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# Disease activity in rheumatoid arthritis is inversely related to cerebral TSPO binding assessed by [$^{11}$C]PBR28 positron emission tomography

Forsberg A.[a,*,1], Lampa J.[b,1], Estelius J.[b], Cervenka S.[a], Farde L.[a,c], Halldin C.[a], Lekander M.[d,g], Olgart Höglund C.[e,h], Kosek E.[f]

[a] Department of Clinical Neuroscience, Centre for Psychiatry Research, Karolinska Institutet and Stockholm County Council, SE-171 76 Stockholm, Sweden
[b] Department of Medicine, Rheumatology Unit, Center for Molecular Medicine (CMM), Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden
[c] PET Science Centre, Precision Medicine and Genomics, IMED Biotech Unit, AstraZeneca, Karolinska Institutet, Sweden
[d] Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden
[e] Department of Medicine and Center for Molecular Medicine (CMM), Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden
[f] Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden
[g] Stress Research Institute, Stockholm University, Stockholm, Sweden
[h] Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

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ABSTRACT

Reumatoid Arthritis (RA) is an autoimmune disorder characterized by peripheral joint inflammation. Recently, an engagement of the brain immune system has been proposed. The aim with the current investigation was to study the glial cell activation marker translocator protein (TSPO) in a well characterized cohort of RA patients and to relate it to disease activity, peripheral markers of inflammation and autonomic activity.

Fifteen RA patients and fifteen healthy controls matched for age, sex and TSPO genotype (*rs6971)* were included in the study. TSPO was measured using Positron emission tomography (PET) and the radioligand [$^{11}$C]PBR28. The outcome measure was total distribution volume ($V_T$) estimated using Logan graphical analysis, with grey matter (GM) as the primary region of interest. Additional regions of interest analyses as well as voxel-wise analyses were also performed. Clinical evaluation of disease activity, symptom assessments, serum analyses of cytokines and heart rate variability (HRV) analysis of 24 h ambulatory ECG were performed in all subjects.

There were no statistically significant group differences in TSPO binding, either when using the primary outcome $V_T$ or when normalizing $V_T$ to the lateral occipital cortex ($p > 0.05$). RA patients had numerically lower $V_T$ values than healthy controls (Cohen's D for GM = −0.21). In the RA group, there was a strong negative correlation between [$^{11}$C]PBR28 $V_T$ in GM and disease activity (DAS28)($r = -0.745$, $p = 0.002$, corrected for rs6971 genotype).

Higher serum levels of IFNγ and TNF-α were found in RA patients compared to controls ($p < 0.05$) and several measures of autonomic activity showed significant differences between RA and controls ($p < 0.05$). However, no associations between markers of systemic inflammation or autonomic activity and cerebral TSPO binding were found.

In conclusion, no statistically significant group differences in TSPO binding as measured with [$^{11}$C]PBR28 PET were detected. Within the RA group, lower cerebral TSPO binding was associated with higher disease activity, suggesting that cerebral TSPO expression may be related to disease modifying mechanisms in RA. In light of the earlier confirmed neuro-immune features of RA, these results warrant further investigations regarding neuro-immune joint-to-CNS signalling to open up for potentially new treatment strategies.

## 1. Introduction

Rheumatoid arthritis (RA) is a chronic, autoimmune inflammatory disease mainly affecting the joints and leading to pain, fatigue and impairment of function (Firestein, 2003). Several aspects of RA indicate an involvement of neuro-immune pathophysiological mechanisms. An animal model of RA, such as collagen antibody-induced arthritis (CAIA) in rodents was reported to be associated with microglia and astrocyte

* Corresponding author at: Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden, Stockholm Spine Center, Stockholm, Sweden.
*E-mail address:* anton.forsberg@ki.se (A. Forsberg).
[1] Both authors contributed equally

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activation as well as mechanical hypersensitivity (Agalave et al., 2014; Bas et al., 2012). Tallying these results, collagen-induced arthritis (CIA) in mice has been shown to induce increased pain sensitivity and astrocyte activation, both of which were reversed with intraperitoneal anti TNF therapy (Inglis et al., 2007). There are several reports of pro-inflammatory cytokine upregulation in the spinal cord of arthritic mice (Bao et al., 2001; Lampa et al., 2012a). Additionally, intrathecal suppression of IL-1 or TNF signalling was shown to hamper arthritis, suggesting interaction between resident cells of the CNS on one hand, and systemic immune regulation on the other (Boyle et al., 2006; Fiorentino et al., 2008).

Additional support for a neuro-immune link in RA is the cholinergic anti-inflammatory pathway, which is characterized by systemic immune regulation through the vagal nerve (for review, see: (Pavlov and Tracey, 2017)). In RA, decreased parasympathetic (vagal) function has been observed (Bruchfeld et al., 2010), and there have been indications that neuro-inflammatory mechanisms may interact with vagal activity (Kosek et al., 2015). Importantly, electrical vagus nerve stimulation has previously been shown to reduce experimental arthritis in animals (Levine et al., 2014) and recently, Koopman et al. demonstrated that vagus stimulation also inhibited systemic TNF production in humans and reduced disease severity in RA patients (Koopman et al., 2016).

