, we will have 95% power to detect a significant difference at the significance level of 0.05 (95% confidence level).

Regarding objective 2, to assess causes of cerebral ischemia in patients with COVID-19, based on the expected 110 cases with symptomatic (n=70) or asymptomatic (expected n=40) cerebral ischemia and (expected) 160 controls without (a)symptomatic cerebral ischemia we have a power of 95% to demonstrate a relative risk of 3 (alpha 0.05). We will still have 80% power to identify less frequent exposures, for example for exposures with a prevalence of 5% in the controls, we can demonstrate a relative risk of 3.4.

For objective 3 and 4, measuring functional outcome and cognitive function after 3 and 12 months and determining the incidence of new cerebral ischemia on MRI after 3 months of follow-up in patients with COVID-19, the sample size will be equal to that of the population of patients with COVID-19 in the previous sub studies. The precision of the descriptive results for this study will be determined by this study size (no statistical comparisons are made here). A loss to follow-up rate of 10% is taken into account for all the sample size calculations.

Analysis of primary outcomes

For objective 1 we will determine the prevalence of silent cerebral ischemia among patients with COVID-19 admitted to or discharged from the hospital and in age and sex matched controls without (previous) COVID-19 infection from the general population including corresponding 95% confidence intervals.

For objective 2, cases, patients with COVID-19 with cerebral ischemia (symptomatic and asymptomatic) and controls (without cerebral ischemia) will be compared with respect to the prevalence of possible risk factors using logistic regression models. Odds ratios will be estimated as measures for the relative risks associated with each

possible risk factor. Each risk factor will be analysed in univariable and multivariable analysis to correct for confounders as age, sex and comorbidities.

In a follow-up study after 3 and 12 months, we will describe functional performance and cognitive function in COVID-19 patients. We will stratify for subgroups in analysis (symptomatic ischemic stroke, asymptomatic cerebral ischemia, no cerebral ischemia).

After 3 months we will investigate the long-term incidence of asymptomatic (silent) cerebral ischemia in patients with COVID-19. The incidence rate will be determined as the number of new (or first) silent cerebral ischemia divided by the total amount of person-time.

Discussion

The CORONIS study is a multicentre prospective cohort study investigating the prevalence, risk factors and long-term incidence of (silent) cerebral ischemia in patients hospitalized with COVID-19 and to determine long-term functional outcome.

Little is known about the occurrence and consequences of clinically 'silent' cerebral ischemia in patients hospitalized with COVID-19. Research on ischemic stroke as a complication of COVID-19 showed a prevalence ranging from 1 to 3%. (15, 17, 19, 21) The rate of ischemic stroke as complication of COVID-19 seems to be higher than in other respiratory viruses such as influenza. (37, 38) Recent neuroradiologic studies described multiple brain MRI abnormalities such as (micro)haemorrhage, ischemic lesions and signs of encephalitis in patients with COVID-19. (39) Post-mortem pathology studies showed vascular damage, including hypoxic damage, ischemic lesions and (micro)haemorrhages, and inflammatory infiltrates in brain tissue.(40, 41). However, most of our knowledge on these cerebrovascular complications of COVID-19 is derived from retrospective data. Several studies have suggested coagulopathy and endotheliopathy both to be as possible mechanisms of COVID-related ischemic stroke, however no prospective risk factor analysis has been done. To anticipate on the possible consequences of both symptomatic and silent ischemic brain lesions, prospective studies in patients with COVID-19 investigating the effects of COVID-19 in the brain are urgently needed.

Our study is the first to prospectively conduct a brain MRI in hospitalized patients with COVID-19 during the acute phase of their infection. This study will therefore provide more knowledge about the possible effects of COVID-19 on the brain and on cerebrovascular damage in this patient population. Combined with the follow-up MRI we will gain knowledge on dynamics of cerebral ischemia in patients with COVID-19 as well. This can help clinicians to understand mechanisms/causes of COVID-19 related functional loss.

Among the strengths of this study is the multicentre design, leading to a large sample size of patients included from multiple regions throughout the Netherlands. Moreover, the prospective design with two follow-up assessments allows us to collect longitudinal and detailed standardized information, including demographics, vascular risk factors, cognitive tests and imaging measurements of the patients. Due to our limited exclusion criteria, we will be able to include a patient group with high external validity. Adding matched controls enables us to compare the prevalence of silent cerebral ischemia and other cerebrovascular lesions in both groups of patients.

Summary and conclusions

In conclusion, CORONIS is a pivotal study to investigate the prevalence, long-term incidence and risk factors of silent cerebral ischemia in hospitalized COVID-19 patients and will determine long-term functional outcome in this population.

Part II

Conventional cerebrovascular MRI markers in COVID-19 patients

Chapter 3

Prevalence and 3-month follow-up of cerebrovascular MRI markers in hospitalized patients with COVID-19: the CORONIS study

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Abstract

Purpose

To investigate the prevalence of cerebrovascular MRI markers in unselected patients hospitalized for COVID-19 (Coronavirus disease 2019), we compared these with healthy controls without previous SARS-CoV-2 infection or hospitalization and subsequently, investigated longitudinal (incidental) lesions in patients after three months.

Methods

CORONIS (CORONavirus and Ischemic Stroke) was an observational cohort study in adult hospitalized patients for COVID-19 and controls without COVID-19, conducted between April 2021 and September 2022. Brain MRI was performed shortly after discharge and after 3 months. Outcomes included recent ischemic (DWI-positive) lesions, previous infarction, microbleeds, white matter hyperintensities (WMH) and intracerebral hemorrhage and were analysed with logistic regression to adjust for confounders.

Results

125 patients with COVID-19 and 47 controls underwent brain MRI a median of 41.5 days after symptom onset. DWI-positive lesions were found in one patient (1%) and in one (2%) control, both clinically silent. WMH were more prevalent in patients (78%) than in controls (62%) (adjusted OR: 2.95 [95% CI: 1.07-8.57]), other cerebrovascular MRI markers did not differ. Prevalence of markers in ICU vs non-ICU patients was similar. After three months, five patients (5%) had new cerebrovascular lesions, including DWI-positive lesions (1 patient, 1.0%), cerebral infarction (2 patients, 2.0%) and microbleeds (3 patients, 3.1%).

Conclusion

Overall, we found no higher prevalence of cerebrovascular markers in unselected, hospitalized COVID-19 patients compared to controls. The few incident DWI-lesions were most likely to be explained by risk-factors of small vessel disease. In the general hospitalized COVID-19 population, COVID-19 shows limited impact on cerebrovascular MRI markers shortly after hospitalization.

Keywords

COVID-19, MRI, Cerebrovascular disorders, Brain ischemia, WMH, SARS-CoV-2

Introduction

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) is associated with both venous and arterial thrombo-embolic events including ischemic stroke. (15, 16, 20) Pulmonary embolism is the most frequent thrombo-embolic complication, but ischemic stroke was also frequently reported in patients with Coronavirus disease 2019 (COVID-19) ranging from 0.9 through 2.0%. (16, 17, 21, 42-44) This was a higher incidence of ischemic stroke compared to hospitalized patients with influenza (0.2-0.9%). An even higher incidence (up to 2.7%) was reported in critically ill COVID-19 patients admitted to an intensive care unit (ICU). (18, 45, 46) Other MRI markers of cerebrovascular disease such as microbleeds, intracerebral hemorrhage and intracranial vessel wall enhancement have also been found in retrospective cohorts of patients with COVID-19. (45, 47-50) The procoagulant and proinflammatory response of COVID-19 may result in 'clinically silent' or 'covert' ischemic lesions and other cerebrovascular MRI abnormalities during the disease course, in addition to symptomatic 'overt' ischemic stroke (associated with clear neurological deficits). Brain imaging in previous studies in patients with COVID-19 was often performed in selected critically-ill patients, presenting with overt neurological symptoms with a clinical indication for imaging. The prevalence of cerebrovascular lesions on MRI without overt symptoms in unselected patients admitted with COVID-19 remains unknown and this has never been compared to controls from the general population with proven absence of COVID-19. In addition, in none of the previous studies follow-up imaging weeks to months after infection to detect incident subacute cerebrovascular changes has been performed. Therefore, we investigated prevalence and 3-month incidence of asymptomatic (silent) cerebral ischemia and other cerebrovascular MRI markers in the CORONavirus and Ischemic Stroke (CORONIS) study, a prospective cohort of unselected hospitalized patients with COVID-19. To evaluate the effect of severe COVID-19 (requiring hospitalization) on cerebrovascular MRI markers, we compared this prevalence with healthy controls without proven infection and without hospitalization.

Methods

Study design

This study is part of the CORONIS study, a prospective observational cohort study that investigates the prevalence, risk factors and long-term effects of (silent) MRI markers of cerebrovascular disease in hospitalized patients with COVID-19. The study protocol has been published previously. (51) The Medical Review Ethics

Committee region Arnhem-Nijmegen approved the study (NL75780.091.20). All patients provided written informed consent.

Study population

All adult patients admitted between April 2021 and September 2022 to one of three Dutch academic hospitals with laboratory-confirmed COVID-19 infection, with or without clinically overt ischemic stroke during admission, regardless of any clinical (neurological) symptoms were eligible for inclusion. For the present analysis, we excluded patients with COVID-19 and ischemic stroke or a transient ischemic attack (TIA) during admission as the primary aim of this study was to detect cerebrovascular MRI markers in patients with COVID-19 without clinically overt stroke. Exclusion criteria included contraindications to MRI or intravenous gadolinium, pregnancy, life expectancy shorter than three months, major disease interfering with study participation or follow-up or inability to provide informed consent.Controls from the general population with a clinically and laboratory proven absence of COVID-19 infection, matched on age and sex), were recruited among the patients' next of kin and social environment. We matched the controls at an approximately 1:3 ratio with the patients, as we considered this number sufficient for exploratory analyses. Since no information from brain MRIs in COVID-19 patients was yet available, we decided to focus mostly on this group, hence the 1:3 ratio. Because recruitment of controls without a history of COVID-19 infection slowed down during the pandemic, we did not reach the target of 60 included controls as described in the study protocol. To ensure the controls were COVID-19 negative, all controls were asked whether they had COVID-19 during the pandemic, or if they had COVID-19 related symptoms but refrained from testing. Additionally, we performed an Anti-Sars-CoV-2 nucleocapsid laboratory test to exclude subjects with a previous asymptomatic COVID-19 infection. In unvaccinated controls, an additional Anti-Sars-CoV-2 spike test was performed.

Data collection

We collected information on demographics, comorbidities, medication use and vaccination status at baseline, which was the moment of enrolment in the study (for patients during admission or shortly after discharge). This included information on cardiovascular risk factors e.g. hypertension, diabetes mellitus, smoking, hypercholesterolemia, atrial fibrillation and a previous TIA or ischemic stroke. For patients with COVID-19, laboratory test results and clinical data (e.g. hospital stay in days, complications, mechanical ventilation need, medication) during admission were collected.

Brain MRI analysis

At baseline, patients and controls underwent 3-Tesla brain MRI including T1-weighted imaging, 2D or 3D fluid-attenuated inversion recovery (FLAIR), diffusion-weighted imaging (DWI) including an apparent diffusion coefficient (ADC) and susceptibility-weighted imaging (SWI). Details of the scanning protocol in each participating center are shown in **supplemental Table 1**. To investigate the dynamics of silent ischemia in patients with COVID-19, they were asked to undergo follow-up MRI three months after baseline MRI (with the same scanning protocol). The controls did not undergo follow-up MRI as we did not expect incident asymptomatic cerebral ischemia over a three months course in these individuals.

MRI markers of cerebrovascular disease

The standardized evaluation protocol consisted of specific cerebrovascular MRI markers of interest, described in **supplemental Table 2**. Markers of cerebral small vessel disease were rated according to the Standards for Reporting Vascular Changes on nEuroimaging (STRIVE-2) criteria. (31, 52) An acute ischemic lesion (incidental DWI-positive lesion) was defined by the presence of diffusion restriction on DWI. Cerebral microbleeds were defined as small (2-10mm) round areas of signal void with blooming seen on gradient-echo sequences and rated according to the microbleed anatomical rating scale, those with a larger diameter were referred to as an intracerebral hemorrhage. (53)

All brain MRIs were anonymized and evaluated by one of four experienced neuroradiologists (FM,KKvU, JdB and JWD) using a standardized, structured protocol. Because of considerable variations in SWI sequences among the different centers and to ensure the coherence of the assessments, one experienced rater (TJvL) evaluated all SWI scans for microbleeds. In cases where uncertainty arose, these assessments were subjected to review and confirmation by an experienced neurologist (FEdL).

Outcome

The primary objectives were to conduct a cross-sectional comparison of the prevalence of MRI markers of cerebrovascular disease in patients with COVID-19 compared with controls at baseline. Second, our aim was to investigate longitudinal changes (incident cerebrovascular lesions) over time (three months) in patients hospitalized for COVID-19.

Statistical analysis

Frequencies and counts were described for categorical data (n, %). For quantitative data we reported means with standard deviation (SD) and medians with interquartile range (IQR). Demographics, medical history and brain MRI markers were compared between groups (COVID-19 vs. controls and ICU vs. non-ICU) with Student's t-test or chi-square test as appropriate with corresponding 95% confidence intervals (95% CI) for (difference in) proportions. Missing values were not imputed. Uni- and multivariable (ordinal) logistic regression was used to analyze the relationship between COVID-19 infection and the primary and secondary outcomes displayed as crude odds ratios (OR) and adjusted odds ratios (aOR) with corresponding 95% CIs adjusted for age, sex, hypertension, diabetes mellitus, hypercholesterolemia, BMI, smoking behaviour and study site. P-values less than 0.05 were considered statistically significant. All analyses were performed with R, version 4.2.2. We reported this article in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines (Supplemental Material). (54)

Results

Study population

In total, 154 patients with COVID-19 and 48 controls were enrolled in CORONIS. After exclusion of participants who withdrew their consent before MRI was performed (n=18), patients with a contra-indication for MRI (n=3), patients with a symptomatic stroke or TIA during admission (n=8), and controls with a positive Anti-Sars-CoV-2 nucleocapsid test (n=1), 125 patients with COVID-19 and 47 controls were analyzed **(Figure 1).**

Patient characteristics

Patients with COVID-19 were comparable to controls regarding age and sex, with a mean age of 58 (SD: 12.8) years and 40.0% (50/125) were female after frequency matching during inclusion. There was a difference in vaccination rate between patients and controls (37.9% vs. 91.7%, *p=0.001)* **(Table 1).** Additional clinical information is presented in **supplemental Table 3.** Median time between positive PCR in patients and inclusion was 16.0 days [IQR: 5.0-12.0].

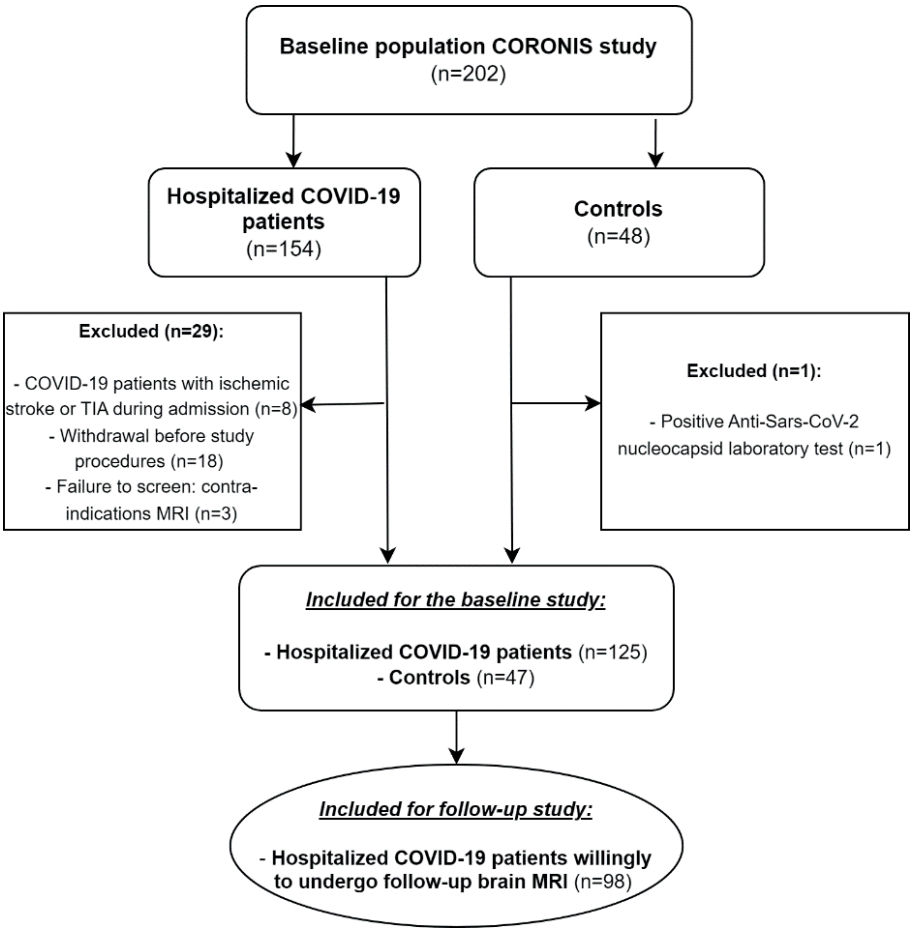

Figure 1. Flowchart of inclusion

Outcomes

Prevalence of MRI markers of cerebrovascular disease

At baseline, silent cerebral ischemia (DWI-positive lesions) was seen in one patient with COVID-19 (0.8%) and in one (2.1%) healthy control (1.3% difference; [95% CI; -5.7%, 3.1 %]; p=0.47). The MRI of the patient with COVID-19 showed multiple incidental DWI-positive lesions located in the white matter of the frontal and parietal lobe. This patient also had extensive segmental pulmonary embolism during hospital admission.

Table 1. Baseline characteristics COVID-19 patients vs. controls

	COVID-19 +	Controls	P-value
Total number of patients (n)	125	47	
Female, n (%)	50 (40.0)	22 (46.8)	0.420
Age at inclusion, years (mean, (SD))	58.1 (12.8)	60.7 (12.6)	0.235
BMI, kg/m2 (median [IQR])	27.8 [24.8, 32.1]	26.73[23.6, 28.5]	***0.024***
Vaccinated before admission/inclusion, n (%)	47 (37.9)	11 (91.7)	***<0.001***
Race, n (%)			0.193
Caucasian/white	103 (84.4)	43 (91.5)	
Black	3 (2.5)	0 (0.0)	
North-African	10 (8.2)	1 (2.1)	
Hispanic	2 (1.6)	0 (0.0)	
Asian	2 (1.6)	0 (0.0)	
Mixed	2 (1.6)	3 (6.4)	
Medical history			
Previous or current smoker, n (%)	76 (60.8)	26 (55.3)	0.514
Previous or current alcohol use, n (%)	98 (78.4)	43 (91.5)	***0.047***
Diabetes Mellitus, n (%)	18 (14.4)	3 (6.4)	0.152
Hypertension, n (%)	48 (38.4)	7 (14.9)	***0.003***
Hypercholesterolemia, n (%)	46 (36.8)	8 (17.0)	***0.013***
Atrial fibrillation, n (%)	8 (6.4)	2 (4.3)	0.592
TIA, n (%)	5 (4.0)	0 (0.0)	0.164
Ischemic stroke, n (%)	5 (4.0)	0 (0.0)	0.164
Malignancy, n (%)	11 (8.8)	6 (12.8)	0.437
Venous thromboembolism (e.g. pulmonary embolism), n (%)	15 (12.0)	1 (2.1)	***0.047***
Pulmonary disease (e.g. COPD, asthma), n (%)	43 (34.4)	5 (10.6)	***0.002***
ICU admission, n (%)	27 (21.6)	N/A	N/A
Time of hospitalization, days (median [IQR])	8.0 [5.0-12.0]	N/A	N/A
Anticoagulant therapy during admission, n (%)	124 (99.2)	N/A	N/A
LMWH prophylactic dose	64 (51.2)	N/A	N/A
LMWH therapeutic dose	52 (41.6)	N/A	N/A
Other anticoagulant therapy (DOAC, vitamin K antagonist, factor Xa inhibitors)	8 (6.4)	N/A	N/A
Antiplatelet therapy during admission, n (%)	14 (11.2)	N/A	N/A
Pulmonary embolism during admission, n (%)	22 (17.6)	N/A	N/A

Abbreviations: COVID-19 = Coronavirus Disease 2019, BMI = Body Mass Index, TIA = transient ischemic stroke, COPD = chronic obstructive pulmonary disease, ICU = intensive care unit, DOAC = direct oral anticoagulation,, N/A = not applicable.

The prevalence of WMH was higher in patients (77.6%) compared to controls (61.7%) (15.9% difference; [95% CI, 0.2%, 531.6%]; *p=0.036*). There was no difference in other MRI markers between cases and controls (**Table 2).** This was also the case when comparing patients admitted to an ICU with patients who were not **(Supplemental Table 4).** In one patient, as an incidental finding, an old asymptomatic cerebral venous sinus thrombosis (sigmoid sinus) was found that required no treatment.

Table 2. Cerebrovascular MRI markers in COVID-19 patients vs. controls

	COVID-19 +	Controls	P-value
Total number of participants, n	125	47	
Incidental DWI-positive lesions, n (%)	1 (0.8)	1 (2.1)	0.469
WMH, n (%)	97 (77.6)	29 (61.7)	***0.036***
WMH - Fazekas score, n (%) Fazekas 0 Fazekas 1 Fazekas 2 Fazekas 3	28 (22.4) 74 (59.2) 18 (14.4) 5 (4.0)	18 (38.3) 25 (53.2) 4 (8.5) 0 (0.0)	0.100
Previous cerebral infarction, n (%)	18 (14.4)	5 (10.6)	0.518
Delayed hypoxemia, n (%)	1 (0.8)	0 (0.0)	0.539
Cerebral hemorrhage, n (%)	6 (4.8)	3 (6.4)	0.678
Microbleeds, n (%)	29 (23.2)	6 (12.8)	0.130
Location, n (%)			0.460
Lobar	14 (48.3)	4 (66.7)	
Deep	6 (20.7)	0 (0.0)	
Cerebellar	2 (6.9)	0 (0.0)	
Lobar and deep	3 (10.3)	2 (33.3)	
Lobar and cerebellar	2 (6.9)	0 (0.0)	
Lobar, deep and cerebellar	2 (6.9)	0 (0.0)	
Count, n (%)			0.317
0	96 (76.8)	41 (87.2)	
1-10	24 (19.2)	5 (10.6)	
>10	5 (4.0)	1 (2.1)	

Abbreviations: COVID-19 = Coronavirus Disease 2019, DWI = diffusion-weighted imaging, WMH = white matter hyperintensities

New/incident MRI markers of cerebrovascular disease at 3 month follow-up

The subset of patients (n=98, 78,4%) with a follow-up MRI had a mean age of 59.0 years (SD 12.3) and 37% were female. Five (5.1%) patients had new, silent cerebrovascular MRI markers after 3 months, including incidental DWI-positive lesions (1 patient, 1.0%), new cerebral infarction (2 patients, 2.0%) and new microbleeds (3 patients, 3.1%) (**Table 3; Figure 2 + 3**). Apart from these five, 2 additional patients had a new WMH, without a change in Fazekas score. After 3 months, the incidental DWI-positive lesions identified in the patient at baseline had converted to a WMH.

Table 3. Cerebrovascular MRI markers in COVID-19 patients on 3-month follow-up

	COVID-19 patients Follow-up MRI
Total number of patients (n)	98
Patients with new MRI markers, n (%)	5 (5.1%)
Time between baseline and follow-up MRI, days (median [IQR])	105.0 [92.0, 119.0]
Incidental DWI-positive lesions, n (%)	1 (1.0)
New white matter hyperintensities (measured as increase of Fazekas score), n (%)	0 (0.0)
New cerebral infarction, n (%)	2 (2.0)
New delayed hypoxemia, n (%)	0 (0.0)
New cerebral hemorrhage, n (%)	0 (0.0)
New microbleeds, n (%)	3 (3.1)
Count, n (%)	
1-10	3 (100.0)
>10	0 (0.0)
Location, n (%)	
Lobar	3 (100.0)
Deep	0 (0.0)
Cerebellar	0 (0.0)
Lobar and deep	0 (0.0)
Lobar and cerebellar	0 (0.0)
Lobar, deep and cerebellar	0 (0.0)

Abbreviations: COVID-19 = Coronavirus Disease 2019, DWI = diffusion-weighted imaging

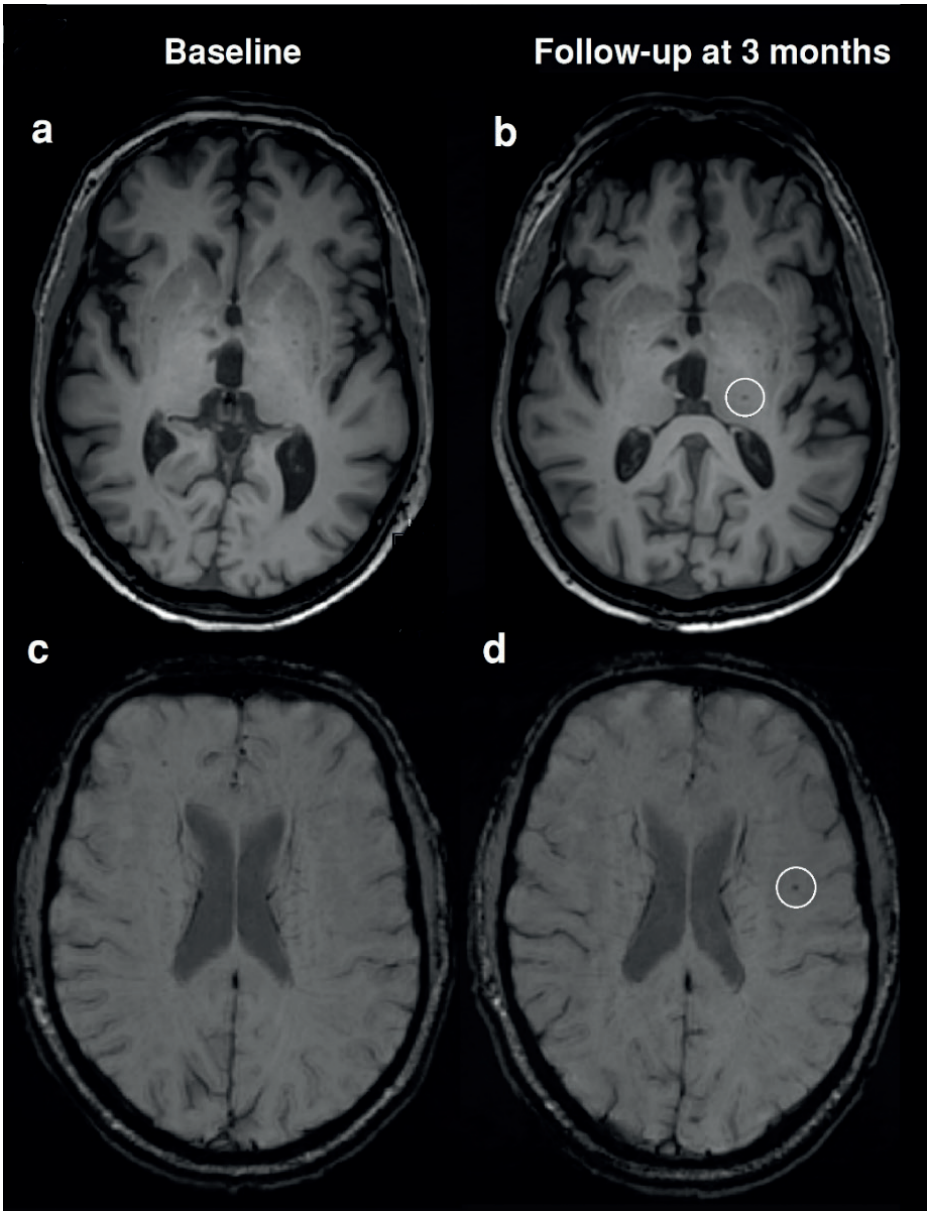

Figure 2. New cerebrovascular MRI markers in two COVID-19 patients; baseline MRI versus 3-month follow-up MRI. (a) Baseline T1 MRI scan patient 1 **(b)** Follow-up T1 MRI scan showing new cerebral infarction (oval) in patient 1 **(c)** Baseline SWI MRI scan in patient 2 **(d)** Follow-up SWI MRI scan showing new microbleed (white circle)) in patient 2. *Abbreviations: MRI = magnetic resonance imaging, COVID-19 = Coronavirus Disease 2019, SWI = susceptibility weighted imaging*

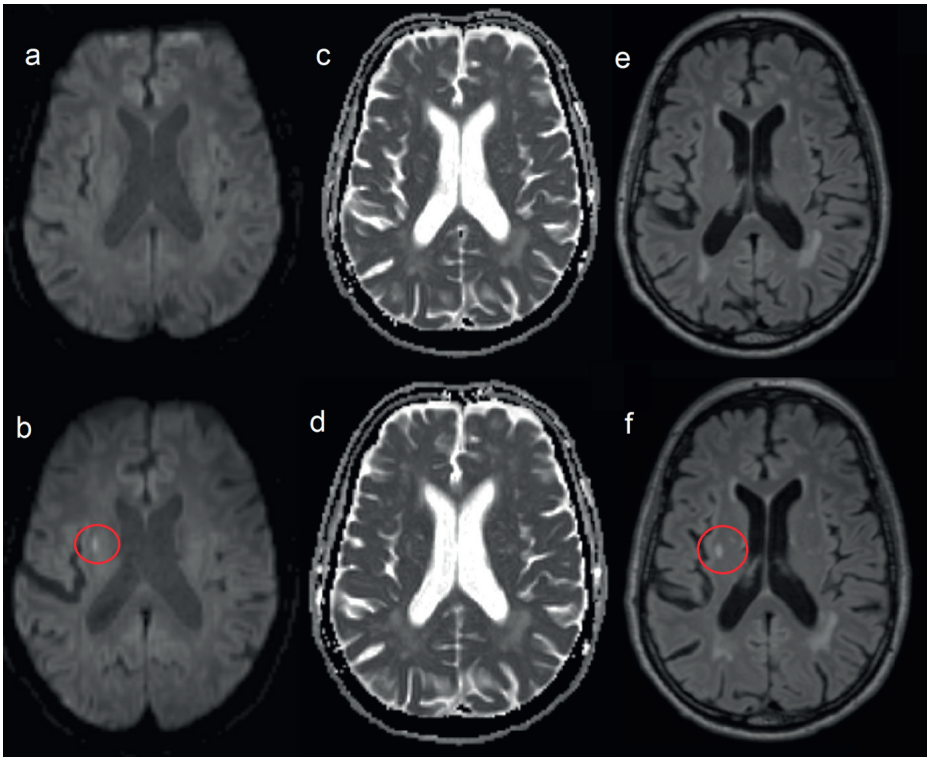

Figure 3. New cerebral infarction on 3-month follow-up MRI in 1 patient with COVID-19. (a) Baseline DWI-MRI scan in patient with COVID-19 **(b)** Follow-up DWI-MRI scan in same patient, with lacunar infarction (hyperintensity) right (red circle) **(c)** Baseline ADC-MRI scan in same patient **(d)** Follow-up ADC-MRI scan, no signs of recent ischemia (no corresponding hypointensity) **(e)** Baseline FLAIR MRI scan in same patient **(f)** Follow-up MRI showing corresponding hyperintense lesion in **(a)** indicating semi-recent lacunar infarction right, converted to WMH. *Abbreviations: MRI = magnetic resonance imaging, COVID-19 = Coronavirus Disease 2019, DWI = diffusion-weighted imaging, ADC = apparent diffusion coefficient, FLAIR = fluid-attenuated inversion recovery, WMH = white matter hyperintensity*

After correction for potential confounders, hospitalization with COVID-19 was associated with a higher incidence of WMH (OR, 2.95 [95% CI: 1.07-8.57]) (**Table 4**). No association with COVID-19 was found for other MRI markers. Due to the low number of events of incidental DWI-positive lesions at baseline MRI and thus limited reliability in multivariable analysis, we only performed univariable analysis for incidental DWI-positive lesions.

Table 4. Association of COVID-19 with MRI markers at baseline corrected for confounders using multivariable logistic regression

MRI markers of cerebrovascular disease at baseline	Number of events (%)		Univariable analysis		Multivariable analysis	
	COVID+ n=125	Controls n=47 (reference)	OR (95% CI)	P-value	OR (95% CI)	P-value
DWI-positive lesions	1 (0.8)	1 (2.1)	0.37 (0.01 – 9.51)	0.486	N/A	***N/A***
White Matter Hyperintensities (any)	97 (77.6)	29 (61.7)	2.15 (1.04 – 4.43)	**0.038**	2.72 (1.04-7.41)	**0.044**
Microbleeds	29 (23.2)	6 (12.8)	2.06 (0.84 – 5.84)	0.136	2.20 (0.81-6.81)	0.143
Cerebral hemorrhage	6/ (4.8)	3 (6.4)	0.74 (0.19 – 3.62)	0.679	0.67 (0.09-5.54)	0.701

Abbreviations: COVID-19 = Coronavirus Disease 2019, DWI = diffusion-weighted imaging.

