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Neuroinflammation in cognitive decline post-cardiac surgery (the FOCUS study): an observational study protocol

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ABSTRACT

Introduction

Postoperative cognitive dysfunction (POCD) occurs frequently after coronary artery bypass grafting (CABG). The underlying mechanisms remain poorly understood, but neuroinflammation might play a pivotal role. We hypothesize that systemic inflammation induced by the surgical trauma could activate the innate immune (glial) cells of the brain. This could lead to an exaggerated neuroinflammatory cascade, resulting in neuronal dysfunction and loss of neuronal cells. Therefore, the aims of this study are to assess neuroinflammation *in vivo* pre- and post-surgery in patients undergoing major cardiac surgery and investigate whether there is a relationship of neuroinflammation to cognitive outcomes, changes to brain structure and function, and systemic inflammation.

Methods and analysis

The FOCUS study is a prospective, single-centre observational study, including 30 patients undergoing elective on-pump CABG. Translocator protein (TSPO) PET neuroimaging will be performed pre- and postoperatively using the second generation tracer ^{18}F-DPA-714 to assess the neuroinflammatory response. In addition, a comprehensive cerebral MRI will be performed pre- and post-surgery, in order to discover newly developed brain and vascular wall lesions. Up to 6 months postoperatively, serial extensive neurocognitive assessments will be performed and blood will be obtained to quantify systemic inflammatory responses and peripheral immune cell activation.

Ethics and dissemination

Patients do not benefit directly from engaging in the study, but imaging neuroinflammation is considered safe and no side effects are expected. The study protocol obtained ethical approval by the Medical Research Ethics Committee (MREC) region Arnhem-Nijmegen. This work will be published in peer-reviewed international medical journals and presented at medical conferences.

Article summary

Strengths and limitations of this study
• This study is the first to examine *in vivo* neuroinflammation both prior to and after cardiac surgery and combines state of the art neuroimaging with longitudinal neuropsychological examinations and detailed immunological analyses
• This study could provide insights whether neuroinflammation plays a key role in POCD, which would make the neuroinflammatory response a future treatment target
• Extensive neuropsychological examinations at four different timepoints enable us to detect even subtle changes in cognitive function
• This study is powered to detect the association between neuroinflammation and cognitive decline between baseline and hospital discharge, the period of time the incidence of cognitive dysfunction is highest.

Keywords

Postoperative cognitive dysfunction (POCD), coronary artery bypass grafting (CABG), neuroinflammation, microglia, TSPO neuroimaging

INTRODUCTION

Background and rationale

Coronary artery bypass grafting (CABG) has been associated with postoperative cerebral complications.[1-4] These may occur directly post-surgery, like stroke or delirium, but can also have long-term sequelae such as postoperative cognitive dysfunction (POCD) and dementia. The incidence of POCD ranges from 20-70% in the first week after surgery, to 10-40% in the following months but may increase again from one postoperative year onwards.[1, 5] Furthermore, a 1.7-fold increased risk to develop new incident Alzheimer's disease within 6 years after CABG was found, compared to patients undergoing a percutaneous coronary intervention.[6]

Development of POCD is presumably related to perioperative brain hypoperfusion, cerebral microembolization, haemodilution, hypercoagulability, cerebral hyperthermia and inadequate glucose homeostasis.[2] In addition, systemic inflammation is hypothesized as an important (and possibly treatable) factor for POCD pathogenesis.[7-9] Cardiac surgery produces substantial systemic inflammation due to multiple stimuli such as sternotomy, extracorporeal circulation (ECC), associated transient endotoxemia, and aortic cross-clamping. Severe systemic inflammation can result in increased permeability of the blood-brain barrier (BBB), enabling systemic inflammatory cytokines to enter the brain.[10, 11] As a result, systemic inflammation may induce activation of the innate immune cells of the brain, the microglia and astrocytes, leading to a neuroinflammatory response.[12] Animal models demonstrated that systemic administration of low-dose endotoxin even leads to long-term inflammatory reprogramming of microglia.[13] Interestingly, research has shown that neuroinflammation is associated with cognitive dysfunction and neurodegenerative disease.[14, 15] Given these associations, we hypothesize that occurrence of POCD in patients undergoing CABG is mediated through the occurrence of neuroinflammation.

Positron Emission Tomography (PET) enables the imaging of glial activation in the CNS using radiolabelled antagonists of translocator protein (TSPO).[16, 17] TSPO is mainly expressed as a transmembrane protein on mitochondria of microglia and astrocytes in the CNS.[18] As TSPO expression is upregulated during neuroinflammatory processes, it makes a promising biomarker for imaging neuroinflammation.[19] Up to now, TSPO neuroimaging has been applied in a wide variety of neurodegenerative and psychiatric conditions, showing associations between neuroinflammation and cognitive decline.[20-22] Recently, first *in vivo* evidence of microglial activity in response to systemic inflammation has been shown in patients and healthy volunteers.[23-25] However, human *in vivo* data on neuroinflammation in patients undergoing major cardiothoracic surgery are still lacking.

This study therefore aims to assess neuroinflammation pre- and post-surgery *in vivo* in patients undergoing elective cardiac surgery. Given the presumed association between neuroinflammation and cognitive decline, quantifying the extent of neuroinflammation post-surgery and its relation to POCD will provide us with important insights for future (interventional) research.

Objectives

Our primary objective is to assess whether neuroinflammation is more pronounced in patients with cognitive decline at hospital discharge after CABG surgery, compared to patients without cognitive decline.

Secondary objectives:

1. To study the relation between the neuroinflammatory response and structural or functional changes to the brain postoperatively.
2. To study the relation between neuroinflammation and cognitive decline at 6 weeks and 6 months post-cardiac surgery.
3. To study the relation between neuroinflammation and postoperative delirium.
4. To study the relation between the perioperative systemic immune responses and neuroinflammation.

METHODS AND ANALYSIS

Study design

The FOCUS study (neuroinflammation in cognitive decline post cardiac surgery) is a single-centre observational time-series design study investigating neuroinflammation, systemic inflammation and neuropsychological performance before and after CABG.

Study population and recruitment

All patients planned for elective CABG in an academic hospital in the Netherlands are screened. Table 1 presents the inclusion- and exclusion criteria. The age criterion of 50 years or older is chosen for two reasons. First, older adults are more prone to subsequent long-term cognitive decline after cardiac surgery.[26, 27] Second, ageing is associated with a more exaggerated neuroinflammatory response following systemic inflammation.[28]

Screening and enrolment logs will be maintained for all patients. After written informed consent is obtained, a blood sample will be genotyped for rs6971 polymorphism using Taqman analysis on a 7500 Fast Real-Time PCR System (ThermoFisher Scientific, Waltham, USA). Low-affinity TSPO binding patients for the radiotracer used in PET imaging will be excluded from participation. In our predominantly Caucasian cohort, the estimated percentage of low affinity binders is below 10%.[29]

Table 1. Inclusion and exclusion criteria

Inclusion criteria	
	• Written informed consent • Age >50 years • Planned for elective on-pump coronary artery bypass grafting surgery • High- or mixed-affinity binders based on rs6971 polymorphism for translocator protein (TSPO) • Pre-hospital use of statins
Exclusion criteria	
Neurological	• Neurodegenerative disease, including mild cognitive impairment • Brain or spinal surgery within the last 6 months • Meningitis or brain infection within the last 6 months • Brain injury (e.g. acute stroke, or subarachnoid haemorrhage) within the last 6 months • Severe brain trauma in previous medical history • Presence of a cerebrospinal fluid catheter or shunt • Presence of a known brain tumour • Pre-hospital use of neuroleptics
Inflammatory	• Active infection (defined as fever >38.5°C or antibiotic treatment) within the last 2 weeks prior to surgery • Immunocompromised state • Auto-immune or auto-inflammatory disease
Cardiological	• Previous cardiac surgery • Cardiovascular event within the last 3 months
Other	• Contra-indication to undergo a PET/CT or MRI scan • Known contrast allergy for gadolinium • Kidney failure (defined by a MDRD-GFR <15ml/min/1.73m^2) • Illiteracy or the inability to speak Dutch • Presence of disabilities that prevent accurate delirium diagnosis • Low TSPO binding affinity (based on rs6971 polymorphism)

Data collection

Patients' demographics and information regarding their surgery indication, treatments, pre-existing comorbidity (Charlson Comorbidity Index),[30, 31] (re)admission, disease severity and mortality risk, length of mechanical ventilation, length of stay at the Intensive Care Unit (ICU) and occurrence of delirium will be retrieved from the patients' medical files. Table 2 shows an overview of events for this study, which will be defined subsequently in more detail.

Table 2. Schedule of events

	Pre-clinical	Pre-operative	CABG	Postoperative			Follow-up	
Timing:		T0 Baseline	T1 Stop ECC	T2 Stop ECC +6h	T3 Incision +24h	T4 CABG + 3-7d	T5 6 w	T6 6 m
Inclusion/exclusion criteria	X							
Informed consent	X							
TSPO binding affinity	X							
PET/CT cerebrum		X				X		
MRI cerebrum		X				X		
Blood sampling		X	X	X	X	X	X	
Neuropsychological assessment		X				X	X	X
Delirium assessment		3 times daily during hospital stay						

CABG = coronary artery bypass grafting; ECC = extracorporeal circulation; h=hours, d=days, w=weeks, m=months,
TSPO = translocator protein, PET/CT = positron emission tomography/computed tomography, MRI: magnetic resonance imaging.

Cerebral imaging

Dynamic brain PET/CT scans will be obtained preoperatively and on the fourth (range: 3rd-7th) postoperative day. Dynamic imaging of the head will be performed for 60 minutes after intravenous injection of the radiolabelled TSPO antagonist ^{18}F-DPA-714, a second generation TSPO ligand. A dose of ~200 MBq of ^{18}F-DPA-714 will be administered as a slow bolus injection during 40 seconds. Scans are obtained on a Siemens Biograph mCT hybrid PET/CT scanner (Siemens, Erlangen, Germany). A low-dose CT will be acquired for attenuation correction and anatomical reference. For pharmacokinetic analysis of the PET data arterial blood sampling will be used.

In addition, a 3 Tesla brain MRI (Siemens TIM TRIO, Erlangen, Germany) will be performed on the same days as the PET/CT scans. T1-weighted MR images will be obtained to co-register with the PET images for anatomical reference. Whole brain grey matter, as well as regions of interest will be delineated using probabilistic brain region templates. The slice thickness will be 1 mm. Additionally, the following MRI sequences will be performed: T2-weighed, susceptibility weighted imaging (SWI), FLAIR, diffusion tensor imaging (DTI), resting state fMRI, TOF MR angiogram of the circle of Willis and pre- and postcontrast 3D high-resolution T1-weighted SPACE sequence to visualize vessel wall abnormalities. In order to visualize cerebral vessel wall abnormalities, 0.1 mmol/kg gadobutrol (Gadovist) contrast agent will be administered intravenously. Details on MRI settings are provided in online supplementary appendix 1.

A senior neuroradiologist blinded to all other data will systematically quantify newly developed lesions post-surgery. This enables us to analyse the relationship between neuroinflammation, neuropsychological decline and cerebral lesion load. To evaluate changes in brain functional connectivity due to an acute immune response, a resting-state fMRI measurement is implemented comparing resting state connectivity in stress-related brain circuits pre- and post-surgery.[32, 33]

Blood sampling

Blood samples will be obtained at baseline preoperatively (T0, concomitant with the PET-scan), intra-operatively at the stop of ECC (T1), 6 hours (T2) and 24 hours (T3) after incision. The timing of blood draws is based on the peak of the systemic inflammatory response post-cardiac surgery, as shown in previous studies we performed in patients undergoing cardiac surgery.[34, 35] Additionally, blood samples will be collected concomitant with the second PET/CT-scan (T4) and six weeks postoperatively (T5) to explore to what extent the inflammatory response persists in time.