The concept of central nervous system abnormalities in RA disease has been suggested by a number of findings. First, RA has been associated with structural and functional changes in the brain (Flodin et al., 2016; Wartolowska et al., 2012) and cerebral functional connectivity patterns were associated to the degree of systemic inflammation (Schrepf et al., 2018). Also, longstanding RA disease is associated with central pain sensitization (Leffler et al., 2002), which is clinically manifested as chronic pain despite adequately suppressed joint inflammation (Altawil et al., 2016; Boyden et al., 2016). Apart from joint inflammation and pain, RA is also associated with fatigue. Although pain has been shown to have important impact on fatigue (Pollard et al., 2006) also central inflammatory mechanisms have been suggested, and TNF-blockade has been shown to reduce RA-associated fatigue to some extent (Yount et al., 2007). Fatigue is a prominent symptom of the “sickness response” normally triggered by tissue injury, pain or infections and believed to promote recovery in acute disorders, but if unabated negative long-term effects such as the development of chronic pain have been proposed (Kadetoff et al., 2012; Karshikoff et al., 2016; Watkins and Maier, 2005). Animal studies show that the sickness response is associated with glia cell activation, and interestingly, IL-1β–dependent sickness behaviour in systemically inflamed rodents has been discussed as a model of human fatigue (Dantzer, 2009). In agreement with this, we previously demonstrated a positive correlation between IL-1β in the CSF and ratings of fatigue in RA patients (Kosek et al., 2015; Lampa et al., 2012a). Furthermore, neuro-immune regulation where activation of the vagus nerve exerts an inhibitory role over the immune system counteracts the systemic “sickness response”, reducing cytokine release and attenuating arthritis (Chavan et al., 2017; Koopman et al., 2016).

One way to study the immune-brain interactions involved in the sickness response in humans is to trigger systemic inflammation by intravenous injection of the endotoxin lipopolysaccharide (LPS). Previous studies have shown that LPS-induced inflammation increased pain sensitivity, spontaneous pain (Lekander et al., 2016), reduced descending pain inhibition and altered cerebral processing of painful stimuli in healthy individuals, aberrations that were similar to findings in patients suffering from fibromyalgia (Karshikoff et al., 2016; Karshikoff et al., 2015). PET studies using radioligands for translocator protein (TSPO) allow for evaluation of glial activity in vivo. Interactions between periphery and CNS have been demonstrated in previous PET studies in humans as increases in cerebral TSPO binding after peripheral LPS-induced acute inflammation (Sandiego et al., 2015) as well as correlations between peripheral and central TSPO binding (Kanegawa et al., 2016). In addition, Kanegawa et al. also showed relationship between the change in [$^{11}$C]PBR28 binding in brain and periphery comparing two PET measurements at different occasions strengthening the suggested interaction.

In patients, studies using the second generation TSPO radioligand [$^{11}$C]PBR28, with a better signal-to-noise ratio than prototypic TSPO radioligands (Fujita et al., 2008; Kreisl et al., 2010), have shown increased cerebral TSPO binding in inflammatory neurological disorders (Herranz et al., 2016; Kreisl et al., 2013b; Zürcher et al., 2015) as well as in patients suffering from chronic low back pain (Loggia et al., 2015) and fibromyalgia (Albrecht et al., 2018a). Interestingly, in the fibromyalgia patients a positive association between cerebral TSPO binding and ratings of fatigue was reported, which is in agreement with symptoms of the sickness response (Albrecht et al., 2018a).

The aim of this study was to evaluate glial activation in RA patients using PET and the TSPO radioligand [$^{11}$C]PBR28, in comparison to healthy controls matched for age, sex and genetically inferred TSPO binding affinity. Given our previous findings of neuro-inflammation in RA patients and the strong indication of peripheral- to central immune interaction in RA, we hypothesized that cerebral TSPO binding would be significantly increased in RA patients compared to HC. Furthermore, we expected cerebral TSPO binding to be associated with increased disease activity. Finally, we wanted to investigate the relation of TSPO binding to systemic inflammation and autonomic activity.

## 2. Materials and methods

### 2.1. Subjects

Patients with RA, all positive for Anti-citrullinated protein antibodies (ACPA$^+$ RA) were recruited through the rheumatology clinic at the Karolinska University Hospital in Stockholm, Sweden. Age and sex matched healthy controls (HC) were recruited through notice board advertisements primarily at the hospital campus and all were physically examined. In total, 15 RA patients and 15 healthy controls were recruited. Baseline characteristics are displayed in Table 1. All RA patients were ACPA$^+$ and fulfilled the ACR 1987 classification criteria for RA (Arnett et al., 1988). Exclusion criteria included fibromyalgia comorbidity, neurological disease, ongoing treatment with antidepressants or cortisone, severe cardiovascular disease or other motives based on the judgment of the responsible physician. Exclusion criteria for the HC were identical to the RA patients, with the additional exclusion criteria of recurrent pain problems, including RA and fibromyalgia. The study was approved by the Regional Ethics Committee on Human Research at Karolinska Institutet, Stockholm, Sweden and the local Radiation Safety Committee, Karolinska University Hospital, Stockholm, Sweden. The protocol conformed to the standard of the Declaration of Helsinki, Finland and all participants were given both written and oral information regarding the study design and provided written informed consent.

During screening, a structural MRI examination was performed. T1 and T2-weighted MR images were obtained for each individual, using the 1.5 Tesla Siemens Avanto scanner (at Medicinsk Röntgen, Odenplan). T2 images were evaluated for pathology by a neuroradiologist and T1 images were used for subsequent coregistration to PET and definition of regions of interest (ROIs), see below.

Participants were instructed to avoid NSAIDs, alcohol and hard physical exercise two days before PET examinations, to drink coffee as usual and to sleep regularly two days before participation. No other change in medication was allowed the week before PET examination.