Discussion

In this study, we found no difference in prevalence of silent cerebral ischemia and other cerebrovascular MRI markers in unselected, hospitalized COVID-19 patients compared to healthy controls (with proven absence of previous SARS-CoV-2 infection without hospitalization) at baseline, apart from a higher burden of WMH. The prevalence of these markers in ICU vs non-ICU patients was similar. After three months, 5.1% of the patients with COVID-19 had new brain MRI markers of cerebrovascular origin including incidental DWI-positive lesions, cerebral infarction and microbleeds.

We did not expect to find a comparable prevalence of silent cerebral ischemia in both patients and controls, considering that previous studies reported an increased risk of ischemic stroke in patients with COVID-19. (16, 17, 21, 42-44) There are several possible explanations for this discrepancy. First, the pathophysiological mechanism of clinically overt 'COVID-19-associated' stroke may differ from silent ischemia. Although both types share risk factors, covid-associated stroke is often caused by a large vessel occlusion while silent ischemia is often associated with small vessel disease. (19, 55-57). Second, patients in our cohort were enrolled after the first two waves (with two dominant variants: alfa b.1.1.7 & delta b.1.617.2) of COVID-19 in the Netherlands. After the first two waves the therapeutic guidelines had changed, and therefore the majority of our patients were treated with either low-

molecular weight heparin, antiplatelet therapy or anticoagulation, which could have reduced thrombo-embolic complications. (58) Treatment with dexamethasone and tocilizumab was also introduced and large-scale vaccination campaigns were made available to the general population which could have led to a lower disease burden than in the first two waves. Together with possibly less pathogenic variants this could have reduced the risk of both clinically overt and silent cerebral ischemia. (59, 60) Third, we enrolled a low number of critically ill patients. Patients who are critically ill and admitted to an ICU are more likely to develop cerebrovascular disease (including critical illness encephalopathy and delayed cerebral ischemia) than patients with a less severe disease course. (21, 45, 46) Fourth, new ischemic lesions with diffusion restriction usually convert to small vessel disease markers such as WMH after 2-3 weeks. Due to local regulations preventing us from scanning patients for research while still infectious, the median time between onset of symptoms and baseline MRI was 6 weeks. The median time from onset of COVID-19-related symptoms to a COVID-related stroke has been reported to be approximately two weeks. (42) Therefore, it is possible that some, but not all, cases of silent cerebral ischemia have been missed which could have led to an underestimation of the true prevalence of silent ischemia.

Previous COVID-19 studies reported an increased prevalence of other cerebrovascular brain MRI markers (i.e. incidence of cerebral microbleeds up to 58.8%) on MRI in patients during the acute phase. (49, 50, 61, 62) Also, several studies showed an even higher prevalence of microbleeds (up to 71%) in patients admitted to an ICU compared to patients on the general ward. (47, 61-63) These findings are not in line with the results of our study, as we observed no difference in MRI markers between patients in the ICU and those in the general ward. A possible explanation might be the fact that patients selected in these studies underwent neuroimaging because of retrospective selection on neurological symptoms. (64) In CORONIS, all included patients were scanned regardless of neurological symptoms during admission. This gave us an insight in the prevalence of MRI markers in a regular and more generalizable population of hospitalized patients with COVID-19.

The prevalence of WMH in our study population is, with a mean age of 58 years, relatively high (77.6%), which may be explained by the high burden of vascular risk factors. Previous studies in the general population, with less vascular risk factors, described a prevalence ranging from 60% up to 90%, but these studies were mainly conducted in older patients (above 60). (65, 66) One previous study that investigated adults between 50 and 59 years old reported a prevalence of 35.3%. (67) It is known that patients with cardiovascular risk factors have a higher risk at admission due

to diseases as COVID-19. (68) These risk factors are also associated with WMH and the association of COVID-19 with WMH could therefore be explained by such confounding factors. For important and well-known risk factors, we could adjust in the multivariable analysis, but additional and even unknown risk factors could have been missed and not been accounted for. Nevertheless, extensive WMH and signs of cerebral small vessel disease can be associated with cognitive, mental and physical function and these patients might be at risk of experiencing subsequent cognitive complaints described in patients with post COVID-19 condition. (69-71)

During follow-up, in five patients new clinically silent MRI markers of cerebrovascular disease were found, including two patients with (silent) cerebral infarction (with in one of them also multiple incidental DWI-positive-lesions). One of these patients had pulmonary embolism during hospitalization, which could imply a hypercoagulable state. This patient developed silent ischemic lesions whilst receiving anticoagulation therapy for three months after discharge. The other patient reported no clinical symptoms and had an extensive cardiovascular medical history. An exact etiology for these brain abnormalities remains undetermined, since these infarcts were asymptomatic and therefore not investigated. The three patients with new microbleeds during follow-up already had prevalent microbleeds. Apart from the underlying cardiovascular risk factors which all of these patients had, it could be hypothesized that COVID-19 could have additionally triggered an ongoing prothrombotic state after the acute phase (including endothelial dysfunction) leading to these signs of cerebrovascular disease. This might persist for several weeks or even months after resolution of the infection, but this warrants further investigation. (72-74) A prior study showed a 90-day cumulative incidence of arterial thrombosis (including myocardial infarction and ischemic stroke) in hospitalized patients with COVID-19 of 3%, which is slightly lower than the incidence we found in our study. (2) These silent ischemic lesions could occur even more often than clinically overt ischemic stroke but due to their lack of symptoms they evade detection. (75) The presence of brain abnormalities in post-COVID patients has been associated with a lower risk of good recovery, so correlating clinically silent lesions to impaired outcome after COVID-19 could be a worthwhile pathophysiological target for research on these long remaining symptoms. (76, 77)

To our knowledge, this is the first study to prospectively perform brain MRI in hospitalized COVID-19 patients, who were not exhibiting neurological symptoms. Strengths of our study included that patients were largely unselected, which reflects a comprehensive representation of the general hospitalized COVID-19 population in the Netherlands. Second, healthy controls were recruited throughout

patients' relatives and acquaintances, generating groups comparable in societal and environmental factors. Third, we repeated MRI after 3 months, which enabled us to investigate a possible ongoing disease process due to COVID-19.

Some limitations need to be considered. First, the median time between symptom onset and MRI was approximately 6 weeks, which could have led to an underestimation of silent ischemia. Second, as patients needed to provide written informed consent, the most critically ill patients may have been underrepresented, who either died during hospitalization, refused or were otherwise unable to provide written consent. Third, by using the Fazekas score as a qualitative assessment scale of white matter hyperintensities, it is possible that subtle changes in white matter hyperintensity volumes within patients may have been missed. Fourth, the relatively small sample size may have contributed to the inability to detect a significant difference resulting in seemingly neutral outcomes. Still, as far as we know, this is the largest sample size in an unselected prospective cohort study on MRI outcomes in hospitalized patients compared to controls. Fifth, constraint of the MRI budget has led to enrollment of a limited number of participants without previous SARS-CoV-2 infection without a formal pre-specified sample size calculation. The main focus of our study was on cerebrovascular abnormalities in patients with COVID-19, therefore we prioritized enrolling and scanning this group. The exploratory nature of our study means the precision of our prevalence estimates is limited and may affect the generalizability of our findings. Finally, a possible limitation is our inability to distinguish between the effects of COVID-19 and those attributed to the hospital admission itself. Our research question was to assess the impact of a severe COVID-19 infection, inherently linked to hospitalization—a clinical question. Addressing the more pathophysiological focus, regarding specifically the effects of COVID-19, would have required individuals hospitalized with a severe non-COVID illness undergoing MRI solely for research purposes, a prospect that raises ethical concerns but could be explored in a future study. However, our comparison with a healthy control group, where the contrasts were most pronounced and significant differences were not observed besides WMH, suggests that it is unlikely that including such a control group (hospitalized non-COVID patients) would have given any new insights.

Conclusion

In this prospective multicenter cohort study of unselected hospitalized COVID-19 patients, we found overall no higher prevalence of cerebrovascular MRI markers, apart from WMH. The few incident DWI-lesions were most likely to be explained by well-known risk-factors for progression of small vessel disease. These findings suggest that severe COVID-19 infection has limited effects on cerebral small vessel disease in the general hospitalized patient without overt neurological symptoms.

Chapter 4

Intracranial vessel wall enhancement on MRI in patients with COVID-19

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Intracranial vessel wall enhancement on MRI in patients with COVID-19

Under review

Abstract

Introduction

COVID-19 is associated with an increased risk of ischemic stroke and on the long-term post-COVID condition. Inflammation of cerebral vessels could be an underlying cause. We investigated the frequency of intracranial vessel wall enhancement (VWE) as a possible sign of inflammation on MRI in patients after hospitalization with COVID-19 and tried to identify risk factors for VWE after COVID-19 to obtain an indication of underlying pathophysiologic mechanisms.

Materials and Methods

We included hospitalized patients with COVID-19 from the CORONIS cohort study. 3T MRI including vessel wall imaging was performed within three months after positive PCR and repeated three months later. We determined the prevalence of VWE and assessed the association between intracranial VWE and possible risk factors with logistic regression analyses.

Results

We included 122 patients (mean age 58 years [SD:13], 60% male) within a median of 41 days after positive PCR for COVID-19. Twenty-six (21%) had 38 sites of VWE on MRI; 7(18%) in the internal carotid artery, 1 (3%) in the middle cerebral artery, and 30 (79%) in the vertebrobasilar arteries. Follow-up MRI in 96 patients (78%) at a median of 105 days after the baseline scan showed VWE in 26 (27%) patients with a total of 40 lesions. Increasing age (OR: 1.08 (per year) [95%CI: 1.03-1.13]), male sex (OR: 2.70, [95%CI: 1.00-7.33]) and history of stroke or TIA (adjOR: 8.35, [95%CI: 1.78-39.15]) were associated with VWE.

Discussion

About one in five patients with a severe COVID-19 infection has intracranial VWE in the first weeks after positive PCR. Whether VWE is causally related to COVID-19 remains to be studied.

Introduction

Coronavirus disease 2019 (COVID-19) infection is associated with an increased risk of stroke with an estimated prevalence of 1.8% in a Dutch study of patients hospitalized with COVID-19 and a pooled prevalence of 1.4% in a large meta-analysis including mild to severe cases internationally. (19, 78) These studies show a persisting increased risk of stroke after correction for comorbidities. The underlying mechanisms for this increased risk, however are unclear. It is hypothesized that stroke in patients with COVID-19 is caused by either a vasculopathy, a coagulopathy, or a combination of both. (79)

The hypothesis of vasculopathy is supported by findings in several small studies, showing vasculopathies on MRI in COVID-related encephalopathy patients, COVID-related cryptogenic stroke patients and in a small cohort of 15 COVID patients 238 days after infection. (80-82) This hypothesis is further strengthened by a pathology case series, that found signs of inflammation in medium-sized intracerebral vessels in patients who died from COVID-19. (83) However, these studies show highly varying frequencies and aspects of vascular involvement of COVID-19 depending on patient selection and study design. How often intracranial vasculopathy is present in unselected patients with acute COVID-19 infections is currently unknown.

Intracranial vessel wall MRI uses pre-and post-contrast series to visualize contrast enhanced areas in the vessel wall that may reflect active/ongoing inflammation. Vessel wall imaging has been used previously to detect vascular involvement in (cerebral) viral infections showing vessel wall enhancement (VWE) in patients with encephalopathies based on herpes zoster virus (HZV), human immunodeficiency virus (HIV) and varicella zoster virus (VZV). (84-86)

To obtain more insight into the presence of vasculopathy in patients with COVID-19 we aimed to investigate the frequency of intracranial vessel wall enhancement during the first weeks after hospitalization for COVID-19 and three months after the baseline. Secondly, we investigated risk factors for vessel wall enhancement after COVID-19, to give us more insight into the pathophysiology of these abnormalities.

Materials and methods

Study design

Participants were recruited from the CORONIS (CORONavirus and Ischemic Stroke) prospective cohort study between April 2021 and September 2022. (51) The CORONIS study aimed to investigate MRI-related brain changes in patients with COVID-19 in three University Medical Centers in the Netherlands: the Leiden University Medical Center (LUMC), the University Medical Center Utrecht (UMCU) and the Radboud University Medical Center in Nijmegen (RadboudUMC). Patients were included during or shortly after hospitalization with COVID-19. Inclusion criteria were age ≥18 years and hospitalization primarily for COVID-19. Exclusion criteria were contra-indications for MRI, life expectancy <3 months, medical conditions interfering with study participation or follow-up (such as health issues preventing patients from being able to come to the hospital) and inability to give informed consent. Clinical data were collected during and after hospitalization including full medical history, cardiovascular risk factors, intoxications, medication use and weight and height at admission. Clinical details of COVID-19 infection, such as date of positive PCR, onset of symptoms, treatments, and intensive care unit (ICU) admission were collected from hospital records. Data on SARS-Cov-2 variants were obtained from the Dutch National Institute of Public Health and the Environment (RIVM), that investigated presence of variants by random sampling of COVID-19 tests nationally. (87) The CORONIS study protocol has been published previously. (51) Ethical approval for this study was obtained from the Medical Ethics Committee Arnhem-Nijmegen. Written informed consent was obtained from all subjects.

Imaging

Vessel wall imaging (VWI) of the intracranial large arteries was acquired with 3-Tesla MRI scans; Siemens Prisma 3T (RUMC), Philips Ingenia 3T (LUMC), and Philips Ingenia Elition 3T X (UMCU).

T1 series were acquired before and after gadolinium-containing contrast agent administration using local protocols. Technical details of imaging per center are shown in supplementary table 1. Participants underwent MRI with a maximum interval between the positive PCR for COVID-19 and MRI of three months. Patients could not undergo MRI scanning while they were still infectious for safety reasons (protection of staff and other patients from infection). Infectious risk was determined according to hospital standards for isolation at the time of hospitalization, based on symptomatology and PCR status. Three months after the first MRI patients were invited for a second brain MRI. The flow chart of inclusions for baseline and

follow-up scans is shown in **supplementary figure 1**. All scans were assessed by experienced neuroradiologists for intracranial vessel wall abnormalities including location (internal carotid artery (ICA), anterior cerebral artery (ACA), middle cerebral artery (MCA) or vertebrobasilar arteries and type of abnormality (presumed atherosclerosis, mural hematoma or other non-specific irregularity), VWE and type of enhancement (concentric or eccentric). Only intracranial arteries were examined. Known artefacts mimicking VWE (such as enhancement at the dural segment of the vertebral arteries because of dilated vasa vasorum) were taken into account during the rating process. Methods concerning the rating system are available in the supplementary material. The MRI scans were rated by one rater in the LUMC (JdB) and the UMCU (JWD). In the RadboudUMC the scans were rated by two raters (AM, KKvU). All raters were experienced neuroradiologists with extensive knowledge of vessel wall imaging in their respective centers.

Statistical analysis

The overall prevalence of VWE was determined at baseline and after three months. The association between risk factors and VWE was assessed with univariable and multivariable logistic regression. For the latter, potential confounding factors were selected using a directed acyclic graph (DAG) for each respective variable. (88) The number of potential variables that could be adjusted for was limited due to the number of outcome events. Odds ratios (OR) with 95%-confidence intervals were calculated. Patient level data (and not lesion level data) from baseline scans were used for regression analyses.

Subgroup analyses were performed to investigate the different types of VWE (location, aspect and pre-contrast correlation). Regression analysis using the same method as in the full VWE group was performed to study the association between possible risk factors and these subgroups. The first subgroup analysis was performed on the most prevalent phenotype of enhancement. Subsequently, patients were divided in a group with suspected atherosclerotic lesions and a group with suspected non-atherosclerotic lesions based on their appearance on MRI (atherosclerotic being VWE lesions which correlated to mixed plaques on T1 before contrast enhancement). Data were analyzed with Statistical Package for the Social Sciences (SPSS) version 25 (IBM Corp. Armonk, NY, USA).

Results

In the CORONIS study, a total of 146 participants were enrolled. Of these patients, 122 (mean age 58 years [SD 13], 40% women, 21% admission to ICU) underwent a brain MRI with contrast agent and were eligible for the current study. No patient had a symptomatic stroke during admission. One patient had clinically silent ischemia on MRI, but no VWE. Thirty (25%) patients were included during predominance of Alpha variants of the coronavirus, 78 (64%) during predominance of Delta variants and 14 (11%) during predominance of Omicron variants in the Netherlands. Full characteristics of the study population are shown in **Table 1**.

Table 1. Baseline characteristics of included patients per VWE subgroup

		All patients (n=122)	Patients with VWE (n=26; 38 lesions)
Age [mean, [SD]]		58 [13]	66 [8]
Sex, male (n,(%))		73 (60)	20 (77)
ICU admission (n,(%))		26 (21)	7 (27)
Medical History (n,(%))	Hypertension	46 (38)	12 (46)
	Diabetes Mellitus	18 (15)	7 (27)
	Hyperlipidemia	44 (36)	14 (54)
	Atrial fibrillation	7 (6)	3 (12)
	TIA***	5 (4)	3 (12)
	Ischemic stroke***	5 (4)	4 (15)
	Myocardial infarction	15 (12)	5 (19)
	Thrombosis	14 (12)	3 (12)
	Smoking history	73 (60)	16 (62)
	History of Malignancy	10 (8)	2 (8)
	Active Malignancy	1 (1)	0 (0)
BMI [mean, [SD]]		29 [5]	28 [5]
Vaccination status before infection (n,(%))	Fully vaccinated****	37 (30)	12 (46)
	Partially vaccinated	9 (7)	3 (12)
	No vaccination	76 (62)	11 (42)

* *Subgroup of patients with vertebrobasilar concentric VWE without abnormalities on T1 before contrast*

** *Non-atherosclerotic is defined as not fitting criteria of mixed-plaque aspect on T1 before contrast, it is possible that beginning atherosclerotic plaques without clear characteristics yet are included in this group*

*** *Before COVID infection*

**** *Defined as two shots of Moderna, AstraZeneca, or Pfizer/BioNTech or one shot of Janssen ≥14 days before positive PCR*

VWE vertebro-basilar subgroup* (n=15; 21 lesions)	VWE athero-sclerotic subgroup (n=9; 10 lesions)	VWE non-athero-sclerotic subgroup** (n=18; 28 lesions)
65 [5]	66 [12]	65 [5]
13 (87)	5 (56)	16 (89)
3 (20)	2 (22)	5 (28)
8 (53)	4 (44)	9 (50)
5 (33)	2 (22)	5 (28)
7 (47)	6 (67)	9 (50)
2 (13)	2 (22)	2 (11)
1 (7)	2 (22)	1 (6)
4 (27)	2 (22)	3 (17)
3 (20)	3 (33)	3 (17)
1 (7)	1 (11)	2 (11)
10 (67)	5 (56)	12 (67)
0 (0)	1 (11)	1 (6)
0 (0)	0 (0)	0 (0)
29 [5]	27 [5]	29 [5]
8 (53)	4 (44)	9 (50)
1 (7)	2 (22)	1 (6)
6 (40)	3 (33)	8 (44)

Baseline MRI

MRI was performed a median of 41 days after positive PCR for COVID-19 (IQR: 29-56). Twenty-six (21% [95%CI: 16%-33%]) patients had VWE on MRI with a total of 38 lesions. Of the 38 lesions, 30 (79%) were located in the vertebral or basilar artery, 7 (18%) in the intracranial internal carotid artery (ICA) and 1 (3%) in the middle cerebral artery. Twenty-six VWE lesions (68%) were concentric and 12 (32%) were eccentric. Ten VWEs were at the site of pre-contrast abnormalities most fitting atherosclerosis (mixed plaques), 4 could be linked to pre-contrast vessel wall irregularities of which 2 were stenotic and 24 could not be linked to vessel wall abnormalities on the T1 scan before contrast. More than half of the VWE lesions (21, 55%) comprised of concentric vertebrobasilar enhancement without pre-contrast abnormalities. Examples of VWE are shown in **figure 1**.

Only two lesions were stenotic (both in the vertebral arteries); others showed no intraluminal abnormalities. An overview of these results is shown in **table 2** and **table 3** shows a patient and lesion level overview.

Fifteen patients showed the same type of vertebrobasilar enhancement that was concentric and could not be related to pre-contrast abnormalities on T1 and were therefore suspect for non-atherosclerotic lesions. Nine patients had VWE which could be linked to atherosclerosis (10 VWE lesions) and 18 had VWE that were not suspect for an atherosclerotic origin (28 VWE lesions). One patient was included in both groups because he showed both atherosclerotic and non-atherosclerotic lesions. Baseline characteristics of all subgroups are shown in **table 1**. No substantial differences in vessel wall enhancement rates were seen between the three centers (RadboudUMC 21%, LUMC 20%, UMCU 27%).

Follow-up MRI

MRI was repeated in 96 (79%) patients after a median of 105 days (IQR: 92-119) from baseline MRI. Follow-up imaging showed 40 sites of VWE in 26 patients (27%; [95%CI: 18% to 36%]). Thirty-three lesions found on the first MRI were still visible; 29 were unchanged, three showed increased enhancement, and one showed decreased enhancement. Of the 26 patients with VWE at baseline, 3 did not undergo a repeat MRI. Of the other 23, 22 (96%) patients had VWE on follow-up (all patients on the same location and 2 patients in an additional location). A total of seven new sites of VWE were visible; two in patients who already had another VWE lesion at baseline and five in patients who had no baseline VWE. One site of baseline VWE was no longer present at follow-up (**table 3 and table 4** for patient and lesion level details). Examples of changes from baseline MRI to follow-up MRI are shown in **figure 2**.

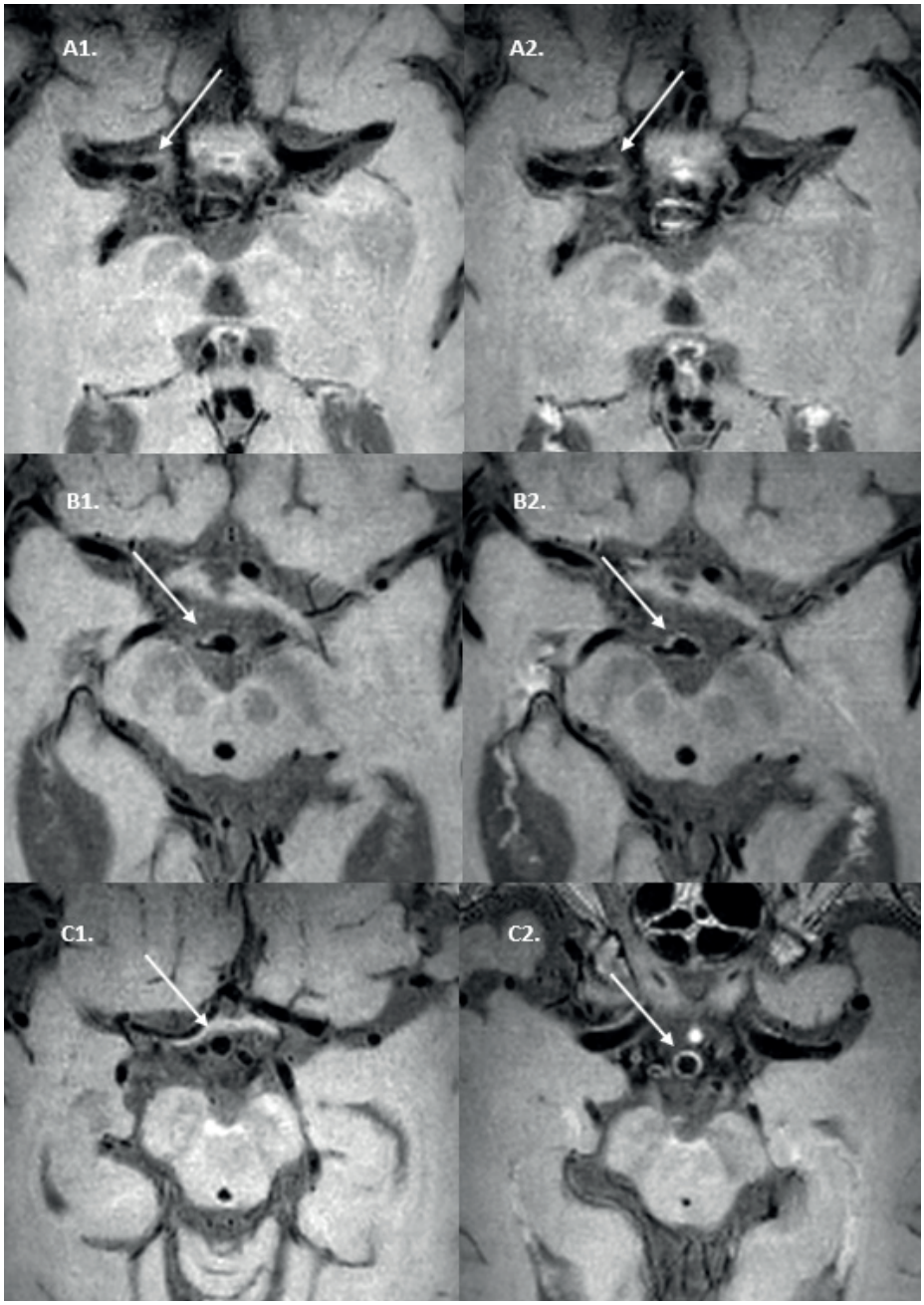


Figure 1. Examples of vessel wall enhancement on MRI before (1) and after (2) contrast administration. A. Eccentric vessel wall enhancement of the right ICA fitting atherosclerosis: atherosclerotic group (subject 7). **B.** Eccentric vessel wall enhancement of the basilary artery without atherosclerosis before contrast enhancement: non-atherosclerotic group (subject 9) **C.** Concentric vessel wall enhancement of the vertebrobasilar artery without any abnormalities on T1: vertebrobasilar subgroup (& non-atherosclerotic group) (subject 5)

Table 2. Vessel Wall Imaging results in number of VWE lesions (N=38) and number of patients with VWE (N=26*)

Area	Vessel	Aspect	Pre-contrast T1 abnormalities at site of enhancement	N of lesions/ [%]	N of patients/ [%]
Anterior	ICA	Eccentric	Atherosclerosis*	2 [5%]	1 [4%]
	Top of ICA	Eccentric	None	3 [8%]	2 [8%]
		Eccentric	Atherosclerosis*	2 [5%]	2 [8%]
	Media	Eccentric	Vessel wall irregularity**	1 [3%]	1 [4%]
Posterior	Vertebral artery unilateral	Concentric	None	4 [11%]	4 [15%]
		Concentric	Atherosclerosis*	4 [11%]	4 [15%]
		Eccentric	Atherosclerosis*	1 [3%]	1 [4%]
		Concentric	Stenosis**	1 [3%]	1 [4%]
		Eccentric	Stenosis**	1 [3%]	1 [4%]
	Vertebral artery bilateral	Concentric	None	5 [26%]****	5 [19%]
	Vertebral artery to basilar artery	Concentric	None	3 [8%]	3 [12%]
	Basilar artery	Concentric	None	4 [11%]	4 [15%]
		Eccentric	Atherosclerosis**	1 [3%]	1 [4%]
		Eccentric	Vessel wall irregularity***	1 [3%]	1 [4%]

Abbreviations: N: number ICA: internal carotid artery

* *Some patients are included in multiple groups if having multiple VWE lesions, therefore percentages add up to more than 100%*

** *Typical mixed plaque fitting typical atherosclerosis*

*** *Not with typical 'mixed plaque' aspect of atherosclerosis*

**** *Bilateral vertebral lesions are counted as two lesions in the total count and percentages*

Table 3. Detailed characteristics of VWE at patient and lesion levels

Subject	Location	Aspect	Correlation*	Change at FU1
1	Vertebral artery [R]	concentric	no abnormalities	no change
	Vertebral artery [L]	concentric	no abnormalities	no change
2	Basilar artery	eccentric	atherosclerosis	no change
3	ACI [R]	eccentric	atherosclerosis	no change
	ACI [L]	eccentric	atherosclerosis	no change
4	Top of ACI [L]	eccentric	no abnormalities	no change
	Top of Basilar artery	concentric	no abnormalities	no change
5	Top of ACI [R]	eccentric	no abnormalities	no change
	Top of ACI [L]	eccentric	no abnormalities	no change
	Basilar artery	concentric	no abnormalities	increase in enhancement
6	Vertebral arteries to basilar artery	concentric	no abnormalities	no change
7	Top of ACI [L]	eccentric	atherosclerosis	no change
	Basilar artery	concentric	no abnormalities	increase in enhancement
				new lesion: Top of ICA [R] eccentric at atherosclerosis
8	Vertebral artery [L]	concentric	vessel wall irregularity	no change
9	Basilar artery	eccentric	vessel wall irregularity	increase in enhancement
10	Vertebral artery [L]	concentric	no abnormalities	no change
11	Vertebral artery [R]	concentric	no abnormalities	no change
	Vertebral artery [L]	concentric	no abnormalities	no change
12	Vertebral artery [L]	concentric	atherosclerosis	no change
				new VWE: vertebral artery [R] concentric at atherosclerosis
13	Vertebral artery	concentric	atherosclerosis	no FU scan
14	ACM [R]	eccentric	vessel wall irregularity	no change
	Vertebral arteries to basilar artery	concentric	no abnormalities	no change
15	Vertebral artery [L]	concentric	atherosclerosis	decrease in enhancement
16	Vertebral artery	concentric	no abnormalities	no change
	Basilar artery	concentric	no abnormalities	no change
17	Vertebral artery [R]	concentric	no abnormalities	no change
	Vertebral artery [L]	concentric	no abnormalities	no change
18	Vertebral artery [R]	concentric	no abnormalities	no longer enhanced
	Vertebral artery [L]	concentric	no abnormalities	no longer enhanced

Table 3. Continued

Subject	Location	Aspect	Correlation*	Change at FU1
19	Vertebral artery [R]	concentric	no abnormalities	no change
	Vertebral artery [L]	concentric	no abnormalities	no change
20	Vertebral artery [L]	concentric	no abnormalities	no FU scan
21	Vertebral artery [R]	concentric	no abnormalities	no change
22	Vertebral artery [R] to basilar artery	concentric	no abnormalities	no change
23	Top of ACI [L]	eccentric	atherosclerosis	no FU scan
24	Vertebral artery [R]	eccentric	atherosclerosis	no change
25	Vertebral artery [L]	concentric	atherosclerosis	no change
26	Vertebral artery [L]	eccentric	vessel wall irregularity	no change
27				new VWE: ACI [R] concentric without correlation
28				new VWE: Vertebrobasilar eccentric without correlation
29				new VWE: ACI [L] concentric at vessel irregularity
30				new VWE: Vertebrobasilar concentric without correlation
31				new VWE: Basilar artery eccentric at atherosclerosis

Abbreviations: [R] right side; [L] left side; FU1= follow-up 1 (3 month follow-up)

* *Correlation to pre-contrast T1 abnormalities*

Table 4. Changes in vessel wall enhancement compared to baseline

Changes after 3 months	VWE lesions	Patients with VWE*
Scan not repeated (N(%**))	3 (8)	3 (12)
Enhancement unchanged (N(%))	29 (76)	21 (81)
New site of enhancement (N)***	7	7
Site no longer enhanced (N(%))	2 (5)	1 (4)
Enhancement ↑ (N(%))	3 (8)	3 (12)
Enhancement ↓ (N(%))	1 (3)	1 (4)

* *Some patients are included in multiple groups if having multiple VWE lesions, numbers add up to more than 26*

** *Percentages refer to percentage of baseline patients/lesions (not percentage of follow-ups)*

*** *Not included in percentages because these were not seen as baseline lesions*

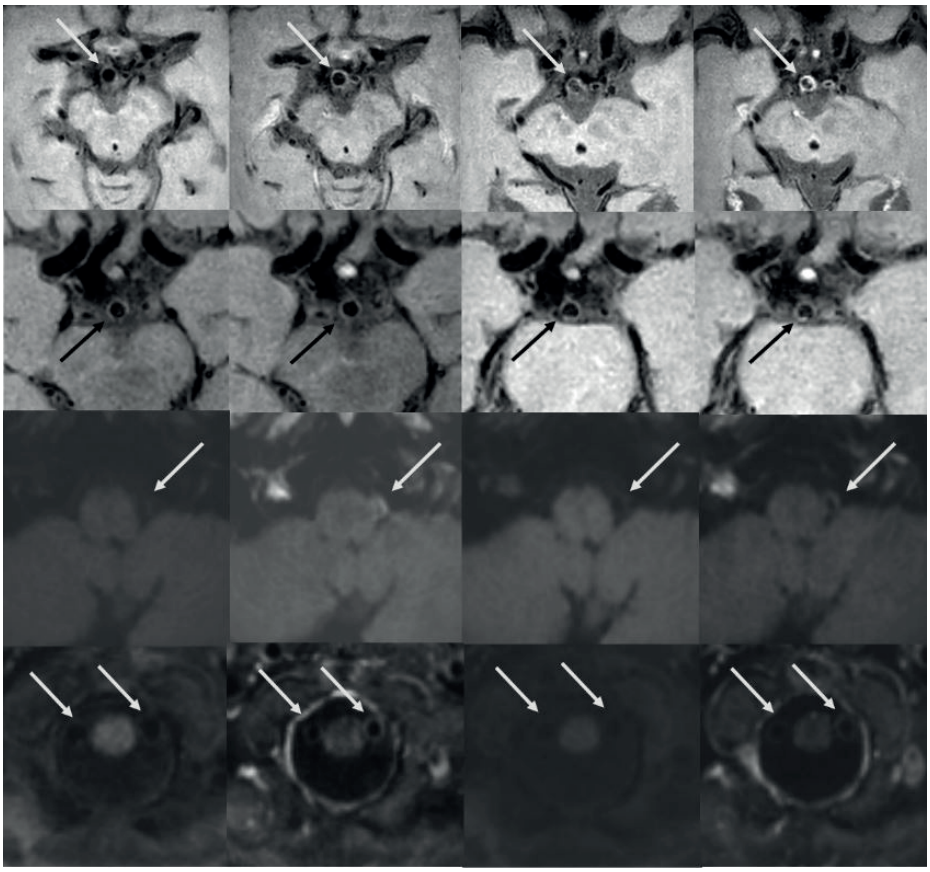


Figure 2. Examples of changes in vessel wall enhancement from baseline to follow-up MRI A. More enhancement at follow-up than at baseline (basilar artery subject 7). **B.** New enhancement at follow-up, not present at baseline (basilar artery subject 28). **C.** Decrease in enhancement at follow-up compared to baseline (left vertebral artery subject 15). **D.** Lesion at baseline no longer enhanced at follow-up (vertebral arteries subject 18)

Risk factor analysis

Male sex (OR: 2.70, 95%CI: 1.00-7.33), age (OR: 1.08 for 1 year age increase; 95%CI: 1.03-1.13) and history of stroke or TIA (adjusted OR: 8.35, 95%CI: 1.78-39.15) were associated with presence of VWE. The full results of the univariable and multivariable analysis are shown in **table 5**. The subgroup analysis investigating risk factors for the specific type of vertebrobasilar concentric enhancement in 15 patients showed similar associations as for the whole VWE group, i.e., age, sex and history of TIA or stroke (shown in supplemental table 2), as did the subgroup analyses focused on the non-atherosclerotic group.