At all timepoints, common blood parameters will be measured, including haemoglobin, leucocyte and thrombocyte count, and circulating pro-and anti-inflammatory cytokines (including tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), interleukin-10 (IL-10) and interleukin-1 receptor antagonist (IL-1RA)). Blood will be centrifuged at 2000g for 10 minutes and the plasma will be stored at -80°C for possible future additional testing in line with the objective of this protocol. In addition, danger-associated molecular patterns (DAMPs, including high mobility group box 1 (HMGB1), heat shock protein-70 (HSP70), calgranulin-C (S100A12), calprotectin (S100A8/9), nuclear DNA, and mitochondrial DNA) will be measured in plasma centrifuged for a second time at high speed (16000 g).

Additionally, *ex vivo* production of cytokines by stimulated isolated peripheral blood mononuclear cells (PBMCs) will be measured, including TNF-α, IL-6, IL-1β, monocyte chemoattractant protein-1 (MCP1) and IL-10. Flow cytometry analysis of whole blood will be performed to study the inflammatory phenotype of the cells (including expression of human leukocyte antigen-DR (HLA-DR), C-C chemokine receptor type 2 (CCR2), CD11b, CD14 and CD16).

Arterial blood samples will be taken immediately before the ^{18}F-DPA-714 injection and during the PET-scan to measure the time course of radioactivity in plasma. In addition to this pharmacokinetic sampling, blood samples will be used to assess the ratio of ^{18}F-DPA-714 and its metabolites in order to correct the arterial input function for metabolite formation.

Neuropsychological assessment

A trained psychologist will perform neuropsychological assessments preoperatively (T0), at hospital discharge (T4), after six weeks (T5) and six months (T6) follow-up. These assessments are in line with the recommendation for neuropsychological research in cardiac surgery patients.[36] Table 3 lists the full test battery used for the neuropsychological assessments as well as the self-report questionnaires. Tests were selected based on sensitivity to detect even subtle deterioration in cognitive performance, with a focus on the domains executive functioning, memory, speed of processing and language. We will use parallel versions of these tests to account for material-specific practice effects after repeated assessment.[37]

During hospitalisation, screening of delirium is standard of care. Confusion Assessment Method for the ICU (CAM-ICU) or Delirium Observation Screening scores (DOS) at the cardiothoracic ward will be performed three times a day. A dedicated senior delirium researcher or neurologist will validate the diagnosis of delirium, using *DSM V criteria for delirium*.[38]

Cardiac surgery

Perioperative care will be executed conform the standard of care protocols for CABG, minimizing confounders due to medical management.

Table 3. Summary of neuropsychological testing

Neuropsychological assessments

Test name	Assessment of
At timepoints T0	
National Adult Reading Test – IQ[48]	Estimation of pre-morbid intelligence level
At timepoints T0, T4, T5, T6	
Trail Making Test A&B[49, 50, 51]	Visual attention and task switching
Stroop Colour-Word Test I, II, III[52]	Susceptibility to interference
Wechsler Adult Intelligence Scale-IV (WAIS) – Digit Span[53, 54]	Working memory
Letter Digit Substitution Test (LDST)[55, 56]	Information processing speed
Rey Auditory Verbal Learning Test (RAVLT)[57, 58]	Verbal episodic memory
Rey/Taylor Complex Figure Test – copy and recall trials (RCFT)[59, 60]	Visuoconstructive ability and visual episodic memory
Rivermead Behavioural Memory Test-3 (RBMT-3), Face recognition[61]	Visual episodic memory
Letter Fluency Test (LFT)[62]	Semantic memory and executive function
Token Test (short form)[63]	Language comprehension

Neuropsychological questionnaires

Test name	Assessment of
At timepoints T0	
Informant Questionnaire on Cognitive Decline in the Eldery (IQCODE)[64]	Subjective cognitive impairment, filled out by significant other
At timepoints T0, T4, T5, T6	
Clinical Frailty Scale[65]	Frailty screening
RAND-36[66]	Health-related quality of life survey
Cognitive Failure Questionnaire (CFQ)[67]	Subjective cognitive complaints
Hospital Anxiety and Depression Scale (HADS)[68]	Anxiety and depressive complaints
At timepoints T4, T5, T6	
Impact of Events Scale-Revised (IES-R)[69]	Distress caused by traumatic events

Data analysis

Pharmacokinetic modelling of dynamic PET data will be performed in PMOD software (PMOD Technologies LLC, Zürich, Switzerland). Binding potential (BP_{nd}) and volume of distribution (V_T) in several regions of interest (ROIs) will be determined using the 2-tissue compartmental model (2TCM) for each scan. Predefined ROIs include the frontal, temporal, parietal and occipital lobes, amygdala, hippocampus, thalamus, cerebellum and the brain stem.

For each patient, ^{18}F-DPA-714 BP_{nd} will be measured in the ROI for baseline and post-surgery PET scans. Since BP_{nd} is proportional to the availability of TSPO binding sites in the brain, an increase in BP_{nd} reflects an increase in glial activation. In addition, we will measure V_T which recently has been shown to mainly reflect changes in peripheral tracer binding during systemic inflammation, rather than changes in TSPO expression in the brain.[25][and unpublished data from our group] Therefore, both outcome measurements are required to validate these recent findings and assess glial activation accurately. Study investigators who analyse the cerebral imaging data will be blinded for inflammatory mediator results and cognitive outcomes.

No consensus about the definition of POCD has been achieved in the literature thus far.[5, 39, 40] At all timepoints, neuropsychological tests of our patients will be compared to available normative data, adjusted for age and educational level, resulting in standardized z-scores. Domain scores will be calculated by averaging the z-scores of the individual tests within a specific domain. Calculation of test performance will be supervised by an experienced clinical neuropsychologist. Performance on each individual test will also be classified as being either within the normal range (0), below average (1) or impaired (2) compared to the aforementioned normative data. "Normal performance" is defined as performance above -1 SD from the normative mean. "Below average" as between -1 SD and -1.65 SD from the normative mean (the lowest 16% of the normal population), and "impaired" as below -1.65 SD from the normative mean (the lowest 5% of the normal population).[41, 42] Furthermore, performance on a cognitive domain as a whole can be calculated as the mean of test rates in each domain. A cognitive domain will be classified as impaired when the average rating of tests is >1. We will define the presence of POCD when patients are *newly* impaired in one or more domains at hospital discharge, or when the average test rating has declined in more than one domain compared to baseline. This way we can dichotomize our patients into two groups: with or without POCD at hospital discharge.

The percentage change in TSPO expression (postoperative versus preoperative) will be calculated and reported as mean ± standard error of the mean (SEM) if normally distributed. This value can be compared using unpaired t-tests between both groups with Bonferroni post-hoc correction, and additionally, linear mixed models will be performed with the presence of POCD and brain region modelled as fixed effects, and subject ID as random effect. Multiple linear regression models will be used to study the relationship between tracer uptake and neuropsychological outcomes. Age, sex, Clinical Frailty Scale, Charlson Comorbidity Index, Hospital Anxiety and Depression Scale (HADS) and the RAND-36 item health survey at inclusion can be included as covariates. Multiple logistic regression analysis is performed to correct for possible confounders such as newly developed structural brain lesions on MRI.

The trajectory of systemic inflammatory parameters and differences in timepoints will be measured with repeated measures one-way ANOVA or linear mixed models in case of missings. In addition, correlations between systemic inflammatory markers and TSPO expression will be studied using Pearson for parametric or Spearman for non-parametric data. Multiple linear regression can be applied to correct for possible confounding factors. Unpaired t-tests will be applied to compare TSPO expression between patients with and without a delirious episode during hospital admission.

All MRI images will be evaluated without prior notice of any clinical parameter. White matter lesions are defined as hyperintense lesions on FLAIR MRI without corresponding cerebrospinal fluid like hypo-intense lesions on the T1 weighted image. Lacunar infarcts are defined as hypo-intense areas >2mm and ≤15mm on FLAIR and T1.[43] Territorial infarcts are defined as hyperintense lesions on FLAIR, and hypointense lesions on T1 image.[43] And finally, microbleeds are defined as small, homogenous, round foci of low signal intensity on T2* weighted images of <10mm in diameter.[44]
Analyses of functional and structural brain images will be performed using FSL (FMRIB's Software Library, Oxford, United Kingdom).[45] After pre-processing and denoising, subject-wise spatial maps of with-in network brain connectivity of the salience, executive control and default mode networks will be compared with non-parametric tests for mean differences. Vascular wall enhancement will be compared between the preoperative and postoperative assessments with paired t-tests or the Wilcoxon signed rank test if the data are non-parametric.

Descriptive statistics will be performed using IBM-SPSS software. Mean (±SEM) or median (IQR) will be presented depending on the distribution of the data. Alpha will be set at 0.05 throughout.

After the first 5 patients we will schedule a technical interim analysis to establish whether a tissue reference model is a reliable, non-invasive method for pharmacokinetic analysis of TSPO neuroimaging during systemic inflammation-induced neuroinflammation. If this or other (mathematical) methods can be reliably validated in our cohort, there will be no longer need for arterial blood sampling.

Sample size calculation

No data on the degree of glial activation after systemic inflammatory responses in post-cardiac surgery patients are available. Research on cognitive dysfunction after cardiac surgery estimates a prevalence of approximately 50% at hospital discharge.[1, 5, 39] Therefore, we will define two groups based on the presence or absence of cognitive dysfunction at hospital discharge.

Previous studies in dementia patients observed a 15-35% higher PET tracer uptake in patients with cognitive impairment compared to healthy controls, with a standard deviation of 30%.[46, 47] Therefore, we assume that patients with cognitive dysfunction at hospital discharge after cardiac surgery will have a 30% higher delta tracer uptake compared to patients without cognitive decline. To assess a 30% higher delta tracer uptake in patients with cognitive dysfunction, an unpaired two-sample t-test results in 13 patients per group with a power of 80% and a one-sided alpha of 0.05. In order to account for lower increments we will include 15 patients per group resulting in a power of 85% with an alpha of 0.05 to differentiate an increase of 30% in delta tracer uptake. Consequently, we will include a total of 30 patients, assuming that 50% will have cognitive decline at hospital discharge. The investigator can decide to withdraw a subject from the study for a) urgent medical reasons, or b) if a protocol violation occurs or c) if the subject is lost to follow-up. Replacement of individuals will not be necessary in this observational cohort once both PET scans are performed (i.e. primary objective has been met).

Sample size calculations were conducted for the primary objective solely, considering cognitive decline at hospital discharge. Assessment of long-term cognitive outcomes (up to 6 months) together with assessment of our secondary outcomes has been set up in an exploratory setting. The data of this study will be important to calculate the power and feasibility of a subsequent prospective project focussed on long-term outcomes.

Interim analysis with futility stop

An interim analysis will be performed after the first 15 subjects to validate that CABG surgery indeed induces a neuroinflammatory response, which will be assessed by TSPO PET imaging. An independent data safety and monitoring board (DSMB), composed by two clinical experts in the field of nuclear medicine and neurology, and a biostatistician, will analyse whether increased PET tracer uptake is observed after CABG surgery compared to the preoperative baseline tracer uptake. If no trend towards a significant difference ($p>0.10$) will be observed, the inclusion will be stopped to prevent futility. Furthermore an adaptive power analysis will be performed by the unblinded statistician DSMB member during the interim analysis to

determine whether the study is underpowered to fulfil the primary objective, and whether the sample size should be adapted. The pooled standard deviation (SD) of the delta PET tracer uptake will be calculated from the first 15 patients of which approximately 50% have cognitive disorders at hospital discharge. Using this pooled SD the sample size calculation will be performed again. If the ratio between this new sample size and the original is greater than 1, the sample size will be adapted if deemed feasible. The investigators will remain blinded for cognitive outcomes until the end of the study.

Study period

The study started enrolling patients in March 2019. The estimated study enrolment completion date is anticipated in the beginning of 2022. Please note that this manuscript was finalised prior to the interim analyses.