All participants were genotyped using provided saliva samples (Tacman SNP genotyping assay) for the genetic polymorphism *rs6971* which affects binding to the TSPO radioligand [$^{11}$C]PBR28, both in vitro and in vivo (Collste et al., 2016; Kreisl et al., 2013a; Owen and Matthews, 2011). This information was also used for matching of RA and HC according to the genotypes high affinity binding (HAB) and mixed affinity binding (MAB), whereas participants with low affinity

**Table 1**
Demographics and characteristics of RA patients and controls. Continuous variables are reported as mean ± SD and categorical variables are reported with percentages.

| | RA | HC | p |
|---|---|---|---|
| Number of subjects | 15 | 15 | |
| Demographics | | | |
| Age (y) | 51 ± 11.5 | 50 ± 11.5 | 0.62 |
| Sex (females) | 13 (86,7%) | 13 (86,7%) | |
| BMI | 24.1 ± 3.4 | 23.5 ± 3.1 | 0.76 |
| TSPO Genotype | | | |
| HABs | 9 (60,0%) | 9 (60,0%) | |
| MABs | 6 (40,0%) | 6 (40,0%) | |
| Disease characteristics | | | |
| DAS28 | 3.5 ± 1.4 | N/A | |
| SJC | 3 ± 3 | N/A | |
| TJC | 4 ± 3 | N/A | |
| PGA (mm) | 25.5 ± 27.0 | 0.6 ± 0.9 | < 0.001 |
| ESR (mm/h) | 17.4 ± 7.9 | 9.5 ± 5.5 | 0.006 |
| CRP (g/L) | 2.2 ± 1.7 | 1.5 ± 1.0 | 0.28 |
| FSS | 3.0 ± 1.4 | 1.2 ± 0.8 | 0.021 |
| Pain VAS (mm) | 27 ± 24 | 1 ± 1 | < 0.001 |
| Fatigue VAS (mm) | 39 ± 30 | 16 ± 15 | 0.019 |
| Treatment | | | |
| MTX/AMA | 11 (73,3%)/ 1 (6,7%) | N/A | |
| Biologics total | 12 (80,0%) | N/A | |
| TNF-α/ABA/RUX | 9 (60,0%)/2 (13,3%)/ 1 (6,7%) | N/A | |
| Analgesics | | | |
| NSAID daily | 1 (6.7%) | N/A | |
| NSAID/COX2 if needed | 6 (40,0%) | N/A | |

ABA, Abatacept; AMA,: Anti-malarials; BMI, Body Mass Index; CRP, C-reactive protein; COX2, cyclooxygenase 2 inhibitors; DAS28, Disease activity score 28; ESR, Erythrocyte sedimentation rate; FSS, Fatigue severeity scale; HAB, High affinity binders; MAB, Mixed affinity binders; MTX, Methotrexate; N/A, not applicable; NSAID, non-steroidal anti-inflammatory drugs; PGA, Patient's Global assessment; SJC, Swollen joint count; TJC, Tender joint count; RUX, Rituximab; TNF-α,TNF-blockade. Missing data: PGA 3 HC, ESR 1 RA, 4 HC, CRP 4 HC, BMI 2 RA, 8 HC.

binding (LAB) genotype were excluded from further study participation.

### 2.2. Questionnaires

To assess disease activity in the RA patients the composite measure of disease activity score 28 (DAS28) was used. The score is based on the combination of erythrocyte sedimentation rate (ESR), patient global assessment of disease on a 100 mm visual analogue scale (PGA) and the number of swollen (SJ) and tender (TJ) joints at 28 locations (Prevoo et al., 1995). DAS28 score was assessed by a trained rheumatologist seeing the patients after completion of the PET scan.

Measures of pain and fatigue were assessed prior to the PET examination. For measurements of pain, participants were asked to rate their overall perception of pain on a 100 mm visual analogue scale (Pain VAS) ranging from “no pain” to “worst imaginable pain” (Huskisson, 1974). For measures of fatigue participants were asked to rate their perception of fatigue during the last week on a 100 mm visual analogue scale (Fatigue VAS) ranging from “no fatigue” to “worst imaginable fatigue” (Rohekar and Pope, 2009). The nine item fatigue severity scale (FSS), known to be able to describe the change of fatigue over time, was additionally used to assess the state of fatigue in participants (Krupp et al., 1989; Whitehead, 2009).

### 2.3. Positron emission tomography (PET) examinations

PET examinations were performed using the High Resolution Research Tomograph (Siemens Molecular Imaging, Knoxville, TN, USA) at the PET centre at Karolinska Institutet, Stockholm, Sweden. For each subject, the PET examination was conducted in the morning/before lunch or just after lunch/afternoon to minimize a possible diurnal influence (Collste et al., 2016). The procedures for [$^{11}$C]PBR28 preparation, injection and PET data acquisition have been described in detail previously (Collste et al., 2016; Kanegawa et al., 2016). Briefly, patients received a radial artery catheter to allow automated arterial blood sampling and a cubital vein catheter in the contralateral arm for intravenous radio ligand administration. Patients were then positioned in the PET system using an individually designed helmet placed in a frame holder as a means of head fixation during the PET data acquisition. The average radioactivity administered was 405 ± 68 MBq (mean ± SD) and 421 ± 36 MBq; with a specific radioactivity of 257 ± 228 GBq/μmol and 317 ± 168 GBq/μmol and an injected mass of 0.84 ± 0.50 μg and 0.61 ± 0.33 μg in patients and controls respectively. There were no significant differences in these measures between the groups ($p > 0.05$). PET data were acquired for 93 min.

### 2.4. Arterial input function

Arterial blood was sampled using an automated system (ABSS) for the first 5 min. Manual samples were drawn at 2, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50, 70 and 90 min. Pre-processing of arterial blood data was performed using Kaleidagraph 4.1 software (Synergy Software) as described in detail before (Kanegawa et al., 2016). Correction for radioligand metabolism was performed in PMOD v3.3 (pixel-wise modelling software; PMOD Technologies Ltd., Zurich, Switzerland) using the parent fraction. Individual parent fraction data were fitted using a 3-exponential model.