Table 5. The association between potential risk factors and vessel wall enhancement in non-stroke patients using univariable and multivariable logistic regression

Risk factor		Patients Without VWE	Patients With VWE
Sex (n, (%))	Female	43 (45)	6 (23)
	Male	53 (55)	20 (77)
Age (mean,(SD))		55.8 (13.2)	65.5 (8.1)
BMI (at admission in kg/m^2) (mean, (SD))		28.2 (5.0)	28.8 (5.7)
Vaccination status (n,(%))	Unvaccinated	65 (68)	11 (42)
	Partially vaccinated	6 (6)	3 (12)
	Fully vaccinated*	25 (26)	12 (46)
Admission to ICU (n,(%))	No	77 (80)	19 (73)
	Yes	19 (20)	7 (27)
History of (before COVID-19)			
Hypertension (n,(%))	No	62 (65)	14 (54)
	Yes	34 (35)	12 (46)
Diabetes Mellitus (n,(%))	No	85 (86)	19 (73)
	Yes	11 (11)	7 (27)
Hyperlipidemia (n,(%))	No	66 (69)	12 (46)
	Yes	30 (31)	14 (54)
Atrial fibrillation (n,(%))	No	92 (96)	23 (88)
	Yes	4 (4)	3 (12)
History of TIA or stroke (n,(%))	No	93 (97)	20 (77)
	Yes	3 (3)	6 (23)
Myocardial infarction (n,(%))	No	86 (90)	21 (81)
	Yes	10 (10)	5 (19)
Venous thrombosis (n,(%))	No	85 (86)	23 (88)
	Yes	11 (11)	3 (12)
Smoking history (n,(%))	No	39 (41)	10 (38)
	Yes	57 (59)	16 (62)
Auto-immune disease (n,(%))	No	78 (81)	23 (88)
	Yes	18 (19)	3 (12)
Peripheral artery disease (n,(%))	No	95 (99)	24 (92)
	Yes	1 (1)	2 (8)
Malignancy (n,(%))	No	87 (91)	24 (92)
	Yes	9 (9)	2 (8)

Abbreviations: BMI= body maxx index, ICU= intensive care unit, N= number, ORcrude = crude odds ratio, aOR= adjusted odds ratio, CI= confidence interval

* *Defined as two shots of Moderna, AstraZeneca, or Pfizer/BioNTech or one shot of Janssen ≥14 days before positive PCR*

[1] *Corrected for age and sex*

Univariable		Multivariable	
ORcrude	**95% CI**	**aOR**	**95% CI**
REF			
2.70	1.00-7.33	-	-
1.08	1.03-1.13	-	-
0.98	0.90-1.06	1.04[1]	0.95-1.14
REF	-	REF	-
2.96	0.64-13.59	3.52[2]	0.58-21.52
2.84	1.11-7.26	1.73[2]	0.56-5.30
REF	-	REF	-
1.49	0.55-4.07	1.75[3]	0.61-5.01
REF	-	REF	-
1.56	0.65-3.76	1.28[1]	0.51-3.22
REF	-	REF	-
2.85	0.98-8.30	1.72[1]	0.57-5.20
REF	-	REF	-
2.57	1.06-6.21	2.20[1]	0.86-5.61
REF	-	REF	-
3.00	0.63-14.35	1.81[1]	0.35-9.43
REF	-	REF	-
9.30	2.14-40.35	8.35[1]	1.78-39.15
REF	-	REF	-
2.05	0.63-6.63	1.27[1]	0.37-4.30
REF	-	REF	-
1.01	0.26-3.92	0.81[4]	0.19-3.49
REF	-	REF	-
1.10	0.45-2.66	0.67[1]	0.27-1.69
REF	-	REF	-
0.57	0.15-2.09	0.53[1]	0.15-1.91
REF	-	REF	-
7.92	0.69-91.00	4.59[1]	0.39-54.61
REF	-	REF	-
0.81	0.16-3.98	0.53[5]	0.10-2.83

[2] *Corrected for age, sex, BMI, immunosuppressive use during admission, glucocorticosteroid use during admission and number of comorbidities*

[3] *Corrected for age, sex, BMI and number of comorbidities*

[4] *Corrected for: age, sex, malignancy5 Corrected for age, sex and smoking history*

Discussion

We found that VWE was present in approximately a fifth of patients who had been hospitalized for COVID-19 six weeks after the start of the infection. Three months later the frequency of VWE increased to more than a quarter of patients with COVID-19. Age, male sex and history of TIA or stroke were associated with presence of VWE on the baseline scan. This finding held up in the subgroup analyses of lesions not clearly associated with atherosclerosis. In the literature, limited data are available on the frequency of VWE after COVID-19. A prior small prospective study found only eccentric VWE consistent with atherosclerosis. There was no statistically significant difference in frequency of these lesions suspect for atherosclerosis between COVID-19 patients 238 days after infection 6/15 [40%] and healthy controls 1/10 [10%], probably because of the small sample size. (80) The frequency of atherosclerosis-related VWE in our study was a bit lower, but our study also showed non-atherosclerotic lesions. This is possibly because in the other study the sample size was small and the interval between the infection and the scan was much longer. (80) As far as we are aware, there are no other prospective studies with intracranial vessel wall imaging after COVID-19. Previous retrospective studies in selected patient groups suggest that VWE is frequent in patients with neurologic symptoms or complications from COVID-19. (81, 82)

To our knowledge, the previously mentioned study on COVID-19 related vessel wall enhancement is the only one that reports the prevalence of VWE in healthy individuals on 3T-MRI [1/10]. (80) The only other studies that previously investigated VWE on 3T-MRI focused on patients with stroke or vasculitis. (89)

In our study at baseline ten VWE lesions were located at a site clearly showing atherosclerosis on the corresponding T1 series before contrast enhancement. Such lesions are most likely associated with active atherosclerosis. (90, 91) Lesions with concentric VWE, all in the vertebrobasilar arteries, were at sites where no pre-contrast abnormalities were visible, fitting the traditional aspect of inflammation or endothelial damage. (90-93) The other VWEs were heterogenic in aspects and cannot be categorized into one pathophysiologic category. All categories, however, hold uncertainty because we cannot be sure about the non-atherosclerotic origin of these lesions as this conclusion was based on MRI instead of histopathology. The fact that new lesions were visible at 3 month follow-up could indicate that the effects of COVID-19 were still ongoing or that these lesions are not linked to the disease. Although we cannot exclude that the progression is due to age related atherosclerosis, in our opinion this is not a likely scenario considering the short

time interval between the scans. All associated risk factors found in the regression analysis are risk factors for or indicators of atherosclerosis. This association remained in the subgroups of VWE not directly tied to atherosclerosis. Remarkably, not all cardiovascular risk factors were associated with VWE, possibly because the effect of these factors was limited or because the study was underpowered to detect these associations. The association could either point towards pre-existing VWE in a population with a high prevalence of cardiovascular risk factors or towards a combination of pre-existing atherosclerosis combined with local inflammation related to COVID-19. (94)

Cerebral VWE is seen in several diseases including active atherosclerosis, dissections, infectious and auto-immune vasculitis and unstable aneurysms. Based on our data and previous studies it is clear that at least a degree of vessel wall enhancement is associated with age and cerebrovascular risk factors. Vessel wall enhancement is seen in patients with COVID-19, a finding demonstrated across multiple reports. (80-82, 89) The significance and underlying mechanisms of this finding and the persistence or evolution therof is still unknown.

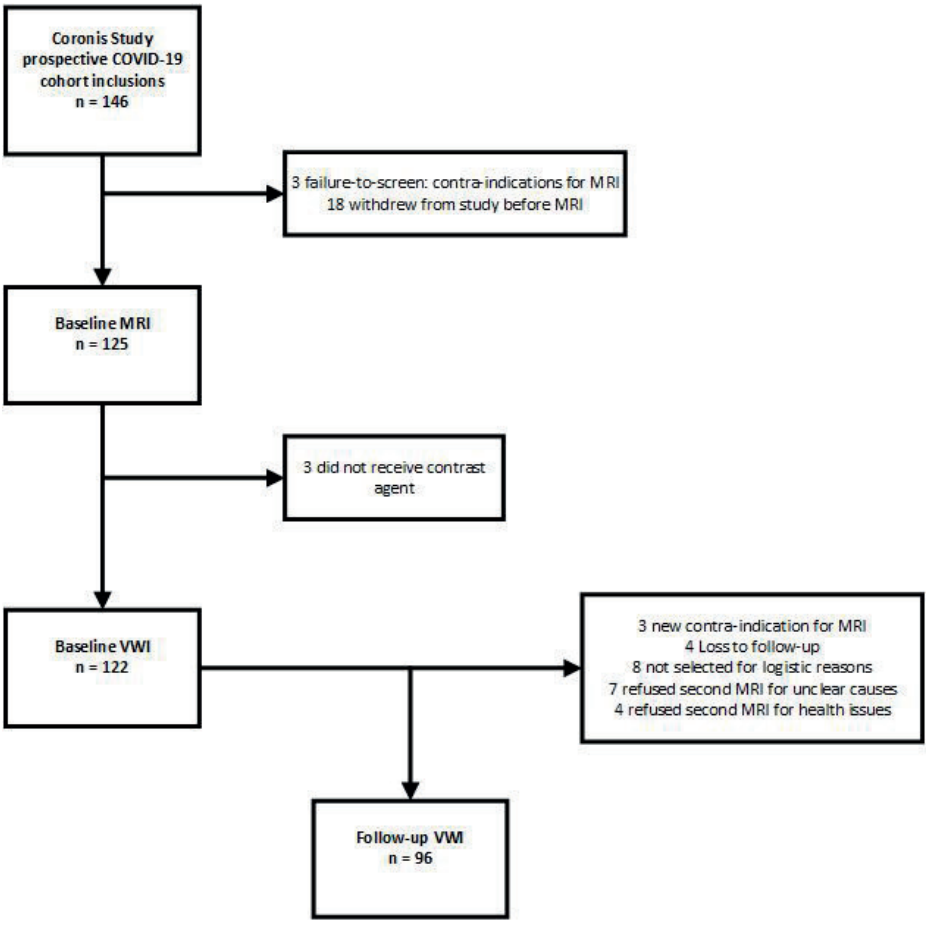
Strengths of our study are the unselected cohort of patients, giving a realistic representation of hospitalized (moderate to severe) COVID-19 patients and complete data on cardiovascular risk factors per patient. The prospective nature of our study and the use of interval imaging further strengthens our results. Our study also has several limitations. Our results are only generalizable to hospitalized COVID-19 cases. We cannot comment on VWE presence in a population with mild disease. Furthermore, MRI scans were not performed during active COVID-19 infection and transient VWE in the acute phase may have been missed. Also, because there were no MRI scans performed before the COVID-19 infection, we cannot tell which lesions with enhancement were pre-existing. The small sample size of this study significantly limits the ability to detect associations with possible associated factors. Finally and most importantly, the administration of intravenous contrast was deemed too invasive for a healthy control group by the medical ethics committee. Therefore, we were not able to include a direct control group for this sub study of CORONIS. This limitation thus precludes any conclusions on causality. When more information on the prevalence of VWE in other populations is published, it will be possible to put our results in a clearer clinical perspective.

In conclusion; about one in five patients with a severe COVID-19 infection has intracranial VWE in the first weeks after positive PCR. This finding could possibly reflect presence of intracranial vascular inflammation. Three months after this

baseline scan the frequency of VWE increases to more than a quarter of patients. It is uncertain whether there is a causal relationship between COVID-19 and VWE.

Further research is necessary to establish the prevalence of VWE in other clinical contexts (most importantly in healthy adults), and to determine the clinical consequences of VWE.

Supplementary materials



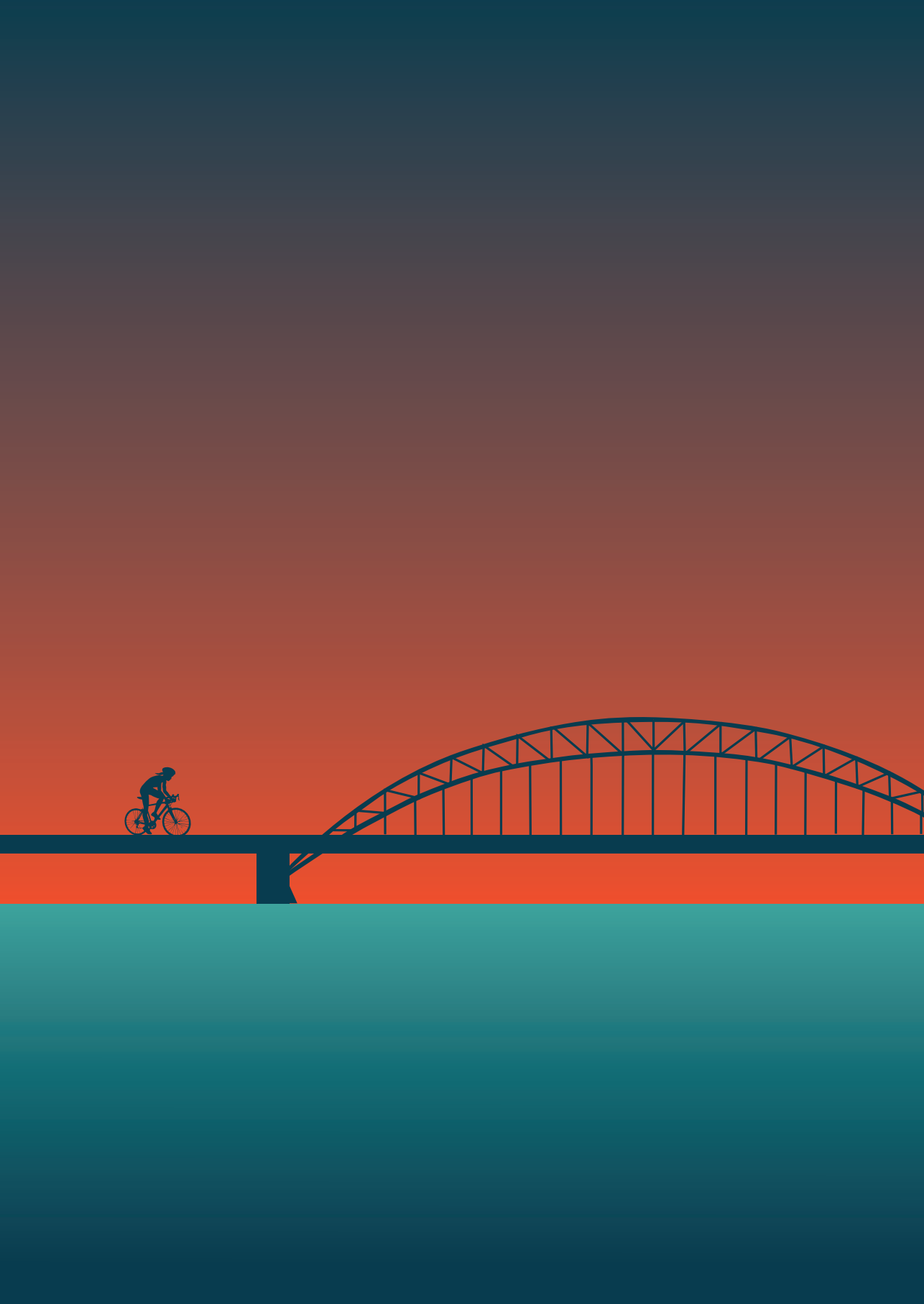
Supplementary Figure 1. Flow chart of inclusions

Supplementary Table 1. MRI scan protocol details per center

		RadboudUMC	LUMC	UMCU
Hardware	Type of MRI scan	Siemens 3T Prisma	Philips 3T Ingenia	Philips Ingenia Elition 3T X
	Type of head coil	32 channel	32 channel	32 channel
Intracranial vessel wall imaging with and without contrast	Orientation	3D space fatsat	3D	Axial
	Technique (spin/gradient)	Spin echo	Spin echo	Spin echo
	Field of view	231 x 231	200 x 176 x 45	200 x 200
	Matrix	516 x 512	332 x 292	332 x 334
	Resolution Acquired	0.9 mm isotropic	0.6 × 0.6 × 1.0 mm	0.5 mm isotropic
	Resolution Reconstructed	0.9 mm isotropic	0.5 x 0.5 x 0.5 mm	0.5 x 0.5 x 0.5 mm
	TR/TE(/TI) (msec)	750/20	1500/38	1500/32,4
	Flip angle (degrees)	120	90	90
	Acquisition time	4:38	7:05	5:57
Contrast agent		15 ml Dotarem®	Clariscan (0.2 ml/kg)	0.1 ml Gadovist/kg

Part III

White matter integrity in COVID-19 patients



Chapter 5

White matter integrity in hospitalized COVID-19 patients is not associated with short- and long-term clinical outcomes

Published as: Theresa J van Lith, Hao Li, Marte W van der Wijk, Naomi T Wijers, Wouter M Sluis, Marieke JH Wermer, Frank-Erik de Leeuw1, Frederick JA Meijer, Anil M Tuladhar

White matter integrity in hospitalized COVID-19 patients is not associated with short- and long-term clinical outcomes

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Abstract

Objectives

SARS-CoV-2 infection is associated with a decline in functional outcomes and many patients experience persistent symptoms, while the underlying pathophysiology remains unclear. This study investigated white matter (WM) integrity on brain MRI in hospitalized COVID-19 patients and its associations with clinical outcomes, including long COVID.

Materials and methods

We included hospitalized COVID-19 patients and controls from CORONIS (CORONavirus and Ischemic Stroke), an observational cohort study, who underwent MRI-DWI imaging at baseline shortly after discharge (<3 months after positive PCR) and three months after baseline scanning. We assessed WM integrity using diffusion tensor imaging (DTI) and neurite orientation dispersion and density imaging (NODDI) and performed comparisons between groups and within patients. Clinical assessment was conducted at three and twelve months with functional outcomes including (Modified Rankin Scale (mRS), Post-COVID-19 Functional Status scale (PCFS), Visual Analogue Scale (VAS) and long COVID, for cognitive assessment the Modified Telephone Interview for Cognitive Status (TICS-M) and for mood the Hospital Anxiety and Depression Scale (HADS). Associations between WM integrity and clinical outcomes were evaluated using logistic and linear regression.

Results

49 patients (mean age 59.5 years) showed higher overall peak width of skeletonized mean diffusivity (PSMD) ($p=0.030$) and lower neurite density index (NDI) in several WM regions compared to 25 controls at baseline ($p<0.05$; FWE-corrected), but did not remain statistically significant after adjusting for WM hyperintensities. Orientation dispersion index (ODI) increased after 3-month follow-up in several WM regions within patients ($p<0.05$). Patients exhibited worse clinical outcomes compared to controls. Low NDI at baseline was associated with worse performance on the Post-COVID-19 Functional Status scale after 12 months ($p=0.018$).

Conclusion

After adjusting for WMH, hospitalized COVID-19 patients no longer exhibited lower WM integrity compared to controls. WM integrity measured shortly after discharge was generally not associated with clinical assessments, suggesting that factors other than underlying WM integrity play a role in worse clinical outcomes or long COVID.

Introduction

Coronavirus disease 2019 (COVID-19) is associated with a wide range of symptoms. (95-97) Many patients experience reduced functional performance and persistent symptoms several months after infection, which includes fatigue, cognitive impairment, mood disorders, and anosmia, often referred to as long COVID. (98) The underlying pathophysiological mechanism for these ongoing symptoms, however, remains unknown. Previous studies investigating the white matter (WM) microstructure with diffusion weighted imaging (DWI) in COVID-19 patients, conducting scans several months to two year post-discharge, yielded conflicting results. (99-101) Two studies, with scans conducted several months after discharge, reported an association between lower WM integrity in several brain regions and a decline in cognition. (100, 101) Meanwhile, a long-term follow-up study reported WM integrity recovery (or increased) two years after COVID-19 infection, in comparison to measurements conducted one year post-infection. (100-102) However, it is unknown whether these changes in alterations in WM integrity reflect COVID-19 pathology or age-related processes as previous studies performed baseline and follow-up MRI months to years after the acute, inflammatory symptomatic phase of the infection. In addition, earlier studies did not consider comorbidity with pre-existing brain damage, such as white matter hyperintensities (WMH) due to underlying cerebral small vessel disease (SVD), which is highly associated with decreased WM integrity. (7) Finally, previous studies mainly focused on cognition but the relation with functional outcomes, mood disorder or long COVID has not yet been investigated.

Our aim was to investigate WM integrity using diffusion metrics, derived from both diffusion tensor imaging (DTI) and neurite orientation dispersion and density imaging (NODDI) models, in patients hospitalized for COVID-19 compared to unaffected controls (with no signs of previous SARS-CoV-2 infection in laboratory results), while taking into account the presence of WMH. In addition, we examined changes in WM integrity over time (3 months) within patients and lastly, investigated the relation between diffusion metrics and short- and long-term clinical outcomes (3 and 12 months after discharge).

Materials and methods

Participants

This study is part of the CORONavirus and Ischemic Stroke (CORONIS) study, a multicenter prospective observational cohort study in the Netherlands examining MRI markers of cerebrovascular disease in patients hospitalized for COVID-19. The study aimed to prospectively perform brain MRI in unselected hospitalized COVID-19 patients, who were not exhibiting neurological symptoms. A detailed description of the study protocol has been described elsewhere. (51) Participants (n=202) were recruited in three academic hospitals between April 2021 and October 2022. Inclusion criteria for patients included (a) hospitalization due to COVID-19, (b) PCR-confirmed diagnosis, and (c) ≥18 years old. Healthy controls, age and sex-matched, were recruited from patients' relatives or through the hospital, requiring a negative Anti-Sars-CoV-2 IgG test at inclusion. Exclusion criteria for both groups were MRI contra-indications, pregnancy, or limited life expectancy (<3 months). Additionally, to address for potential confounders, we excluded patients with conditions such as cardiac arrest or PRES, which were not present in our cohort. For this study, we only included participants from a single site (Radboudumc) who underwent multi-shell DWI, which was not conducted in the other participating centers. COVID-19 patients with overt ischemic stroke were excluded. This study was approved by the local the Medical Review Ethics Committee region Arnhem-Nijmegen on 01-04-2021. All participants provided written informed consent.

Data collection

Baseline data collection included demographics, lifestyle, education, medical history, and hospitalization details. (10) Brain MRI scans were performed at baseline; for COVID-19 patients, either during admission or shortly after discharge within three months following a positive PCR, and for healthy controls, on the day they signed the informed consent. Follow-up MRIs were performed for COVID-19 patients three months later. Healthy controls did not undergo follow-up MRI. Telephone follow-up questionnaires were collected at three and twelve months after inclusion for all participants (for COVID-19 patients during 3-month MRI visit). Education levels were categorized on a validated scale from 1 (below primary school) to 7 (academic degree) and grouped into 'Low' (levels 1-3), 'Middle' (level 4), and 'High' (above level 4) following the Verhage scaling system. (103) The type of ventilation was classified into three categories to indicate the severity of COVID-19: 1) non-invasive respiratory support, including nasal cannula or non-rebreathing mask; 2) non-invasive ventilation, such as Optiflow; and 3) invasive ventilation, involving intubation.

Neuroimaging protocol

All participants underwent the same scanning protocol on a 3 Tesla MRI scanner (Siemens Prisma) at baseline and during follow-up. The protocol included the following sequences: 3D T1 weighted (T1W) space fatsat with the following parameters: 0.9ms isotropic voxel size, and repetition time (TR) = 700ms, Echo Time (TE) = 9mm; 3D fluid-attenuated inversion recovery (FLAIR) with following parameters: 1mm isotropic voxel size and TR = 500ms, TE = 394ms; multi-shell DWI with the following parameters: 80 diffusion-weighted directions (40 × b = 1,000, and 40 × b = 2,000s/mm2), 6 × b = 0 images, 2.0mm isotropic voxels, and TR = 4600ms, TE = 80ms. (51) Imaging details have been described previously. (51)

White matter hyperintensity (WMH) volume

We used a validated segmentation method based on k-nearest neighbors algorithm (UBO Detector) to automatically segment WMH and calculate WMH volumes using bias-corrected T1 and FLAIR images. (104) Segmentations were visually reviewed for errors or artifacts. Note that UBO detector calculated WMH volumes in SPM's DARTEL space. Therefore, there is no need to adjust for intracranial volume.

Diffusion MRI preprocessing and metrics

Diffusion MRI data were pre-processed to remove the noise and Gibbs artifacts, correct head motion, eddy currents-induced distortion, susceptibility-induced distortion (top-up) and intensity bias using MRtrix 3.0 software (105), Functional Magnetic Resonance Imaging of the Brain Software Library (FSL) software (106) and Advanced Normalization Tools (99). Due to the absence of a b0 image with reversed phase encoding in our DWI scans, 'topup' was performed based on a synthesized b0 image from the T1 image using Synb0-DISCO. (107) Diffusion metrics derived from different diffusion models (DTI and NODDI) were calculated. While DTI-derived measures (including fractional anisotropy (FA) and mean diffusivity (MD) have been widely used, they only provide a composite view of contributions from multiple tissue components (intra-neurite, extra-neurite, and cerebral spinal fluid (CSF) within the voxel. In contrast, NODDI model can delineate contributions from each compartment, offering the measures neurite density index (NDI), orientation dispersion index (ODI) and cerebrospinal fluid volume fraction (fCSF) of each tissue components within one voxel. (100) First, two DTI metrics were calculated with the pre-processed diffusion data (only b=0 and b=1000): mean diffusivity (MD) and fractional anisotropy (FA) maps of each participant using the 'dtifit' function within FSL. (108) Second, we used the entire multi-shell DWI data to fit the NODDI model using NODDI toolbox in MATLAB (http://mig.cs.ucl.ac.uk/index.php?n=Tutorial.NODDImatlab). Using this tool, we calculated three NODDI parameters: NDI, ODI and fCSF maps for each participant. (8)

Peak width of Skeletonized Mean Diffusivity (PSMD) values was calculated using the PSMD tool (http://www.psmd-marker.com/). (108)

Tract-Based Spatial Statistics

Voxel-wise statistical analysis of FA maps was conducted using the Tract-Based Spatial Statistics (TBSS) pipeline (Smith, Jenkinson et al. 2006), which is a component of FSL software (version 6.0.1). (106, 109, 110) FA maps from all participants (including scans of patients at baseline and follow-up and healthy controls) were fed into TBSS pipeline tool to create a mean FA skeleton representing the centres of all tracts shared across the population. Next, MD, NDI, ODI, fCSF maps were projected into the WM skeleton using the 'tbss_non_FA' function within TBSS tool of FSL, resulting in aligned maps of FA, MD, NDI, ODI, fCS. Lastly, the resultant five maps were analysed using voxel-wise cross-subject statistics.

Clinical outcomes during follow-up

Functional outcomes included the modified Rankin Scale (mRS), Post-COVID-19 Functional Status scale (PCFS), Visual Analogue Scale (VAS) and long COVID (defined following the WHO definition and Delphi (2021) consensus). (28, 29, 111, 112) Symptoms reported by patients were collected during the two follow-up moments (physical and telephone interviews). Since participants were explicitly asked for fatigue and dyspnea, we only adjudicated these symptoms to long COVID if they had an impact on everyday functioning. This was expressed as a decline on the PCFS scale between study procedures (baseline, 3-month and 12-month follow-up). Cognitive assessment included the Modified Telephone Interview for Cognitive Status (TICS-M). To assess symptoms of anxiety and depression (mood), the Hospital Anxiety and Depression Scale (HADS) was used. (27)

Statistical analysis

Patients and controls' baseline characteristics and clinical outcomes were compared using Chi-square tests for categorical variables (n, %) and the t-test (mean, SD) or Wilcoxon rank-sum (median, IQR) tests for continuous data. mRS and PCFS were categorized in the same two groups (0-1: good vs. $\geq$2: poor outcome). For the cross-sectional comparison (patients vs. controls) of diffusion metrics (PSMD and WMH volume), linear regression adjusted for age and sex was performed. Voxel-wise group comparisons of FA, MD, NDI, ODI and fCSF maps between 1) patients and controls (adjusted for age and sex (model 1) and additionally for WMH volume (model 2)) and 2) within patients (baseline vs. follow-up) were performed using the FSL Randomise tool (permutation-based inference: 5000 permutations). Significant clusters were identified using threshold-free cluster enhancement-based family-

wise error correction for multiple comparison in TBSS analyses ($p<0.05$). (113) Longitudinal analysis of clinical outcomes within patients included McNemar's test (n, %) for categorical variables, two-sample t-tests and Wilcoxon tests (non-normal distributions) for continuous data. DTI and NODDI metrics values of regions that differed between groups were extracted. Logistic and linear regression were used to analyse the relationship between significant MRI parameters (PSMD and NDI) and clinical outcomes, reported as crude odds ratios (OR) and adjusted odds ratios (aOR) (95% CIs) corrected for age (model 1) and additionally WMH (model 2) and multiple testing using false discovery rate (FDR). (114) Subgroup analyses of diffusion metrics were performed between a) ICU and non-ICU patients and b) patients with long COVID and those without. Education was grouped into three levels for the baseline characteristics comparison and the original seven levels for logistic and linear regression. Two-sided p values <0.05 were considered statistically significant. Data was analyzed using R version 4.3.1.

Results

Participants

73 patients and 27 controls were eligible for participation of whom 24 patients and 2 controls were excluded (**Figure 1**). This study resulted in 49 patients with COVID-19 (mean age: 59.5 years (SD 12.6); 32.7% female, 30.6% admitted to ICU) and 25 healthy controls (mean age: 58.5 years (SD 10.1); 48.0% female). Baseline characteristics are shown in **Table 1.** There were no differences between patients and controls regarding age or sex. Regarding all participants, time between baseline assessments and follow-up 1 was 111 days (median, IQR [93.0-140.0) and 249 days between follow-up 1 and follow-up 2 [IQR 224.0-271.0] (+- 8.2 months).

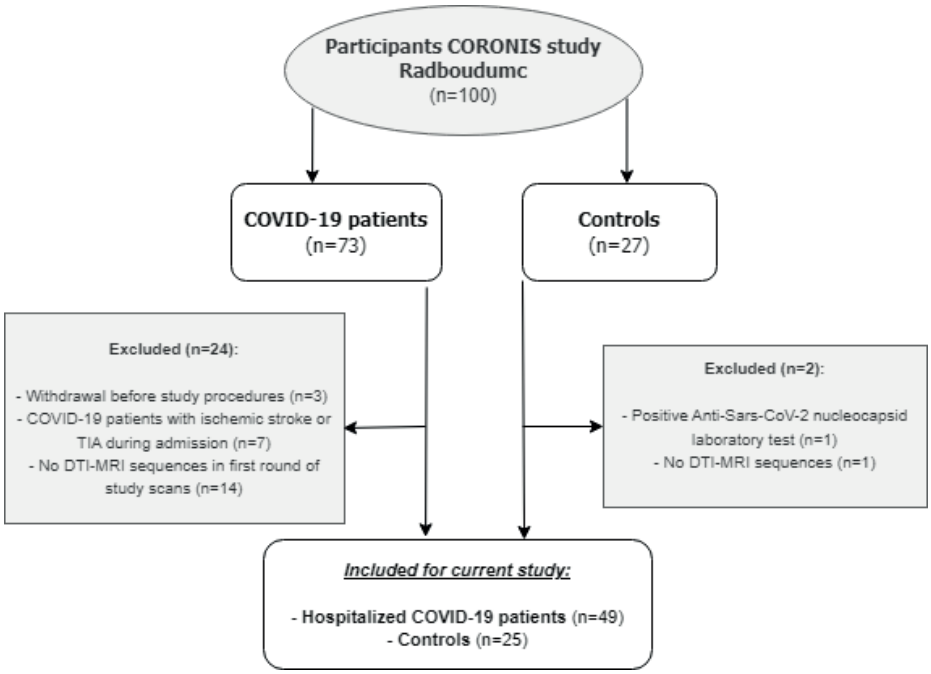

Figure 1. Flowchart of study population

Abbreviations: TIA = transient ischemic attack, DTI = diffusion tensor imaging

WM integrity - cross-sectional analyses

At baseline, patients with COVID-19 exhibited higher age and sex adjusted PSMD values (mean = 1.70 $*10^{-4}$ mm^2/s, SD = 0.29 $*10^{-4}$ mm^2/s) than controls (mean = 1.56 $*10^{-4}$ mm^2/s, SD = 0.13$*10^{-4}$ mm^2/s) *(p=0.030)*. After additional correction for WMH volume, this difference was not statistically significant anymore (*p=0.590*). There were no significant differences in FA and MD values using voxel-wise analyses between groups (p-corrected values >0.05). Compared to controls, adjusted for age and sex, patients demonstrated significantly lower NDI values in the right anterior thalamic radiation (ATR), forceps minor and right inferior fronto occipital fasiciculus (**Figure 2**). These differences disappeared after additionally adjusting for WMH volumes. Voxel-based analyses showed no significant differences of ODI and fCSF between patients and controls. In a subgroup analysis, there were no significant differences in diffusion metrics between COVID-19 patients with long COVID and those without. Additionally, no significant differences were found in diffusion metrics between ICU patients and non-ICU patients.