Ethics and dissemination

The study is conducted according to the principles of the Declaration of Helsinki and in accordance with the Dutch Medical Research Involving Human Subjects Act (WMO) and Good Clinical Practice guidelines. The study obtained ethical approval by the Medical Research Ethics Committee (MREC) region Arnhem-Nijmegen (CMO 2016-2598). The study is registered in the ClinicalTrials.gov database (NCT 04520802). The burden of the study protocol consists of two PET/CT-scans and two cerebral MRIs, additional blood sampling before, during and after surgery and four neuropsychological examinations, two during hospital admission and two follow-up visits during the first 6 months after hospital discharge.

Patient and public involvement statement

The hypothesis of this study was conceived with the help of patients through outpatient clinical follow-up after an ICU admission. Through patient experience of different long-term consequences, this study's endpoints involves cognitive performance, psychological symptoms and quality of life. A patient member of the MREC judged the study protocol for feasibility, burden and understandability of patient information.

Data management

Data will be handled confidentially and pseudonymously. Study data will be de-identified and a secured subject identification code list will be kept and stored separately from the data. This observational study uses an electronic remote data capture system. All missing and ambiguous data will be queried. The investigator will permit study-related monitoring, audits and regulatory inspection at their site, providing access to source data/documents. In all cases, it remains the responsibility of the investigator to ensure that data are accurate. Coded data will be kept after closure of the study and can only be used for ancillary studies after strict approval of the principal investigator. Anonymized data can be shared with other organizations for academic research, consensus development or other projects aimed at advancement of knowledge in this area. Body materials consisting of blood will be preserved in a coded form

for 10 years for possible follow-up studies. The MREC will be consulted before body material is used for follow-up research.

Public disclosure and publication policy

The results of this study will be published, regardless of whether these are positive, negative or inconclusive, in peer-reviewed international (open access) medical journals and presented at medical conferences. In addition, a summary of the results of this study will be published on the website of the funding agency The Netherlands Organization for Health Research and Development (ZonMw).

Relevance of findings

Cerebral dysfunction after cardiac surgery occurs frequently and may severely affect patients' daily lives. Due to the lack of research data within this area, the pathophysiology of cerebral dysfunction post-surgery is unknown. Therefore, there are currently no interventions available to prevent or treat this deterioration. The FOCUS study will quantify glial activation, which is suggested to be important in this pathophysiology, and relate this to cognition, structural and functional changes to the brain and systemic inflammation. This study combines state of the art molecular and MR neuroimaging techniques, elaborates longitudinal neuropsychological examinations, and comprehensive immunological laboratory tests. Objective neurocognitive examinations will be performed at four different timepoints, up to six months postoperatively, enabling us to detect even subtle changes in cognition. Better understanding of the pathogenesis of POCD could direct neuroscientists towards the development of targets for future interventions. This imaging paradigm could provide an approach to examine the efficacy of such interventions in clinical studies.

In addition, the knowledge obtained with this study is of importance for patients and healthcare professionals as well. The participating patient will not directly benefit from study participation. However, they will be followed with regard to possible complaints in light of potential post-cardiac surgery cerebral dysfunction. This will increase the awareness of the participating patients and caregivers with respect to such complaints. This might benefit the patient and caregivers as it will decrease uncertainty about the nature of the complaints when they occur. Furthermore this study searches for a biological explanation for post-surgery cerebral complaints which are often not understood or classified as functional.

Several limitations need to be addressed. First, as this study is powered at cognitive decline at hospital discharge, it is not powered to study the association between glial activation and cognitive decline after 6 months. The second limitation concerns the arterial sampling during the dynamic PET-scans. Automated arterial sampling, which is frequently used in other studies using outpatient clinic patients, leads to a loss of 200-300 ml of blood per scan. Loss of such an amount of blood in cardiac surgery patients is not preferable as it could result in a significant health risk for these critically ill patients. Therefore we will sample manually, which reduces blood volume loss. Manual sampling results in less sampling points and could therefore lead to a slightly less accurate plasma activity curve.

Imaging neuroinflammation as proposed is safe, as corroborated by existing human and animal data. In addition, imaging neuroinflammation could lead to potential prognostic and interventional targets that could revolutionize healthcare for this large group of patients.

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Contributors:

WFA conceived the study idea. APvT and WFA developed the study design to which JvT, WL, HJD, NPR, FdL, FJAM, RPCK, NK, JvdH, PP and MR contributed. APvT and HBD drafted the manuscript. All authors were involved in the editing of the manuscript and read and approved the final manuscript.

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Supplemental material to:

A.M. Peters van Ton et al.,
Neuroinflammation in cognitive decline post-cardiac surgery (the FOCUS study): an observational study protocol

Appendix 1: MRI scan protocol – parameter settings

	Protocol Name	Sequence/Contrast parameters	Resolution(mm) Matrix Size Parallel Imaging	Duration (min:secs)
T1-weighted MPRAGE	MPRAGE	TR/TI/TE=2300/900/2.32ms Flip angle=8º	0.9x0.9x0.9mm 240x240x192 iPAT=2	5:21
T2-weighted TSE TRA	T2_tse_tra	TR/TE=3500/92 ms Flip angle = 120 º	0.4x0.4x5mm 230x173x154	2:01
Susceptibility weighted	Fl3D_SWI	TR/TE=27/20ms Flip angle = 15º	1x1x3mm 250x188x156 iPAT=2	2:43
FLAIR	T2_flair	TR/TI/TE=9000/87/2500ms Flip angle=150º	0.6x0.6x5mm 230x173x143	4:32
Field map	Gre_field	TR/TE1/TE2=400/5.19/7.65 Flip angle= 60º	3.8x3.8x3mm 240x240x135	0:54
DTI	MDDW64	TR/TE=6900/67 Directions=108 b-value=1000 s/mm^2	2x2x2mm 240x240x128 iPAT=2	12:47
fMRI	Ep2d	TR/TE=2390/30ms Flip angle= 90º	3.5x3.5x3.5 224x224x144	6:06
TOF MR angiogram	ToF	TR/TE=24/3.93ms Flip angle= 15º	0.5x0.5x0.6mm 200x150x154 iPAT=2	11:10
T1-weighted SPACE *pre-contrast & post-contrast*	Tse3d_spc	TR/TE=750/20ms	0.5x0.5x0.9mm 231x231x51 iPAT=2	2x 3:49

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

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		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	#3	State specific objectives, including any prespecified hypotheses	4
Methods			

Study design	#4	Present key elements of study design early in the paper	4
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-11
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	4-5
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-11
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6-7
Bias	#9	Describe any efforts to address potential sources of bias	4
Study size	#10	Explain how the study size was arrived at	10
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	9
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	8-10
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	8-10
Statistical methods	#12c	Explain how missing data were addressed	8-10
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	10
Statistical methods	#12e	Describe any sensitivity analyses	NA

Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	NA
Participants	#13b	Give reasons for non-participation at each stage	NA
Participants	#13c	Consider use of a flow diagram	NA
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	5
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	NA
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	NA
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	NA
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
Main results	#16b	Report category boundaries when continuous variables were categorized	NA
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	#18	Summarise key results with reference to study objectives	NA

Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	12
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	12
Generalisability	#21	Discuss the generalisability (external validity) of the study results	12
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

BMJ Open

Neuroinflammation in cognitive decline post-cardiac surgery (the FOCUS study): an observational study protocol

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Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Immunology (including allergy), Neurology, Mental health
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Neuroinflammation in cognitive decline post-cardiac surgery (the FOCUS study): an observational study protocol

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ABSTRACT

Introduction

Postoperative cognitive dysfunction (POCD) occurs frequently after coronary artery bypass grafting (CABG). The underlying mechanisms remain poorly understood, but neuroinflammation might play a pivotal role. We hypothesize that systemic inflammation induced by the surgical trauma could activate the innate immune (glial) cells of the brain. This could lead to an exaggerated neuroinflammatory cascade, resulting in neuronal dysfunction and loss of neuronal cells. Therefore, the aims of this study are to assess neuroinflammation *in vivo* pre- and post-surgery in patients undergoing major cardiac surgery and investigate whether there is a relationship of neuroinflammation to cognitive outcomes, changes to brain structure and function, and systemic inflammation.

Methods and analysis

The FOCUS study is a prospective, single-centre observational study, including 30 patients undergoing elective on-pump CABG. Translocator protein (TSPO) PET neuroimaging will be performed pre- and postoperatively using the second generation tracer ^{18}F-DPA-714 to assess the neuroinflammatory response. In addition, a comprehensive cerebral MRI will be performed pre- and post-surgery, in order to discover newly developed brain and vascular wall lesions. Up to 6 months postoperatively, serial extensive neurocognitive assessments will be performed and blood will be obtained to quantify systemic inflammatory responses and peripheral immune cell activation.

Ethics and dissemination

Patients do not benefit directly from engaging in the study, but imaging neuroinflammation is considered safe and no side effects are expected. The study protocol obtained ethical approval by the Medical Research Ethics Committee (MREC) region Arnhem-Nijmegen. This work will be published in peer-reviewed international medical journals and presented at medical conferences.

Article summary

Strengths and limitations of this study
• This study is the first to examine *in vivo* neuroinflammation using TSPO PET neuroimaging both prior to and after cardiac surgery
• This study combines state of the art neuroimaging with longitudinal neuropsychological examinations and detailed immunological analyses
• Extensive neuropsychological examinations at four different timepoints enable us to detect even subtle changes in cognitive function
• This study is powered to detect the association between neuroinflammation and cognitive decline between baseline and hospital discharge, the period of time the incidence of cognitive dysfunction is highest.
• The power of this pilot study is inadequate to adjust for confounders.

Keywords

Postoperative cognitive dysfunction (POCD), coronary artery bypass grafting (CABG), neuroinflammation, microglia, TSPO neuroimaging

INTRODUCTION

Background and rationale

Coronary artery bypass grafting (CABG) has been associated with postoperative cerebral complications.[1-4] These may occur directly post-surgery, like stroke or delirium, but can also have long-term sequelae such as postoperative cognitive dysfunction (POCD) and dementia. The incidence of POCD ranges from 20-70% in the first week after surgery, to 10-40% in the following months but may increase again from one postoperative year onwards.[1, 5] Furthermore, a 1.7-fold increased risk to develop new incident Alzheimer's disease within 6 years after CABG was found, compared to patients undergoing a percutaneous coronary intervention.[6]

Development of POCD is presumably related to perioperative brain hypoperfusion, cerebral microembolization, haemodilution, hypercoagulability, cerebral hyperthermia, and inadequate glucose homeostasis.[2] In addition, systemic inflammation is hypothesized as an important (and possibly treatable) factor for POCD pathogenesis.[7-9] Cardiac surgery produces substantial systemic inflammation (reflected by leucocytosis and significant dysregulation of cytokines and other inflammatory mediators, affecting various physical processes) due to multiple stimuli such as sternotomy, extracorporeal circulation (ECC), associated transient endotoxemia, and aortic cross-clamping. Systemic inflammation can result in increased communication and signalling from the periphery to the brain.[10, 11] As a result, systemic inflammation may induce activation of the innate immune cells of the brain, the microglia and astrocytes, leading to a neuroinflammatory response.[12] Animal models demonstrated that systemic administration of low-dose endotoxin even leads to long-term inflammatory reprogramming of microglia.[13] Interestingly, research has shown that neuroinflammation is associated with cognitive dysfunction and neurodegenerative disease.[14, 15] Given these associations, we hypothesize that occurrence of POCD in patients undergoing CABG is mediated through the occurrence of neuroinflammation.

Positron Emission Tomography (PET) enables the imaging of glial activation in the CNS using radiolabelled antagonists of translocator protein (TSPO).[16, 17] TSPO is mainly expressed as a transmembrane protein on mitochondria of microglia and astrocytes in the CNS.[18] As TSPO expression is upregulated during neuroinflammatory processes, it makes a promising biomarker for imaging neuroinflammation.[19] Up to now, TSPO neuroimaging has been applied in a wide variety of neurodegenerative and psychiatric conditions, showing associations between neuroinflammation and cognitive decline.[20-22] Recently, first *in vivo* evidence of microglial activity in response to systemic inflammation has been shown in patients and healthy volunteers.[23-25] However, human *in vivo* data on neuroinflammation in patients undergoing major cardiothoracic surgery are still lacking.