#### 2.4.1. Image analysis and quantification

Image processing and the definition of ROIs using T1 MR images were performed as described previously (Collste et al., 2016; Kanegawa et al., 2016). The primary ROI was brain grey matter (GM). Moreover, an exploratory analysis of additional regions were performed to be able to relate our results to previous findings with [$^{11}$C]PBR28 in pain disorders (Albrecht et al., 2018a; Loggia et al., 2015). These regions included: lateral frontal cortex (LFC), lateral parietal cortex (LPC), putamen (PUT), and thalamus. Additionally, ROIs related to vagus nerve induced activation/deactivation, found in various neuroimaging studies, were identified to explore TSPO binding in these specific networks (Chae et al., 2003). These included one combined ROI of regions related to vagus nerve activation: thalamus, Insula, OFC, temporal pole and cerebellum. Additionally a combined ROI related to vagus nerve deactivation included the regions amygdala and hippocampus. In total seven ROIs were investigated.

Quantification of [$^{11}$C]PRB28 binding was performed using Logan graphical analysis with a metabolite corrected plasma input function to fit the TAC data and estimate total distribution volume ($V_T$) in each ROI (Logan et al., 1990). The estimation of $V_T$ was based on five frames from 33 to 63 min. In addition, for each PET examination a parametric $V_T$ image was generated using the stationary wavelet aided parametric imaging (WAPI) approach (Cselényi et al., 2002) WAPI analysis of TSPO binding has previously shown to be sensitive to within-subject changes in $V_T$ (Jucaite et al., 2015), and shown good reliability for 63 min data (Collste et al., 2016). In order to compare to previous studies using [$^{11}$C]PBR28 (Albrecht et al., 2018c) regional $V_T$ values where normalized to lateral occipital cortex to investigate regional relative changes in [$^{11}$C]PBR28 binding resulting in distribution volume ratio (DVR) values. Occipital cortex was chosen since it has recently been shown to be the preferred pseudo reference region in two clinical cohorts of patients with back pain, fibromyalgia or amyotrophic lateral sclerosis (ALS) (Albrecht et al., 2018c).

### 2.5. Measures of peripheral inflammation including cytokines

Two vials of blood collected in analysis appropriate tubes during

blood sampling prior to the PET scan were directly sent to the central hospital lab at Karolinska University Hospital, Stockholm, Sweden for the respective analysis of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) content.

Prior to, and on the same day as the PET measurements a sample of venous blood was collected. The sample was left undisturbed for 1–1.5 h in room temperature and was then centrifuged for either 10 min at 3200*g* or 20 min at 1500 g. Serum was aliquoted and stored in −80 °C until use. Cytokine levels in serum samples were measured using the sandwich immunoassay kits human V-PLEX proinflammatory panel 1 (K15049D, Meso Scale Diagnostics) (IFNγ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α) and Human V-PLEX MCP-1 (K151NND, Meso Scale Diagnostics) (MCP-1) according to the manufacturer's instructions using overnight incubation at 4 °C. Cytokines were analysed as duplicates on three separate sets of plates. Pairs of matched patient and control samples were always analysed on the same plate. Detection limit values for each respective plate were used to replace any values below the detection limit. Based on findings in previous research (Kosek et al., 2015) the following cytokines were evaluated and related to brain measures of [$^{11}$C]PBR28 and autonomic activity: tumor necrosis factor alpha (TNFα), Interleukin 6 (IL-6), Interleukin 8 (IL-8), Interleukin 10 (IL-10), Interferon gamma (IFNγ) and monocyte chemoattractant protein 1 (MCP1).

### 2.6. Autonomic activity

To assess autonomic activity via heart rate variability a 24 h ambulatory recording of ECG (CardioMem CM 4000 multichannel ECG recorder, GE healthcare) was collected for each individual after the completed PET scan. Participants were instructed to follow their normal daily routine, but to refrain from strenuous physical exercise during the period of the ECG recording. All ECG recordings were uploaded to the program CardioDay v2.3.2 (GE Healthcare) where they were visually inspected to ensure correct identification of QRS complexes. Any areas of interference as well as ectopic heart beats were excluded from analysis. To ensure high quality of the resulting HRV parameters ECG recordings were only considered for HRV analysis of time and frequency domain variables if recording length was above 20 h after quality inspection. ECG recordings from all study participants passed quality inspection.

After inspection HRV parameters of both time domain and frequency domain (fast Fourier transform) were calculated by the program in accordance with the European society of cardiology and north American society of pacing and electrophysiology task force established guidelines (1996). The 24 h average of the following parameters of time domain: standard deviation of the NN interval (SDNN), standard deviation of the average NN intervals in 5 min segments (SDANN), heart rate variability triangular index (HRVTI), square root of the mean squared differences of successive NN intervals (RMSSD) and frequency domain: total power (Power), low frequency power (LF), high frequency power (HF), LF/HF ratio (LF/HF), normalized units of LF (LF nu) and normalized units of HF (HF nu) were considered measures of interest and used for further analysis. The HRV measurements of SDNN, HRVTI and total power are considered to reflect overall autonomic activity. The SDANN measure is considered to reflect long term HRV components corresponding to ultralow frequency variations in heart rate. The RMSSD has been shown to reflect short term components of HRV corresponding to HF variations in heart rate and both RMSSD and HF measures are considered measures of parasympathetic, i.e. vagal, activity. The LF measure instead reflects low frequency HRV events and is considered a combined measure of both sympathetic and parasympathetic activity while the LF/HF ratio is considered a measure of sympathovagal balance.