Table 1. Baseline characteristics COVID-19 patients vs. controls

	COVID-19 patients n=49	Controls n=25	p*
Female, n (%)	16 (32.7)	12 (48.0)	0.301
Age at inclusion, mean (SD)	59.53 (12.63)	58.48 (10.06)	0.719
COVID vaccine before admission/inclusion, n (%)	21 (42.9)	22 (88.0)	***0.011***
ICU admission, n (%)	15 (30.6)	N/A	N/A
Respiratory or ventilation therapy required at maximum during admission, n (%)			
Nasal cannula, oxygen mask, non-rebreathing mask	26 (53.2)	N/A	N/A
Non-invasive ventilation (Optiflow)	15 (30.5)		
Invasive ventilation (intubation)	8 (16.3)		
Days hospital admission, median [IQR]	11.0 [7.0, 15.0]	N/A	N/A
Days between positive PCR and baseline MRI, median [IQR]	40.0 [30.0, 54.0]	N/A	N/A
Days between positive PCR and inclusion study, median [IQR]	16.0 [8.0, 27.0]	N/A	N/A
Days between hospital admission and baseline MRI, median [IQR]	32.00 [27.0-48.0]	N/A	N/A
Education level, n (%)			0.085
Low	8 (16.3)	6 (24.0)	
Middle	27 (55.1)	7 (28.0)	
High	14 (28.6)	12 (48.0)	
Cardiovascular history:			
Body Mass Index (BMI) in kg/m^2, mean (SD)	28.40 (4.31)	27.46(3.35)	0.344
Smoking, n (%)	29 (59.2)	11 (44.0)	0.321
Diabetes Mellitus, n (%)	10 (20.4)	2 (8.0)	0.300
Hypertension, n (%)	19 (38.8)	3 (12.0)	***0.034***
Hypercholesterolemia, n (%)	21 (42.9)	6 (24.0)	0.181
Pulmonary disease (e.g. COPD, asthma), n (%)	21 (42.9)	1 (4.0)	***0.001***
MRI characteristics			
White Matter Hyperintensity (WMH) volume (mm^3), median [IQR]	1.481 [0.884-3.435]	0.857 [0.392-1.512]	***0.021***
Microbleeds, n (%)	5 (10.2)	2 (8.0)	0.759

**Unadjusted p-values*

Abbreviations: ICU = intensive care unit, PCR = Polymerase chain reaction, COPD = chronic obstructive pulmonary disease, N/A = not applicable.WM integrity - cross-sectional analyses

Changes of WM integrity in patients over time

Of the 49 patients at baseline, 39 (79.6%) patients underwent brain MRI during follow-up after three months. Ten patients were excluded because they did not undergo follow-up MRI due to various reasons (illness (n=1), lost to follow-up (n=5), claustrophobia (n=3), moved to foreign country (n=1)). ODI values increased after three months of follow-up in patients compared to baseline in the ATR, bilateral corticospinal tract, cingulum (cingulate gyrus and hippocampus), forceps major and minor, inferior fronto-occipital fasciculus on both sides, bilateral inferior fronto-occipital fasciculus left, superior longitudinal fasciculus left and right and the right uncinate fasciculus **(Figure 3),** which remained significant after correction for the change of WMH volume. However, other diffusion metrics and WMH volumes did not change over time.

Clinical outcomes during follow-up

COVID-19 patients had worse functional outcomes compared to controls three and twelve months after discharge. Patients had more symptoms of depression (*p=0.008*), lower scores on cognitive functions, measured by TICS-M at 12 months, and lower scores on the VAS scale (*p=0.006*) (**Table 2**). Long COVID was present in 62.2% of the patients after three months and in 40.9% after twelve months. Apart from a decrease in frequency of long COVID, no differences over time in functional and cognitive outcomes were detected in patients between the 3- and 12-month follow-up **(Supplementary Table 2)**.

Association between WM integrity and clinical outcomes

The diffusion metrics at baseline and the changes in ODI values during follow-up were not related to the clinical outcomes **(Supplementary tables, 4-6).** However, lower NDI values of the regions that differed between patients and controls at baseline, were significantly associated with lower scores on the PCFS scale (functional outcome) at the 12-month follow-up **(Supplementary Table 3)**, which remained significant after additional adjustment for WMH volumes *(p=0.018)*.

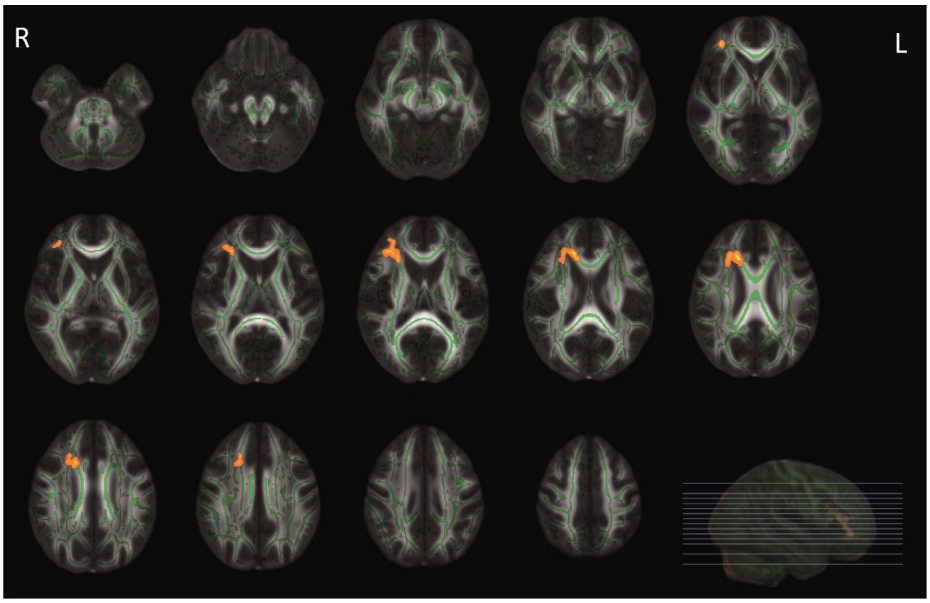

Figure 2. Tract-Based Spatial Statistics (TBSS) analyses of neurite density index (NDI) values between patients with COVID-19 and healthy controls. WM tracts in orange represent regions with significantly lower NDI values in patients with COVID-19 compared with healthy controls, after adjusting for age and sex, and for multiple comparisons ($P < 0.05$, family-wise error corrected). These differences disappeared after additionally adjusting for WMH volumes.

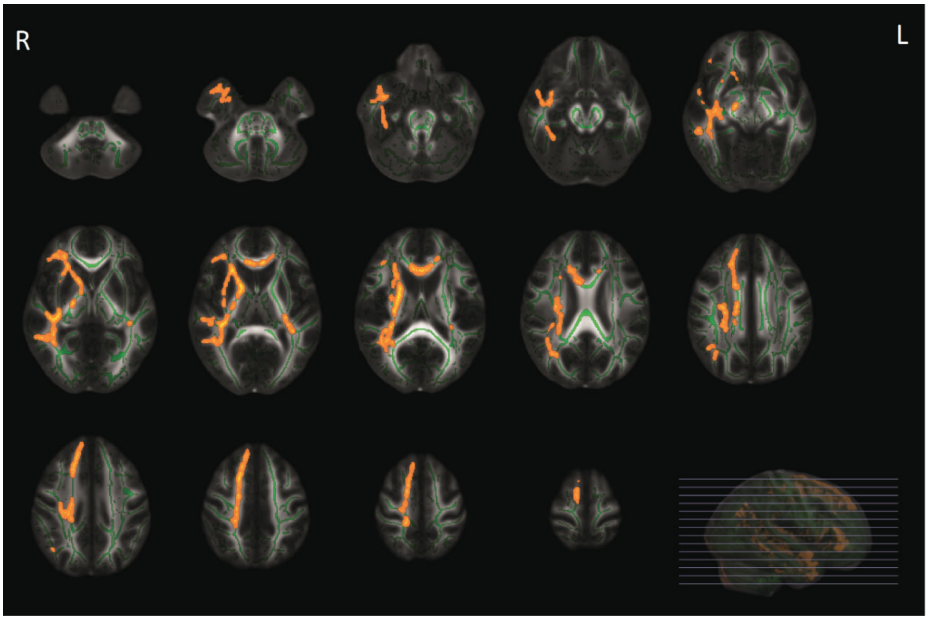

Figure 3. Tract-Based Spatial Statistics (TBSS) analyses of orientation dispersion index (ODI) values in patients with COVID-19 between baseline and follow-up MRI. WM tracts in yellow-orange represent regions with significant ODI values in COVID-19 at baseline compared with ODI values after 3-month follow-up, adjusted for multiple comparisons ($P < 0.05$, family-wise error corrected).

Table 2. Functional, cognitive outcome and mood symptoms between groups

Outcomes		COVID-19 patients		Controls	
	n total		*n total*		*p**
3-month follow-up					
Cognitive function					
TICS-M score, mean (SD)	45	36.22 (4.16)	20	37.20 (3.09)	0.386
Functional outcomes					
mRS (reference 0-1) ≥2-6, n (%)	45	15 (33.3)	20	0 (0.0)	***0.006***
PCFS (reference 0-1) ≥2-4, n (%)	45	22 (48.9)	20	2 (10.0)	***0.006***
Mood					
Hospital Anxiety and Depression Scale					
HADS-Anxiety, median [IQR]	45	3.0 [1.0, 6.0]	20	3.0 [0.8, 5.5]	0.474
HADS-Depression, median [IQR]	45	2.0 [1.0, 7.0]	20	1.0 [0.0, 2.0]	0.095
Long COVID[a], n (%)	45	28 (62.2)	-	-	-
12-month follow-up					
Cognitive function					
TICS-M, mean (SD)	42	35.24 (4.66)	21	38.81 (3.60)	***0.006***
Functional outcomes					
mRS (reference 0-1) ≥2-6, n (%)	44	16 (36.4)	22	0 (0.0)	***0.004***
PCFS (reference 0-1) ≥2-4, n (%)	44	22 (50.0)	22	0 (0.0)	***<0.001***
VAS scale (0-100), mean (SD)	44	73.77 (16.41)	24	85.92 (8.73)	***0.004***
Mood					
Hospital Anxiety and Depression Scale (HADS)					
HADS-Anxiety, median [IQR]	44	3.5 [0.0, 7.0]	24	2.5 [0.0, 4.0]	0.147
HADS-Depression, median [IQR]	44	3.5 [1.0, 8.0]	24	1.0 [0.0, 2.0]	***0.008***
Long COVID[a], n (%)	44	18 (40.9)	-	-	-

**Adjusted for multiple comparisons using false discovery rate (FDR).*

[a] Description symptoms reported in Supplementary Table 1

Abbreviations: TICS-M = Modified Telephone Interview for Cognitive Status, mRS = modified Rankin Scale, PCFS = Post-COVID functional scale, VAS = Visual Analogue Scale, HADS = Hospital Anxiety and Depression Scale, N/A = not applicable

Discussion

In our study, we showed that after adjusting for WMH volume hospitalized COVID-19 patients no longer exhibited higher PSMD and lower NDI values compared to controls. Our longitudinal study revelead decreased ODI in several regions of the WM in patients three months post-COVID hospitalization. Patients exhibited worse clinical outcomes compared to controls months after infection, but only decreased NDI at baseline was associated with worse performance on the Post-COVID-19 Functional Status scale after 12 months. In addition, we found no associations between diffusion metrics and long COVID. Our results suggest that other factors play a role in poorer clinical outcomes and long COVID in patients several months after COVID-19 infection.

We found lower WM integrity (indicated by higher values of PSMD and lower values of NDI) in patients compared to controls, which disappeared after adjusting for WMH. Several previous studies demonstrated that COVID-19 patients have alterations of the cerebral WM identified by DWI that are still present one year after infection. (115, 116) Some of these changes of WM integrity have been attributed to the SARS-CoV-2 infection. COVID-19 could potentially contribute to an increase of WM damage (loss of WM integrity and WMH), as microvascular pathology has been observed with the evidence of infected brain endothelial cells in the histopathology of the brains of COVID-19 patients. (117) However, due to the absence of MRI scans conducted before infection in these studies, as well as in our study, it is not possible to demonstrate or rule out the presence of pre-existing WM damage, for which these previous studies also did not correct in analysis. In our opinion, the alterations of the WM found in our study are more likely to be attributed to underlying WMH, since the differences disappeared after correction for this confounder in our study. Patients with COVID-19 had higher WMH volume at baseline, a hallmark of SVD, and were more frequently diagnosed with hypertension compared to the controls. Patients with cardiovascular risk factors, such as hypertension, are at an increased risk for admission for COVID-19. (2) This may explain the higher prevalence of hypertension, and consequently the higher prevalence of SVD and higher WMH volume in hospitalized COVID-19 patients compared to controls. Therefore, the observed differences are more likely to be explained by the presence of SVD and less likely by the SARS-CoV-2 infection itself.

We found an increase in ODI over time within patients. Lower ODI values in the cerebral WM indicate that the fibers are less dispersed within this voxel, which in most cases suggest higher WM microstructural integrity. (8) These findings of an increase in ODI might therefore be indicative of ongoing WM loss, which is not

captured by other diffusion metrics. This finding, however, contradicts the results of an earlier study that examined COVID-19 patients one and two years after their discharge. (101, 102) This study reported a decrease in ODI at the two year time point, suggesting a recovery of the WM over time. The observed discrepancies in results could possibly be attributed to different disease phases of the SARS-CoV-2 infection – MRI interval, potentially capturing different phases of the disease. Due to the lack of follow-up MRI in the control group, we were unable to confirm whether this finding could be considered as a deterioration specific to SARS-CoV-2 infection, or might be apparent as a ''normal" process in the brain. We acknowledge the potential influence of factors such as for example metabolic syndrome on our results, given the well-known associations between hypertension, hypercholesterolemia, and SVD which are related to WM integrity. This underscores the importance of considering these factors (such as metabolic syndrome) but also other factors such as asthma and COPD (beyond the scope of our current study) in future research.

Our study revealed that patients hospitalized for COVID-19 exhibited worse clinical outcomes during follow-up assessments after three and twelve months, compared to the control group. Here, we only found an association between lower NDI values (in the MRI shortly after discharge) and worsened PCFS (global functional outcome scale) at twelve months. In contrast to previous studies on DTI and clinical outcomes, we found no associations with cognitive scores. (101, 102) Firstly, this could be due to the fact that the cognitive assessment tool we used (TICS-m) may lack an in-depth evaluation of cognitive function. Second, the relatively small sample size may have led to a type II error. Lastly, the relative WMH damage to regions with abnormal NDI values might not have been enough to cause a noticeable, symptomatic decline in cognitive decline or other clinical outcomes during follow-up.

We observed reduced white matter integrity at baseline, indicated by lower NDI, in the anterior thalamic radiation (ATR), forceps minor and right inferior fronto-occipital fasciculus (IFOF) of patients. In addition, we found significant association between lower NDI of these regions and PCFS. Previous research has linked the ATR and forceps minor regions to executive function and processing speed. (2) In addition, WMH and reduced white matter integrity in ATR and IFOF were also associated with reduced processing speed. (3) Executive function and processing speed can influence overall functioning, for example possibly the PCFS; however, this relationship requires more thorough investigation using more extensive cognitive assessments.We observed no difference in WM integrity diffusion metrics between patients with long COVID and without long COVID and did not

find associations between baseline WM integrity and long COVID during follow-up. To date, no studies have explored this aspect; however, we hypothesize that our sample size may have been too small to detect a significant difference. Additionally, we did not perform MRI scans after 12 months, on which we determined the frequency of long COVID, which would have been particularly beneficial in this context. Given the lack of a clear association between clinical outcomes and diffusion metrics in a group with a high frequency of poor clinical outcomes, it is likely that factors such as respiratory problems at disease onset, length of hospital stay, and ICU admission, may play a more significant role in explaining the clinical status, including long COVID.

There are some limitations that need to be addressed. First, in patients, we performed baseline and post-discharge brain MRI after three months. Considering the time in which WM damage, including loss of microstructural WM integrity and WMH arises, a 3-month follow-up period may be too short to capture changes and it would have been valuable to include a follow-up MRI one year after infection for analysis and comparison with clinical outcomes. Second, our sample was relatively small, consisting of hospitalized patients varying from short stay to ICU admission and it included a small control group. Moreover, the smaller sample size that underwent follow-up MRI may further limit our ability to identify significant associations. Third, patients had not undergone brain MRI before COVID-19, making it impossible to adjudicate WM integrity assessed after the SARS-CoV-2 infection and establish the relationship with the actual infection. Fourth, the cognitive examination was limited. Extended cognitive evaluation may have an added value to identify impairment in specific cognitive domains or more subtle cognitive problems. Fifth, no follow-up imaging was performed in the control group which limits our comparibility of the WM integrity with the patients.

Conclusions

To conclude, our study revealed lower WM integrity in patients hospitalized for COVID-19 compared to healthy controls, which is likely explained by the presence of WMH and not by SARS-CoV-2 infection itself. After three months, we found deterioration of WM integrity within patients. WM integrity at baseline, or the changes after 3 months thereof, were generally not associated with poorer clinical outcomes after one year, suggesting that other factors play a more important role in the clinical outcomes and long COVID in patients after SARS-CoV-2 infection.

Supplementary materials

Supplementary table 1. Ongoing symptoms after COVID-19, reported by the WHO definition of Long COVID

Symptom reported	Follow-up 1 (3 months)	Follow-up 2 (12 months)
Fatigue, n (%)	20 (44.4)	14 (31.8)
Dyspnea, n (%)	19 (42.2)	12 (27.2)
Memory loss, n (%)	3 (6.67)	5 (11.4)
Difficulty finding words, n (%)	2 (4.44)	0 (0.0)
Myalgia, n (%)	1 (2.22)	1 (2.8)
Concentration problems, n (%)	1 (2.22)	4 (9.1)
Tinnitus, n (%)	1 (2.22)	0 (0.0)
Joint pain, n (%)	1 (2.22)	0 (0.0)
Numbness fingers, n (%)	1 (2.22)	0 (0.0)
Dizziness, n (%)	1 (2.22)	0 (0.0)
Sensory sentivity, n (%)	0 (0.0)	1 (2.3)

Supplementary table 2. Longitudinal changes of clinical outcomes between 3- and 12-month follow-up in COVID-19 patients

Outcomes		**3-month follow-up**		**12-month follow-up**	
Cognitive function	*n total*	*Mean (SD)*	*n total*	*Mean (SD)*	*p-value*
TICS-M score	45	36.22 (4.16)	42	35.24 (4.66)	0.301
Functional outcomes	*n total*	*n with outcome (%)*	*n total*	*n with outcome (%)*	
Modified Rankin Scale (mRS) (reference 0-1) ≥2-6, n (%)	45	15 (33.3)	44	16 (36.4)	0.706
PCFS (reference 0-1) ≥2-4, n (%)	45	22 (48.9)	44	22 (50.0)	0.739
Long COVID	45	28 (62.2)	44	18 (40.9)	***0.005***
Mood	*n total*	*Median [IQR]*	*n total*	*Median [IQR]*	
Hospital Anxiety and Depression Scale (HADS)					
HADS - Anxiety, median [IQR]	45	3.0 [1.0, 6.0]	44	3.5 [0.0, 7.0]	0.814
HADS - Depression, median [IQR]	45	2.0 [1.0, 7.0]	44	3.5 [1.0, 8.0]	0.357

Abbreviations: COVID-19 = coronavirus disease 2019, TICS-M = Modified Telephone Interview for Cognitive Status, mRS = modified Rankin Scale, PCFS = Post-COVID-19 Functional Status scale, HADS = Hospital Anxiety and Depression Scale

Supplementary table 3. Association between Peak width of skeletonized mean diffusivity (PSMD) at baseline and functional clinical outcomes after 3- and 12-month follow-up in COVID-19 patients

	Number of patients (%)	Model 1[a]		Model 2	
		OR (95% CI)	P-value[b]	OR (95% CI)	P-value
3-month follow-up					
Modified Rankin Scale (mRS)					
0-1	30 (66.7)	Reference		Reference	
2-6	15 (33.3)	1.29 [0.64 – 2.62]	0.557	3.04 [1.01 – 11.49]	0.396
Post-COVID-19 Functional Status scale (PCFS)					
0-1	23 (51.1)	Reference		Reference	
2-4	22 (48.9)	1.30 [0.67 – 2.67]	0.557	1.60 [0.59 – 4.91]	0.664
Long COVID	28 (62.2)	1.15 [0.59-2.43]	0.690	2.01 [0.69-7.40]	0.664
12-month follow-up					
mRS					
0-1	28 (63.6)	Reference		Reference	
2-6	16 (36.4)	2.01 [0.98 – 4.85]	0.360	2.32 [0.84 – 7.51]	0.605
PCFS					
0-1	22 (50.0)	Reference		Reference	
2-4	22 (50.0)	1.88 [0.91 – 4.61]	0.360	2.08 [0.76 – 6.82]	0.664
Long COVID	18 (40.9)	1.41 [0.71-2.94]	0.557	2.07 [0.77-6.39]	0.664

[a] Model 1: adjusted for age, model 2: adjusted for age + WMH volume

[b] All P-values are adjusted for multiple comparison using false discovery rate (FDR)

Abbreviations: PSMD = Peak width of Skeletonized Mean Diffusivity, mRS = modified Rankin scale, PCFS = Post-COVID-19 Functional Status scale, Long COVID, COVID = coronavirus disease

Supplementary table 4. Association between Neurity density index (NDI) at baseline and functional clinical outcomes after three and twelve months of follow-up in COVID-19 patients

	Number of patients (%)	Model 1[a]		Model 2	
		OR (95% CI)	P-value[b]	OR (95% CI)	P-value
3-month follow-up					
Modified Rankin Scale (mRS)					
0-1	30 (66.7)	Reference		Reference	
2-6	15 (33.3)	0.74 [0.37 – 1.40]	0.357	0.52 [0.22 – 1.14]	0.235
Post-COVID-19 Functional Status scale (PCFS)					
0-1	23 (51.1)	Reference		Reference	
2-4	22 (48.9)	0.55 [0.27 – 1.04]	0.312	0.47 [0.20 – 0.97]	0.235
Long COVID	28 (62.2)	0.61 [0.30-1.16]	0.312	0.44 [0.19-0.95]	0.235
12-month follow-up					
mRS					
0-1	28 (63.6)	Reference		Reference	
2-6	16 (36.4)	0.46 [0.21 – 0.91]	0.185	0.46 [0.19 – 0.99]	0.235
PCFS					
0-1	22 (50.0)	Reference		Reference	
2-4	22 (50.0)	0.26 [0.10 – 0.58]	***0.018***	0.23 [0.08 – 0.55]	***0.018***
Long COVID	18 (40.9)	0.55 [0.26-1.05]	0.312	0.45 [0.19-0.95]	0.235

[a] Model 1: adjusted for age, model 2: adjusted for age + WMH volume

[b] All P-values are adjusted for multiple comparison using false discovery rate (FDR)

Abbreviations: NDI = neurity density index, mRS = modified Rankin scale, PCFS = Post-COVID-19 Functional Status scale, COVID = coronavirus disease

Supplementary Table 5. Associations between Peak width of skeletonized mean diffusivity (PSMD) and Neurity density index (NDI) at baseline and cognition at 3- and 12-month follow-up

Diffusion metrics		TICS-M after 3 months		TICS-M after 12 months	
		Standardized β [95% CI]	p-value[b]	Standardized β [95% CI]	p-value
PSMD at baseline	Model 1[a]	-0.015 [-0.344 – 0.313]	0.925	-0.214 [-0.547 – 0.118]	0.200
	Model 2	-0.034 [-0.533 – 0.465]	0.890	-0.371 [-0.854 – 0.111]	0.127
NDI at baseline	Model 1	-0.020 [0.327 – 0.287]	0.897	0.168 [-0.145 – 0.481]	0.285
	Model 2	-0.027 [-0.389 – 0.335]	0.881	0.189 [-0.174 – 0.553	0.298

[a] Model 1: adjusted for age + education (7 levels), model 2: adjusted for age + education (7 levels) + white matter hyperintensities volume

[b] All P-values are adjusted for multiple comparison using false discovery rate (FDR)

Abbreviations: PSMD = Peak width of skeletonized mean diffusivity, NDI = neurity density index, TICS-M = Modified Telephone Interview for Cognitive Status

Supplementary Table 6. Associations between Peak width of skeletonized mean diffusivity (PSMD) and Neurity density index (NDI) at baseline and mood disorder symptoms at 3- and 12-month follow-up

Diffusion metrics		HADS-Anxiety after 3 months		HADS-Depression after 3 months	
		Standardized β [95% CI]	p-value[b]	Standardized β [95% CI]	p-value
PSMD at baseline	Model 1[a]	0.047 [-0.287 – 0.380]	0.824	0.037 [-0.295 – 0.368]	0.824
	Model 2	0.356 [-0.136 – 0.848]	0.339	0.140 [-0.365 – 0.644]	0.773
NDI at baseline	Model 1	-0.198 [-0.502 – 0.107]	0.339	-0.190 [-0.493 – 0.113]	0.339
	Model 2	-0.370 [-0.715 – -0.024	0.296	-0.283 [-0.638 – 0.072]	0.339
Diffusion metrics		**HADS-Anxiety after 12 months**		**HADS- Depression after 12 months**	
		Standardized β [95% CI]	**p-value**	**Standardized β [95% CI]**	**p-value**
PSMD at baseline	Model 1	0.020 [-0.334 – 0.374]	0.988	-0.003 [-0.361 – 0.355]	0.988
	Model 2	0.211 [-0.296 – 0.718]	0.648	0.060 [-0.458 – 0.579]	0.988
NDI at baseline	Model 1	-0.206 [-0.523 – 0.112]	0.396	-0.267 [-0.583 – 0.050]	0.256
	Model 2	-0.337 [-0.695 – 0.021]	0.256	-0.376 [-0.736 – -0.01]	0.256

[a] Model 1: adjusted for age, model 2: adjusted for age + white matter hyperintensities volume

[b] All P-values are adjusted for multiple comparison using false discovery rate (FDR)

Abbreviations: PSMD = Peak width of skeletonized mean diffusivity, NDI = neurity density index, HADS = Hospital Anxiety and Depression Scale

Supplementary table 7. Association between Peak width of skeletonized mean diffusivity (PSMD) and Neurity density index (NDI) at baseline and Visual Analogue Scale (VAS) at 12-month follow-up

Diffusion metrics		Visual Analogue Scale (VAS) after 12 months	
		Standardized β [95% CI]	p-value[b]
PSMD at baseline	Model 1[a]	-0.225 [-0.575 – 0.126]	0.270
	Model 2	-0.130 [-0.636 – 0.377]	0.608
NDI at baseline	Model 1	0.344 [0.035 – 0.652]	0.120
	Model 2	0.319 [-0.039 – 0.677]	0.158

[a] Model 1: adjusted for age, model 2: adjusted for age + white matter hyperintensities volume

[b] All P-values are adjusted for multiple comparison using false discovery rate (FDR)

Abbreviations: PSMD = Peak width of skeletonized mean diffusivity, NDI = neurity density index, VAS = Visual Analogue Scale

Chapter 6

Higher blood pressure variability during hospitalization is associated with lower cerebral white matter integrity in COVID-19 patients

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Higher blood pressure variability during hospitalisation is associated with lower cerebral white matter integrity in COVID-19 patients
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Abstract

Background

High blood pressure variability (BPV) is associated with cerebrovascular damage and dementia, but it is unknown whether high short-term BPV during hospitalization is also associated with cerebral white matter (WM) damage. We examined whether BPV, measured in-hospital using continuous monitoring, is associated with WM microstructural integrity in COVID-19 patients.

Methods

We included hospitalized COVID-19 patients from the CORONavirus and Ischemic Stroke (CORONIS) study who underwent continuous vital signs monitoring using a wearable device during general ward admission and MRI-DWI shortly after discharge. Systolic BPV was calculated as Average Real Variability (ARV) and Coefficient of Variation (CV) with 1-, 5- and 20-minute intervals. WM integrity was assessed with diffusion tensor imaging (DTI). Associations between BPV and WM integrity were examined with linear regression adjusted for age, mean systolic blood pressure, number of measurements and type of respiratory support.

Results

We included 47 COVID-19 patients (mean age: 59.6 years). BP was measured 6306±4343 times per patient (median admission: 11 days [IQR 7.5-15.0]). Both higher ARV and CV were associated with lower fractional anisotropy (FA) (ARV1: β=-0.40, p=0.010; CV1: β=-0.33, p=0.026) while higher CV was associated with higher peak width of skeletonized mean diffusivity (PSMD) after adjustment for confounders (CV1: β=0.28, p=0.038). Correction for WM hyperintensities did not change these results.

Conclusions

High BPV during hospitalization is associated with lower WM integrity in COVID-19 patients, suggesting that BPV may be a target for preserving WM integrity and improving cerebrovascular outcomes. Our findings need validation in hospitalized patients without COVID-19 to examine generalizability.

Key words

Cerebral small vessel disease, Blood pressure variability, White matter hyperintensity, Magnetic Resonance Imaging, COVID-19

Introduction

Hypertension is a major risk factor for cardiovascular disease and dementia. (118) Accumulating evidence suggests that large fluctuations in blood pressure (BP), often referred to as blood pressure variability (BPV), increase the risk of cardiovascular disease, dementia and ischemic stroke independent of mean BP. (119-123) Several systematic reviews have linked BPV with MRI markers of cerebral small vessel disease (SVD) such as white matter hyperintensities (WMH), but also with lower white matter (WM) integrity, assessed with diffusion tensor imaging (DTI). (124-126) Areas of WM with reduced microstructural integrity are shown to converse into WMH, which are associated with worse cognitive and functional outcomes. (127) Lower WM integrity after COVID-19 infection has been reported, which is possibly associated with worse outcomes, but the association with BPV remains unknown. (128) Investigation of this relationship can provide insights into the etiology of adverse outcomes or help with therapeutic strategies for both the general population and those affected by COVID-19.

BPV includes short-term (during 24 hours, including beat-to-beat, minute-to-minute and hour-to-hour), mid-term (multiple days) and long-term (weeks, months, or years) variability with different underlying mechanisms and clinical consequences. (123) Previous studies mainly investigated relationships between WM integrity and BPV measured over months or years. The clinical applicability of this approach is limited, since data measured over several years is often not available. The emergence of devices that can reliably measure BP continuously has facilitated the assessment of (short-term) BPV over prolonged durations, both in clinical settings and at home, offering great promise for monitoring and management of BP and BPV. (129)

BPV assessment during hospital admission has emerged as a valuable tool. A recent study in over 80.000 hospitalized patients showed that high BPV during hospital admission is linked to increased risk of dementia within two years. (130) In hospitalized stroke and COVID-19 patients, BPV was associated with in-hospital mortality and ICU admission. (12, 131) How increased BPV during hospitalization affects the brain and if this is clinically relevant, especially in COVID-19 patients, remains unclear. Therefore, our objective was to investigate whether high BPV, measured using continuous monitoring during hospitalization in patients with COVID-19 is associated with lower microstructural integrity of the brain.

Methods

Study design

This study was part of the CORONavirus and Ischemic Stroke (CORONIS) study, a multicenter, prospective observational study investigating MRI markers of cerebrovascular disease and long-term clinical outcomes in patients hospitalized with COVID-19. A detailed study protocol has been published previously. (132) The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study on 01-04-2021 (NL75780.091.20). All patients provided written informed consent.