This study therefore aims to assess neuroinflammation pre- and post-surgery *in vivo* in patients undergoing elective cardiac surgery. Given the presumed association between neuroinflammation and cognitive decline, quantifying the extent of neuroinflammation post-surgery and its relation to POCD will provide us with important insights for future (interventional) research.

Objectives

Our primary objective is to assess whether neuroinflammation is more pronounced in patients with cognitive decline at hospital discharge after CABG surgery, compared to patients without cognitive decline.

Secondary objectives:

1. To study the relation between the neuroinflammatory response and structural or functional changes to the brain postoperatively.
2. To study the relation between neuroinflammation and cognitive decline at 6 weeks and 6 months post-cardiac surgery.
3. To study the relation between neuroinflammation and postoperative delirium.
4. To study the relation between the perioperative systemic immune responses and neuroinflammation.

METHODS AND ANALYSIS

Study design

The FOCUS study (neuroinflammation in cognitive decline post cardiac surgery) is a single-centre observational time-series design study investigating neuroinflammation, systemic inflammation and neuropsychological performance before and after CABG.

Study population and recruitment

All patients planned for elective CABG in an academic hospital in the Netherlands are screened. Table 1 presents the inclusion- and exclusion criteria. The age criterion of 50 years or older is chosen for two reasons. First, older adults are more prone to subsequent long-term cognitive decline after cardiac surgery.[26, 27] Second, ageing is associated with a more exaggerated neuroinflammatory response following systemic inflammation.[28]

Screening and enrolment logs will be maintained for all patients. After written informed consent is obtained, a blood sample will be genotyped for rs6971 polymorphism using Taqman analysis on a 7500 Fast Real-Time PCR System (ThermoFisher Scientific, Waltham, USA). Low-affinity TSPO binding patients for the radiotracer used in PET imaging will be excluded from participation. In our predominantly Caucasian cohort, the estimated percentage of low affinity binders is below 10%.[29]

Data collection

Patients' demographics and information regarding their surgery indication, treatments, pre-existing comorbidity (Charlson Comorbidity Index),[30, 31] (re)admission, disease severity and mortality risk, length of mechanical ventilation, length of stay at the Intensive Care Unit

(ICU) and occurrence of delirium will be retrieved from the patients' medical files. Table 2 shows an overview of events for this study, which will be defined subsequently in more detail.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	
	• Written informed consent • Age >50 years • Planned for elective on-pump coronary artery bypass grafting surgery • High- or mixed-affinity binders based on rs6971 polymorphism for translocator protein (TSPO) • Pre-hospital use of statins
Exclusion criteria	
Neurological	• Neurodegenerative disease, including mild cognitive impairment • Brain or spinal surgery within the last 6 months • Meningitis or brain infection within the last 6 months • Brain injury (e.g. acute stroke, or subarachnoid haemorrhage) within the last 6 months • Severe brain trauma in previous medical history • Presence of a cerebrospinal fluid catheter or shunt • Presence of a known brain tumour • Pre-hospital use of neuroleptics
Inflammatory	• Active infection (defined as fever >38.5°C or antibiotic treatment) within the last 2 weeks prior to surgery • Immunocompromised state (due to immunomodulatory drugs or underlying conditions) • Auto-immune or auto-inflammatory disease
Cardiological	• Previous cardiac surgery • Cardiovascular event within the last 3 months
Other	• Contra-indication to undergo a PET/CT or MRI scan • Known contrast allergy for gadolinium • Kidney failure (defined by a MDRD-GFR <15ml/min/1.73m^2) • Illiteracy or the inability to speak Dutch • Presence of disabilities that prevent accurate delirium diagnosis • Low TSPO binding affinity (based on rs6971 polymorphism)

Table 2. Schedule of events

	Pre-clinical	Pre-operative	CABG	Postoperative			Follow-up	
Timing:		T0 Baseline	T1 Stop ECC	T2 Stop ECC +6h	T3 Incision +24h	T4 CABG + 3-7d	T5 6 w	T6 6 m
Inclusion/exclusion criteria	X							
Informed consent	X							
TSPO binding affinity	X							
PET/CT cerebrum		X				X		
MRI cerebrum		X				X		
Blood sampling		X	X	X	X	X	X	
Neuropsychological assessment		X				X	X	X
Delirium assessment		3 times daily during hospital stay						

CABG = coronary artery bypass grafting; ECC = extracorporeal circulation; h=hours, d=days, w=weeks, m=months,
TSPO = translocator protein, PET/CT = positron emission tomography/computed tomography, MRI: magnetic resonance imaging.

Cerebral imaging

Dynamic brain PET/CT scans will be obtained preoperatively and on the fourth (range: 3rd-7th) postoperative day. Dynamic imaging of the head will be performed for 60 minutes after intravenous injection of the radiolabelled TSPO antagonist ^{18}F-DPA-714, a second generation TSPO ligand. This generation of TSPO ligands outperforms the first generation TSPO tracer, isoquinoline carboxamide (^{11}C-PK11195), on TSPO binding affinity and PET imaging properties, but requires polymorphism genotyping and stratifying according to binding affinity status.[32] A dose of ~200 MBq of ^{18}F-DPA-714 will be administered as a slow bolus injection during 40 seconds. Scans are obtained on a Siemens Biograph mCT hybrid PET/CT scanner (Siemens, Erlangen, Germany). A low-dose CT will be acquired for attenuation correction and anatomical reference. For pharmacokinetic analysis of the PET data arterial blood sampling will be used.

In addition, a 3 Tesla brain MRI (Siemens TIM TRIO, Erlangen, Germany) will be performed on the same days as the PET/CT scans. T1-weighted MR images will be obtained to co-register with the PET images for anatomical reference. Whole brain grey matter, as well as regions of interest will be delineated using probabilistic brain region templates. The slice thickness will be 1 mm. Additionally, the following MRI sequences will be performed: T2-weighed, susceptibility weighted imaging (SWI), FLAIR, diffusion tensor imaging (DTI), resting state fMRI, TOF MR angiogram of the circle of Willis and pre- and postcontrast 3D high-resolution T1-weighted SPACE sequence to visualize vessel wall abnormalities. In order to visualize cerebral vessel wall abnormalities, 0.1 mmol/kg gadobutrol (Gadovist) contrast agent will be administered intravenously. Details on MRI settings are provided in online supplementary appendix 1.

A senior neuroradiologist blinded to all other data will systematically quantify newly developed lesions post-surgery. This enables us to analyse the relationship between neuroinflammation, neuropsychological decline and cerebral lesion load. To evaluate changes in brain functional connectivity due to an acute immune response, a resting-state fMRI measurement is implemented comparing resting state connectivity in stress-related brain circuits pre- and post-surgery.[33, 34] A previous study found positive associations between alterations in resting-state functional connectivity in the brain's default mode network and global cognitive change after cardiac surgery[35], and the current study enables us to extend these findings by studying the relationship with (neuro)inflammation.

Blood sampling

Blood samples will be obtained at baseline preoperatively (T0, concomitant with the PET-scan), intra-operatively at the stop of ECC (T1), 6 hours (T2) and 24 hours (T3) after incision. The timing of blood draws is based on the peak of the systemic inflammatory response post-cardiac surgery, as shown in previous studies we performed in patients undergoing cardiac surgery.[36, 37] Additionally, blood samples will be collected concomitant with the second PET/CT-scan (T4) and six weeks postoperatively (T5) to explore to what extent the inflammatory response persists in time.

At all timepoints, common blood parameters will be measured, including haemoglobin, leucocyte and thrombocyte count, and circulating pro-and anti-inflammatory cytokines (including tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), interleukin-10 (IL-10), and interleukin-1 receptor antagonist (IL-1RA)) by using simultaneous

Luminex assays. Blood will be centrifuged at 2000g for 10 minutes and the plasma will be stored at -80°C for possible future additional testing in line with the objective of this protocol. In addition, danger-associated molecular patterns (DAMPs, including high mobility group box 1 (HMGB1), heat shock protein-70 (HSP70), calgranulin-C (S100A12), calprotectin (S100A8/9), nuclear DNA, and mitochondrial DNA) will be measured as reported previously,[38] in plasma centrifuged for a second time at high speed (16000 g).
Additionally, *ex vivo* production of cytokines by stimulated isolated peripheral blood mononuclear cells (PBMCs) will be measured, including TNF-α, IL-6, IL-1β, monocyte chemoattractant protein-1 (MCP1) and IL-10. Flow cytometry analysis of whole blood will be performed as described previously,[39, 40] to study the inflammatory phenotype of the cells (including expression of human leukocyte antigen-DR (HLA-DR), C-C chemokine receptor type 2 (CCR2), CD11b, CD14, and CD16).

Arterial blood samples will be taken immediately before the ^{18}F-DPA-714 injection and during the PET-scan to measure the time course of radioactivity in plasma. In addition to this pharmacokinetic sampling, blood samples will be used to assess the ratio of ^{18}F-DPA-714 and its metabolites in order to correct the arterial input function for metabolite formation.

Neuropsychological assessment

A trained psychologist will perform neuropsychological assessments preoperatively (T0), at hospital discharge (T4), after six weeks (T5) and six months (T6) follow-up. These assessments are in line with the recommendation for neuropsychological research in cardiac surgery patients.[41] Table 3 lists the full test battery used for the neuropsychological assessments as well as the self-report questionnaires. Tests were selected based on sensitivity to detect even subtle deterioration in cognitive performance, with a focus on the domains executive functioning, memory, speed of processing and language. We will use parallel versions of these tests to account for material-specific practice effects after repeated assessment.[42]

During hospitalisation, screening of delirium is standard of care. Confusion Assessment Method for the ICU (CAM-ICU) or Delirium Observation Screening scores (DOS) at the cardiothoracic ward will be performed three times a day. A dedicated senior delirium researcher or neurologist will validate the diagnosis of delirium, using *DSM V criteria for delirium*.[43]

Cardiac surgery

Perioperative care will be delivered according to the regular clinical protocol for CABG. This minimizes the risk of potential confounders due to variability in medical management.

Table 3. Summary of neuropsychological testing

Neuropsychological assessments

Test name	Domain	Subdomain
At timepoints T0		
National Adult Reading Test – IQ[44]	Premorbid intelligence (descriptive)	Estimation of pre-morbid intelligence level
At timepoints T0, T4, T5, T6		
Trail Making Test B[45-47]	Executive function	Visual attention and task switching
Stroop Colour-Word Test I, II, III[48]	Executive function	Susceptibility to interference
Wechsler Adult Intelligence Scale-IV (WAIS) – Digit Span[49, 50]	Executive function	Working memory
Letter Digit Substitution Test (LDST)[51, 52]	Information processing speed	Information processing speed
Trail Making Test A[45-47]	Information processing speed	Information processing speed
Rey Auditory Verbal Learning Test (RAVLT)[53, 54]	Episodic memory	Verbal episodic memory
Rey/Taylor Complex Figure Test –recall trials (RCFT)[55, 56]	Episodic memory	Visual episodic memory
Rivermead Behavioural Memory Test-3 (RBMT-3), Face recognition[57]	Episodic memory	Visual episodic memory
Rey/Taylor Complex Figure Test – copy trial (RCFT)[55, 56]	Visuoconstructive ability	Visuoconstructive ability
Letter Fluency Test (LFT)[58]	Language	Semantic memory
Token Test (short form)[59]	Language	Language comprehension

Neuropsychological questionnaires

Test name	Assessment of
At timepoints T0	
Informant Questionnaire on Cognitive Decline in the Eldery (IQCODE)[60]	Subjective cognitive impairment, filled out by significant other
At timepoints T0, T4, T5, T6	
Clinical Frailty Scale[61]	Frailty screening
RAND-36[62]	Health-related quality of life survey
Cognitive Failure Questionnaire (CFQ)[63]	Subjective cognitive complaints
Hospital Anxiety and Depression Scale (HADS)[64]	Anxiety and depressive complaints
At timepoints T4, T5, T6	
Impact of Events Scale-Revised (IES-R)[65]	Distress caused by traumatic events

Data analysis

Pharmacokinetic modelling of dynamic PET data will be performed in PMOD software (PMOD Technologies LLC, Zürich, Switzerland). Binding potential (BP_{ND}) and volume of distribution (V_T) in several regions of interest (ROIs) will be determined using the 2-tissue compartmental model (2TCM) for each scan. Predefined ROIs include the frontal, temporal, parietal and occipital lobes, amygdala, hippocampus, thalamus, cerebellum and the brain stem.