### 2.7. Statistical analysis

Differences in $V_T$ values for [$^{11}$C]PBR28 between RA patients and controls were evaluated using repeated measures ANOVA using the age and genotype matched subjects as pairs (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). For all statistical analyses including absolute $V_T$ values, genotype was entered as a covariate. Statistical significance for regional paired *t*-tests were set to 0.007 (Bonferroni corrected) since in total seven ROIs were investigated. Effect size expressed as Cohen's D was calculated for absolute $V_T$ values and all seven ROIs was evaluated.

To assess the distribution of cytokine and HRV data Shapiro-Wilk's test of normality and visual inspection of Q-Q plots was used. Several cytokine and HRV variables were found to be non-normally distributed wherefore differences between RA and control groups was assessed in SPSS v.24 using Mann Whitney *U* test. Results were considered significant if $p < 0.05$.

Relationships between [$^{11}$C]PBR28 binding vs symptoms, disease activity, cytokine concentrations and HRV parameters were evaluated using partial correlative analysis for normally distributed data, correcting for genotype, and Spearman correlation for non-normally distributed data using SPSS 24. To assess the distribution of this data Shapiro-Wilk's test of normality and visual inspection of Q-Q plots was used.

As supplementary analysis to the ROI-based comparisons, voxel-based analysis was performed using SPM5. A custom MRI template in MNI (Montreal Neurological Institute, McGill University, Montreal, Canada) space was created by normalizing all subjects individual T1-w MRI to the standard MRI template in SPM5, then smoothed with a 12 mm FWHM Gaussian kernel and averaged Parametric images of $V_T$ were then normalized to the custom MRI template based on warping parameters calculated using each individual's T1-w MRI. Normalized $V_T$ PET images were smoothed with an 8 mm FWHM Gaussian kernel. An explicit mask created by multiplying the mean $V_T$ PET image (thresholded at $V_T > 6$) with the averaged T1-w MRI image (thresholded at intensity > 0.5). To investigate differences in $V_T$ between groups in the voxel-based analysis a paired *t*-test model was created both without global normalization and normalizing each individual brain to its respective $V_T$ value in the lateral occipital cortex. The analyses included data for 15 RA patients and 15 healthy controls. Statistical significance at the voxel level was set at $p < 0.001$ (uncorrected). Clusters with an extent of 50 voxels and $p < 0.05$ (corrected for multiple comparisons), were considered significant. Statistical maps were corrected for multiple comparisons using cluster level inference derived from random field theory as implemented in SPM.

## 3. Results

The demographic information and subject characteristics are presented in Table 1. As expected RA patients had significantly higher ratings of pain and fatigue and also elevated measures of systemic inflammation. There were no statistically significant differences between the genetically inferred TSPO HAB and MAB groups within the RA cohort regarding VAS pain, VAS fatigue, DAS28 and ESR. CRP was higher in the MAB group compared to HAB in RA patients (3.32 vs 1.41 g/l, $p = 0.05$).

### 3.1. PET

All subjects successfully underwent PET examinations using [$^{11}$C]PBR28. One subject only had 51 min of valid PET data but could still be reliably quantified using Logan graphical analysis and parametric imaging could also be performed. Therefore no data was excluded from statistical analyses. The spatially normalized mean images of [$^{11}$C]PBR28 in RA respective HC are shown in Fig. 1.

Repeated measures ANOVA including $V_T$ values revealed no

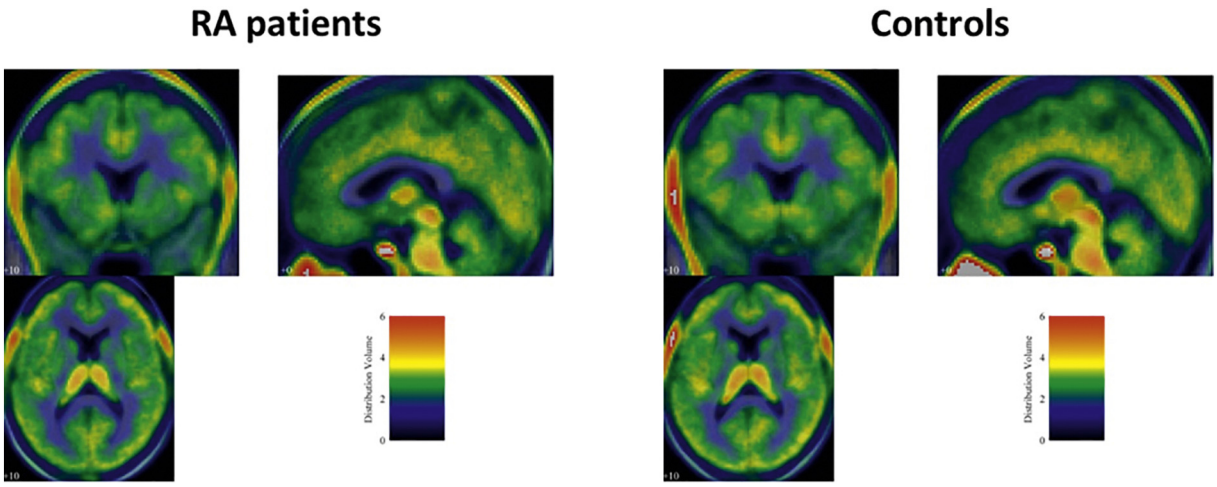


**Fig. 1.** Mean spatially normalized parametric images of absolute $V_T$ values of [$^{11}$C]PBR28 binding in RA patients and controls. No significant group differences were found.