Study population and procedures

In the CORONIS study, adult patients hospitalized between April 2021 and September 2022 in one of three centers in the Netherlands because of laboratory-confirmed SARS-CoV-2 infection were included (**Figure 1**). Exclusion criteria comprised: MRI and contrast contraindications, pregnancy, limited life expectancy (<3 months), major disease interfering with study participation or follow-up or inability to provide informed consent. For the current analysis, we only included participants from the Radboudumc hospital (n=73) who wore a non-invasive wearable vital signs monitoring system (ViSi mobile) during hospital admission and underwent multi-shell MRI diffusion weighted imaging (DWI), which was not performed in the other participating centers. Patients with clinically overt stroke were excluded from this analysis. Demographic and lifestyle information, education, medical history and data regarding hospital admission were collected at the initial assessment as reported previously. (132)

Blood pressure (variability) monitoring and assessment

BP was continuously monitored on the general ward using an FDA-cleared non-invasive wearable vital signs monitoring system (ViSi mobile, Sotera Wireless LTD, San Diego, California) of which the validity has been described previously. (133) BP was measured cuffless by a pulseoxy sensor with calibration using an upper arm cuff two times a day. Pseudonymized data were retrieved from the research storage (Sotera's Amazon Web server) for analysis. BP readings that were considered potentially non-physiologic (systolic BP > 300 or <40 mmHg) were excluded. ViSiMobile was not available in the intensive care unit (ICU), so in case of ICU admission during hospitalization, BP data was only used until transfer to the ICU for better comparison of data. To ensure accuracy, we excluded three minutes before and after VisiMobile calibration from the BPV calculations.

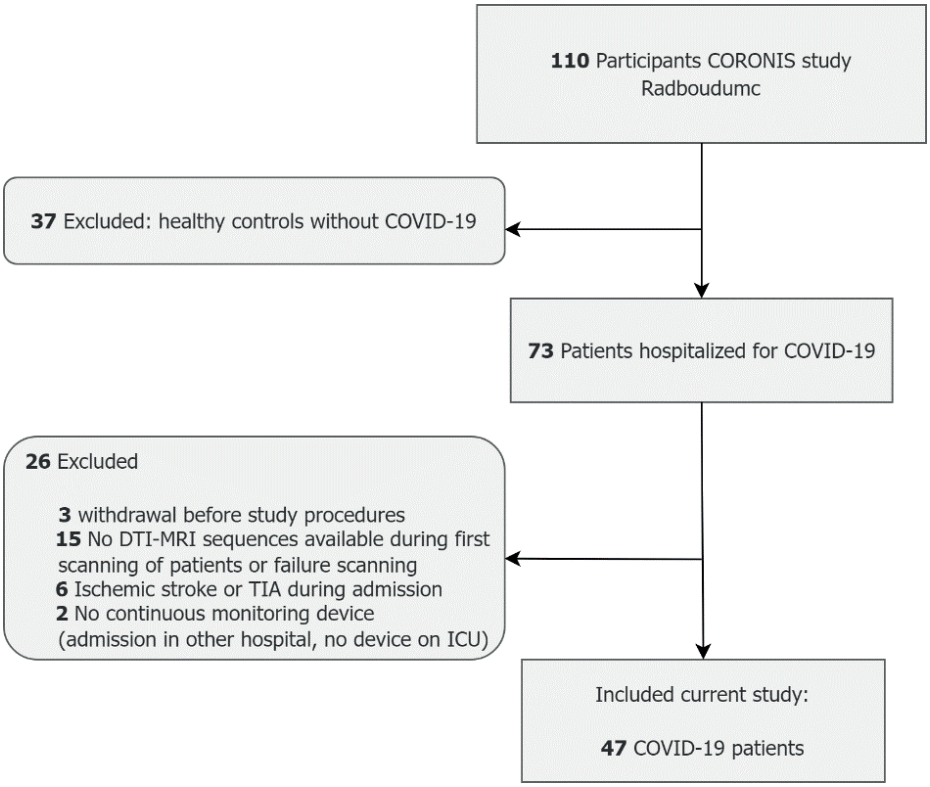

Figure 1. Flowchart of participant inclusion

Abbreviations: CORONIS = CORONavirus and Ischemic Stroke, TIA = transient ischemic attack, ICU = intensive care unit

Two indices of BPV were calculated: Coefficient of Variation (CV) and the Average Real Variability (ARV), to examine consistency of results independent of BPV metric since there is currently no consensus on the most reliable metric to use. We used the following formulas to calculate these measures:

$$CV = \left(\frac{SD}{mean}\right) \times 100$$

$$ARV = \frac{\sum |BP_{k+1} - BP_k|}{n}$$

where n is the number of valid BP measurements and k is the order of measurements. Both measures were calculated across various time intervals to examine the robustness of this measure in continuous data. First, they were calculated using minute-to-minute BP measurements. Additionally, calculations were performed for 5-minute intervals, considering values only at the first and last minutes within each 5-minute span, for example 12:00, 12:05, 12:10 and so on. Similarly, analyses were conducted for 20-minute intervals.

MRI protocol

Brain MRI scans were conducted during hospital stay or shortly after discharge (<3 months after positive PCR-test). The imaging details have been described previously. (132) MR images were acquired on a 3T MRI (Siemens, Prisma), using the following scan protocol: 3D T1 weighted (T1W) space fatsat (0.9ms isotropic voxel size, repetition time (TR) = 700ms, Echo Time (TE) = 9mm); 3D fluid-attenuated inversion recovery (FLAIR) (1mm isotropic voxel size, TR=500ms, TE=394ms); multi-shell DWI (80 diffusion-weighted directions (40 × b=1,000, and 40 × b=2,000s/mm2), 6 × b=0 images, 2.0mm isotropic voxels, TR=4600ms, TE=80ms).

MRI processing and outcomes

We pre-processed diffusion MRI data starting with denoising and Gibbs artifacts. Additionally, we corrected for head motion, eddy currents-induced distortion, susceptibility-induced distortion (top-up) and intensity bias using the MRtrix 3.0 software, Functional Magnetic Resonance Imaging of the Brain Software Library (FSL) software and Advanced Normalization Tools. (99, 105, 106) Top-up was conducted based on synthesized b0 image from the T1 image using Synb0-DISCO, since no reversed phase-encoding b0 DWI image was available. (107) We calculated two DTI metrics with the pre-processed diffusion data (only b=0 and 1000 volumes): 1) mean diffusivity (MD) and 2) fractional anisotropy (FA). Tract-Based Spatial Statistics (TBSS) pipeline within FSL was employed to create a WM skeleton representing the centers of all tracts shared across the population. (106, 109, 110) Next, MD was projected into the WM skeleton using the 'tbss_non_FA' function within TBSS tool of FSL, resulting in aligned maps of MD. Next, this WM skeleton was used to extract the mean MD/FA values, representing the global average WM microstructural abnormalities for each participant. In addition, we calculated Peak width of Skeletonized Mean Diffusivity (PSMD) values using the PSMD tool (http://www.psmd-marker.com/). (108) For segmentation and calculation of WMH volumes, we used the automated k-nearest neighbors algorithm (UBO Detector) using bias-corrected T1 and FLAIR images. (104) The segmentations were visually

reviewed for errors and artifacts. We did not adjust for intracranial volume (ICV), since the UBO detector calculated WMH volumes in SPM's DARTEL space.

Statistical analysis

Baseline characteristics are presented as numbers and proportions (%) and continuous measurements are reported as mean (SD) or median (IQR), based on whether they were normally distributed. To assess whether the three intervals (1/5/20 minutes) measures consistently represent BPV or if they differ significantly across different intervals, we examined the intercorrelations among the BPV metrics using correlation coefficients. Univariable and multivariable linear regression was used to analyse the relationship between BPV measures and continuous MRI diffusion outcomes (FA, MD, PSMD). Multivariable models were adjusted for baseline age, mean systolic BP during hospitalization, number of BP measurements and type of ventilation. Type of ventilation was categorized into three categories thought to reflect the severity of COVID-19: 1) non-invasive respiratory support (nasal cannula or non-rebreathing mask), 2) non-invasive ventilation (Optiflow) and 3) invasive ventilation (intubation). Non-invasive ventilation was also available on the general COVID ward instead of ICU only. Several sensitivity analyses were conducted. First, we used multivariable linear regression with additional adjustment for WMH volume to correct for pre-existing MRI markers of SVD. Second, we examined effect modification of WMH volume and pre-existing hypertension using interaction terms. Missing data was not imputed. We considered two-sided p-values less than 0.05 statistically significant. Data were analysed with R version 4.3.1.

Results

We included 47 COVID-19 patients with a mean age of 59.6 years (SD 12.9) of whom 16 (34.0%) were female (**Table 1**). Patients were hospitalized with COVID-19 infection for 11 days [median, IQR 7.5-15.0]. During this hospitalization, BP was measured on average 6306 times (SD 4343). Fourteen participants (30%) were admitted to the ICU. Median time between positive SARS-CoV-2 PCR test and baseline MRI was 39 days [IQR 27.0, 51.0].

Table 1. Baseline characteristics

	Patients
n, (%)	47 (100.0)
Age in years, mean (SD)	59.6 (12.9)
Women, n (%)	16 (34.0)
Education, n (%)	7 (14.9)
Low (≤ low-level secondary education)	26 (55.3)
Middle (average-level secondary education	14 (29.8)
High (≥high level secondary education/university)	
Duration of hospital admission in days, median [IQR]	11.0 [7.50, 15.0]
Time between admission and baseline MRI, median [IQR]	32.0 [21.5, 42.5]
Highest required respiratory/ventilation therapy during admission, n (%)	
Nasal cannula, oxygen mask, non-rebreathing mask	24 (51.1)
Non-invasive ventilation (Optiflow)	15 (31.9)
Invasive ventilation (intubation)	8 (17.0)
ICU admission, n (%)	14 (29.8)
Cardiovascular risk factors	
Hypertension, n (%)	19 (40.4)
BMI in kg/m^2, mean (SD)	28.50 (4.34)
Hyperlipidemia, n (%)	20 (42.6)
Diabetes Mellitus, n (%)	9 (19.1)
(Previous) smoker, n (%)	27 (57.4)
Pulmonary diseases (COPD/asthma), n (%)	21 (44.7)
Antihypertensive medication during admission, n (%)	19 (40.4)
Type of antihypertensive medication used during admission (multiple options are possible per patient), n (%)	
Angiotensin-converting enzyme inhibitor (ACE-i)	12 (25.5)
Angiotensin receptor blocker (ARB)	4 (8.5)
β-blocker	13(27.7)
Calcium channel blocker (CCB)	14 (29.8)
Diuretic	8 (17.0)
Blood pressure metrics	
Days of continuous blood pressure monitoring, median [IQR]	6 [4-10]
Systolic blood pressure during hospitalization (mmHg), mean (SD)	125.12 (13.6)
Average Real Variability (ARV) 1-minute interval, mean (SD)	2.53 (0.90)
ARV 5-minutes interval, mean (SD)	5.04 (1.67)
ARV 20-minutes interval, mean (SD)	7.34 (2.26)
Coefficient of Variation (CV) 1-minute interval, mean (SD)	11.7 (2.8)
CV 5-minutes interval, mean (SD)	11.8 (2.8)
CV 20-minutes interval, mean (SD)	11.9 (2.9)
White matter hyperintensities volume (WMH) mm^3, median [IQR]	1.337 [0.845-3.733]

Abbreviations: ARB = angiotensin receptor blocker, ACE-I = angiotensin-coverting enzyme inhibitor, CCB = calcium channel blocker, BMI = Body Mass Index, ICU = intensive care unit, COPD = chronic obstructive pulmonary disease, ARV = Average Real Variability, CV = Coefficient of Variation, WMH = White matter hyperintensities, N/A = not applicable.

In **Figure 2**, correlations between CV and ARV measured over different intervals are shown. CV measured over 1, 5 or 20 minutes are very highly correlated (r > 0.98). ARV1 and 5 are strongly correlated (r=0.97), but correlate less strong with ARV20 (r=0.75/r=0.78). CV and ARV are only moderately correlated (correlation coefficients between 0.35-0.43).

Figure 2. Correlations between BPV measures over different timespans.
Abbreviations: CV = Coefficient of Variation, ARV = Average Real Variability

Association BPV and DTI outcomes

All measures of BPV were associated with lower FA in both univariable and multivariable models, as shown in **Table 2**. Only ARV20 was associated with higher MD after adjusting for confounders (**Table 2**). All measures of CV were associated with higher PMSD after adjusting for confounders, while no significant association between ARV and PSMD was observed (**Table 2**).

BPV and WMH volume

To examine if the association between BPV and DTI outcomes was independent of brain macrostructural changes, we performed an additional analysis adjusting for WMH volume. Results are shown in **Supplementary Table 1**. Associations between all measures of BPV and FA are significant after additional adjustment for WMH volume. ARV1/5/20 was associated with higher MD, while ARV1 and CV1/5/20 was associated with higher PSMD after adjustment for WMH (**Supplementary Table 1**).

Table 2. Association of BPV metrics during hospital stay with white matter microstructural integrity metrics

	FRACTIONAL ANISOTROPY (FA)			
BPV Metrics	Unadjusted Standardized β [95% CI]	p-value	Adjusted* Standardized β [95% CI]	p-value
ARV1	-0.32 [-0.60 - -0.03]	***0.029***	-0.40 [-0.70 - -0.10]	***0.010***
ARV5	-0.39 [-0.67 - -0.12]	***0.006***	-0.44 [-0.73 - -0.15]	***0.004***
ARV20	-0.42 [-0.69 - -0.15]	***0.003***	-0.47 [-0.75 - -0.19]	***0.002***
CV1	-0.38 [-0.66 – -0.11]	***0.008***	-0.33 [-0.61 - -0.04]	***0.026***
CV5	-0.41 [-0.69 – -0.14]	***0.004***	-0.36 [-0.64 – -0.08]	***0.014***
CV20	-0.43 [-0.70 – -0.16]	***0.003***	-0.38 [-0.67 - -0.10]	***0.008***
	MEAN DIFFUSIVITY (MD)			
BPV Metrics	Unadjusted Standardized β [95% CI]	p-value	Adjusted* Standardized β [95% CI]	p-value
ARV1	0.32 [0.03 – 0.60]	***0.033***	0.23 [-0.06 – 0.53]	0.122
ARV5	0.38 [0.10 – 0.66]	***0.009***	0.26 [-0.03 – 0.55]	0.074
ARV20	0.41 [0.14 – 0.69	***0.004***	0.31 [0.02 – 0.59]	***0.034***
CV1	0.17 [-0.13 – 0.46]	0.256	0.14 [-0.14 – 0.42]	0.315
CV5	0.20 [-0.09 – 0.49]	0.179	0.17 [-0.11 – 0.45]	0.224
CV20	0.22 [-0.07 – 0.51]	0.137	0.20 [-0.08 – 0.48]	0.152
	PEAK WIDTH OF SKELETONIZED MEAN DIFFUSIVITY (PSMD)			
BPV Metrics	Unadjusted Standardized β [95% CI]	p-value	Adjusted* Standardized β [95% CI]	p-value
ARV1	0.20 [-0.10 – 0.49]	0.187	0.12 [-0.19 – 0.42]	0.447
ARV5	0.24 [-0.05 – 0.53]	0.104	0.12 [-0.18 – 0.42]	0.424
ARV20	0.26 [-0.03 – 0.55]	0.078	0.15 [-0.14 – 0.45]	0.296
CV1	0.36 [0.09 – 0.64]	***0.012***	0.28 [0.02 – 0.55]	***0.038***
CV5	0.39 [0.11 – 0.66]	***0.007***	0.31 [0.04 – 0.58]	***0.024***
CV20	0.41 [0.14 – 0.69]	***0.004***	0.35 [0.09 – 0.61]	***0.011***

**Adjusted for age, mean systolic BP, number of BP measurements and respiratory support*

The interaction term for BPV and WMH was not significant in any models assessing BPV measures and FA or MD as outcome (**Supplementary Table 2**). However, there was a significant interaction between BPV*WMH volume in models assessing CV and PSMD, indicating that the association between CV and PSMD is stronger with greater WMH volumes. The BPV*hypertension interaction term is only significant in the model assessing association between CV20 and PSMD (**Supplementary Table 3**). No other significant differences in the relationship between BPV and WM integrity were observed between individuals with and without hypertension.

Discussion

In this study, we showed that high BPV measured with continuous BP monitoring was associated with lower WM microstructural integrity (i.e. lower FA and higher PSMD) in hospitalized COVID-19 patients, independent of WMH. Our findings indicate that both CV and ARV as measurements of BPV are associated with WM damage. In the population-based Rotterdam Study, higher visit-to-visit BPV was significantly associated with lower FA and higher MD in the whole WM, while others have found lower FA only in the hippocampus and not in the whole WM. (126, 134) We expand these findings by demonstrating that short-term BPV measured during hospitalization because of COVID-19 is also associated with worse WM integrity.

BPV was associated with lower FA and higher PSMD, but not with MD. While FA quantifies the degree of anisotropy or directionality of water diffusion, known to be higher in organized WM pathways, MD is a measure of diffusion in each direction. (135) It is possible that alterations in tissue microstructure affect directionality of water diffusion (i.e. decreased FA) but do not substantially increase overall water diffusion (i.e. unchanged MD). (136) In PSMD, histogram analysis is used to capture diffuse pathological changes based on the distribution of MD values across the brain. (108) This is suggested to be especially sensitive to microstructural damage observed in SVD, which may explain why we do observe an association with PSMD but not with MD. (108) Overall, these changes in DTI measures suggest that high BPV is associated with lower integrity of the WM.

The relationship between COVID-19 infection, BPV and cerebrovascular damage is complex and there are several potential explanations for our results. First, infection with COVID-19 may cause an excessive immune reaction in some patients which causes a cytokine storm. (137) This cytokine storm can disturb several physiological systems, including BP regulation, and cause higher BPV. (138) In turn, high BPV caused by COVID-19 may damage the brain by causing mechanical stress on vessel walls, leading to greater arterial stiffness and therefore less dampening of blood flow. This may lead to microvascular damage. Second, COVID-19 infection may cause cerebral damage, in which case BPV may not have a causal effect on the brain but is simply a marker of worse COVID-19 infection. Much remains unknown about the effects of COVID-19 on the brain, but some studies report lower DTI metrics after COVID-19 infection. (139) However, as discussed previously, higher BPV is also associated with cerebral damage and worse clinical outcomes in many other studies evaluating individuals without COVID-19, for example with ischemic stroke. (124, 140) Moreover, lower BPV in COVID-19 patients was associated with less mortality,

suggesting that BPV does have an adverse effect, although this was a retrospective study. (138) Third, reverse causality is possible, where subclinical brain damage may cause central autonomic dysfunction, resulting in higher BPV. It is possible that our participants already had cerebrovascular damage prior to hospitalization, causing higher BPV. This is especially relevant if we consider that hospitalized COVID-19 patients often have a worse cardiovascular risk profile. (141) However, our results remained statistically significant after adjusting for WMH volumes, which results from long-term cerebrovascular damage, and there was no interaction with WMH volumes. This suggests that the association between BPV and microstructural integrity is independent of pre-existing cerebral damage. Despite the absence of MRI scans prior to COVID-19 to conform this, our findings align with a previous study that also describe this association independent of WMH. (126)

Cuffless continuous BP monitoring systems, such as ViSiMobile used in this study, have great potential for measuring BPV. (142) As BP is highly dynamic and regular BP measurements only provide snapshots of BP in static conditions, cuffless devices can be used to detect BP changes during daily activities and sleep. However, the European Society of Hypertension does not recommend using cuffless devices based on pulse wave analysis to measure BPV yet since this technique is inadequately validated. (123) Nonetheless, continuous vital sign monitoring is already integrated in many hospitals without requiring additional effort from medical staff. When cuffless BP measurement is further adopted, these devices could become the preferred method to assess average BP levels and BPV.

Many different indices can be used to quantify BPV and there is currently no gold standard, especially in continuous cuffless BP monitoring. (143, 144) The CV is commonly used but does not account for the order in which the BP measurements were obtained. (123) ARV may therefore more accurately reflect the time series nature of BP data and is more commonly used in ambulatory BP monitoring. (123) While both ARV and CV were associated with lower FA, ARV was associated with higher MD while CV was associated with higher PSMD in our study. ARV and CV indices were only moderately correlated, which suggests that these indices capture different aspects of BPV. It remains to be determined which BPV index should be used in which context. Furthermore, we showed a consistent BPV based on CV and ARV calculated during any time interval (1/5/20 minutes). This is useful information for clinical practice, where minute-to-minute data is often not available.

There are some limitations we need to address. First, COVID-19 may affect BPV during admission and WM integrity itself. Although we adjusted for type of ventilation during admission as a reflection of COVID-19 severity, it is crucial to validate our findings in a distinct study population without COVID-19 or other viral infections to examine the generalizability of our findings. Second, we have a relatively small sample size and our findings should be confirmed in a larger cohort. Third, based on our results we cannot conclude if there is a causal relationship. This should be examined in longer studies with sequential MRI scans with BPV measured in between. Fourth, we measured BP using pulse wave analysis, and as described earlier, this method has not been adequately validated to measure BP. Finally, we did not adjust our p-values for multiple comparisons, since the measures and outcomes we analysed are inherently related and not completely independent. Applying such a correction could therefore increase the risk of type II errors, thereby masking real effects. However, we believe this is not an issue as our results show the same trends across all measures.

Future research should examine if our findings apply to hospitalized patients without COVID-19. If so, BPV during hospitalization could help identify those at risk of brain damage or ICU admission. BP management in hospitalized patients is currently focused on the absolute BP values whereas our study emphasizes the potential additional importance of controlling BP fluctuations to achieve stable BP. There is evidence that calcium channel blockers and non-loop diuretics are most effective in reducing long-term BP. (145) In hospitalized COVID-19 patients, use of CCBs was associated with lower BPV and maintaining low BPV had a protective effect on mortality. (138) It remains unknown if lowering BPV also has a protective effect on brain integrity. If so, an optimal therapeutic target for BPV would need to be determined, since some BP fluctuations are normal in healthy individuals.

In conclusion, we demonstrated that high BPV, measured using continuous cuffless BP monitoring during hospital admission for COVID-19, was associated with lower microstructural integrity of the WM in the brain. If a causal relationship would be established, continuous BP monitoring during acute care hospitalization for determining BPV may be useful to identify patients at risk of cerebral damage.

Supplementary materials

Supplementary Table 1. Association of BPV metrics with white matter microstructural integrity, additionally adjusted for white matter hyperintensities.

	FRACTIONAL ANISOTROPY (FA)		MEAN DIFFUSIVITY (MD)		PEAK WIDTH OF SKELETONIZED MEAN DIFFUSIVITY (PSMD)	
BPV Metrics	**Standardized β [95% CI]**	**p-value**	**Standardized β [95% CI]**	**p-value**	**Standardized β [95% CI]**	**p-value**
ARV1	-0.44 [-0.72 – -0.11]	***0.002***	0.28 [0.02 – 0.54]	***0.035***	0.19 [0.01 – 0.38]	***0.041***
ARV5	-0.47 [-0.77 - -0.21]	***0.001***	0.30 [0.05 – 0.55]	***0.021***	0.18 [0.00 – 0.36]	0.053
ARV20	-0.47 [-0.75 - -0.19]	***0.002***	0.29 [0.04 – 0.54]	***0.022***	0.13 [-0.05 – 0.32]	0.151
CV1	-0.29 [-0.57 – -0.02]	***0.034***	0.10 [-0.15 – 0.35]	0.417	0.22 [0.06 – 0.39]	***0.010***
CV5	-0.33 [-0.59 – -0.06]	***0.019***	0.13 [-0.13 – 0.38]	0.315	0.24 [0.07 – 0.40]	***0.006***
CV20	-0.34 [-0.61 – -0.07]	***0.016***	0.14 [-0.11 – 0.39]	0.273	0.25 [0.08 – 0.41]	***0.004***

Supplementary Table 2. Results for interaction term BPV metric*WMH volume.

	FRACTIONAL ANISOTROPY (FA)		MEAN DIFFUSIVITY (MD)		PEAK WIDTH OF SKELETONIZED MEAN DIFFUSIVITY (PSMD)	
BPV metric	**Standardized β [95% CI]**	**p-value**	**Standardized β [95% CI]**	**p-value**	**Standardized β [95% CI]**	**p-value**
ARV1	-0.24 [-0.67-0.18]	0.251	0.25 [-0.15-0.65]	0.214	-0.15[-0.44-0.13]	0.288
ARV5	-0.23 [-0.64-0.17]	0.248	0.26 [-0.12-0.64]	0.174	-0.11[-0.39-0.17]	0.419
ARV20	-0.24 [-0.63-0.15]	0.220	0.24 [-0.13-0.61]	0.197	-0.03[-0.31-0.25]	0.831
CV1	-0.10 [-0.33-0.14]	0.408	0.12 [-0.10-0.34]	0.266	0.15 [0.02-0.29]	***0.026***
CV5	-0.08 [-0.31-0.15]	0.479	0.11 [-0.10-0.32]	0.313	0.15 [0.02-0.28]	***0.025***
CV20	-0.07 [-0.28-0.15]	0.547	0.09 [-0.11-0.30]	0.370	0.15 [0.02-0.27]	***0.021***

Supplementary Table 3. Results for interaction term BPV metric*hypertension status.

	FRACTIONAL ANISOTROPY (FA)		**MEAN DIFFUSIVITY (MD)**		**PEAK WIDTH OF SKELETONIZED MEAN DIFFUSIVITY (PSMD)**	
BPV metric	**Standardized β [95% CI]**	**p-value**	**Standardized β [95% CI]**	**p-value**	**Standardized β [95% CI]**	**p-value**
ARV1	0.19 [-0.12-0.49]	0.222	-0.16 [-0.46-0.13]	0.275	-0.18 [-0.49-0.12]	0.233
ARV5	0.12 [-0.21-0.44]	0.475	-0.11 [-0.43-0.21]	0.500	-0.15 [-0.48-0.19]	0.377
ARV20	-0.06 [-0.38-0.26]	0.703	-0.06 [-0.38-0.26]	0.703	-0.04 [-0.37-0.29]	0.798
CV1	0.02 [-0.28-0.32]	0.888	0.04 [-0.26-0.33]	0.806	0.24 [-0.04-0.51]	0.087
CV5	0.02 [-0.28-0.31]	0.910	0.05 [-0.24-0.34]	0.748	0.26 [-0.01-0.53]	0.059
CV20	0.01 [-0.28-0.30]	0.946	0.06 [-0.23-0.34]	0.693	0.27 [0.01-0.53]	***0.046***

Part IV

PET-CT imaging in COVID-19 patients

Chapter 7

Gallium-68 labelled RGD PET/CT imaging of endothelial activation in COVID-19 patients

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Gallium-68 labelled RGD PET/CT imaging of endothelial activation in COVID-19 patients

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Abstract

In coronavirus disease 2019 (COVID-19), endothelial cells play a central role and inadequate response is associated with vascular complications. PET imaging with gallium-68 labelled RGD-peptide (^{68}Ga-RGD) targets $\alpha_v\beta_3$ integrin expression which allows quantification of endothelial activation. In this single-center, prospective observational study, we included ten hospitalized patients with COVID-19 between October 2020 and January 2021. Patients underwent ^{68}Ga-RGD PET/CT followed by iodine mapping of lung parenchyma. CT-based segmentation of lung parenchyma, carotid arteries and myocardium was used to quantify tracer uptake by calculating standardized uptake values (SUV). Five non-COVID-19 patients were used as reference. The study population was 68.5 (IQR 52.0-74.5) years old, with median oxygen need of 3 l/min (IQR 0.9-4.0). ^{68}Ga-RGD uptake quantified as SUV±SD was increased in lungs (0.99±0.32 *versus* 0.45±0.18, $p<0.01$) and myocardium (3.44±1.59 *versus* 0.65±0.22, $p<0.01$) of COVID-19 patients compared to reference but not in the carotid arteries. Iodine maps showed local variations in parenchymal perfusion but no correlation with SUV. In conclusion, using ^{68}Ga-RGD PET/CT in COVID-19 patients admitted with respiratory symptoms, we demonstrated increased endothelial activation in the lung parenchyma and myocardium. Our findings indicate the involvement of increased and localized endothelial cell activation in the cardiopulmonary system in COVID-19 patients.

Introduction

Coronavirus disease 2019 (COVID-19), caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), starts in the respiratory tract followed by a varying course and severity of disease, ranging from asymptomatic to multiple organ failure. (15, 146, 147) The distribution of the angiotensin converting enzyme 2 (ACE2) receptor plays a crucial role in the course of the disease because it is known that the receptor is a target for cellular entry for the coronavirus. (72, 148, 149) The ACE2 receptor is expressed on pneumocytes and endothelial cells (ECs) in the lungs, as well as on ECs in the vascular system. (72, 148, 149) The presence of viral particles has been demonstrated in multiple organs such as the lungs, brain, and myocardium. (150-153) It has been reported that infection by SARS-CoV-2 can modulate the expression of ACE2 receptor. (72, 149) Subsequently, this can result in loss of the inhibitory role of ACE2 on the activation of local acting vasoactive peptides and the initiation of an inflammatory cascade, including the recruitment of immune cells and vascular leakage. (72, 149, 154, 155) Increased endothelial activation also results in a procoagulant state that could lead to arterial and venous thrombi, causing pulmonary embolism (PE) and ischemic stroke. (72, 73) These events are frequently observed in COVID-19 patients and are strongly associated with poor outcomes. (72, 156) The increased incidence of PE and ischemic stroke in COVID-19 as compared to other viral infections hints towards systemic involvement of ECs, rather than local processes. (14, 157, 158) Therefore, endothelial activation and dysfunction might be a critical step in the pathogenesis of COVID-19 and may explain the observed phenomena of a procoagulant state, tissue oedema and ischemic events in multiple organ systems. (73, 149, 156)

In vivo localization and quantification of endothelial activation in COVID-19 patients is pivotal in developing novel treatment strategies and optimizing patient management. Various radiolabelled arginine-glycine-aspartate tripeptide (RGD)-based compounds have been developed to quantify the expression of integrins in vivo. (159-161) Integrins are cell adhesion molecules that are expressed on the surface of endothelial cells and pericytes**.** Gallium-68 labelled RGD (^{68}Ga-RGD), binding to integrin $\alpha_v\beta_3$, was previously examined in head and neck cancer and arterious-venous malformations. (162, 163) As increased tracer uptake on PET/CT is correlated to areas with increased endothelial activation, we hypothesized that ^{68}Ga-RGD PET/CT would provide further insight into the role of the capillary and larger vessel endothelium in COVID-19.

In this prospective study, we quantified endothelial activation in lung parenchyma, myocardium and carotid arteries in hospitalized COVID-19 patients using ^{68}Ga-RGD PET/CT imaging. In addition, Iodine mapping of the lungs with CT subtraction, as a surrogate of pulmonary perfusion, was used to determine whether endothelial activation affects lung parenchyma perfusion. (164)

Results

Patients

From October 2020 until January 2021, ten hospitalized patients with COVID-19 were enrolled in this study. Five patients with similar distributions in age and sex, enrolled in a previous trial (EudraCT 2015-000917-31), were used as reference. (163) Baseline characteristics are shown in **Table 1**. Patients were admitted to the hospital for a minimum of 6 and a maximum of 23 days. The mean (±SD) D-dimer and CRP values on day of acquisition were 1244 (± 821) and 38 (± 41). During the inclusion period the alpha (also B1.1.7) variant was the predominant SARS-CoV-2 variant in the Netherlands.

Table 1. Baseline characteristics and pre-existing comorbidities of COVID-19 patients versus reference patients.