For each patient, ^{18}F-DPA-714 BP_{ND} will be measured in the ROI for baseline and post-surgery PET scans. Since BP_{ND} is proportional to the availability of TSPO binding sites in the brain, an increase in BP_{ND} reflects an increase in glial activation. In addition, we will measure V_T which recently has been shown to mainly reflect changes in peripheral tracer binding during systemic inflammation, rather than changes in TSPO expression in the brain.[25][and unpublished data from our group] Therefore, both outcome measurements are required to validate these recent findings and assess glial activation accurately. Study investigators who analyse the cerebral imaging data will be blinded for inflammatory mediator results and cognitive outcomes.

No consensus about the definition of POCD has been achieved in the literature thus far.[5, 66, 67] At all timepoints, neuropsychological tests of our patients will be compared to available normative data, adjusted for age and educational level, resulting in standardized z-scores. Overall domain z-scores will be calculated by averaging the z-scores of the individual tests within a specific domain. Calculation of test performance will be supervised by an experienced clinical neuropsychologist. Additionally, z-scores of each individual test will be clinically classified as either being within the normal range (0), below average (1), or impaired (2), compared to the aforementioned normative data. “Normal performance” is defined as performance above -1 SD from the normative mean. “Below average” as between -1 SD and -1.65 SD from the normative mean (the lowest 16% of the normal population), and “impaired” as below -1.65 SD from the normative mean (the lowest 5% of the normal population).[68, 69] Thus, an overall weighted performance score will be calculated for all five cognitive domains. A cognitive domain will be clinically classified as impaired when the average weighted score of all tests within the domain is >1. We will define the presence of POCD when 1) patients are *newly* impaired in one or more domains at hospital discharge, compared to baseline, or 2) when the overall weighted performance score deteriorated in more than one domain at hospital discharge compared to baseline. Accordingly, all patients will be dichotomized into two groups: with or without POCD at hospital discharge.

The percentage change in TSPO expression (postoperative versus preoperative) will be calculated and reported as mean ± standard error of the mean (SEM) if normally distributed. This value can be compared using unpaired t-tests between both groups with Bonferroni post-hoc correction, and additionally, linear mixed models will be performed with the presence of POCD and brain region modelled as fixed effects, and subject ID as random effect. Multiple linear regression models will be used to study the relationship between tracer uptake and neuropsychological outcomes. Age, sex, Clinical Frailty Scale, Charlson Comorbidity Index, Hospital Anxiety and Depression Scale (HADS) and the RAND-36 item health survey at inclusion can be included as covariates. Multiple logistic regression analysis is performed to correct for possible confounders such as newly developed structural brain lesions on MRI.

The trajectory of systemic inflammatory parameters and differences in timepoints will be measured with repeated measures one-way ANOVA or linear mixed models in case of missings. In addition, correlations between systemic inflammatory markers and TSPO expression will be

studied using Pearson for parametric or Spearman for non-parametric data. Multiple linear regression can be applied to correct for possible confounding factors. Unpaired t-tests will be applied to compare TSPO expression between patients with and without a delirious episode during hospital admission.

All MRI images will be evaluated without prior notice of any clinical parameter. White matter lesions are defined as hyperintense lesions on FLAIR MRI without corresponding cerebrospinal fluid like hypo-intense lesions on the T1 weighted image. Lacunar infarcts are defined as hypo-intense areas >2mm and ≤15mm on FLAIR and T1.[70] Territorial infarcts are defined as hyperintense lesions on FLAIR, and hypointense lesions on T1 image.[70] And finally, microbleeds are defined as small, homogenous, round foci of low signal intensity on T2* weighted images of <10mm in diameter.[71]
Analyses of functional and structural brain images will be performed using FSL (FMRIB's Software Library, Oxford, United Kingdom).[72] After pre-processing and denoising, subject-wise spatial maps of with-in network brain connectivity of the salience, executive control and default mode networks will be compared with non-parametric tests for mean differences. Vascular wall enhancement will be compared between the preoperative and postoperative assessments with paired t-tests or the Wilcoxon signed rank test if the data are non-parametric.

Descriptive statistics will be performed using IBM-SPSS software. Mean (±SEM) or median (IQR) will be presented depending on the distribution of the data. Alpha will be set at 0.05 throughout.

After the first 5 patients we will schedule a technical interim analysis to establish whether a tissue reference model is a reliable, non-invasive method for pharmacokinetic analysis of TSPO neuroimaging during systemic inflammation-induced neuroinflammation. If this or other (mathematical) methods can be reliably validated in our cohort, there will be no longer need for arterial blood sampling.

Sample size calculation

No data on the degree of glial activation after systemic inflammatory responses in post-cardiac surgery patients are available. Research on cognitive dysfunction after cardiac surgery estimates a prevalence of approximately 50% at hospital discharge.[1, 5, 66] Therefore, we will define two groups based on the presence or absence of cognitive dysfunction at hospital discharge.

Previous studies in dementia patients observed a 15-35% higher PET tracer uptake in patients with cognitive impairment compared to healthy controls, with a standard deviation of 30%.[73, 74] Therefore, we assume that patients with cognitive dysfunction at hospital discharge after cardiac surgery will have a 30% higher delta tracer uptake compared to patients without cognitive decline. To assess a 30% higher delta tracer uptake in patients with cognitive dysfunction, an unpaired two-sample t-test results in 13 patients per group with a power of 80% and a one-sided alpha of 0.05. In order to account for lower increments we will include 15 patients per group resulting in a power of 85% with an alpha of 0.05 to differentiate an increase of 30% in delta tracer uptake. Consequently, we will include a total of 30 patients, assuming that 50% will have cognitive decline at hospital discharge. The investigator can decide to withdraw a subject from the study for a) urgent medical reasons, or b) if a protocol violation occurs or c) if the subject is lost to follow-up. Replacement of individuals will not be necessary in this observational cohort once both PET scans are performed (i.e. primary objective has been met).

Sample size calculations were conducted for the primary objective solely, considering cognitive decline at hospital discharge. Assessment of long-term cognitive outcomes (up to 6 months) together with assessment of our secondary outcomes has been set up in an exploratory setting. The data of this study will be important to calculate the power and feasibility of a subsequent prospective project focussed on long-term outcomes.

Interim analysis with futility stop

An interim analysis will be performed after the first 15 subjects to validate that CABG surgery indeed induces a neuroinflammatory response, which will be assessed by TSPO PET imaging. An independent data safety and monitoring board (DSMB), composed by two clinical experts in the field of nuclear medicine and neurology, and a biostatistician, will analyse whether increased PET tracer uptake is observed after CABG surgery compared to the preoperative baseline tracer uptake. If no trend towards a significant difference ($p>0.10$) will be observed, the inclusion will be stopped to prevent futility. Furthermore an adaptive power analysis will be performed by the unblinded statistician DSMB member during the interim analysis to determine whether the study is underpowered to fulfil the primary objective, and whether the sample size should be adapted. The pooled standard deviation (SD) of the delta PET tracer uptake will be calculated from the first 15 patients of which approximately 50% have cognitive disorders at hospital discharge. Using this pooled SD the sample size calculation will be performed again. If the ratio between this new sample size and the original is greater than 1, the sample size will be adapted if deemed feasible. The investigators will remain blinded for cognitive outcomes until the end of the study.

Study period

The study started enrolling patients in March 2019. The estimated study enrolment completion date is anticipated in the beginning of 2022. Please note that this manuscript was finalised prior to the interim analyses.

Ethics and dissemination

The study is conducted according to the principles of the Declaration of Helsinki and in accordance with the Dutch Medical Research Involving Human Subjects Act (WMO) and Good Clinical Practice guidelines. The study obtained ethical approval by the Medical Research Ethics Committee (MREC) region Arnhem-Nijmegen (CMO 2016-2598). The study is registered in the ClinicalTrials.gov database (NCT 04520802). The burden of the study protocol consists of two PET/CT-scans and two cerebral MRIs, additional blood sampling before, during and after surgery and four neuropsychological examinations, two during hospital admission and two follow-up visits during the first 6 months after hospital discharge.

Patient and public involvement statement

The hypothesis of this study was conceived with the help of patients through outpatient clinical follow-up after an ICU admission. Through patient experience of different long-term

consequences, this study's endpoints involves cognitive performance, psychological symptoms and quality of life. A patient member of the MREC judged the study protocol for feasibility, burden and understandability of patient information.

Data management

Data will be handled confidentially and pseudonymously. Study data will be de-identified and a secured subject identification code list will be kept and stored separately from the data. This observational study uses an electronic remote data capture system. All missing and ambiguous data will be queried. The investigator will permit study-related monitoring, audits and regulatory inspection at their site, providing access to source data/documents. In all cases, it remains the responsibility of the investigator to ensure that data are accurate. Coded data will be kept after closure of the study and can only be used for ancillary studies after strict approval of the principal investigator. Anonymized data can be shared with other organizations for academic research, consensus development or other projects aimed at advancement of knowledge in this area. Body materials consisting of blood will be preserved in a coded form for 10 years for possible follow-up studies. The MREC will be consulted before body material is used for follow-up research.

Public disclosure and publication policy

The results of this study will be published, regardless of whether these are positive, negative or inconclusive, in peer-reviewed international (open access) medical journals and presented at medical conferences. In addition, a summary of the results of this study will be published on the website of the funding agency The Netherlands Organization for Health Research and Development (ZonMw).

Relevance of findings

Cerebral dysfunction after cardiac surgery occurs frequently and may severely affect patients' daily lives. Due to the lack of research data within this area, the pathophysiology of cerebral dysfunction post-surgery is unknown. Therefore, there are currently no interventions available to prevent or treat this deterioration. The FOCUS study will quantify glial activation, which is suggested to be important in this pathophysiology, and relate this to cognition, structural and functional changes to the brain and systemic inflammation. This adds to previous and ongoing observational work perioperatively combining blood and cerebrospinal fluid parameters with MR neuroimaging, cognition and electroencephalogram recordings.[75, 76] Our study combines state of the art molecular and MR neuroimaging techniques, elaborates longitudinal neuropsychological examinations, and comprehensive immunological laboratory tests. Objective neurocognitive examinations will be performed at four different timepoints, up to six months postoperatively, enabling us to detect even subtle changes in cognition. Better understanding of the pathogenesis of POCD could direct neuroscientists towards the development of targets for future interventions. This imaging paradigm could provide an approach to examine the efficacy of such interventions in clinical studies.

In addition, the knowledge obtained with this study is of importance for patients and healthcare professionals as well. The participating patient will not directly benefit from study participation. However, they will be followed with regard to possible complaints in light of potential post-

cardiac surgery cerebral dysfunction. This will increase the awareness of the participating patients and caregivers with respect to such complaints. This might benefit the patient and caregivers as it will decrease uncertainty about the nature of the complaints when they occur. Furthermore this study searches for a biological explanation for post-surgery cerebral complaints which are often not understood or classified as functional.

Several limitations need to be addressed. First, as this study is powered at cognitive decline at hospital discharge, it is not powered to study the association between glial activation and cognitive decline after 6 months. The second limitation concerns the arterial sampling during the dynamic PET-scans. Automated arterial sampling, which is frequently used in other studies using outpatient clinic patients, leads to a significant loss of blood per scan. Loss of such an amount of blood in cardiac surgery patients is not preferable as it could result in a significant health risk for these critically ill patients. Therefore we will sample manually, which reduces blood volume loss. Manual sampling results in less sampling points and could therefore lead to a slightly less accurate plasma activity curve. Finally, the power of this pilot study is inadequate to allow adjustment for confounding factors.