**Table 2A**
Mean ± SD VT values and paired t-test results from selected ROIs in HABs and MABs together as well as separated.

A

| Genotype | Group | GM | LFC | LPC | PUT | THA | Activate | De-activate |
|---|---|---|---|---|---|---|---|---|
| HAB + MAB | RA (mean ± SD) | 3.21 ± 1.54 | 3.33 ± 1.65 | 3.31 ± 1.60 | 2.94 ± 1.49 | 3.70 ± 2.08 | 3.14 ± 1.51 | 3.24 ± 1.72 |
| | HC (mean ± SD) | 3.49 ± 1.48 | 3.52 ± 1.50 | 3.46 ± 1.41 | 3.20 ± 1.46 | 4.11 ± 1.93 | 3.47 ± 1.51 | 3.53 ± 1.69 |
| | Paired *t*-test* | 0.567 | 0.718 | 0.753 | 0.593 | 0.537 | 0.510 | 0.620 |
| | Cohen's D** | −0.21 | −0.13 | −0.12 | −0.20 | −0.23 | −0.25 | −0.18 |
| MAB | RA (mean ± SD) | 1.98 ± 0.79 | 2.05 ± 0.89 | 2.00 ± 0.75 | 1.79 ± 0.76 | 2.06 ± 0.98 | 1.93 ± 0.76 | 1.89 ± 0.94 |
| | HC (mean ± SD) | 2.51 ± 0.52 | 2.48 ± 0.61 | 2.48 ± 0.57 | 2.24 ± 0.49 | 2.84 ± 0.73 | 2.48 ± 0.50 | 2.49 ± 0.51 |
| | Paired *t*-test* | 0.196 | 0.322 | 0.210 | 0.196 | 0.123 | 0.189 | 0.225 |
| | Cohen's D** | −0.86 | −0.64 | −0.83 | −0.86 | −1.07 | −0.88 | −0.80 |
| HAB | RA (mean ± SD) | 4.04 ± 1.36 | 4.19 ± 1.48 | 4.18 ± 1.42 | 3.71 ± 1.37 | 4.80 ± 1.90 | 3.96 ± 1.33 | 4.14 ± 1.53 |
| | HC (mean ± SD) | 4.15 ± 1.57 | 4.21 ± 1.53 | 4.11 ± 1.44 | 3.85 ± 1.56 | 4.96 ± 2.04 | 4.13 ± 1.62 | 4.22 ± 1.87 |
| | Paired t-test* | 0.889 | 0.980 | 0.925 | 0.868 | 0.879 | 0.827 | 0.934 |
| | Cohen's D** | −0.07 | −0.01 | 0.05 | −0.08 | −0.07 | −0.11 | −0.04 |

GM = Grey Matter; LFC = Lateral Frontal Cortex; LPC = Lateral Parietal Cortex; PUT = Putamen; THA = Thalamus. *Significance level: $p < .007$ (Bonferroni corrected).
**0.2 = Small eff size, 0.5 = Median eff size, 0.8 = Strong eff size, > 1.2 = Very strong eff size.

**Table 2B**
Mean ± SD VT values normalized to Occipital cortex and paired t-test results from selected ROIs in HABs and MABs together as well as separated.

B

| Genotype | Group | LFC | LPC | PUT | THA | Activate | De-activate |
|---|---|---|---|---|---|---|---|
| HAB + MAB | RA (mean ± SD) | 0.97 ± 0.09 | 0.97 ± 0.07 | 0.85 ± 0.08 | 1.05 ± 0.14 | 0.98 ± 0.06 | 0.93 ± 0.15 |
| | HC (mean ± SD) | 0.96 ± 0.09 | 0.94 ± 0.07 | 0.87 ± 0.09 | 1.11 ± 0.13 | 0.99 ± 0.04 | 0.95 ± 0.12 |
| | Paired *t*-test* | 0.680 | 0.398 | 0.591 | 0.208 | 0.683 | 0.693 |
| MAB | RA (mean ± SD) | 0.95 ± 0.10 | 0.94 ± 0.07 | 0.82 ± 0.13 | 0.94 ± 0.16 | 0.96 ± 0.09 | 0.87 ± 0.21 |
| | HC (mean ± SD) | 0.93 ± 0.08 | 0.93 ± 0.07 | 0.84 ± 0.08 | 1.07 ± 0.13 | 0.97 ± 0.04 | 0.92 ± 0.11 |
| | Paired *t*-test* | 0.788 | 0.936 | 0.791 | 0.262 | 0.786 | 0.606 |
| HAB | RA (mean ± SD) | 0.98 ± 0.09 | 0.98 ± 0.07 | 0.87 ± 0.04 | 1.10 ± 0.09 | 0.99 ± 0.05 | 0.97 ± 0.07 |
| | HC (mean ± SD) | 0.97 ± 0.10 | 0.95 ± 0.07 | 0.88 ± 0.10 | 1.13 ± 0.14 | 1.00 ± 0.05 | 0.97 ± 0.13 |
| | Paired t-test* | 0.773 | 0.329 | 0.642 | 0.608 | 0.787 | 0.951 |

WB = Whole Brain; GM = Grey Matter; LFC = Lateral Frontal Cortex; LPC = Lateral Parietal Cortex; PUT = Putamen; THA = Thalamus. *Significant level: $p < 0.007$ (Bonferroni corrected).

significant difference between groups across the ROIs included ($F(0.281) = 0.980$, $p = 0.604$). Numerically lower values were observed in RA patients compared healthy controls but not reaching statistical significance in any of the ROIs analysed (Table 2A). Calculation of effect size revealed a Cohen's D of −0.21 constituting a small effect size (regional effect sizes are shown in Table 2A). We found significant correlations between $V_T$ values in GM and all other ROIs within both cohorts (RA; $r \geq 0.982$, $p < 0.0001$, HC; $r \geq 0.943$, $p < 0.0001$). DVR showed no statistically significant differences between groups as determined by repeated measure ANOVA utilizing the matched subjects in each group as pairs ($F(0.125) = 0.991$, $p = 0.729$) (Table 2BB).