	COVID-19 patients	Reference patients
No. of patients in total	10	5
Male, n (%)	7 (70.0)	4 (80.0)
Age, median [IQR]	68.5 [52.0-74.5]	69.0 [64.0-72.5]
BMI, mean (SD)	30.4 (4.5)	25.2 (5.3)
Hypertension, n (%)	2 (20.0)	2 (40.0)
Diabetes Mellitus, n (%)	2 (20.0)	1 (20.0)
Hypercholesterolemia, n (%)	3 (30.0)	3 (60.0)
COPD, n (%)	2 (20.0)	4 (80.0)
Myocardial infarction, n (%)	1 (10.0)	0 (0.0)
Stroke or TIA, n (%)	2 (20.0)	0 (0.0)
Peripheral arterial disease, n (%)	0 (0.0)	1 (20.0)
Coronary Artery Revascularization (PCI/CABG), n (%)	1 (10)	0 (0)
Total hospital stay in days, median (IQR)	8 [6.8-12.0]	-
Time between admission and PET/CT in days, median [IQR]	5 [4.0-6.3]	-
Time between onset of symptoms and PET/CT in days, median [IQR]	15 [11.0-16.3]	-
Oxygen therapy required during hospital stay, n (%)		
Oxygen suppletion therapy: Nasal cannula/non rebreathing mask	9 (90.0)	-
Non-invasive ventilation: Optiflow	1 (10.0)	-
Invasive ventilation: Intubation	0 (0.0)	-
O_2 need in L/min at start PET/CT, median [IQR]	3 [0.9-4.0]	-
Complications during hospital stay		
Ischemic stroke or myocardial infarction	0 (0.0)	-
Pulmonary embolism	2 (20.0)	-

Abbreviations: BMI: body mass index; IQR: interquartile range; COPD: chronic obstructive pulmonary disease; TIA: transient ischemic attack; PCI: Percutaneous coronary intervention; CABG: coronary artery bypass grafting

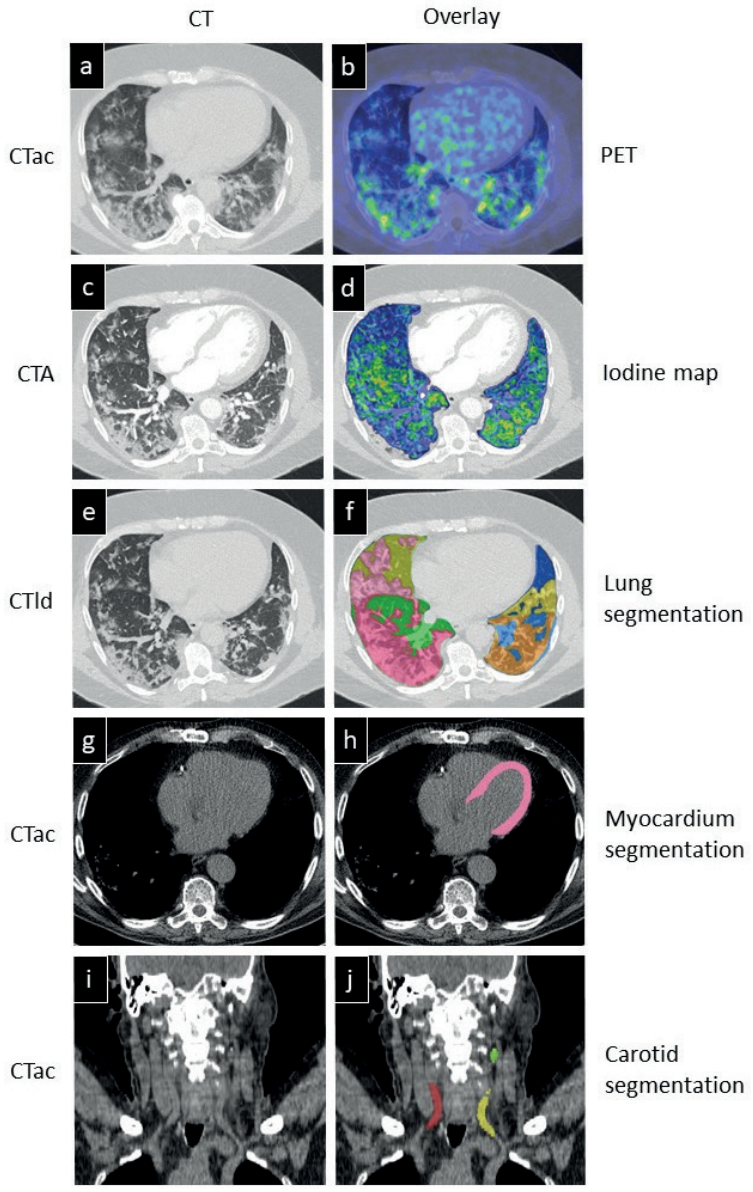

Figure 1. CT reconstructions with correlating PET, subtraction CT iodine mapping and segmentation overlays. Axial view of CTac imaging of the thorax. **(b)** CTac imaging of the thorax with attenuation corrected PET overlay. **(c)** CTA of the thorax with breath hold after contrast injection. **(d)** Subtraction CT of the lungs: iodine map overlay. **(e)** CTld of the thorax with breath hold instruction. **(f)** CTld with lung segmentation overlay; Unaffected parts of lobes are colored green and blue. Lilac, pink, orange and yellow are segmented affected parts of the lobes **(g)** CTac imaging of the myocardium. **(h)** CTac with myocardium segmentation overlay. **(i)** Coronal view of CTac imaging of the head and neck. **(j)** CTac with carotid arteries segmentation overlay (red = right carotid artery, yellow = left carotid artery, green = left internal carotid artery).

^{68}Ga-RGD PET/CT imaging of the lungs

To quantify RGD uptake, we calculated the standardized uptake value (SUV) to compensate for differences in net injected activity, incubation time and body weight (CTac, used for attenuation correction, shown in figure 1a and PET overlay in **Figure 1f**). Mean uptake of ^{68}Ga-RGD in the whole lung parenchyma of COVID-19 patients (mean SUV 0.99±0.32) was increased compared to reference patients (mean SUV 0.45±0.18) (*p<0.01*) (**Figure 2a**). A deep learning algorithm for automatic segmentation of parenchymal involvement per lobe was used to define regions of affected lung parenchyma (ground glass opacities (GGOs), consolidations and reticular opacities), and regions without these features (unaffected parenchyma) (**Figure 1h**). (165) Tracer uptake in affected lung parenchyma (mean SUV 1.43±0.30) was increased compared to unaffected parenchyma in all patients (*p<0.01*). Moreover, mean SUV in unaffected lung parenchyma (0.83±0.22), was also increased compared to reference patients (*p<0.01*) (**Figure 2a**).

As a measure of severity of involvement of the lung parenchyma in COVID-19 patients, the CT severity score (CTSS) was calculated automatically on basis of the amount of affected parenchyma on the CTld (**Figure 1c**). (165) The CTSS was scored for each lung lobe individually and is found to be correlated (R = 0.80; *p<0.01*) to the mean SUV of ^{68}Ga-RGD uptake per lobe, visualized in **Figure 3**.

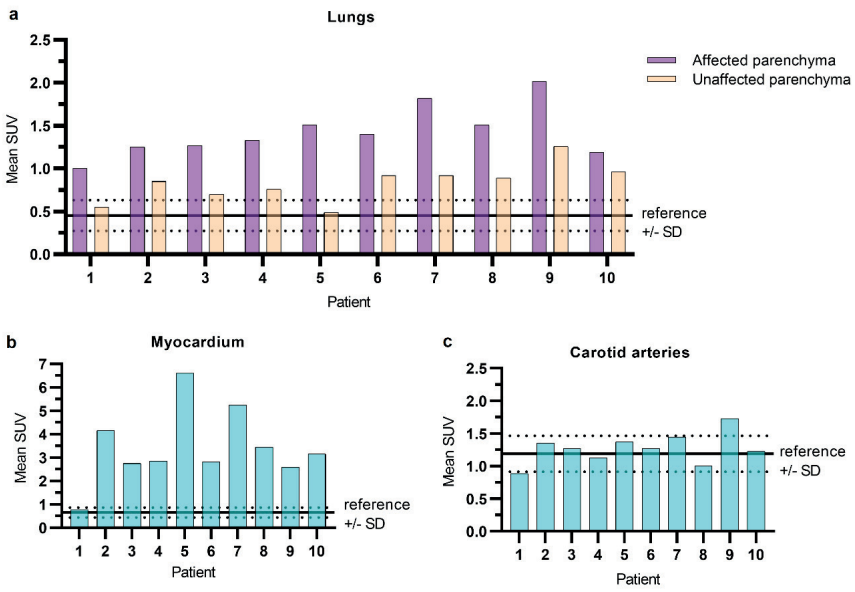

Figure 2. Mean SUV as compared to mean +/- SD (dotted lines) SUV of reference patients in the (a) automatically segmented affected and unaffected pulmonary parenchyma per patient, (b) myocardium and (c) carotid arteries. *Abbreviations: SUV: Standardized Uptake Value; SD: Standard Deviation*

CT subtraction lungs

The association of endothelial activation with perfusion of lung parenchyma was evaluated on a CT subtraction scan (**Figure 1g**) with regional differences in distribution of iodinated IV contrast in pulmonary arterial phase (**Figure 1b**) used as a marker of pulmonary perfusion. In 2 out of 10 patients the CT subtraction scans were not analyzed due to registration artefacts and an incomplete acquisition of the lungs. Consequently, in 8 patients, 40 lobes were first scored based on their image quality. 0% of lobes were regarded poor, 58% acceptable and 42% good. The main reasons to downgrade diagnostic acceptability were contrast-related beam hardening and scattering around the mediastinum and subclavian artery. Additionally, some parenchymal areas with severe consolidations were not included in the automated delineation of lung parenchyma by the subtraction algorithm and were subsequently excluded from analyses.

Visual scores on a scale of 1-5 of presence and grade of perfusion inhomogeneities in the resulting 37 segmented regions of affected and unaffected parenchyma are presented in **Figure 4**. Three out of 40 regions of affected parenchyma were too small to score. Increased perfusion was more often observed in affected lung parenchyma (16/37 regions) as compared to unaffected regions (2/40 regions).

^{68}Ga-RGD PET/CT imaging of the myocardium

Dyspnea in COVID-19 may not only result from pulmonary causes, but also from cardiogenic causes. Therefore, endothelial activation in the myocardium of the left ventricle was quantified. The mean SUV of the myocardium in the COVID-19 patients was significantly increased in all COVID-19 patients (3.44±1.59) as compared to reference patients (0.65±0.22) ($p<0.01$) (**Figure 2b**). We found no significant difference between the mean SUV derived from the AI-based algorithm (3.44±1.59) compared to the manual delineation (2.70±1.04) of the COVID-19 patients ($p=0.09$). Figure 1i shows an example of the myocardium segmentation using the CTac scans (**Figure 1d**).

^{68}Ga-RGD PET/CT imaging of the carotid arteries

To study whether endothelial activation was confined to the capillaries, or also larger vessels suppling the brain, the mean SUV of the carotid arteries was calculated and compared with the reference group (**Figure 2c**). There was no difference in mean SUV in carotid arteries between COVID-19 patients (1.27±0.23) and reference patients (1.19±0.27) ($p=0.39$). **Figure 1j** shows an example of the carotid segmentation using the CTac scans (Figure 1e). Furthermore, mean SUV values of the left (1.25±0.27) and right (1.29±0.27) carotid artery were compared between patients and did not differ ($p=0.94$).

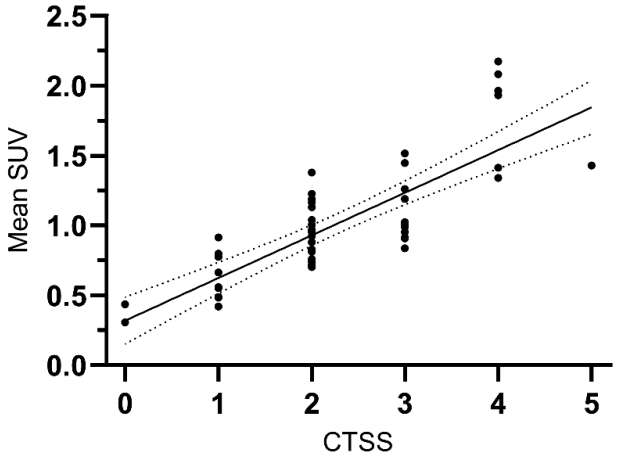

Figure 3. Correlation of CT severity score (CTSS) per lung lobe and mean SUV in COVID-19 patients. *Abbreviations: SUV: Standardized Uptake Value. The dotted lines represent 95% confidence interval*

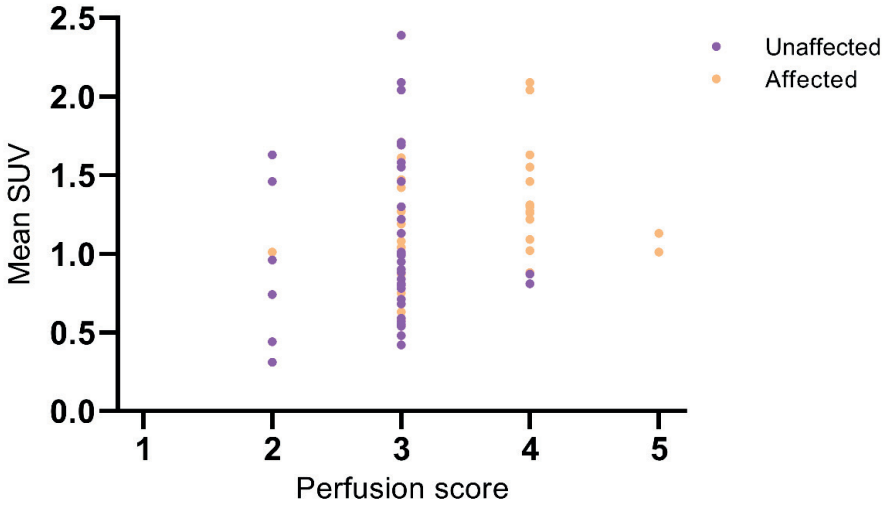

Figure 4. Mean SUV compared to visually assessed perfusion score of automatically segmented affected and unaffected regions per lung lobe. *Abbreviations: SUV: Standardized Uptake Value*

^{68}Ga-RGD tracer distribution

To investigate if there were differences in tracer distribution between COVID-19 patients and reference patients, mean SUV in the blood pool (aorta), muscle and spleen were calculated. The mean SUV in the blood pool was similar between

COVID-19 patients and references (*p=0.1*), as well as uptake in the spleen (*p=0.9*) and muscle tissue (*p=0.6*).

^{68}Ga-RGD uptake compared to clinical parameters

The severity of COVID-19 can be assessed by a variety of clinical parameters, including laboratory markers, medical history and demographic factors (e.g. increased BMI). (5) We investigated if there was a correlation between tracer uptake and clinical parameters associated with severe COVID-19 disease. BMI was significantly positively correlated with mean SUV (RGD uptake) of the lungs of COVID-19 patients (r=0.68; *p=0.03*), in the reference group this correlation was not significant (r=0.81; *p=0.10*) (**Figure 5**). However, a negative correlation between BMI and mean SUV of the myocardium (r=-0.45; *p=0.20*) was found in COVID-19 patients (see additional information in supplementary Table S2). The biomarker C-reactive protein as well as the total hospital stay in days of the COVID-19 patients were not significantly associated with tracer uptake in the lungs, carotid arteries or myocardium. For D-dimer, the positive association with mean SUV in the myocardium was significant (r=0.80; *p=<0.01*) contradictory to the association with lungs or carotid arteries.

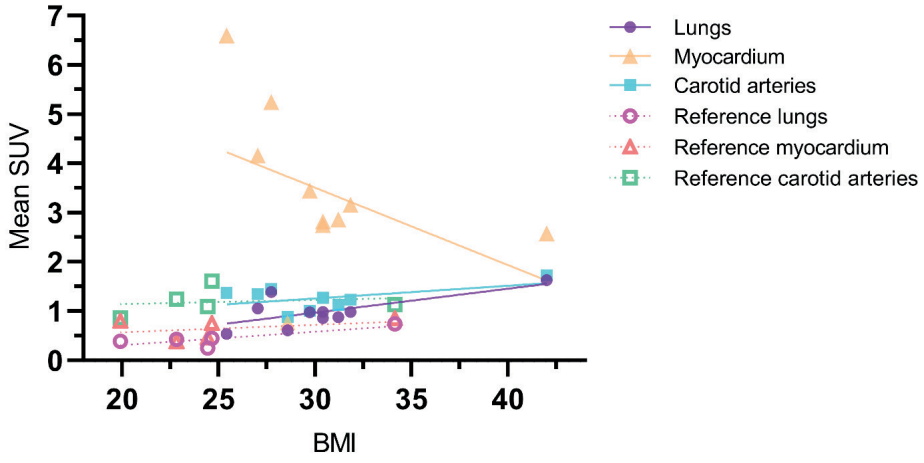

Figure 5. Pearson's R correlation between BMI and the mean SUV calculated in the lungs, myocardium and carotid arteries per COVID-19 and reference patient. *Abbreviations: BMI: Body mass index; SUV: Standardized Uptake Value*

Discussion

In this imaging study, we quantified endothelial activation of lung parenchyma, myocardium and the carotid arteries using ^{68}Ga-RGD PET/CT imaging in hospitalized COVID-19 patients with respiratory symptoms. ^{68}Ga-RGD uptake was significantly increased in lung parenchyma and myocardium in patients with COVID-19 compared to reference patients. This observation is consistent with endothelial activation in the cardiopulmonary system. Furthermore, endothelial activation was also observed in lung parenchyma that was unaffected on CT images, suggesting a larger involvement of the pulmonary vasculature than is assessed by anatomical imaging by CT. In contrast, no increased uptake in larger peripheral vessels, i.e. carotid arteries, or other organs systems was observed. Therefore, our results suggest specific endothelial activation in the cardiopulmonary system in COVID-19 patients with respiratory symptoms.

COVID-19 induces systemic inflammation (166) including endothelial activation (149, 167) as part of the physiological response to infection (168). However, endothelial dysfunction and hypercoagulability are associated with COVID-19 severity (149) and progression to organ failure (169). Dysregulation and activation of the endothelium can contribute to the pathological response and may cause collateral damage. Our results confirm the presence of endothelial activation, based on the increased uptake of the tracer in both affected and unaffected lung parenchyma as well as in the myocardium. Because COVID-19 predominantly involves the respiratory tract, these effects in the affected lung parenchyma were expected. Interestingly, we found that in unaffected lung parenchyma (parenchyma without abnormalities on CT) of COVID-19 patients there was still a significantly higher uptake of the tracer compared to lung parenchyma of the reference group. This suggests that endothelial activation is part of the inflammatory response and very likely precedes structural changes in lung tissue in these regions. (170) This may lead to an underestimation of lung involvement in COVID-19 at the time of CT-scanning.

Furthermore, our data indicates that endothelial activation, as part of the inflammatory response, occurs in capillaries throughout the cardiopulmonary system, concluding from significantly increased uptake in the left ventricle. The activation of vascular endothelial cells is also suggested by Nägele et al. (171) This observed endothelial activation may be one of the reasons for the increased incidence of myocardial infarction, arrythmia and myocarditis during hospitalization and after recovery. (172, 173) Moreover, dysfunction of the endothelium due to viral illnesses is associated with long term risk of cardiovascular events. (174) In

light of these observations, it is very interesting to notice that we did not observe increased tracer uptake in the carotid arteries. The systemic inflammatory response does not activate the endothelial bed throughout the whole body, but seems to be localized to the cardiopulmonary system.

During the pandemic a high rate of ischemic strokes was reported in hospitalized COVID-19 patients compared to influenza patients (1.5% versus 0.2%). (18) Although tracer uptake was not different between patients and references in our cohort, this result does not necessarily imply absence or presence of endothelial activation. For example, the partial volume effect which will affect measurements in small ROIs, has a larger effect for ^{68}Ga-labeled tracers than in previously reported studies with ^{18}F-labeled RGD-peptides. (175) The resulting underestimation of tracer accumulation hampers a conclusive observation of carotid artery activation in COVID-19 patients.

Generally, obesity is related to endothelial dysfunction (176), and large retrospective cohort studies previously identified high BMI as risk factor for poor outcome in hospitalized COVID-19 patients. (177) In our limited dataset, in subjects with higher BMI we observed higher tracer uptake in lungs and carotid arteries, but discrepantly lower uptake in the myocardium.

Chest CT was frequently used during the COVID-19 pandemic for diagnosing and risk stratification methods (165, 178, 179) Ventilation/perfusion single photon emission tomography (V/Q SPECT) is an alternative to CT angiography, which is able to assess cumulative, real lung perfusion. Several small studies using V/Q SPECT demonstrated heterogenous perfusion patterns in affected lung parenchyma, occasionally colocalizing with COVID-19 related abnormalities on CT. (180-183) In line with these series, our results support the notion that local perfusion defects found in molecular imaging can precede structural changes on CT. Alternatively, the one phase CT subtraction CT protocol with fluctuating image quality might not have been sensitive enough to detect all lung perfusion abnormalities.

There are however several limitations for our study. The study has a relatively small sample size and reference cohort, due to challenging logistics of a molecular imaging study with a short-lived radiotracer in hospitalized COVID-19 patients. Also, the segmentation algorithm used for discriminating affected from unaffected parenchyma may have been influenced by pre-existing pulmonary abnormalities or atelectasis, interstitial lung abnormalities or pulmonary edema, which could not be checked as no previous scans were available. This may have led to a larger

area of parenchyma classified to be ''affected'' and therefore possibly lower SUV in the calculation of mean SUV of the affected areas. It is currently unknown if COPD in the reference patients may lead to a higher SUV due to endothelial activation or a lower SUV due to emphysema in the reference measurements. If any, this might underestimate the effect of activated endothelium in affected segments in COVID-19 patients.

In conclusion, we demonstrate that ^{68}Ga-RGD PET/CT imaging allows to assess the localization and magnitude of endothelial activation in the cardiopulmonary system in hospitalized COVID-19 patients. Our findings support the hypothesis that endothelial activation is a critical step in the inflammatory response to SARS-CoV-2 infection.

Materials and methods

Patients

Ethical permission was obtained from the medical ethical committee Arnhem-Nijmegen (ClinicalTrials.gov identifier: NCT04596943). All study proceedings were performed in accordance with Dutch clinical trials guidelines and all participants provided written informed consent prior to participation. In this single-center proof-of-concept prospective observational study, we included hospitalized adult patients with PCR proven SARS-CoV-2 infection, admitted to the nursing ward. Exclusion criteria included previously documented severe lung abnormalities, glomerular filtration rate $\leq$30 ml/min, contra-indications for PET/CT (pregnancy, breast-feeding or severe claustrophobia) or contra-indications for administration of iodine-containing agents. Patient data, including demographics, medical history, clinical parameters, laboratory examinations, treatment and complications during hospital stay were collected. No adverse events were reported.

Non-COVID-19 patients

Five patients with oral cavity squamous cell carcinomas were used as reference. ^{68}Ga-RGD PET/CT scans were performed according to the protocol described in the 2016 study of Lobeek et al. (163)

Image acquisition

^{68}Ga-RGD PET/CT

[^{68}Ga]Ga-DOTA-E-[c(RGDfK)]$_2$ was synthesized at the Radboudumc (Nijmegen, the Netherlands) as described in Lobeek et al. (163) A mean dose of 196±20 MBq ^{68}Ga-RGD was injected intravenously as a bolus over 1 minute followed by saline flushing. All PET/CT scans were performed on a Biograph mCT 4-ring clinical scanner without ECG or respiratory gating (Siemens). PET acquisition of patients 2-10 commenced median 31 minutes (IQR 24-38) post-injection at 5 minutes per bed position. Patient 1 was scanned at a significantly later time point post-injection (118 minutes) due to patient transport logistics, this subject showed a remarkably lower ^{68}Ga-RGD uptake across all analyses. The scan range included the thorax, head and neck of the patients. Reconstruction of PET images with vendor specific software comprised of attenuation correction with CT and TrueX algorithm with point spread function and time of flight measurement using 3 iterations and 21 subsets (Siemens). Slice thickness was 3 mm, pixel spacing 4.07 mm, matrix size 200x200 voxels and pixel full-width half maximum 3 mm. A 3D Gaussian filter kernel of 3 mm was used for postprocessing.

Low-dose CT scans for attenuation correction (CTac) and anatomical reference were acquired with automatically modulated X-ray tube voltage and current (120kV, 50 mA). Scan range was equal to PET, slice thickness 3 mm, pixel spacing 0.98 mm, matrix size 512x512 voxels and images were reconstructed using a B31f kernel.

Subtraction CT

Directly following PET/CT, patients underwent subtraction CT on the same scanner: A CT of the thorax before and after iodinated intravenous contrast administration (iomeprol 300mgI/ml), to evaluate one-phase iodine enhancement of the pulmonary parenchyma. An unenhanced CT (CTld, mean DLP 124 mGy. cm) was made after breath hold instruction with automatically modulated X-ray tube current (reference 75mAs, >66). Subsequently, injection of a bolus (112±12 ml) of 300 mg/ml iodine-contrast at 5 ml/s was followed by a 40 ml saline chaser at the same injection rate. One patient received a contrast bolus of 3 ml/s and consequently a triggering delay. After a threshold of 100 hounsfield units (HU) was measured in the pulmonary trunk, a breath hold instruction was given to the patient for the acquisition of CT angiography (CTA) of the thorax using modulated current (reference 100 mAs, >86, mean DLP = 169 mGy.cm). Both CT images were acquired with tube voltage of 100 kV and reconstructed with kernel I30f/3, a slice

thickness of 1.5 mm with pixel spacing of 0.72 mm and matrix voxel size 512x512. Median HU in de pulmonary trunk was 414 HU.

From these two scans, iodine maps were calculated by subtracting CtId from the CTA scans after motion correction and mask segmentation as described in Grob et al. (164)

Image Analysis

Lung segmentation

We used a previously developed COVID-CT artificial intelligence algorithm for segmentation of the five lung lobes in the CTId images. (165) Additionally, this algorithm segmented affected areas (with GGOs and consolidations) from unaffected areas per lobe. This resulted in 10 ROIs per patient, and the corresponding CT severity score per lung lobe as described in Lessmann et al. (165) The CTSS was not calculated for the reference group, since this score is validated for COVID-19 and not for COPD associated changes in lung parenchyma. Rigid registration of the CTId to the CTac was performed using MevisLab (Fraunhofer Mevis, Bremen, Germany). This transformation was used to register the segmentation to the PET images and subsequently calculate the SUV within the 10 ROIs.

As the PET scan was made during free breathing, one investigator manually adjusted the segmentations to exclude the liver and spleen signal from quantification if their activity concentration was projected over the lower lung.

^{68}Ga-RGD PET/CT scans of the reference patients were used as reference PET signal in lung parenchyma unaffected by COVID-19 infection. The CTac images of the references were segmented using the same lung lobe segmentation artificial intelligence algorithm. (165) This segmentation was registered to the PET images and subsequently adjusted to exclude the liver and spleen signal before calculating mean SUV per lobe.

Subtraction CT

One investigator (EvG) and one chest radiologist with 6 years of experience in thoracic radiology (MB) evaluated image quality and presence and grade of perfusion inhomogeneities on subtraction CT. They graded image quality of the perfusion maps per lung lobe on a visual grading scale from 1-3 (1: bad, 2: acceptable, 3: good). In each of the 10 ROIs per patient perfusion was assessed on a scale from 1-5 (1: severely decreased, 2: decreased, 3: as expected, 4: increased, 5: severely increased) compared to what was expected in a corresponding part

of healthy parenchyma. In case of discrepancy between the two readers, this was solved in consensus.

Myocardium segmentation

The myocardium of the left ventricle (LV) was delineated on the CTId using the artificial intelligence based algorithm "Whole-heart segmentation in non-contrast-enhanced CT" in all patients. (184) On basis of this segmentation, the mean SUV was calculated for the myocardium. Additionally, one investigator manually (FVDH) delineated the LV myocardium on the CTA of the COVID-19 patients. The mean SUV derived from the algorithm was compared per patient with the mean SUV derived from the manual delineation in order to verify the algorithm. The relatively thin wall of the right ventricle was not delineated as SUV quantification would be unreliable on a scan without ECG or respiratory gating.

Carotid artery segmentation

One investigator manually (RvL) delineated bilateral carotid vessel structures of the patients and references on co-registered PET/CT slices (Inveon Research Workplace version 4.2, Siemens). She segmented the common carotid artery (extending from the aortic arch until the carotid bulb), internal carotid artery, external carotid artery and the carotid canal and put ROIs in the lumen, vessel wall and atherosclerotic plaques. The carotid bifurcation was excluded from the ROI to prevent influence of the partial volume effect. ROIs were additionally reviewed by a neuroradiologist with 12 years of experience in neuroradiology. The mean SUV was calculated per ROI and for all regions combined.

Tracer distribution

We set ROIs for blood pool, muscles and spleen to investigate whether variations in tracer distribution between COVID-19 patients and references occurred and calculated SUV mean values. As a representation for blood pool, an ellipsoid ($5cm^3$) was drawn in the lumen of the descending aorta. Muscle activity (where uptake is expected to be low) was calculated using an ellipsoid ($5cm^3$) in the trapezius muscle and an ellipsoid ($10cm^3$) in the spleen.

Statistical analysis

Patient characteristics are displayed as counts and percentages and median with IQR. The mean standardized uptake value was calculated and the standard deviation. Differences in mean SUV between COVID-19 patients and references were analyzed using the Mann-Whitney U test. A paired T-test was used to calculate differences in affected versus unaffected parenchyma. Correlations between mean SUV and

clinical parameters were calculated using Pearson *r*. Two-sided P values of less than 0.05 were considered statistically significant. Statistical analysis was performed using SPSS 25 (IBM) and Graphpad Prism 5 software (GraphPad Software).

Supplementary materials

Table S1. Clinical parameters + outcomes per COVID-19 patient. Age and sex are not included due to privacy reasons.

Clinical parameters	COVID-19 patients									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
BMI (kg/m^2)	29	27	30	31	25	30	28	30	42	32
Saturation (%)	93	96	95	93	91	94	98	95	93	94
O2 need (L/min)	0.5	2	4	4	0	3	2.5	4	4.5	3
D-dimer (ng/ml)	640	1410	1540	1360	3300	500	1450	790	740	710
Ferritin (ug/ml)	2352	949	1600	2491	2251	720	224	56	1441	957
CRP (mg/l)	39	12	123	9	35	2	49	7	9	95
LDH (U/l)	257	240	429	323	279	308	369	226	279	348
ALC (U/l)	1.10	1.39	0.92	2.15	0.41	3.61	1.00	1.11	2.74	0.72
Hospital stay (in days)	6	6	9	7	23	12	7	12	10	7
Onset symptoms to admission to hospital (time, in days)	5	12	10	5	9	13	11	11	9	8
Admission to PET/CT (time, in days)	5	4	4	6	4	11	5	6	7	3
PET/CT to discharge (time, in days)	1	2	5	1	19	1	2	6	3	4
ICU admission (time, in days)	1	0	0	0	0	0	1	0	0	0
SUV lungs (mean)	0.61	1.06	0.86	0.88	0.54	0.98	1.39	0.98	1.63	0.99
SUV myocardium (mean)	0.77	4.16	2.75	2.86	6.60	2.82	5.25	3.45	2.58	3.16
SUV carotid arteries (mean)	0.89	1.35	1.27	1.13	1.37	1.27	1.45	1.01	1.73	1.23

Abbreviations: BMI: body mass index; CRP: C-reactive protein; LDH: Lactate dehydrogenase; ICU: intensive care unit; SUV: standardized uptake value

Table S2. Correlation between clinical parameters and SUV mean per organ (using Pearson R two-tailed)

	Lungs		Myocardium		Carotid arteries	
Clinical parameters	**R-value**	**R-value**	**P-value**	**P-value**	**R-value**	**P-value**
BMI	0.68	-0.45	0.196	0.031	0.50	0.071
CRP	-0.19	-0.04	0.919	0.604	-0.05	0.901
D-dimer	-0.40	0.80	0.006	0.248	0.22	0.538
Total hospital stay	-0.36	0.62	0.057	0.307	0.19	0.608

**Correlation is significant at a p-value of 0.05 (2-tailed)*

Part V

General discussion and summary

Chapter 8

General discussion

General discussion

The COVID-19 pandemic has profoundly impacted global healthcare, marking a pivotal moment in modern medical history. During the first wave of the pandemic, it became clear that infection with SARS-CoV-2 was associated with thrombo-embolic events including pulmonary embolism, but also ischemic stroke had been reported. Large cohort studies or systematic reviews, however, on the incidence of ischemic stroke, silent cerebral ischemia but also other cerebrovascular markers in COVID-19 patients in general had not been conducted yet. Most of the research focussed mainly on patients with clinical (neurological) symptoms with clear indications for scanning of the brain. Conversely, no studies had been undertaken on patients who do not exhibit these overt neurological symptoms. The potential neurological impacts of COVID-19 and its effect on the brain, beyond acute ischemic stroke, are of great importance for better understanding of the consequences of viral infection, clinical implications and strategies for the improvement of patients' outcomes.

The overall aim of this thesis was therefore to:

- Explore the prevalence and incidence of cerebrovascular markers and vessel wall changes in hospitalized COVID-19 patients using MRI
- Analyze the integrity of WM structure and its relationship with long-term clinical outcomes and BPV
- To explore PET/CT tracer uptake in the carotid arteries as a proxy for carotid endothelial activation in COVID-19 patients through PET/CT imaging

The results described in this thesis are based on two different studies: the CORONIS study and a study conducted in one hospital (Radboudumc), which performed PET/CT imaging in COVID-19 patients.

In this chapter, we will start by addressing methodological considerations, followed by a discussion of the main findings. We will conclude by exploring the clinical implications of the studies within the context of this thesis and consider potential future directions.

Methodological considerations

Analyzing the study's methodological strengths and weaknesses, including aspects such as study design, internal validity, precision, and external validity, is crucial for a proper interpretation of the findings of a study.

1. Study design

The studies in **chapter 2-6** are based on CORONIS, a multicentre, prospective, observational cohort study. In this study, we included patients ≥ 18 years hospitalized for COVID-19 (with (aim: n=200) or without (aim: n=70) ischemic stroke or transient ischemic attack (TIA)) during the pandemic and controls without COVID-19 infection in three academic hospitals in the Netherlands between 2021 and 2023 and performed brain MRI. This study enabled us to investigate the prevalence and incidence of cerebrovascular MRI markers, examine VWE and WM integrity in patients hospitalized for COVID-19, collect data on BPV during their admission, and establish long-term functional outcomes in both patients and controls. At the end of the inclusion period, we were only able to include n=7 patients with ischemic stroke or TIA of the aimed/planned n=70 patients. Due to the unexpectedly low number of COVID-19 patients hospitalized with ischemic stroke and the severe clinical conditions of those who were admitted, many were unable to participate in our study. This restricted us to identify possible risk factors for ischemic stroke and/or silent cerebral ischemia in patients with COVID-19 due to the small sample size.