Imaging neuroinflammation as proposed is safe, as corroborated by existing human and animal data. In addition, imaging neuroinflammation could lead to potential prognostic and interventional targets that could revolutionize healthcare for this large group of patients.

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Contributors:

WFA conceived the study idea. APvT and WFA developed the study design to which JvT, WL, HJD, NPR, FJAM, RPCK, NK, JvdH, PP and MR contributed. APvT and HBD drafted the manuscript. All authors were involved in the editing of the manuscript and read and approved the final manuscript.

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Supplemental material to:

A.M. Peters van Ton et al.,

Neuroinflammation in cognitive decline post-cardiac surgery (the FOCUS study): an observational study protocol

Appendix 1: MRI scan protocol – parameter settings

	Protocol Name	Sequence/Contrast parameters	Resolution(mm) Matrix Size Parallel Imaging	Duration (min:secs)
T1-weighted MPRAGE	MPRAGE	TR/TI/TE=2300/900/2.32ms Flip angle=8º	0.9x0.9x0.9mm 240x240x192 iPAT=2	5:21
T2-weighted TSE TRA	T2_tse_tra	TR/TE=3500/92 ms Flip angle = 120 º	0.4x0.4x5mm 230x173x154	2:01
Susceptibility weighted	Fl3D_SWI	TR/TE=27/20ms Flip angle = 15º	1x1x3mm 250x188x156 iPAT=2	2:43
FLAIR	T2_flair	TR/TI/TE=9000/87/2500ms Flip angle=150º	0.6x0.6x5mm 230x173x143	4:32
Field map	Gre_field	TR/TE1/TE2=400/5.19/7.65 Flip angle= 60º	3.8x3.8x3mm 240x240x135	0:54
DTI	MDDW64	TR/TE=6900/67 Directions=108 b-value=1000 s/mm^2	2x2x2mm 240x240x128 iPAT=2	12:47
fMRI	Ep2d	TR/TE=2390/30ms Flip angle= 90º	3.5x3.5x3.5 224x224x144	6:06
TOF MR angiogram	ToF	TR/TE=24/3.93ms Flip angle= 15º	0.5x0.5x0.6mm 200x150x154 iPAT=2	11:10
T1-weighted SPACE *pre-contrast & post-contrast*	Tse3d_spc	TR/TE=750/20ms	0.5x0.5x0.9mm 231x231x51 iPAT=2	2x 3:49

Reporting checklist for cohort study.

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		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	#3	State specific objectives, including any prespecified hypotheses	4
Methods			

Study design	#4	Present key elements of study design early in the paper	4
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-11
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	4-5
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-11
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6-7
Bias	#9	Describe any efforts to address potential sources of bias	4
Study size	#10	Explain how the study size was arrived at	10
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	9
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	8-10
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	8-10
Statistical methods	#12c	Explain how missing data were addressed	8-10
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	10
Statistical methods	#12e	Describe any sensitivity analyses	NA

Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	NA
Participants	#13b	Give reasons for non-participation at each stage	NA
Participants	#13c	Consider use of a flow diagram	NA
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	5
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	NA
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	NA
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	NA
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
Main results	#16b	Report category boundaries when continuous variables were categorized	NA
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	#18	Summarise key results with reference to study objectives	NA

Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	12
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	12
Generalisability	#21	Discuss the generalisability (external validity) of the study results	12
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

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Neuroinflammation in cognitive decline post-cardiac surgery (the FOCUS study): an observational study protocol

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Secondary Subject Heading:	Immunology (including allergy), Neurology, Mental health
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Neuroinflammation in cognitive decline post-cardiac surgery (the FOCUS study): an observational study protocol

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ABSTRACT

Introduction

Postoperative cognitive dysfunction (POCD) occurs frequently after coronary artery bypass grafting (CABG). The underlying mechanisms remain poorly understood, but neuroinflammation might play a pivotal role. We hypothesize that systemic inflammation induced by the surgical trauma could activate the innate immune (glial) cells of the brain. This could lead to an exaggerated neuroinflammatory cascade, resulting in neuronal dysfunction and loss of neuronal cells. Therefore, the aims of this study are to assess neuroinflammation *in vivo* pre- and post-surgery in patients undergoing major cardiac surgery and investigate whether there is a relationship of neuroinflammation to cognitive outcomes, changes to brain structure and function, and systemic inflammation.

Methods and analysis

The FOCUS study is a prospective, single-centre observational study, including 30 patients undergoing elective on-pump CABG. Translocator protein (TSPO) PET neuroimaging will be performed pre- and postoperatively using the second generation tracer ^{18}F-DPA-714 to assess the neuroinflammatory response. In addition, a comprehensive cerebral MRI will be performed pre- and post-surgery, in order to discover newly developed brain and vascular wall lesions. Up to 6 months postoperatively, serial extensive neurocognitive assessments will be performed and blood will be obtained to quantify systemic inflammatory responses and peripheral immune cell activation.

Ethics and dissemination

Patients do not benefit directly from engaging in the study, but imaging neuroinflammation is considered safe and no side effects are expected. The study protocol obtained ethical approval by the Medical Research Ethics Committee (MREC) region Arnhem-Nijmegen. This work will be published in peer-reviewed international medical journals and presented at medical conferences.

Article summary

Strengths and limitations of this study
• This study is the first to examine *in vivo* neuroinflammation using TSPO PET neuroimaging both prior to and after cardiac surgery
• This study combines state of the art neuroimaging with longitudinal neuropsychological examinations and detailed immunological analyses
• Extensive neuropsychological examinations at four different timepoints enable us to detect even subtle changes in cognitive function
• This study is powered to detect the association between neuroinflammation and cognitive decline between baseline and hospital discharge, when the incidence of cognitive dysfunction is highest.
• The power of this pilot study is inadequate to adjust for confounders.

Keywords

Postoperative cognitive dysfunction (POCD), coronary artery bypass grafting (CABG), neuroinflammation, microglia, TSPO neuroimaging

INTRODUCTION

Background and rationale

Coronary artery bypass grafting (CABG) has been associated with postoperative cerebral complications.[1-4] These may occur directly post-surgery, like stroke or delirium, but can also have long-term sequelae such as postoperative cognitive dysfunction (POCD) and dementia. The incidence of POCD ranges from 20-70% in the first week after surgery, to 10-40% in the following months but may increase again from one postoperative year onwards.[1, 5] Furthermore, patients have a 1.7-fold increased risk to develop new incident Alzheimer's disease within six years after undergoing a CABG, compared to patients undergoing a percutaneous coronary intervention.[6]

Development of POCD is presumably related to perioperative brain hypoperfusion, cerebral microembolization, haemodilution, hypercoagulability, cerebral hyperthermia, and inadequate glucose homeostasis.[2] In addition, systemic inflammation is hypothesized as an important (and possibly treatable) factor for POCD pathogenesis.[7-9] Cardiac surgery produces substantial systemic inflammation (reflected by leucocytosis and significant dysregulation of cytokines and other inflammatory mediators, affecting various physical processes) due to multiple stimuli such as sternotomy, extracorporeal circulation (ECC), associated transient endotoxemia, and aortic cross-clamping. Systemic inflammation can result in increased communication and signalling from the periphery to the brain.[10, 11] As a result, systemic inflammation may induce activation of the innate immune cells of the brain, the microglia and astrocytes, leading to a neuroinflammatory response.[12] Animal models demonstrated that systemic administration of low-dose endotoxin even leads to long-term inflammatory reprogramming of microglia.[13] Interestingly, research has shown that neuroinflammation is associated with cognitive dysfunction and neurodegenerative disease.[14, 15] Given these associations, we hypothesize that occurrence of POCD in patients undergoing CABG is mediated through the occurrence of neuroinflammation.

Positron Emission Tomography (PET) enables the imaging of glial activation in the CNS using radiolabelled antagonists of translocator protein (TSPO).[16, 17] TSPO is mainly expressed as a transmembrane protein on mitochondria of microglia and astrocytes in the CNS.[18] As TSPO expression is upregulated during neuroinflammatory processes, it makes a promising biomarker for imaging neuroinflammation.[19] Up to now, TSPO neuroimaging has been applied in a wide variety of neurodegenerative and psychiatric conditions, showing associations between neuroinflammation and cognitive decline.[20-22] Recently, first *in vivo* evidence of microglial activity in response to systemic inflammation has been shown in patients and healthy volunteers.[23-25] However, human *in vivo* data on neuroinflammation in patients undergoing major cardiothoracic surgery are still lacking.

This study therefore aims to assess neuroinflammation pre- and post-surgery *in vivo* in patients undergoing elective cardiac surgery. Given the presumed association between neuroinflammation and cognitive decline, quantifying the extent of neuroinflammation post-surgery and its relation to POCD will provide us with important insights for future (interventional) research.

Objectives

Our primary objective is to assess whether neuroinflammation is more pronounced in patients with cognitive decline at hospital discharge after CABG surgery, compared to patients without cognitive decline.

Secondary objectives:

1. To study the relation between the neuroinflammatory response and structural or functional changes to the brain postoperatively.
2. To study the relation between neuroinflammation and cognitive decline at 6 weeks and 6 months post-cardiac surgery.
3. To study the relation between neuroinflammation and postoperative delirium.
4. To study the relation between the perioperative systemic immune responses and neuroinflammation.

METHODS AND ANALYSIS

Study design

The FOCUS study (neuroinflammation in cognitive decline post cardiac surgery) is a single-centre observational time-series design study investigating neuroinflammation, systemic inflammation and neuropsychological performance before and after CABG.

Study population and recruitment

All patients planned for elective CABG in an academic hospital in the Netherlands are screened. Table 1 presents the inclusion- and exclusion criteria. The age criterion of 50 years or older is chosen for two reasons. First, older adults are more prone to subsequent long-term cognitive decline after cardiac surgery.[26, 27] Second, ageing is associated with a more exaggerated neuroinflammatory response following systemic inflammation.[28]

Screening and enrolment logs will be maintained for all patients. After written informed consent is obtained, a blood sample will be genotyped for rs6971 polymorphism using Taqman analysis on a 7500 Fast Real-Time PCR System (ThermoFisher Scientific, Waltham, USA). Low-affinity TSPO binding patients for the radiotracer used in PET imaging will be excluded from participation. In our predominantly Caucasian cohort, the estimated percentage of low affinity binders is below 10%.[29]

Data collection

Patients' demographics and information regarding their surgery indication, treatments, pre-existing comorbidity (Charlson Comorbidity Index),[30, 31] (re)admission, disease severity and mortality risk, length of mechanical ventilation, length of stay at the Intensive Care Unit

(ICU) and occurrence of delirium will be retrieved from the patients' medical files. Table 2 shows an overview of events for this study, which will be defined subsequently in more detail.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	
	• Written informed consent • Age >50 years • Planned for elective on-pump coronary artery bypass grafting surgery • High- or mixed-affinity binders based on rs6971 polymorphism for translocator protein (TSPO) • Pre-hospital use of statins
Exclusion criteria	
Neurological	• Neurodegenerative disease, including mild cognitive impairment • Brain or spinal surgery within the last 6 months • Meningitis or brain infection within the last 6 months • Brain injury (e.g. acute stroke, or subarachnoid haemorrhage) within the last 6 months • Severe brain trauma in previous medical history • Presence of a cerebrospinal fluid catheter or shunt • Presence of a known brain tumour • Pre-hospital use of neuroleptics
Inflammatory	• Active infection (defined as fever >38.5°C or antibiotic treatment) within the last 2 weeks prior to surgery • Immunocompromised state (due to immunomodulatory drugs or underlying conditions) • Auto-immune or auto-inflammatory disease
Cardiological	• Previous cardiac surgery • Cardiovascular event within the last 3 months
Other	• Contra-indication to undergo a PET/CT or MRI scan • Known contrast allergy for gadolinium • Kidney failure (defined by a MDRD-GFR <15ml/min/1.73m^2) • Illiteracy or the inability to speak Dutch • Presence of disabilities that prevent accurate delirium diagnosis • Low TSPO binding affinity (based on rs6971 polymorphism)

Table 2. Schedule of events

	Pre-clinical	Pre-operative	CABG	Postoperative			Follow-up	
Timing:		T0 Baseline	T1 Stop ECC	T2 Stop ECC +6h	T3 Incision +24h	T4 CABG + 3-7d	T5 6 w	T6 6 m
Inclusion/exclusion criteria	X							
Informed consent	X							
TSPO binding affinity	X							
PET/CT cerebrum		X				X		
MRI cerebrum		X				X		
Blood sampling		X	X	X	X	X	X	
Neuropsychological assessment		X				X	X	X
Delirium assessment		3 times daily during hospital stay						

CABG = coronary artery bypass grafting; ECC = extracorporeal circulation; h=hours, d=days, w=weeks, m=months,
TSPO = translocator protein, PET/CT = positron emission tomography/computed tomography, MRI: magnetic resonance imaging.