### 3.2. Cytokines and measures of peripheral inflammation

The ESR and CRP values are presented in Table 1. Serum samples were missing for one RA patient and one HC enabling cytokine analysis

in a total of 28 samples. Cytokine measurement observations were above detection limit in 28/28 samples for IFNγ, MCP-1, TNF-α and IL-8. For IL-6 and IL-10 the corresponding number were 23/28 and 24/28 respectively. RA patients displayed significantly higher serum levels of both TNFα ($p = 0.001$), IFNγ ($p = 0.027$), IL-6 (0.021) and IL-10 ($p = 0.014$) compared to HC as expected. However, no differences were detected in serum levels of IL-8 ($p = 0.993$) or MCP-1 ($p = 0.782$). Results are summarized in Supplementary Table 1.

### 3.3. Autonomic activity

As predicted RA patients showed significantly lower values compared to controls in the time domain variables SDNN ($p = 0.004$), SDANN ($p = 0.018$) and HRVTI ($p = 0.027$). Lower values were also observed in RA patients for RMSSD, however, the difference did not reach statistical significance ($p = 0.056$). For frequency domain variables only power ($p = 0.045$) was significantly lower in RA patients compared to controls. No group difference was observed for LF/HF ($p = 0.525$). Furthermore, RA patients displayed higher heart rate compared to controls although this difference did not reach statistical significance. Results are summarized in Supplementary Table 2.

### 3.4. Correlations between parameters

Partial correlations corrected for genotype or Spearman correlations were calculated evaluating relationships between [$^{11}$C]PBR28 $V_T$ in GM and ratings of pain and fatigue, ESR, CRP and DAS28. We restricted the analysis to GM as we found significant correlations between $V_T$ values in GM and all other ROIs. For the cytokines and autonomic parameters also the vagus nerve activation and de-activation areas were included in the analysis. There were no significant correlations between ratings of pain or fatigue to GM [$^{11}$C]PBR28 $V_T$ values ($p > 0.05$; data not shown). RA patients had negative correlations between [$^{11}$C]PBR28 $V_T$ in GM, our primary ROI, and CRP ($r = -0.572$, $p = .026$)(Spearman correlation), however MABs had higher CRP compared to HABs ($p = 0.05$). We found a strong negative correlation between [$^{11}$C]PBR28 $V_T$ in GM and DAS28 ($r = -0.745$, $p = 0.002$)(partial correlation, corrected for genotype) (Fig. 2).

None of the cytokines or HRV parameters examined showed a significant correlation to global or regional [$^{11}$C]PBR28 $V_T$ values ($p > 0.05$; data not shown). However, in the RA group a positive correlation ($r = 0.69$; $p < 0.01$) was observed between serum IL-10 levels and LF/HF, which reflects vagal activity (Stein et al., 1994). No other significant correlations between cytokines and HRV parameters were found in the RA or HC group.

## 4. Discussion

The primary aim of this study was to evaluate the pattern of glial activation in the brain of RA patients compared to age-, sex- and genetically inferred TSPO binding affinity matched healthy controls. Our main hypothesis of increased TSPO binding in patients, to be interpreted as glia activation, was not confirmed. Instead, patients showed consistently numerically lower values than healthy controls and although a small overall effect size was found (Cohen's D for GM = −0.21), larger effect sizes were seen in several regions for the MAB sub-group. Also, no significant differences were found between RA patients and controls when using DVR with occipital cortex as pseudo reference region (Albrecht et al., 2018c). Our second hypothesis, that higher TSPO binding would be related to increased disease activity was also not confirmed. Instead, we found a strong negative association between TSPO binding and the composite measure of disease activity score 28 (DAS28). Finally, RA patients had higher serum concentrations of the pro-inflammatory cytokines TNF, IL-6 and IFNγ, as well as the anti-inflammatory IL-10 and HRV analysis indicated altered autonomic activity, however none of these parameters were associated with TSPO binding.

There are several possible explanations for our unexpected findings. First, experimental studies indicate that TSPO expression does not increase in human microglia following classical pro-inflammatory activation (Owen et al., 2017). In fact, in human monocyte-derived macrophages, pro-inflammatory activation is even associated with a reduction of TSPO gene expression and TSPO binding sites (Narayan et al., 2018; Owen et al., 2017). Therefore, our results of normal, or slightly lower cerebral TSPO binding in RA patients compared to HC, do not necessarily contradict an activation of the proinflammatory type of