Moreover, no MRI scans made before the SARS-CoV-2 infection were available for comparison. Therefore, we were unable to definitively establish whether the observed effects in brain abnormalities in found on brain MRI or PET/CT are solely due to COVID-19 or influenced by pre-existing conditions.

The PET/CT study in **chapter 7** is based on patients from a single-center proof-of-concept prospective observational study in the Radboudumc, including hospitalized adult patients with PCR proven SARS-CoV-2 infection, admitted to the nursing ward. Controls included patients with oral cavity squamous cell carcinomas, enrolled before the pandemic (2016). The study had a relatively small sample size (patients; n=10) and reference (n=5) cohort, due to challenging logistics of a molecular imaging study with a radiotracer in COVID-19 patients admitted to the hospital, although small sample sizes are common in PET/CT research. Consequently, achieving a precise match between patients and references based on cardiovascular risk factors therefore proved to be challenging and therefore impacted the comparability of the outcomes.

2. Internal validity

Internal validity assesses the capacity of a study to establish causality between a determinant and an outcome. This validity can be compromised by selection bias, information bias, and confounding. The focus of internal validity is to ensure that any observed changes in the outcome can be attributed to the exposure (in our case, COVID-19) itself, while carefully accounting for random errors (chance variations) and systematic errors (biases) that may arise during the study's design and execution. (185)

1.1 Selection bias

Selection bias occurs when the measured prevalence in a study cohort differs systematically from the expected estimate in the original population. This form of bias can arise due to differences in patient characteristics at the start of the study or because of certain dropout rates throughout the study duration. (186)

In the CORONIS study, selection bias might have occurred during the inclusion period (**chapter 2**) because critically ill patients may have been underrepresented in this study for several reasons. First, patients were recruited starting from April 2021. This was during the 3th wave in the pandemic, and it is known that patients were more severely ill in the earlier waves. Second, most patients were recruited and included during the pandemic on that we admitted to a low-care wards, and about a third were recruited from/after ICU. Patients had to be able to undergo brain MRI. Since patients needed to provide written informed consent, we may have missed patients that might have either died during hospitalization, refused or were unable to provide written consent. Third, patients who had problems with their health during follow-up were more likely to decline a follow-up brain MRI after three months (follow-up MRI in n=98, 78,4%). These issues (the timing of the start of the study (during less severe 3th wave of the pandemic) and the selection of patients) might have led to an underrepresentation of severely ill COVID-19 patients and subsequently, an underestimation of cerebrovascular MRI markers in our cohort and/or associations with clinical outcomes. This underrepresentation of severely ill patients may also be the case for patients in the PET/CT study where only patients from the general ward were included (non-ICU) because they had to be able to undergo PET/CT imaging while using oxygen supply.

Subsequently, patients in the CORONIS study were recruited from three different hospitals. Specific measurements such as MRI-DTI imaging and continuous BP monitoring, however, were conducted exclusively at one participating center

(Radboudumc). This may have introduced center-specific bias, potentially influencing the generalizability of the findings in all COVID-19 patients throughout the Netherlands. Additionally, only patients in the general ward had a wearable vital signs device during admission, this device was not available during ICU admissions. Therefore we were not able to assess the BPV during ICU admission. We only included the patients' BP measurements until their transfer to the ICU (and not after return to the normal ward) for better comparison of the data.

1.2 Information bias

Information bias might arise if the definitions of outcomes, determinants or confounders are inconsistent or unclear or if they are inaccurately measured across different study sites. This bias might be demonstrated in this thesis by misclassification of events.

Misclassification of cerebrovascular MRI markers

In the CORONIS study, measurement error of cerebrovascular MRI markers could have been introduced since different MRI scanners across the participating hospitals, each with varying field strengths, slice thicknesses and acquisition protocols were used. Cerebrovascular MRI markers were assessed by radiologists within their own participating centers, which could have introduced center-specific measurement bias, resulting in different prevalence of markers between centers. To prevent this, raters used a standardized MRI protocol for evaluation of the SVD markers and additionally, interrater meetings were conducted. Reevaluation of a random sample (n=15) of patients from all centers was conducted during the study by the radiologists. This evaluation showed overall agreement on the prevalence of WMH, but revealed significant variations of the prevalence of microbleeds detected due to varying slice thicknesses of SWI sequences across centers. Consequently, to ensure consistency, all images were reevaluated by a single rater with minimal size of the microbleeds 2 and maximum 10 mm, aiming to achieve the most accurate results. Additionally, using the Fazekas score as a qualitative assessment scale might have led to measurement bias. This scale was originally developed to rate the extent of WMH using a visual quantitative method. (187) Subtle changes in patients with the highest Fazekas Score (=3) however might have been missed using this scaling system due to the ceiling effect, meaning that the maximum score on a scale has been reached and therefore preventing capturing further changes of the disease (in this case progression of WMH). Hereby, significant differences in the prevalence (and incidence after three months) of WMH may still occur among patients with the same Fazekas score whilst these subtle differences can be important for clinical interpretation and prognosis. Although this was only a relatively small group in the

study (n=5), this could have led to an underestimation of the impact of COVID-19 on the brain in patients with extensive WMH.

Measurement bias of BPV by continuous vital signs monitoring system

We investigated BPV in patients from the CORONIS study by using the BP measured by a cuffless continuous monitoring system during hospital admission. The accuracy of the device to capture true BP however can vary (for example depending on posture of the body) or calibration moments with a BP cuff, therefore possibly leading to measurement bias. One example is that the device requires recalibration with each significant change in position. To limit these problems, we excluded BP readings that were considered potentially non-physiologic (systolic BP >300 or <40 mmHg). Additionally, we excluded data of the minute during calibration, minute preceding and minute following the calibration of the continuous monitoring device, to ensure accuracy. Given that we have an average of 6,000 measurements per participant, the impact of a few inaccurate readings on the overall results is likely negligible. Due to these accuracy issues, the use of cuffless continuous BP monitoring systems is not yet validated for measuring BPV according to the European Society Hypertension guidelines. We tackled this by examining different intervals of BPV (1- 5- and 20-minute intervals) for comparison of the intervals and to account for the effect of missing or inaccurate values. Additionally, no gold standard has been set to quantify BPV to this date and therefore we calculated multiple BPV indices (by calculating Average Real Variability (ARV) and Coefficient of Variation (CV)) for comparison.

Measurement bias due to partial volume effect in PET/CT scanning

In this thesis, we discussed the use of the 68Ga-RGD tracer in PET/CT imaging to activate uptake in αvβ3 integrin in the endothelium of the carotid arteries. Limited spatial resolution in PET/CT imaging however leads to the partial volume effect (PVE), where smaller objects such as areas expressing αvβ3 integrin appear blurred. This blurring can lead to information bias because of an underestimation of the observed uptake of the tracer targeting αvβ3 integrin, which is important for accurate SUV estimates. This effect is intensified in larger arteries where tracer uptake is confounded by the presence of blood and other substances, complicating the transparency of the endothelial signals. Subsequently, the lack of significant differences in tracer uptake between COVID-19 patients and reference groups does not definitively indicate an absence of endothelial activation. This bias can lead to underestimation of tracer uptake, which can misrepresent the actual physiological uptake (representing in our case endothelial activation) being studied.

MRI scans and follow-up questionnaires were conducted in patients at baseline and after three months, with additional questionnaires after twelve months in the CORONIS study. We aimed to correlate baseline WM integrity values and changes between baseline and follow-up with patient-reported clinical outcomes. However, the absence of MRI scans after 1 year — during the last follow-up questionnaire — represents a significant limitation. This impedes us to directly correlate long-term WM integrity with clinical outcomes, thereby leading to incomplete conclusions about the disease progression and patient outcomes after 1 year. We were however able to investigate this association after three months between clinical assessments WM integrity using MRI-DTI imaging, which showed us overall no significant associations.

1.3 Temporal bias

Temporal bias describes a mismatch between the time interval research data can only obtained in the course of the disease and the moment clinical data are needed for clinical decision making regarding diagnostics or further treatments. (188) In the CORONIS study, the median time between onset of symptoms and baseline MRI was 6 weeks. Patients could not be scanned earlier during admission due to (preventive) measures during the pandemic. This extended timespan could have led to temporal bias, where the timing our data assessments may have affected the outcomes and might have led to an underestimation of silent cerebral ischemia. Acute (intermittent) abnormalities (f.e. incidental diffusion-weighted imaging (DWI) positive lesions indicating acute ischemia) that might have occurred earlier during admission or later than the timing of the baseline MRI may not be adequately captured. It has been investigated before that the incidental DWI-positive lesions in specific may be visible for only up to three weeks. (189)

1.4 Confounding

Confounding arises when a variable is associated with both the determinant and the outcome of interest, but is not in the causal pathway,. This variable may affect the observed relationship between the outcome and the determinant, potentially misrepresenting the true association. (185)

In the CORONIS study, patients had a higher prevalence of hypertension and pulmonary disease compared to the controls. Hypertension is recognized as the main risk factor associated with cerebral SVD, which includes WMH, as described in several longitudinal cohort studies and reviews, particularly among the elderly ($\geq$65 years). (190, 191) Moreover, cerebral SVD is known to be the most important contributor to ischemic stroke, cognitive impairment and dementia. (7, 191) In

addition, WMH and WM integrity are known to be highly associated. Not only the prevalence of WMH, but hypertension appears to be a predictor for the progression of WMH as well. (192-194)

It is possible that COVID-19 patients already had a cardiovascular profile that predisposed them to cerebral SVD, including WMH and WM integrity, which was evident in our study. The COVID-19 patients exhibited more WMH as well as a higher incidence of hypertension compared to the controls. Although we corrected for hypertension during analysis, we were not able to correct for all confounders and this might have influenced the observed differences. In our WM integrity study, we calculated WMH volumes of all patients as a marker of pre-existing cerebral damage and corrected for this in our analysis. When comparing WM integrity between patients and controls, no significant differences remained. Nonetheless, even after adjusting for these volumes, an association between high BPV during admission and lower WM integrity remained evident.

Additionally, differences in disease severity (e.g. ICU vs. general ward stay) could have influenced the outcomes in patients in all chapters. We took this into account in analysis by adjusting for these factors and performed subgroup analysis, which showed no differences between groups.

2. External validity

External validity refers to the extent to which the results of a study can be generalized beyond the study's specific setting, populations or times. (185) The inclusion of only hospitalized patients, especially those capable of giving consent, may limit the findings' applicability to all patients affected by the condition being studied. The results might not be generalizable to less severely ill patients or those in different healthcare environments. However, our study included hospitalized COVID-19 patients without neurological symptoms, a population that has not previously been investigated. This contrasts with most current studies, which have focused solely on hospitalized patients presenting with neurological symptoms and therefore our study is more representative of the ‘’general” patient hospitalized for COVID-19. Additionally, given that most of the participants in the CORONIS and PET/CT study are Caucasian, the findings are likely to be generalizable to other European populations. However, they are likely less applicable to non-Caucasian patients.

All patients included in the chapters of this thesis were recruited in academic hospitals. Typically, patient demographics normally tend to differ between academic and regional hospitals in the Netherlands. However, during the pandemic, hospitals collaborated extensively, with the focus on sharing and thereby reducing the (logistic) burden of excessive numbers of COVID-19 patients, rather than making any distinction between academic and non-academic settings. Consequently, we believe our results are representative of COVID-19 patients across the Netherlands, as hospital-based bias was likely minimized by these collaborative efforts.

Discussion of main findings

1 | Prevalence and incidence of cerebrovascular MRI markers and vessel wall abnormalities

In this thesis, we reported no increased prevalence of (silent) cerebral ischemia (incidental DWI-positive lesions) or other cerebrovascular MRI markers in patients hospitalized due to COVID-19 compared to healthy controls, apart from WMH. This is in contrast with many other cohorts during the pandemic that report increased prevalence of cerebrovascular markers among others in patients admitted due to COVID-19. During the first waves starting from March 2020 until the end of 2020, several studies showed an increased incidence of ischemic strokes (range between 0.9-2.0%), particularly within the ICU population (up to 2.7%), compared with influenza patients (0.2-0.9%). (16, 18, 21, 45) However, most of these studies were primarily conducted among patients with neurological (overt) symptoms, who underwent additional MRI revealing increased prevalence of SVD markers. We acknowledge the limitations of our study due to its relatively small sample. In our study, the small sample size reduced the statistical power, increasing the risk of missing true associations. Additionally, it made the results more sensitive to random variation. Drawing from existing literature involving both population-based and hospital-based samples, we anticipated that the prevalence of asymptomatic silent brain infarcts would be 6-9 times higher (i.e., 18-25%, assuming a 3% prevalence of symptomatic events) compared to symptomatic brain infarcts. (35)

The low prevalence could be explained, firstly, by differences in pathophysiological mechanisms, with clinically apparent COVID-19-associated strokes often resulting from large vessel occlusion, while silent ischemia is primarily associated with SVD. Secondly, our cohort was enrolled after the first two COVID-19 waves in the Netherlands, when updated treatment guidelines and increased use of anticoagulants and anti-inflammatory drugs likely reduced thromboembolic

complications. Vaccination and less pathogenic variants may have further lowered the disease burden. In addition, the low prevalence may be linked to higher doses of anticoagulant therapy, which affect both arterial and venous systems. Patients in the CORONIS study were enrolled after the first two waves of COVID-19 in the Netherlands, where alpha (B.1.1.7) and delta (B.1.617.2) were the dominant variants. During this period, therapeutic guidelines evolved, leading to widespread anticoagulant use, which contributed to a reduction in pulmonary embolism, as stated previous studies comparing the 1st and 2nd waves. (195, 196)This change likely influenced prothrombotic processes and inflammation, impacting the incidence of ischemic strokes. Additionally, the low number of critically ill patients in our study, who are at higher risk for cerebrovascular complications, and delayed MRI scans due to local regulations may have led to underestimation of silent ischemia prevalence.

The high prevalence of WMH (77.6%) in our study, with a mean age of 58 years, compared to our controls (61.7%) is likely due to the high burden of vascular risk factors in our COVID-19 patients. Other studies conducted in the general population in older patients (>60), with less vascular risk factors, reported a prevalence between 60-90%. (65, 66) Patients with cardiovascular risk factors are known to face a higher risk of complications upon admission due to diseases such as COVID-19. (68) Although we adjusted for these major cardiovascular risk factors, the limitations of multivariable analysis mean that less common confounders could not be included, potentially leaving some residual confounding in the association between COVID-19 and WMH. Subsequently, the WMH may have been present before admission. Without MRI scans prior to infection, it is challenging to link the observed WMH to COVID-19. We suspect that the WMH did not develop over a short period and likely are the result from pre-existing damage rather than COVID-19 itself.

During 3-month follow-up, five patients (5.1%) developed new clinically silent cerebrovascular MRI markers, including two with silent cerebral infarctions, one of whom also had multiple incidental DWI-positive lesions. One patient, who had a pulmonary embolism during hospitalization, developed silent ischemic lesions despite three months of anticoagulation therapy. The other asymptomatic patient had an extensive cardiovascular history. Our results align with a study showing a 3% 90-day cumulative incidence of arterial thrombosis in hospitalized COVID-19 patients during follow-up. (2) The etiology of these brain MRI markers, while undetermined due to their asymptomatic nature, is most likely explained by the context of their medical histories and conditions. However, we must acknowledge

the limitations and generalizability of our study findings due to its relatively small sample.

When investigating vessel wall imaging in patients with COVID-19, we observed VWE in 21% of the initial cohort, which increased to 27% during follow-up after three months. VWE is seen in various diseases, including atherosclerosis, dissections, infectious and autoimmune diseases, or aneurysms, with differing locations and prevalence. (197) Atherosclerotic plaques are typically focal and eccentric, showing wall enhancement on T1W postcontrast images. Previous research has indicated that enhancing atherosclerotic lesions after contrast administration in ischemic stroke patients are associated with active inflammation and new ischemic strokes. (25) In contrast with the focal and eccentric enhancement in atherosclerotic plaques, our study primarily found concentric vertebrobasilar enhancements, with more than half of the lesions showing no pre-contrast plaque abnormalities. Research on infectious diseases remains limited, and specificity is often unknown. One study on VWE in COVID-19 found only eccentric VWE consistent with atherosclerosis, scanned almost a year after infection. (198) There was no control group in the CORONIS study for comparison of the prevalence of VWE found in our patients. To conclude, causality between COVID-19 and VWE remains unknown.

Most studies have focused on ischemic stroke patients or other brain inflammations, particularly in the carotid vessels. Our findings, mainly in the vertebrobasilar arteries, indicate a higher percentage of VWE compared to previous studies, suggesting increased inflammation associated with COVID-19. However, a causal relationship between VWE and COVID-19, and its clinical implications, cannot be made with our study. Further research should aim to establish reference values for VWE across various diseases, including healthy controls, to enhance clinical application and understanding of vessel wall imaging. This would be valuable for diagnosing and monitoring various diseases in the future.

2 | WM integrity and its relation with clinical outcomes and BPV

Our study showed a lower WM integrity in patients with COVID-19 compared to healthy controls, without correction for WMH. This was expressed as higher overall PSMD and lower NDI in several brain regions (in patients vs. controls). Prospective studies on WM integrity (changes) in COVID-19 patients remain scarce and showed alterations of the WM integrity. However, these studies did not adjust for pre-existing WMH, which is highly associated with WM integrity. In our study, the differences between patients and controls disappeared after adjusting for pre-existing cerebral WM damage (expressed in WMH volumes). This can be explained

by the fact that the patients with COVID-19 had higher WMH volume during baseline scanning and cardiovascular risk factors (hypertension, hypercholesterolemia) were more prevalent compared to the controls, as mentioned before. The fact that this difference disappeared after correcting for WMH suggests that underlying SVD was more likely the cause, rather than the infection of SARS-CoV-2.

We observed that after three months, there was decline of WM integrity in patients, expressed by increased orientation dispersion index (ODI) values in several brain regions of the patients. Note that low ODI values indicate higher WM integrity. Changes in WM integrity have been observed in other studies in patients with COVID-19, but they described an improvement of diffusion metrics after one year, contrary to our results. (101) Our results may be explained by the fact that that we had different MRI scanning intervals, capturing different stages of the diseases compared to the other studies, with our imaging interval more in the ''acute" phase of COVID-19. We only found alterations in one metric of WM integrity, whilst these other studies reported several variables to be altered between MRI intervals. It could also be the case that the three month follow-up period may be too short to capture alterations of the WM. Unfortunately, we were not able to compare this decline with control patients since they did not underwent follow-up MRI. Since research on this topic remains scarce, the causality and timing of these changes in relation to COVID-19 remains uncertain.

Understanding the clinical impact of WM integrity alterations in patients is highly relevant, as shown in other neurological conditions such as Multiple Sclerosis (MS), Mild Cognitive Impairment (MCI), and SVD, where WM changes have been associated with clinical outcomes. (10) In light of these findings, we also aimed to investigate whether alterations in WM integrity, both globally and in specific WM tracts, were linked to clinical outcomes in our cohort of COVID-19 patients, and whether these changes could predict worse long-term outcomes. Therefore, we examined short- and long-term clinical outcomes of patients with COVID-19 after 3 and 12 months, such as mood, cognition and daily functioning. Our findings showed that patients with COVID-19 perform worse across various assessments, including cognitive function and mood, compared to controls. This aligns with numerous other cohort studies examining the performance of post-COVID patients, compared to control groups. (95-97) Additionally, one study reported that hospitalized patients with COVID-19 perform even worse compared to non-hospitalized patients, with higher incidences of long COVID. (199)

Contrary to expectations, we observed no correlations with WM integrity changes, indicating that these changes do not explain long COVID or other persistent complaints in our study. This suggests the need for further investigation into the underlying causes of long COVID, but possibly not in the WM as displayed in our study.

Subsequently, we examined BPV during hospitalization of patients and its impact on WM integrity, where we yielded significant findings. BPV – to clarify: fluctuations of the levels of BP – has shown to be an independent risk factor of cardiovascular events. We found that high (short-term) BPV, was associated with lower WM integrity, independent of mean BP measured during admission. Although we cannot establish a causal relationship, the association is noteworthy and is consistent with other studies (investigating long-term BPV with WM integrity), warranting further investigation. (124-126) This may indicate that not only treating hypertension, but also BPV (independent of BP) is an important factor in improving patients' outcomes. Large cohort studies have shown that long-term BPV is associated with increased risk of mortality, dementia and cerebrovascular damage. (119, 122, 144) It would be interesting to verify our results in non-COVID cohorts. We believe that BPV could be a potential marker of brain damage in the future and may provide a valuable tool for clinical practice and therapeutic strategies in patients with high BPV, to reduce the risk of events later on.

3 | PET/CT imaging for the assessment of inflammation

In this thesis, we investigated endothelial activation in the carotid arteries of hospitalized COVID-19 patients using Gallium-68 labelled RGD (68Ga-RGD) PET/CT imaging. SARS-CoV-2 infection activates the angiotensin-converting enzyme 2 (ACE2) receptor, which is expressed on endothelial cells. (72, 148, 149) ACE2 regulates the renin-angiotensin system (RAS), and its dysregulation leads to endothelial activation, vasoconstriction, inflammation and eventually endothelial dysfunction. (72, 149) During COVID-19, the virus induces endothelial activation, which upregulates the expression of αvβ3 integrins on endothelial cells. These integrins play a role in inflammation and the 68Ga-RGD tracer specifically binds to αvβ3 integrins, allowing us to visualize endothelial activation in vivo. (162, 200) This relationship highlights how the ACE2 receptor, integrins, and tracer uptake are interconnected in the context of COVID-19-induced endothelial dysfunction.

Additionally, previous studies on ischemic stroke showed increased PET tracer activity in the carotids, suggesting it is an effective method for capturing dynamic disease activity. We found no significant increase in tracer uptake in the carotid

arteries compared to the reference group (without COVID-19), indicating that there was no significant involvement endothelial activation in the carotid arteries. We did find higher uptake of the tracer in the heart and lungs. The lack of increased tracer uptake in the carotid arteries may imply that the inflammatory response in COVID-19 does not significantly affect large arteries, but points towards a more localized vascular response, mainly in the (smaller) arteries of the cardiopulmonary system rather than extending to the cerebral vasculature. These results are again, reassuring for patients with COVID-19.

Clinical relevance

Brain abnormalities in COVID-19 patients

Our research on COVID-19's impact on cerebrovascular MRI markers provides valuable insights for future medical practice and research. Our study reports the prevalence of cerebrovascular MRI markers in COVID-19 patients admitted to a general hospital without neurological symptoms. Initially, COVID-19 patients appeared to be at high risk for thromboembolic events, but our findings indicate that the risk of cerebrovascular markers is comparable to that in healthy controls. This contrasts with early reports of increased brain abnormalities, such as ischemic stroke and microbleeds. Notably, our study revealed that COVID-19 patients had more cardiovascular risk factors and, consequently, more WMH. Understanding the likelihood of silent cerebral ischemia or other cerebrovascular (MRI) markers in asymptomatic patients is crucial, as these conditions can impact long-term outcomes. Although the lower-than-expected number of abnormalities diverges from previous studies on symptomatic patients, it offers a reassuring perspective for future patients with COVID-19 and similar viral inflammatory diseases.

In addition, we found that differences in WM integrity between COVID-19 patients and controls are primarily associated with pre-existing cerebrovascular damage (WMH) rather than the direct impact of COVID-19. Furthermore, these changes did not strongly predict long-term outcomes, indicating that other factors likely contribute to persistent symptoms and long COVID. Should a causal relationship be established between high BPV and loss of WM integrity (independent of SARS-CoV-2 infection), then continuous BP monitoring during acute care hospitalization to assess BPV may be valuable for identifying patients at risk of cerebral damage.

Furthermore, understanding the behaviour of respiratory viruses, both current and future, is important for preparing for potential pandemics, which remains

a plausible scenario. We can use this knowledge for future pandemics and might assume that the likelihood of abnormalities in viral (pulmonary) diseases, despite systemic inflammation, might primarily be located in the lungs or heart, not necessarily within the brain. Patients with neurological complaints however should always be treated following standard medical guidelines.

Future directions

In this thesis, we did not find any evidence of major abnormalities within the brain or vessels within the brain, nor did we find clear associations between WM integrity and clinical outcome measures (including long COVID) in patients with a COVID-19 infection. This indicates that COVID-19 has a limited effect on cerebrovascular disease shortly after hospitalization, in our study population at least. The absence of this direct link highlights the complexity of the etiology of ongoing post-covid symptoms. Our findings serve as a reminder of the broad nature of this disease and the necessity for a broad, multidisciplinary approach in future research.

Long COVID (Post COVID-19 Condition)

Acute, severe COVID-19 disease has reduced significantly globally. Our society has returned to pre-pandemic manners, without the need for continuous use of face masks anymore. However, long COVID remains a significant challenge for healthcare systems with pressure on health care needs and patients to be unable to work or become disabled. The definition of long COVID is complex and has undergone several revisions by the WHO over the past few years, indicating the difficulties of comprehensive understanding. Long COVID encompasses a spectrum of symptoms that persist post-infection [current definition: *the continuation or development of new symptoms 3 months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least 2 months with no other explanation]*. This phenomenon is not entirely novel, as it resembles other post-viral syndromes observed with infections like Respiratory Syncytial Virus (RSV) or Epstein-Barr virus, with post-viral fatigue as the main overlapping symptom. (38) Treatment approaches for these conditions often converge, focusing on activity modulation, physical therapy, structured routines, exercise, and stress reduction.

Pathophysiological evidence of the disease remains difficult in the diagnostic process. A recent Dutch study found that long COVID patients experience worsened muscle abnormalities and symptoms like severe fatigue and muscle pain following physical exertion. These symptoms are linked to dysfunctional mitochondria in muscle cells, which produce less energy than normal, though conclusions are limited by small sample sizes. Effective treatments remain unavailable. Our studies

have not identified any associations with long COVID nor explanations in the brain, as outlined in our thesis. An overview paper published in Nature (2023) stated that long COVID is believed to be multifactorial, with hospital-related factors such as respiratory problems at onset, duration of hospital stay, and ICU admissions linked to its persistence. (39)

While the specific causes and treatments for long COVID fall outside the scope of this thesis, the strain it places on patients and healthcare workers underscores the need for further research. Understanding and addressing long COVID is crucial for better preparedness in future pandemics.

What to do in new emerging pandemics?

In 2024, conducting scientific research has become increasingly complex. During the COVID-19 pandemic, non-essential research was halted while urgent drug studies, such as those in England, were rapidly set up. The RECOVERY trial, which discovered that dexamethasone reduced mortality in patients, was supported by the National Health Service (NHS) in England and conducted across 176 UK hospitals. The study protocol's first version was created in March 2020 with start of study the same month, with analyses published in New England Journal of Medicine (NEJM) by July 2020 impacting the whole world regarding treatment of patients with COVID-19, highlighting the rapid tempo of the project (5 months). (201)

In contrast to the swift approval process in the UK, our study experienced significant delays. Our study, driven by initial findings of increased clotting in COVID-19 patients, primarily aimed to investigate cerebral thrombosis or silent ischemia in an observational cohort study setting. After requesting medical ethical approval mid-end 2020, our research faced delays due to the prioritization of COVID-related studies and stringent ethical reviews, beginning only in April 2021—one year later, as detailed in the timeline below.

By the time we were finally permitted to include the first patient in our observational cohort study, after revising our study protocol, it was April 2021—nearly a year later. The urgency of our research question had diminished as treatments like dexamethasone became effective and other nations reported similar findings. Despite the prompt response from funding agencies, setting up a study in multiple hospitals encountered additional obstacles. Our observational cohort study, which received prioritization during the pandemic over other research, still faced delays. This underscores the need for more streamlined research protocols.

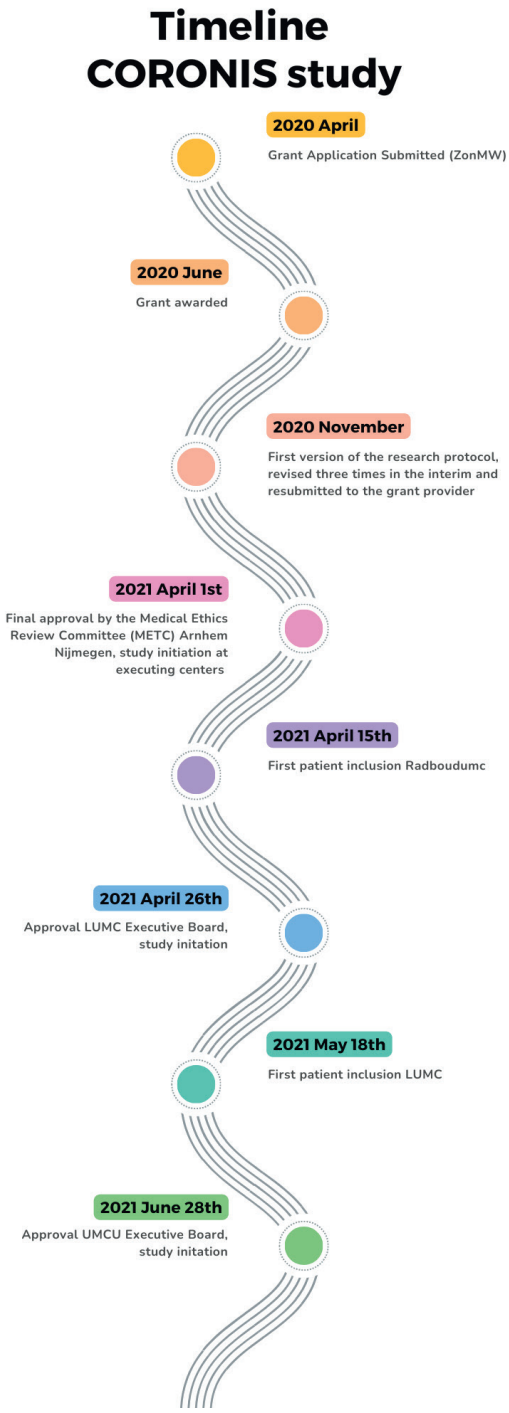

Figure 2. Timeline study approval CORONIS

Clear agreements on requirements and involvement of qualified personnel to ensure safety should allow for exceptions to standard hospital research regulations, prioritizing patient safety.

The government has committed to a structural investment of over €136 million annually in pandemic preparedness for public health, in collaboration with the Ministry of Health, Welfare and Sport, the RIVM, municipal health services, and local governments. (202) One program includes quarterly and annual studies on physical and mental health, focusing on various demographics, using data from general practitioners and large-scale health surveys in the Dutch population. (203) The research aims to provide insights for policymakers and health professionals to mitigate the pandemic's negative health effects and prepare for future health crises. Additionally, it is crucial to reevaluate our current regulations to establish streamlined procedures for study approval and implementation, enabling more effective research responses to public health during future pandemics. Furthermore, it would be of great value to incorporate research conducted during new pandemics as a foundational pillar of this kind of initiatives to achieve better and more efficient findings.

The swift implementation of drug trials in England during the pandemic presents an efficient model. Despite the low likelihood of COVID-19 re-emerging as a pandemic, future pandemics from other (viral) diseases necessitate rapid, pragmatic responses for effective treatments. International collaboration, especially within the European Union (EU), would be essential to simplify research processes rather than adding regulations.

Conclusion

In this thesis, we have ''unmasked" the cerebral and vessel wall consequences of COVID-19 infection and demonstrated that it has a limited impact on cerebrovascular MRI or PET/CT markers shortly after hospitalization. The abnormalities observed were primarily explained by underlying cardiovascular risk factors. We recommend establishing research readiness in advance to avoid wasting valuable time during the early phases of future pandemics.

Chapter 9

Summary

Summary

Overall aim

The overall aim of this thesis was to investigate the prevalence and incidence of cerebrovascular MRI markers and vessel wall abnormalities in hospitalized coronavirus disease 2019 (COVID-19) patients. Subsequently, we provided insights in the structural white matter (WM) integrity of the brain and its relation with blood pressure variability (BPV) in COVID-19 patients and its association with long-term clinical outcomes. Moreover, we aimed to explore PET/CT tracer uptake by αvβ3 integrins in the carotid arteries as a proxy for carotid endothelial activation in COVID-19 patients.

The MRI studies in this thesis **(chapters 2-6)** are based on the CORONavirus and Ischemic Stroke (CORONIS) study, a multicenter observational cohort study on the prevalence, incidence and risk factors of silent cerebral ischemia and other cerebrovascular MRI markers and long-term clinical outcomes after hospitalization. Patients included were hospitalized with COVID-19 (>18 years) and healthy controls with proven absence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, recruited in the Radboudumc, LUMC and UMCU hospital. At baseline (2011-2022), a total of 202 participants enrolled and follow-up was performed 3 and 12 months after baseline study procedures. In **Part I** of this thesis (**chapter 2**) we described the rationale and protocol of the CORONIS study.

In addition, we performed PET/CT imaging in hospitalized COVID-19 patients and compared this with references (**chapter 7**).

In **chapter 3,** we investigated cerebrovascular MRI markers in hospitalized COVID-19 patients compared to healthy controls. Brain MRIs were performed shortly after discharge and three months later. We found a similar prevalence of cerebrovascular MRI markers in COVID-19 patients compared with controls, except for white matter hyperintensities (WMH), which were more prevalent in patients (77.6 vs. 61.7%). New cerebrovascular lesions (5.1%) were observed after three months, primarily attributed to small vessel disease risk factors. Overall, we concluded that COVID-19 has a limited impact on cerebrovascular MRI markers shortly after hospitalization.