Cerebral imaging

Dynamic brain PET/CT scans will be obtained preoperatively and on the fourth (range: 3rd-7th) postoperative day. Dynamic imaging of the head will be performed for 60 minutes after intravenous injection of the radiolabelled TSPO antagonist ^{18}F-DPA-714, a second generation TSPO ligand. This generation of TSPO ligands outperforms the first generation TSPO tracer, isoquinoline carboxamide (^{11}C-PK11195), on TSPO binding affinity and PET imaging properties, but requires polymorphism genotyping and stratifying according to binding affinity status.[32] A dose of ~200 MBq of ^{18}F-DPA-714 will be administered as a slow bolus injection during 40 seconds. Scans are obtained on a Siemens Biograph mCT hybrid PET/CT scanner (Siemens, Erlangen, Germany). A low-dose CT will be acquired for attenuation correction and anatomical reference. For pharmacokinetic analysis of the PET data arterial blood sampling will be used.

In addition, a 3 Tesla brain MRI (Siemens TIM TRIO, Erlangen, Germany) will be performed on the same days as the PET/CT scans. T1-weighted MR images will be obtained to co-register with the PET images for anatomical reference. Whole brain grey matter, as well as regions of interest will be delineated using probabilistic brain region templates. The slice thickness will be 1 mm. Additionally, the following MRI sequences will be performed: T2-weighed, susceptibility weighted imaging (SWI), FLAIR, diffusion tensor imaging (DTI), resting state fMRI, TOF MR angiogram of the circle of Willis and pre- and postcontrast 3D high-resolution T1-weighted SPACE sequence to visualize vessel wall abnormalities. In order to visualize cerebral vessel wall abnormalities, 0.1 mmol/kg gadobutrol (Gadovist) contrast agent will be administered intravenously. Details on MRI settings are provided in online supplementary appendix 1.

A senior neuroradiologist blinded to all other data will systematically quantify newly developed lesions post-surgery. This enables us to analyse the relationship between neuroinflammation, neuropsychological decline and cerebral lesion load. To evaluate changes in brain functional connectivity due to an acute immune response, a resting-state fMRI measurement is implemented comparing resting state connectivity in stress-related brain circuits pre- and post-surgery.[33, 34] A previous study found positive associations between alterations in resting-state functional connectivity in the brain's default mode network and global cognitive change after cardiac surgery[35], and the current study enables us to extend these findings by studying the relationship with (neuro)inflammation.

Blood sampling

Blood samples will be obtained at baseline preoperatively (T0, concomitant with the PET-scan), intra-operatively at the stop of ECC (T1), 6 hours after stop ECC (T2) and 24 hours after incision (T3). The timing of blood draws is based on the peak of the systemic inflammatory response post-cardiac surgery, as shown in previous studies we performed in patients undergoing cardiac surgery.[36, 37] Additionally, blood samples will be collected concomitant with the second PET/CT-scan (T4) and six weeks postoperatively (T5) to explore to what extent the inflammatory response persists in time.

At all timepoints, common blood parameters will be measured, including haemoglobin, leucocyte and thrombocyte count, and circulating pro-and anti-inflammatory cytokines (including tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), interleukin-10 (IL-10), and interleukin-1 receptor antagonist (IL-1RA)) by using simultaneous

Luminex assays. Blood will be centrifuged at 2000g for 10 minutes and the plasma will be stored at -80°C for possible future additional testing in line with the objective of this protocol. In addition, danger-associated molecular patterns (DAMPs, including high mobility group box 1 (HMGB1), heat shock protein-70 (HSP70), calgranulin-C (S100A12), calprotectin (S100A8/9), nuclear DNA, and mitochondrial DNA) will be measured as reported previously,[38] in plasma centrifuged for a second time at high speed (16000 g).
Additionally, *ex vivo* production of cytokines by stimulated isolated peripheral blood mononuclear cells (PBMCs) will be measured, including TNF-α, IL-6, IL-1β, monocyte chemoattractant protein-1 (MCP1) and IL-10. Flow cytometry analysis of whole blood will be performed as described previously,[39, 40] to study the inflammatory phenotype of the cells (including expression of human leukocyte antigen-DR (HLA-DR), C-C chemokine receptor type 2 (CCR2), CD11b, CD14, and CD16).

Arterial blood samples will be taken immediately before the ^{18}F-DPA-714 injection and during the PET-scan to measure the time course of radioactivity in plasma. In addition to this pharmacokinetic sampling, blood samples will be used to assess the ratio of ^{18}F-DPA-714 and its metabolites in order to correct the arterial input function for metabolite formation.

Neuropsychological assessment

A trained psychologist will perform neuropsychological assessments preoperatively (T0), at hospital discharge (T4), after six weeks (T5) and six months (T6) follow-up. These assessments are in line with the recommendation for neuropsychological research in cardiac surgery patients.[41] Table 3 lists the full test battery used for the neuropsychological assessments as well as the self-report questionnaires. Tests were selected based on sensitivity to detect even subtle deterioration in cognitive performance, with a focus on the domains executive functioning, memory, speed of processing and language. We will use parallel versions of these tests to account for material-specific practice effects after repeated assessment.[42]

During hospitalisation, screening of delirium is standard of care. Confusion Assessment Method for the ICU (CAM-ICU)[43] or Delirium Observation Screening scores (DOS)[44, 45] at the cardiothoracic ward will be performed three times a day. A dedicated senior delirium researcher or neurologist will validate the diagnosis of delirium, using *DSM V criteria for delirium*.[46]

Cardiac surgery

Perioperative care will be delivered according to the regular clinical protocol for CABG. This minimizes the risk of potential confounders due to variability in medical management.

Table 3. Summary of neuropsychological testing

Neuropsychological assessments

Test name	Domain	Subdomain
At timepoints T0		
National Adult Reading Test – IQ[47]	Premorbid intelligence (descriptive)	Estimation of pre-morbid intelligence level
At timepoints T0, T4, T5, T6		
Trail Making Test B[48-50]	Executive function	Visual attention and task switching
Stroop Colour-Word Test I, II, III[51]	Executive function	Susceptibility to interference
Wechsler Adult Intelligence Scale-IV (WAIS) – Digit Span[52, 53]	Executive function	Working memory
Letter Digit Substitution Test (LDST)[54, 55]	Information processing speed	Information processing speed
Trail Making Test A[48-50]	Information processing speed	Information processing speed
Rey Auditory Verbal Learning Test (RAVLT)[56, 57]	Episodic memory	Verbal episodic memory
Rey/Taylor Complex Figure Test –recall trials (RCFT)[58, 59]	Episodic memory	Visual episodic memory
Rivermead Behavioural Memory Test-3 (RBMT-3), Face recognition[60]	Episodic memory	Visual episodic memory
Rey/Taylor Complex Figure Test – copy trial (RCFT)[58, 59]	Visuoconstructive ability	Visuoconstructive ability
Letter Fluency Test (LFT)[61]	Language	Semantic memory
Token Test (short form)[62]	Language	Language comprehension

Neuropsychological questionnaires

Test name	Assessment of
At timepoints T0	
Informant Questionnaire on Cognitive Decline in the Eldery (IQCODE)[63]	Subjective cognitive impairment, filled out by significant other
At timepoints T0, T4, T5, T6	
Clinical Frailty Scale[64]	Frailty screening
RAND-36[65]	Health-related quality of life survey
Cognitive Failure Questionnaire (CFQ)[66]	Subjective cognitive complaints
Hospital Anxiety and Depression Scale (HADS)[67]	Anxiety and depressive complaints
At timepoints T4, T5, T6	
Impact of Events Scale-Revised (IES-R)[68]	Distress caused by traumatic events

Data analysis

Pharmacokinetic modelling of dynamic PET data will be performed in PMOD software (PMOD Technologies LLC, Zürich, Switzerland). Binding potential (BP_{ND}) and volume of distribution (V_T) in several regions of interest (ROIs) will be determined using the 2-tissue compartmental model (2TCM) for each scan. Predefined ROIs include the frontal, temporal, parietal and occipital lobes, amygdala, hippocampus, thalamus, cerebellum and the brain stem.

For each patient, ^{18}F-DPA-714 BP_{ND} will be measured in the ROI for baseline and post-surgery PET scans. Since BP_{ND} is proportional to the availability of TSPO binding sites in the brain, an increase in BP_{ND} reflects an increase in glial activation. In addition, we will measure V_T which recently has been shown to mainly reflect changes in peripheral tracer binding during systemic inflammation, rather than changes in TSPO expression in the brain.[25][and unpublished data from our group] Therefore, both outcome measurements are required to validate these recent findings and assess glial activation accurately. Study investigators who analyse the cerebral imaging data will be blinded for inflammatory mediator results and cognitive outcomes.

No consensus about the definition of POCD has been achieved in the literature thus far.[5, 69, 70] At all timepoints, neuropsychological tests of our patients will be compared to available normative data, adjusted for age and educational level, resulting in standardized z-scores. Overall domain z-scores will be calculated by averaging the z-scores of the individual tests within a specific domain. Calculation of test performance will be supervised by an experienced clinical neuropsychologist. Additionally, z-scores of each individual test will be clinically classified as either being within the normal range (0), below average (1), or impaired (2), compared to the aforementioned normative data. “Normal performance” is defined as performance above -1 SD from the normative mean. “Below average” as between -1 SD and -1.65 SD from the normative mean (the lowest 16% of the normal population), and “impaired” as below -1.65 SD from the normative mean (the lowest 5% of the normal population).[71, 72] Thus, an overall weighted performance score will be calculated for all five cognitive domains. A cognitive domain will be clinically classified as *impaired* when the average weighted score of all tests within the domain is >1. We will define the presence of POCD when 1) patients are *impaired* in one or more domains at hospital discharge, compared to baseline, or 2) when the overall weighted performance score deteriorated (from classification score 0 or 1 to 1 or 2, respectively) in more than one domain at hospital discharge compared to baseline. Accordingly, all patients will be dichotomized into two groups: with or without POCD at hospital discharge.

The percentage change in TSPO expression (postoperative versus preoperative) will be calculated and reported as mean ± standard error of the mean (SEM) if normally distributed. This value can be compared using unpaired t-tests between both groups with Bonferroni post-hoc correction, and additionally, linear mixed models will be performed with the presence of POCD and brain region modelled as fixed effects, and subject ID as random effect. Additionally, the (change in) mean of the cognitive domain scores is analysed as continuous dependent outcome, with (change in) TSPO expression and (change in) systemic inflammatory markers as predictors in multiple linear regression models. Age, sex, Clinical Frailty Scale, Charlson Comorbidity Index, Hospital Anxiety and Depression Scale (HADS), and the RAND-36 item health survey at inclusion can be included as covariates. Multiple logistic regression analysis is performed to correct for possible confounders such as newly developed structural brain lesions on MRI.

The trajectory of systemic inflammatory parameters and differences in timepoints will be measured with repeated measures one-way ANOVA or linear mixed models in case of missings. In addition, correlations between systemic inflammatory markers and TSPO expression will be studied using Pearson for parametric or Spearman for non-parametric data. Unpaired t-tests will be applied to compare TSPO expression between patients with and without a delirious episode during hospital admission.