In **chapter 4,** we investigated the frequency of intracranial vessel wall enhancement (VWE) on MRI as a sign of arterial vessel wall inflammation. We included patients with COVID-19 who underwent MRI shortly after discharge and again three months later. VWE was found in 21% of patients, most commonly in the vertebrobasilar

arteries. Follow-up MRI in patients showed VWE in 27%; and in 5 patients new lesions (60% in vertebrobasilar artery, 40% in the a. carotid interna). Increasing age, male sex, and history of stroke or TIA were associated with VWE. The causal relationship between COVID-19 and VWE remains uncertain, necessitating further research to assess the prevalence of VWE in other clinical contexts, particularly among healthy adults.

In **chapter 5**, we investigated WM integrity by brain MRI and its associations with clinical outcomes. Our patients and controls underwent MRI-DWI imaging shortly after discharge and three months later. We assessed WM integrity using diffusion tensor imaging (DTI) and with clinical assessments at three and twelve months. Patients showed higher peak width of skeletonized mean diffusivity (PSMD) and lower NDI in several regions indicating lower WM integrity compared to controls, but these differences were not significant after adjusting for WM hyperintensities. We found that WM integrity shortly after discharge was not linked to clinical outcomes, suggesting other factors contribute to worse outcomes in COVID-19 patients.

In **chapter 6**, we examined whether short-term BPV measured in-hospital is associated with cerebral microstructural integrity in COVID-19 patients. We included 20 hospitalized COVID-19 patients who underwent continuous BP monitoring and MRI-DWI shortly after discharge. A higher BPV was associated with lower fractional anisotropy (FA) and higher PSMD, indicating WM damage. These associations persisted after adjusting for confounders and were not influenced by WMH volume or hypertension. Our findings showed that high BPV is associated with decreased microstructural integrity of the WM. If a causal relationship is established, continuous monitoring during hospitalization could help identify patients at risk for cerebral damage. Further validation is needed in patients without COVID-19 or another viral disease.

In **chapter 7**, we investigated endothelial activation in the carotid arteries of hospitalized COVID-19 patients using Gallium-68 labeled RGD (68Ga-RGD) PET/CT imaging. Our study included ten COVID-19 patients and five non-COVID-19 references. We found no significant increase in tracer uptake in the carotid arteries, but observed significantly higher uptake in the heart) and lungs of COVID-19 patients, suggesting localized endothelial activation in the cardiopulmonary system rather than in the cerebral vasculature. These results indicate that the inflammatory response in COVID-19 primarily affects the cardiopulmonary system.

Conclusion

In this thesis, we have ''unmasked'' the cerebral and vessel wall consequences of COVID-19 infection and demonstrated that it has a limited impact on cerebrovascular MRI or PET/CT markers shortly after hospitalization. The abnormalities observed were primarily explained by underlying cardiovascular risk factors. We recommend establishing research readiness in advance to avoid wasting valuable time during the early phases of future pandemics.

Chapter 10

Dutch summary

Nederlandse samenvatting

Het doel van dit proefschrift was om de prevalentie en incidentie van MRI markers voor cerebrovasculaire ziekte en vaatwandafwijkingen bij klinisch opgenomen patiënten met COVID-19 te onderzoeken. Daarnaast richtte het onderzoek zich op de structurele integriteit van witte stof (WM) in de hersenen, de relatie met bloeddrukvariabiliteit (BPV) en de associatie van deze factoren met lange termijn klinische uitkomsten. Verder werd endotheel activatie in de carotiden in COVID-19-patiënten middels traceropname door αvβ3-integrines onderzocht met PET/CT-beeldvorming.

De MRI-studies in dit proefschrift (**hoofdstukken 2-6**) zijn gebaseerd op de CORonavirus and Ischemic Stroke (CORONIS) studie. Dit is een multicenter, observationele, cohortstudie waarin de prevalentie, incidentie en risicofactoren van MRI markers voor cerebrovasculaire ziekte en lange termijn uitkomsten na ziekenhuisopname door COVID-19 onderzocht is. Patiënten (≥18 jaar) opgenomen in het ziekenhuis vanwege COVID-19 en controle deelnemers zonder COVID-19-infectie werden geïncludeerd in het Radboudumc, LUMC en UMCU. In totaal werden 202 deelnemers geïncludeerd (2021-2022) en gevolgd gedurende 3 en 12 maanden na de baseline onderzoeken. **Hoofdstuk 2** beschrijft de rationale en het protocol van de CORONIS-studie.

Daarnaast werden PET/CT-scans uitgevoerd bij klinisch opgenomen COVID-19-patiënten, waarbij de resultaten werden vergeleken met die van controles (**hoofdstuk 7**).

In **hoofdstuk 3** onderzochten we MRI markers voor cerebrovasculaire ziekte bij COVID-19-patiënten kort na ontslag en drie maanden later, vergeleken met gezonde controles. De prevalentie van de MRI markers voor cerebrovasculaire ziekte was vergelijkbaar met uitzondering van witte stofafwijkingen, die vaker voorkwamen bij patiënten (77,6% versus 61,7% bij gezonde controles). Bij 5,1% van de patiënten werden nieuwe laesies waargenomen, die waarschijnlijk te verklaren zijn door onderliggende cardiovasculaire risicofactoren, predisponerend voor small vessel disease (SVD). We concludeerden dat COVID-19 een beperkte impact heeft op MRI markers voor cerebrovasculaire ziekte kort na ziekenhuisopname.

In **hoofdstuk 4** onderzochten we intracraniële vaatwandaankleuring op MRI, als een marker voor arteriële vaatwandinflammatie. Patiënten ondergingen een MRI kort na ontslag en drie maanden later opnieuw. Vaatwandaankleuring werd

vastgesteld bij 21% van de patiënten, voornamelijk in de vertebrobasilaire arteriën. Bij follow-up bleek vaatwandaankleuring aanwezig in 27% van de patiënten; nieuwe vaatwandaankleuring werd waargenomen bij 5 patiënten (60% in de vertebrobasilaire arteriën, 40% in de a. carotis interna). Oudere leeftijd, mannelijk geslacht en een voorgeschiedenis van een beroerte of TIA waren geassocieerd met vaatwandaankleuring. De causale relatie tussen COVID-19 en VWE blijft onduidelijk en vereist nader onderzoek in bredere populaties, waaronder gezonde volwassenen.

In **hoofdstuk 5** onderzochten we de integriteit van de witte stof bij COVID-19-patiënten en de associatie met klinische uitkomsten. Diffusion-tensor imaging (DTI-MRI) en vragenlijsten over klinische uitkomsten (o.a. dagelijks functioneren, stemmingsklachten en long COVID) werden verricht kort na ontslag en drie en twaalf maanden later. Hoewel patiënten een hogere piekbreedte van peak width of skeletonized mean diffusivity (PSMD) en een lagere witte stof integriteit hadden, waren deze verschillen niet significant na correctie voor witte stofafwijkingen. We vonden geen associatie tussen witte stof integriteit kort na ontslag en klinische uitkomsten. Dit wijst erop dat andere factoren bijdragen aan slechtere prognoses bij COVID-19-patiënten.

In **hoofdstuk 6** onderzochten we de relatie tussen korte termijn variabiliteit van de bloeddruk (BPV), gemeten tijdens ziekenhuisopname, en de integriteit van de witte stof in de hersenen. Bij klinisch opgenomen COVID-19-patiënten, die continue bloeddrukmonitoring (tijdens ziekenhuisopname) en MRI-beeldvorming kort na ontslag ondergingen, was een hogere BPV geassocieerd met lagere fractionele anisotropie (FA) en hogere PSMD (wat duidt op lagere witte stof integriteit van de hersenen). Deze associaties bleven significant na correctie voor confounders. Indien de causale relatie wordt bevestigd, kan continue BPV-monitoring tijdens ziekenhuisopname helpen bij het identificeren van patiënten met risico op cerebrale schade.

In **hoofdstuk 7** onderzochten we endotheel activatie in de carotiden van COVID-19-patiënten met Gallium-68-labeled RGD (68Ga-RGD) PET/CT beeldvorming. We vonden een hogere opname van de tracer in het hart en de longen, maar geen significante toename in traceropname in de carotiden.

Conclusie

In dit proefschrift hebben we de gevolgen van COVID-19 in de hersenen en de intracraniële vaatwanden onderzocht middels MRI en PET onderzoek. De impact van COVID-19 op deze MRI- en PET/CT-markers kort na ziekenhuisopname bleek

beperkt en werd grotendeels verklaard door onderliggende cardiovasculaire risicofactoren. We adviseren proactieve voorbereiding op toekomstig onderzoek bij pandemieën, om kostbare tijd in de vroege fasen te benutten voor effectieve data- en kennisverzameling.

Appendices

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List of abbreviations

Abbreviations	
68Ga-RGD	Gallium-68 labelled RGD
ACA	Ànterior cerebral artery
ACI	Arteria carotid interna
ACE	Angiotensin-converting enzyme
ACE2	Angiotensin-converting enzyme 2
ACE-I	Angiotensin-coverting enzyme inhibitor
ADC	Apparent diffusion coefficient
aOR	Adjusted odds ratio
ARB	Angiotensin receptor blocker
ARV	Average Real Variability
ATR	Anterior thalamic radiation
BMI	Body Mass Index
BA	Basilar artery
BP	Blood pressure
BPV	Blood pressure variability
CABG	Coronary artery bypass grafting
CCB	Calcium channel blocker
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
CORONIS	CORONavirus and Ischemic Stroke (study)
COVID-19	Coronavirus disease 2019
CRP	C-reactive protein
CSF	Cerebral spinal fluid
CT	Computed tomography
CTA	Computed tomography angiography
CTac	Low-dose CT scans for attenuation correction
CTld	Unhanced low dose CT
CV	Coefficient of Variation
DAG	Directed acyclic graph
DOAC	Direct oral anticoagulation
DTI	Diffusion tensor imaging
DWI	Diffusion-weighted imaging
eGFR	Estimated glomerular filtration rate
ECs	Endothelial cells

Abbreviations	
EU	European Union
FA	Fractional anisotropy
FDR	False discovery rate
FLAIR	Fluid-attenuated inversion recovery
FSL	Functional Magnetic Resonance Imaging of the Brain Software Library
HADS	Hospital Anxiety and Depression Scale
HZV	Herpes zoster virus
HIV	Human immunodeficiency virus
HU	Hounsfield units
ICA	Internal carotid artery
ICU	Intensive care unit
IQR	Interquartile range
OR	Odds ratio
LDH	Lactate dehydrogenase
LUMC	Leids Universitair Medisch Centrum
MCA	Middle cerebral artery
MCI	Mild Cognitive Impairment
MD	Mean diffusivity
MOCA	Montreal Cognitive Assessment
MRI	Magnetic Resonance Imaging
mRS	Modified Rankin Scale
MS	Multiple sclerosis
NA or N/A	Not applicable
NEJM	New England Journal of Medicine
NHS	National Health Service
NODDI	Neurite orientation dispersion and density imaging
NDI	Neurite density index
ODI	Orientation dispersion index
PCA	Posterior cerebral artery
PCI	Percutaneous coronary intervention
PCFS	Post-COVID-19 Functional Status scale
PCR (test)	Polymerase chain reaction (test)
PE	Pulmonary embolism
PET/CT	Positron emissie tomografie / computer tomografie

Abbreviations	
PFO	Patent foramen ovale
PRES	Posterior reversible encephalopathy syndrome
PSMD	Peak width of skeletonized mean diffusivity
Radboudumc	Radboud University Medical Center
RIVM	Dutch National Institute of Public Health and the Environment
RAS	Renin-angiotensin system
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SD	Standard deviation
STRIVE	STandards for ReportIng Vascular changes on nEuroimaging
STROBE guidelines	Strengthening the Reporting of Observational Studies in Epidemiology guidelines
SUV	Standardized uptake values
SVD	Small vessel disease
SWI	Susceptibility-weighted imaging
TBSS	Tract-Based Spatial Statistics
TE	Echo time
TIA	Transient ischemic attack
TICS-M	Modified Telephone Interview for Cognitive Status
TR	Repetition time
UMCU	Universitair Medisch Centrum Utrecht
VA	Vertebral artery
VAS	Visual Analogue Scale
VWE	Vessel wall enhancement
VWI	Vessel wall imaging
VZV	Varicella zoster virus
V/Q SPECT	Ventilation/perfusion single photon emission tomography
WM	White matter
WMH	White matter hyperintensities

&

Curriculum vitae

Resa van Lith was born on February 27th 1995 in Nijmegen, the Netherlands. After graduation from secondary school (Stedelijk Gymnasium Nijmegen) in 2013, she started with the study medicine at the Radboud University Nijmegen.

For her master thesis, she investigated the risk of ischemic and bleeding events in young stroke patients of the FUTURE study, under supervision of Jamie Verhoeven and Prof. dr. Frank-Erik de Leeuw. She obtained her medical degree in 2020. Subsequently, she started her PhD project at the Neurology department of the Radboudumc under supervision of Prof. Dr. Frank-Erik de Leeuw and dr. Tuladhar. The results of this project are presented in this thesis.

During the course of the PhD, she also worked as a medical doctor (not in training) in the Rijnstate hospital for seven months whilst continuing her PhD. As of August 2024, Resa worked as a medical doctor (not in training) in Neurology at the Radboudumc.

In January 2025, she started her Neurology residency program at the Radboudumc.

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List of publications

* Shared authorship

This thesis

1. **van Lith TJ***, Sluis WM*, Wijers NT*, Meijer FJ, Kamphuis-van Ulzen K, de Bresser J, Dankbaar JW, van den Heuvel FM, Antoni ML, Mulders-Manders CM, de Mast Q, van de Veerdonk FL, Klok FA, Tuladhar AM, Cannegieter SC, Wermer MJ, van der Worp HB, Huisman MV*, & de Leeuw FE* (2022). Prevalence, risk factors, and long-term outcomes of cerebral ischemia in hospitalized COVID-19 patients - study rationale and protocol of the CORONIS study: A multicentre prospective cohort study. Eur Stroke J, 7(2), 180-187. https://doi.org/10.1177/23969873221092538
2. van Genugten EAJ*, **van Lith TJ***, van den Heuvel FMA*, van Steenis JL, Ten Heggeler RM, Brink M, Rodwell L, Meijer FJA, Lobeek D, Hagmolen Of Ten Have W, van de Veerdonk FL, Netea MG, Prokop M, Nijveldt R, Tuladhar AM, & Aarntzen E (2023). Gallium-68 labelled RGD PET/CT imaging of endothelial activation in COVID-19 patients. Scientific Reports, 13(1), 11507. https://doi.org/10.1038/s41598-023-37390-9
3. **van Lith TJ***, Sluis WM*, Wijers NT, Meijer FJA, Ulzen KK, de Bresser J, Dankbaar JW, de Mast Q, Klok FA, Cannegieter SC, Wermer MJH, Huisman MV, Tuladhar AM, van der Worp HB, & de Leeuw FE (2024). Prevalence and 3-month follow-up of cerebrovascular MRI markers in hospitalized COVID-19 patients: the CORONIS study. Neuroradiology. https://doi.org/10.1007/s00234-024-03411-1
4. **van Lith TJ***, Li H, van der Wijk MW, Wijers NT, Sluis WM, Wermer MJH, de Leeuw FE, Meijer FJA & Tuladhar AM (2024) White matter integrity in hospitalized COVID-19 patients is not associated with short- and long-term clinical outcomes. Frontiers in Neurology; 2024 Aug;15:1440294. https://doi.org/10.3389/fneur.2024.1440294
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Articles submitted in this thesis

1. Wijers NT, **van Lith TJ**, Sluis WM, Meijer FJA, Kamphuis-van Ulzen K, Dankbaar JW, Tuladhar AM, van der Worp HB, Huisman MV, Klok FA, de Leeuw FE, Cannegieter SC, de Bresser J, & Wermer MJH. Intracranial vessel wall enhancement on MRI in patients with COVID-19. *Under review*

Other publications:

1. Verhoeven JI., **van Lith TJ**, Ekker MS, Hilkens NA, Maaijwee NAM, Rutten-Jacobs LCA, Klijn CJM, & de Leeuw FE (2022). Long-term Risk of Bleeding and Ischemic Events After Ischemic Stroke or Transient Ischemic Attack in Young Adults. Neurology, 99(6), e549-e559. https://doi.org/10.1212/WNL.0000000000200808
2. Immens MH, van den Hoeven V, **van Lith TJ**, Duijnhouwer TD, Ten Cate TJ, & de Leeuw FE (2024). Heart-Stroke Team: A multidisciplinary assessment of patent foramen ovale-associated stroke. Eur Stroke J, 9(1), 219-225. https://doi.org/10.1177/23969873231214862

PhD Portfolio

Courses and workshops	Organizer	Year
Introduction Day	Radboudumc	2021
Graduate School Introduction Day	Donders Graduate School	2021
Graduate School Day 1 & 2	Donders Graduate School	2021 + 2023
Basiscursus Regelgeving en Organisatie voor Klinisch onderzoekers (BROK)	NFU BROK Academie	2021
Castor online courses	Castor	2021
R course LUMC	Boerhaave Nascholing LUMC	2021
RU – Writing Scientific Articles	Radboud University	2021
Statistics for Clinical Researchers	Radboudumc	2023
RU – Effective Writings Strategies	Radboud University	2023
Spies & Spreken – Presenteren en promoveren	Spies & Spreken	2023
R Course	Radboudumc	2023
Cursus Zelfinzicht – de sleutel voor je loopbaan	Radboud University	2023
Scientific Integrity Course	Radboudumc	2023
RU – Design and Illustration	Radboud University	2023
RU - Analysing longitudinal and multilevel data using R	Radboud University	2023
NIHSS Course	ErasmusMC/Radboud University	2024
The next step in my career	Radboudumc	2024

External lectures and conferences	Role	Location	Year
International Stroke Conference 2021	Visitor	Virtual/online	2021
ESOC Conference 2022	Poster Presentation	Lyon	2022
De Jonge Specialist Congres 2023	Visitor	Online	2022
ESOC Conference 2023	Poster Presentation	Munchen	2023
ESOC Conference 2024	Poster Presentation	Basel	2024
De Jonge Specialist Congres 2024	Visitor	Den Bosch	2024

Project supervision	Year	Duration
Vincent van der Hoeven (Master)	2021	3 months
Marte van der Wijk (Master)	2022	6 months

&

Research data management

Medical and ethical approval of the studies:

- This thesis is based on the results of research involving human participants (or existing data from published papers), which were conducted in accordance with relevant national and international legislation and regulations, guidelines, codes of conduct and Radboudumc policy.
- The recognized Medical Ethics Review Committee 'METC Oost-Nederland' has given approval to conduct these studies (NL75780.091.20).
- The institutional ethical review committee CMO Radboudumc, Nijmegen, the Netherlands has given approval to conduct these studies (CMO Radboudumc dossier number: 2020-7114, biobank 2020-7139).

Privacy participants:

- According to Dutch legislation, data collection from electronic patient files was performed by personnel with a treatment relationship with the patient or by the researcher(s) upon consent by the study participant.
- The privacy of the participants in these studies was warranted by the use of pseudonymization.
- The pseudonymization key was stored on a secured network drive that was only accessible to members of the project who needed access to it because of their role within the project. The pseudonymization key was stored separately from the research data.
- The privacy of the participants in these studies was warranted by the use of fully anonymous data.

Informed consent:

- Informed consent was obtained from participants to collect and process their data for this research project.
- Consent was also obtained for sharing the (pseudonymized) data after research.
- The sensitivity and confidentiality of the raw qualitative data (i.e. interviews, forum groups) makes sharing of the data without compromising confidentiality and privacy impossible, therefore consent for sharing of the raw data was not asked from the participants.

Data collection and storage:

- Data for all studies in this thesis were collected in the Radboudumc hospital and not re-used from other sources.
- Data and questionnaires for all studies were collected through electronic Case Report Forms (eCRF) of a prospective data collection in Castor EDC. Data were converged from (electronic) health records or Castor EDC to SPSS (SPSS Inc., Chicago, Illinois, USA) or R version 4.3.1.
- Clinical data for all studies were extracted and added in the electronic Case Report Forms (eCRF) in Castor EDC, from (electronic) health records (EPIC).
- Data from chapter 2-6 were stored and analyzed on the Neurology department server (\\umcms011\neuro_onderzoek$\CORONIS1) and in Castor EDC and are only accessible by project members working at the Radboudumc.
- Data from chapter 7 were stored at the Radiology and Nuclear Imaging Department server and are only accessible by project members working at the Radboudumc.
- These secure storage options safeguard the availability, integrity and confidentiality of the data.
- Paper (hardcopy) data is stored in cabinets on the department on the Neurology department.

Data sharing according to the FAIR principles

- Repository where the data will be saved: Radboud Data Repository.
- Accessibility will be regulated via prof. dr. F.E. de Leeuw.
- DOI dataset: https://doi.org/10.34973/e1sa-zs44.
- Metadata standards: available on the Radboud Data Repository.
- The data underlying the published chapters 2-7 are available for reuse through the following data repositories: Radboud Data Repository.
- Data were made reusable by adding sufficient documentation (research protocol, codebook and a readme file), by using preferred and sustainable data formats and by publishing under the CC.BY.4.0 license.
- The datasets from chapters 2-6 were published with restricted access. Requests for access will be checked by prof. dr. F.E. de Leeuw against the conditions for sharing the data as described in the signed Informed Consent.
- The data underlying chapter 7 is not suitable for reuse and will be archived for 15 years in DACs and RDCs of the Radboud Data Repository after termination of the study.

All research data that are presented in this thesis were well archived according to the FAIR (Findable, Accessible, Interoperable and Reusable) principles.

Donders Graduate School for Cognitive Neuroscience

For a successful research Institute, it is vital to train the next generation of scientists. To achieve this goal, the Donders Institute for Brain, Cognition and Behaviour established the Donders Graduate School in 2009. The mission of the Donders Graduate School is to guide our graduates to become skilled academics who are equipped for a wide range of professions. To achieve this, we do our utmost to ensure that our PhD candidates receive support and supervision of the highest quality.

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Beste Mina, dr. Jacob, mr. Egypt, toen ik op 1 januari 2021 de onderzoekskamer in stapte (mss was het 2 januari) was het altijd dezelfde aanblik; Mengfei en jij in de hoek, druk bezig met de R analyses. Toen Esther en ik kwamen hebben we met jullie onderzoekskamer 3 omgetoverd in een feestkamer met heel veel plezier, raphitjes, fietstochtjes, lachen, klagen en steun aan elkaar. Ik vind het super knap hoe je het allemaal hebt gedaan afgelopen jaren en je gaat binnenkort ook nog met je droomvrouw trouwen. Ik kijk uit naar komende jaren nog verder samen.

Beste Maikel en Axel, neurodinnies, samen hebben we vanaf het begin al veel plezier gehad als PhDers in de onderzoekskamer en nadien in het nieuwe gebouw. Zelfs een weekje weg om overdag te schrijven en 's avonds Selling Sunset te bingen is niet te gek voor ons. Wielrennen op fietsvakantie, DTRH, binnenkort zelfs de bruiloft in Egypte en nog veel meer wat we al samen hebben gedaan. Zonder jullie was mijn PhD tijd veel saaier geweest en ik ben blij dat ik komende jaren nog met jullie verder zal gaan als AIOS dinnies.

Mengfei, at the start of my PhD we had a lot of fun with Mina and Esther. I have really nice memories from our mountainbike tours together (with Mina not eating

enough and you taking care of him) as a superduo you both are. I am looking forward to seeing you again or later on as professor together with dr. Jacob!

Hao, I want to thank you for your patience and humor during our projects together. Often we struggled a lot with the DTI analysis but in the end it always came together. I think it is really brave of you to come here for the overseas PhD. You are our big publishing star and I admire you for that. Thank you for your help.

Floor, wij kennen elkaar natuurlijk al geruime tijd (sinds start studie!!!). Ik wist dat je een aanwinst voor het team zou worden en je hebt enorm veel gezelligheid (en kennis!) meegebracht. De congressen zouden zonder jou niets zijn.

Mijntje, toen jij overkwam naar de ''onderzoekershoek'' wisten we dat het goed zat met jou. Met jouw jarenlange ervaring met onderzoek, SPSS en inmiddels R queen kunnen we altijd bij je terecht. Nu verhuisd naar een prachtig huis, maar gelukkig zullen we elkaar in de opleiding nog veel zien!

Jamie, mijn onderzoekscarrière is begonnen bij jou en Frank-Erik. Na mijn onderzoeksstage heb ik de neurologie (gelukkig) niet meer achter me kunnen laten. Ons project is in een prachtig blad gepubliceerd en heeft zijn vruchten afgeworpen. Nu tevens huisgenoten ''over de brug'' en dezelfde ideeën over het vitaal houden van onszelf en elkaar, ik kijk uit naar de komende jaren.

Maaike, Nina en Karlijn, zowel mede-onderzoekers als wielrenners, afgelopen jaren heb ik veel van jullie aanwezigheid genoten op zowel de werkvloer als op de berg. Het is leuk dat we dit elk jaar weer doen en we zetten deze lijn komende jaren gewoon door.

Annemieke, jij bent betrokken geweest bij de opstartfase van de CORONIS studie en hebt al veel voorwerk verricht waar ik op heb voort kunnen borduren. Veel dank hiervoor.

Vincent en Marte, als studenten zijn jullie een van de pijlers van het succes van de CORONIS studie geweest. Jullie hebben actief geholpen met de inclusies en follow-up van de deelnemers en dit in goede banen geleid toen ik er niet was. Heel erg bedankt voor jullie hulp en het prachtige resultaat.

Mede (oud) onderzoekers, Anna, Erin, Esmée, Esther B, Fang, Gemma, Hanna, Janneke, Merel, Lotte, Sanne en andere collega's, bedankt voor al jullie enthousiasme, steun en motiverende woorden. We hebben elkaar altijd goed kunnen oppeppen en helpen, neurovasculair is de place to be!

Mede AIOS, neurologen, CVA nazorg collega's en verpleegkundigen van het Radboudumc, ik kijk uit naar de komende jaren te gaan met jullie!

Jules en Willanka, aka Neuroadboudmusketeers, aka opleidingsgenootjes!!! Ik ben ontzettend blij dat wij samen dit jaar de opleiding zijn gestart. Zowel de voorfase, als ook de PhD afrondstruggles en de kliniekperikelen kan ik altijd heerlijk met jullie bespreken. Op zaal hebben we dan ook veel gestreden en gelachen samen, op nog veel borrels samen!

Melina, als student heb ik je leren kennen maar inmiddels uitgegroeid tot vriendin! Ik ben heel erg blij dat je voor Nijmegen hebt gekozen en uit Amsterdam hiernaartoe bent gekomen. Ik hoop dat we de komende jaren nog veel gaan koffie drinken samen en wandelingen in Live Laugh Lent zullen maken.

Sarah, Joost en de rest van de Rijnstate collega's; bedankt voor de geweldige tijd in de kliniek als ANIOS in het Rijnstate. Ik hoop jullie komende jaren nog vaak terug te zien.

Lieve Vera, aka ''Bae", dada, 70e bestuursbae, lichtingsgenoot, karibu sana voor jouw bestaan in mijn leven. Ik ben ontzettend blij dat we in 2014 met Marente rond de tafel in het studiecentrum zaten om Rowbuust te verenigen. Sinds die tijd bij jij ondenkbaar uit mijn leven. Tanzania in 2024 met jou is een van de mooiste ervaringen in mijn leven. Ninafray na nakupenda sana, asante sana dada, ninataka kurudi na wewe! Ik hoop dat we in de toekomst ooit nog zoiets moois samen zullen gaan doen (met inmiddels jouw ''mini" bae!?). Jij bent een van de constante factoren in mijn leven en ik ben je daar heel erg dankbaar voor. Ik kan niet wachten tot mini Bae er is!

Jelle, aka ''Blacka", praeses, inmiddels kennen we elkaar al meer dan 10 jaar and still going strong. Vanaf het bestuursjaar zat het goed; wij konden op elkaar bouwen. We denken vaak precies hetzelfde en ons ''beleid" komt dan meestal ook overeen met een hoge mate van efficiëntie. Altijd als ik er even doorheen zit weet jij mij weer op te vrolijken met een Haribo en je gezelschap. Ik ben blij dat je weer terug

bent in NL en in Nijmegen bent gebleven, want ik word heel bij van jou. Op nog vele (wielren)jaartjes samen.

Micaela, aka ''Mica", de avonturier. Toen ik Ben en jou mocht opzoeken was dit precies het kantelpunt in mijn leven (ook wel de ''brandende" toren). Voor mij een onvergetelijke en noodzakelijke reis naar Panama. Het is inspirerend hoe jullie aldaar bezig waar de wereld en zorg beter te maken. Ik ben dankbaar dat ik hier een glimps van heb mogen opvangen en ik ben super trots op jullie. Ik geniet van onze donderdagen bij Looplab en ben heel erg blij met jou!

Lieve Jasmijn, Sarah en Sieb, aka ''de lotgenoten van 215", wie had gedacht dat wij samen in de groepsapp onderzoekers van 215 zouden eindigen. We hebben altijd veel steun aan elkaar gehad en bij koffie's onze gedachtes kunnen delen. Inmiddels al 2 van ons klaar en nog steeds zien we elkaar veel. Jas; ik vind het fijn hoe we afgelopen jaren naar elkaar toe zijn gegroeid en je bent een voorbeeld in hoe jij je eigen plan trekt! Saar; al vanaf start als tappertjes samen and still going strong. Je bent een geweldig mens, vergeet dat niet! Sieb; zonder jou zouden we een stuk minder hebben gelachen en ik kan jouw nuchterheid altijd waarderen.

Aaron, ik wil je bedanken voor je geloof in mij in de start van de PhD. Je hebt het grootste traject hiervan meegemaakt en mij vertrouwen gegeven in mezelf. Ook al is het einde anders gelopen, de fijne start van mijn PhD heb ik grotendeels aan jou te danken. Ik hoop dat we hier over een aantal jaar met een fijn gevoel op terug kunnen kijken!

Sophie Tooten, aka Toto/mentorkindje/dinnie/chimaera/medeoudphocasbestuur/klep… je weet het wel. Het begon als kindje en inmiddels has the student become the teacher. Ik ben blij dat ik je bij Phocas heb kunnen trekken en je nu weer zie in het ziekenhuis. Je gaat een geweldige arts worden en ik ben blij dat ik je collega zal blijven in de toekomst!

Emma, koningin van de bopla/borrels/kapla en noem maar op. Ik ben blij dat we afgelopen jaar zo naar elkaar toe zijn gegroeid en geniet van onze momenten samen. Je bent altijd oprecht geïnteresseerd en hebt mij meermaals met een kaartje/tijdschrijft of iets anders liefs verrast. Ik ben blij met jou!

Donya, ookwel ''donnie", je bent mijn levensvriendin en dat zal je voor altijd blijven!

Marieke en Marleen, aka ''Miek en Marly", dank voor jullie onuitputtelijke steun. Vanaf de middelbare volgen jullie al mijn tijdlijn en ondanks we allemaal het roer meermaals hebben omgegooid weten we elkaar altijd terug te vinden!

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Al mijn andere lieve vrienden en familie, waaronder de rest van cogroep 2015, de ''boys", Rowbuust, chimaeren en nimaeren, Ruben B, Cindy, Liora, Myrna en meer, heel erg bedankt voor jullie vertrouwen in mij dat ik deze PhD tot het einde ging brengen!

Lieve Huub, niet meer broertje te noemen maar (grotere) broer. Door de jaren heen is het niet makkelijk geweest en ik ben ontzettend trots op waar je nu staat en waar je vandaan bent gekomen. Je hebt je eigen plan getrokken (amsterdammer geworden?!) en ik ben blij dat we afgelopen jaren zo naar elkaar toe zijn gegroeid (en ik ben blij dat we het roeibloed allebei delen nu). We komen er steeds meer achter hoeveel we op elkaar lijken en dat vind ik heerlijk. We zijn elkaar steun en toeverlaat en ik ben blij dat je me opzoekt voor een rondje ''autorijden" als we weer eens wat te bespreken hebben. Je hebt me afgelopen jaar heel erg gesteund in alles en daar ben ik je dankbaar voor. Je bent daarom ook geen ''broertje" maar broer te noemen en ik ben heel blij dat je in mijn leven bent!

Lieve pap en mam (en Flip), waar moet ik beginnen. Ik denk dat afgelopen jaar voor jullie misschien nog wel meer als een rollercoaster voelde als voor mij. Er is ontzettend veel tegelijk gebeurd en dankzij jullie heb ik alles weer op de rit en woon ik dichtbij jullie in de buurt (met de prachtige badkamer van pap!!!). Alle twijfels en alle levenskeuzes hebben jullie met veel geduld altijd aangehoord en me hierin geadviseerd. Ik had nooit hier gestaan op deze dag zonder jullie. Ik ben blij met jullie onbegrensd vertrouwen in mij, dat ik alles kan bereiken als ik er maar voor ga en alle kansen die jullie ons hebben gegeven om hier te komen. Ik ben ook dankbaar wat jullie hiervoor op hebben gegeven voor ons. Pap, bedankt dat jij altijd mijn (kliniek) verhalen aanhoort en hier ook aandacht en interesse voor hebt,

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