All MRI images will be evaluated without prior notice of any clinical parameter. White matter lesions are defined as hyperintense lesions on FLAIR MRI without corresponding cerebrospinal fluid like hypo-intense lesions on the T1 weighted image. Lacunar infarcts are defined as hypo-intense areas >2mm and ≤15mm on FLAIR and T1.[73] Territorial infarcts are defined as hyperintense lesions on FLAIR, and hypointense lesions on T1 image.[73] And finally, microbleeds are defined as small, homogenous, round foci of low signal intensity on T2* weighted images of <10mm in diameter.[74]
Analyses of functional and structural brain images will be performed using FSL (FMRIB's Software Library, Oxford, United Kingdom).[75] After pre-processing and denoising, subject-wise spatial maps of with-in network brain connectivity of the salience, executive control and default mode networks will be compared with non-parametric tests for mean differences. Vascular wall enhancement will be compared between the preoperative and postoperative assessments with paired t-tests or the Wilcoxon signed rank test if the data are non-parametric.

Descriptive statistics will be performed using IBM-SPSS software. Mean (±SEM) or median (IQR) will be presented depending on the distribution of the data. Alpha will be set at 0.05 throughout.

After the first 5 patients we will schedule a technical interim analysis to establish whether a tissue reference model is a reliable, non-invasive method for pharmacokinetic analysis of TSPO neuroimaging during systemic inflammation-induced neuroinflammation. If this or other (mathematical) methods can be reliably validated in our cohort, there will be no longer need for arterial blood sampling.

Sample size calculation

No data on the degree of glial activation after systemic inflammatory responses in post-cardiac surgery patients are available. Research on cognitive dysfunction after cardiac surgery estimates a prevalence of approximately 50% at hospital discharge.[1, 5, 69] Therefore, we will define two groups based on the presence or absence of cognitive dysfunction at hospital discharge.

Previous studies in dementia patients observed a 15-35% higher PET tracer uptake in patients with cognitive impairment compared to healthy controls, with a standard deviation of 30%.[76, 77] Therefore, we assume that patients with cognitive dysfunction at hospital discharge after cardiac surgery will have a 30% higher delta tracer uptake compared to patients without cognitive decline. To assess a 30% higher delta tracer uptake in patients with cognitive dysfunction, an unpaired two-sample t-test results in 13 patients per group with a power of 80% and a one-sided alpha of 0.05. In order to account for lower increments we will include 15 patients per group resulting in a power of 85% with an alpha of 0.05 to differentiate an increase of 30% in delta tracer uptake. Consequently, we will include a total of 30 patients, assuming that 50% will have cognitive decline at hospital discharge. The investigator can decide to withdraw a subject from the study for a) urgent medical reasons, or b) if a protocol violation occurs or c) if the subject is lost to follow-up. Replacement of individuals will not be necessary

in this observational cohort once both PET scans are performed (i.e. primary objective has been met).

Sample size calculations were conducted for the primary objective solely, considering cognitive decline at hospital discharge. Assessment of long-term cognitive outcomes (up to 6 months) together with assessment of our secondary outcomes has been set up in an exploratory setting. The data of this study will be important to calculate the power and feasibility of a subsequent prospective project focussed on long-term outcomes.

Interim analysis with futility stop

An interim analysis will be performed after the first 15 subjects to validate that CABG surgery indeed induces a neuroinflammatory response, which will be assessed by TSPO PET imaging. An independent data safety and monitoring board (DSMB), composed by two clinical experts in the field of nuclear medicine and neurology, and a biostatistician, will analyse whether increased PET tracer uptake is observed after CABG surgery compared to the preoperative baseline tracer uptake. If no trend towards a significant difference ($p>0.10$) will be observed, the inclusion will be stopped to prevent futility. Furthermore an adaptive power analysis will be performed by the unblinded statistician DSMB member during the interim analysis to determine whether the study is underpowered to fulfil the primary objective, and whether the sample size should be adapted. The pooled standard deviation (SD) of the delta PET tracer uptake will be calculated from the first 15 patients of which approximately 50% have cognitive disorders at hospital discharge. Using this pooled SD the sample size calculation will be performed again. If the ratio between this new sample size and the original is greater than 1, the sample size will be adapted if deemed feasible. The investigators will remain blinded for cognitive outcomes until the end of the study.

Study period

The study started enrolling patients in March 2019. The estimated study enrolment completion date is anticipated in the beginning of 2022. Please note that this manuscript was finalised prior to the interim analyses.

Ethics and dissemination

The study is conducted according to the principles of the Declaration of Helsinki and in accordance with the Dutch Medical Research Involving Human Subjects Act (WMO) and Good Clinical Practice guidelines. The study obtained ethical approval by the Medical Research Ethics Committee (MREC) region Arnhem-Nijmegen (CMO 2016-2598). The study is registered in the ClinicalTrials.gov database (NCT 04520802). The burden of the study protocol consists of two PET/CT-scans and two cerebral MRIs, additional blood sampling before, during and after surgery and four neuropsychological examinations, two during hospital admission and two follow-up visits during the first 6 months after hospital discharge.

Patient and public involvement statement

The hypothesis of this study was conceived with the help of patients through outpatient clinical follow-up after an ICU admission. Through patient experience of different long-term consequences, this study's endpoints involves cognitive performance, psychological symptoms and quality of life. A patient member of the MREC judged the study protocol for feasibility, burden and understandability of patient information.

Data management

Data will be handled confidentially and pseudonymously. Study data will be de-identified and a secured subject identification code list will be kept and stored separately from the data. This observational study uses an electronic remote data capture system. All missing and ambiguous data will be queried. The investigator will permit study-related monitoring, audits and regulatory inspection at their site, providing access to source data/documents. In all cases, it remains the responsibility of the investigator to ensure that data are accurate. Coded data will be kept after closure of the study and can only be used for ancillary studies after strict approval of the principal investigator. Anonymized data can be shared with other organizations for academic research, consensus development or other projects aimed at advancement of knowledge in this area. Body materials consisting of blood will be preserved in a coded form for 10 years for possible follow-up studies. The MREC will be consulted before body material is used for follow-up research.

Public disclosure and publication policy

The results of this study will be published, regardless of whether these are positive, negative or inconclusive, in peer-reviewed international (open access) medical journals and presented at medical conferences. In addition, a summary of the results of this study will be published on the website of the funding agency The Netherlands Organization for Health Research and Development (ZonMw).

Relevance of findings

Cerebral dysfunction after cardiac surgery occurs frequently and may severely affect patients' daily lives. Due to the lack of research data within this area, the pathophysiology of cerebral dysfunction post-surgery is unknown. Therefore, there are currently no interventions available to prevent or treat this deterioration. The FOCUS study will quantify glial activation, which is suggested to be important in this pathophysiology, and relate this to cognition, structural and functional changes to the brain and systemic inflammation. This adds to previous and ongoing observational work perioperatively combining blood and cerebrospinal fluid parameters with MR neuroimaging, cognition and electroencephalogram recordings.[78, 79] Our study combines state of the art molecular and MR neuroimaging techniques, elaborates longitudinal neuropsychological examinations, and comprehensive immunological laboratory tests. Objective neurocognitive examinations will be performed at four different timepoints, up to six months postoperatively, enabling us to detect even subtle changes in cognition. Better understanding of the pathogenesis of POCD could direct neuroscientists towards the development of targets for future interventions. This imaging paradigm could provide an approach to examine the efficacy of such interventions in clinical studies.

In addition, the knowledge obtained with this study is of importance for patients and healthcare professionals as well. The participating patient will not directly benefit from study participation. However, they will be followed with regard to possible complaints in light of potential post-cardiac surgery cerebral dysfunction. This will increase the awareness of the participating patients and caregivers with respect to such complaints. This might benefit the patient and caregivers as it will decrease uncertainty about the nature of the complaints when they occur. Furthermore this study searches for a biological explanation for post-surgery cerebral complaints which are often not understood or classified as functional.

Several limitations need to be addressed. First, as this study is powered at cognitive decline at hospital discharge, it is not powered to study the association between glial activation and cognitive decline after 6 months. The second limitation concerns the arterial sampling during the dynamic PET-scans. Automated arterial sampling, which is frequently used in other studies using outpatient clinic patients, leads to a significant loss of blood per scan. Loss of such an amount of blood in cardiac surgery patients is not preferable as it could result in a significant health risk for these critically ill patients. Therefore we will sample manually, which reduces blood volume loss. Manual sampling results in less sampling points and could therefore lead to a slightly less accurate plasma activity curve. Unfortunately, TSPO expression is not specific to microglia and astrocytes, and the measured PET signal can be affected by recruitment of peripheral monocytes to the brain, adherence of circulating leukocytes to the vascular epithelium, or TSPO expression in neurons or vascular endothelial cells. Finally, the power of this pilot study is inadequate to allow adjustment for all potential confounding factors.

Imaging neuroinflammation as proposed is safe, as corroborated by existing human and animal data. In addition, imaging neuroinflammation could lead to potential prognostic and interventional targets that could revolutionize healthcare for this large group of patients.

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Contributors:

WFA conceived the study idea. APvT and WFA developed the study design to which JvT, WL, HJD, NPR, FJAM, RPCK, NK, JvdH, PP and MR contributed. APvT and HBD drafted the manuscript. All authors were involved in the editing of the manuscript and read and approved the final manuscript.

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Supplemental material to:

A.M. Peters van Ton et al.,

Neuroinflammation in cognitive decline post-cardiac surgery (the FOCUS study): an observational study protocol

Appendix 1: MRI scan protocol – parameter settings

	Protocol Name	Sequence/Contrast parameters	Resolution(mm) Matrix Size Parallel Imaging	Duration (min:secs)
T1-weighted MPRAGE	MPRAGE	TR/TI/TE=2300/900/2.32ms Flip angle=8º	0.9x0.9x0.9mm 240x240x192 iPAT=2	5:21
T2-weighted TSE TRA	T2_tse_tra	TR/TE=3500/92 ms Flip angle = 120 º	0.4x0.4x5mm 230x173x154	2:01
Susceptibility weighted	Fl3D_SWI	TR/TE=27/20ms Flip angle = 15º	1x1x3mm 250x188x156 iPAT=2	2:43
FLAIR	T2_flair	TR/TI/TE=9000/87/2500ms Flip angle=150º	0.6x0.6x5mm 230x173x143	4:32
Field map	Gre_field	TR/TE1/TE2=400/5.19/7.65 Flip angle= 60º	3.8x3.8x3mm 240x240x135	0:54
DTI	MDDW64	TR/TE=6900/67 Directions=108 b-value=1000 s/mm^2	2x2x2mm 240x240x128 iPAT=2	12:47
fMRI	Ep2d	TR/TE=2390/30ms Flip angle= 90º	3.5x3.5x3.5 224x224x144	6:06
TOF MR angiogram	ToF	TR/TE=24/3.93ms Flip angle= 15º	0.5x0.5x0.6mm 200x150x154 iPAT=2	11:10
T1-weighted SPACE *pre-contrast & post-contrast*	Tse3d_spc	TR/TE=750/20ms	0.5x0.5x0.9mm 231x231x51 iPAT=2	2x 3:49

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

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		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	#3	State specific objectives, including any prespecified hypotheses	4
Methods			

Study design	#4	Present key elements of study design early in the paper	4
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-11
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	4-5
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-11
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6-7
Bias	#9	Describe any efforts to address potential sources of bias	4
Study size	#10	Explain how the study size was arrived at	10
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	9
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	8-10
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	8-10
Statistical methods	#12c	Explain how missing data were addressed	8-10
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	10
Statistical methods	#12e	Describe any sensitivity analyses	NA

Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	NA
Participants	#13b	Give reasons for non-participation at each stage	NA
Participants	#13c	Consider use of a flow diagram	NA
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	5
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	NA
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	NA
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	NA
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
Main results	#16b	Report category boundaries when continuous variables were categorized	NA
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	#18	Summarise key results with reference to study objectives	NA

Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	12
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	12
Generalisability	#21	Discuss the generalisability (external validity) of the study results	12
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17