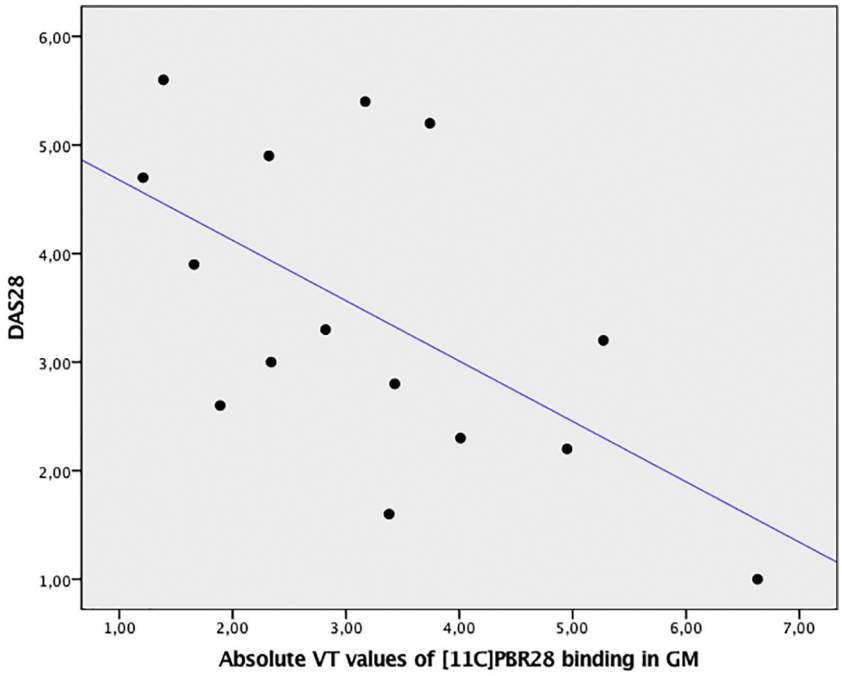


**Fig. 2.** Scatter plot showing the relation between absolute $V_T$ values of [$^{11}$C]PBR28 binding in grey matter (GM) and disease activity score (DAS28) in RA patients ($r = -0.745$, $p = 0.002$, corrected for HAB/MAB genotype).

microglia (or astrocytes). Hypothetically, in accordance with our finding of a strong, negative association between GM TSPO binding and disease activity (DAS28), there may be a shifted balance towards the pro-inflammatory M1-like phenotype of glia in RA. The latter would tally our previous findings of a pro-inflammatory (M1-like) type of cytokine profile in the CSF of RA patients characterized by elevated IL-1β compared to controls (Lampa et al., 2012b) and elevated IL-1β as well as reduced anti-inflammatory cytokines IL-4 and IL-10 compared to the CSF of fibromyalgia patients (Kosek et al., 2015) in which increased cerebral [$^{11}$C]PRB28 binding was recently demonstrated (Albrecht et al., 2018a).

In addition, TSPO may be of pathophysiological significance in other locations than the brain. Narayan et al. documented an increased [$^{11}$C]PBR28 PET signal in RA joints compared to healthy joints, with increased PBR28 ligand binding in the synovial tissue and the highest TSPO mRNA expression and $^{3}$H-PBR28 binding in fibroblast-like synoviocytes as well as inactivated and M2-like activated, reparative macrophages (Narayan et al., 2018). Increased TSPO mRNA expression has also been reported in the disc of patients with lumbar disc herniation and was associated with higher intensity of back pain (Palada et al., 2019) and increased TSPO binding was found in the affected nerve roots of patients suffering from lumbar radiculopathy (Albrecht et al., 2018a). The interaction with the peripheral immune system can be of specific importance in this context, as infiltrating cells may interact with resident CNS cells both by activating and inhibitory actions. Thus it has been shown that interaction between other immune cells, preferably T cells, and microglia may result in anti-inflammatory actions (Wang et al., 2016). Although less is understood regarding the neuroimmune crosstalk between the periphery and CNS, we recently reported neuroimmune blood borne joint-to-CNS signalling in patients with painful osteoarthritis and disc herniation, respectively (Kosek et al., 2018; Palada et al., 2019). Moreover, we have previously shown that TSPO binding in the brain correlated with TSPO binding in blood cells, as measured using [$^{11}$C]PBR28 in healthy human subjects (Kanegawa et al., 2016). In order to understand the pathophysiological role of TSPO in RA, future studies should address potential relationships between CNS and joint TSPO expression and their respective associations with symptoms and treatment effects in RA. Further investigations, preferably using PET ligands with a higher degree of cellular specificity are needed to disentangle the involvement of different glial subtypes in chronic inflammatory diseases, such as RA. A ligand detecting M1-like pro-inflammatory glia activation in humans would be of special relevance for the examination of spinal as well as cerebral glia activation.

In line with RA being a chronic inflammatory disease (Hueber et al., 2007), we observed increased levels of CRP, ESR as well as several pro-inflammatory cytokines in RA patients including TNF-α, IFNγ and IL-6. However, a significant association to cerebral TSPO binding was found only for CRP. The negative correlation between CRP and [$^{11}$C]PBR28 VT in GM was most likely explained by the significant difference in CRP between the genotypes as MABs had higher CRP and lower cerebral TSPO binding compared to HABs. Interestingly RA patients also displayed a higher serum level of IL-10 which is considered immunoregulatory. Increased IL-10 levels have previously been described in both systemic lupus erythematosus (SLE) and RA and have been considered on the one hand to be an effect of B- and T cell hyperactivity or on the other hand as a result of increased levels of circulatory monocytes in the dysregulated immune response of these autoimmune diseases (Al-Janadi et al., 1996; Tsukamoto et al., 2017).

Furthermore, RA patients have frequently been described as having decreased parasympathetic (vagal) activity and increased heart rate (Aydemir et al., 2010; Goldstein et al., 2007; Stojanovich et al., 2007), which we were able to confirm in the present study. Autonomic activity and immunity are closely interconnected as illustrated by the vagus dependent cholinergic anti-inflammatory pathway, the proposed efferent arm of the inflammatory reflex, which is impaired in several autoimmune diseases including RA (Huston and Tracey, 2011). Associations between autonomic activity and inflammatory mediators has been demonstrated by Goldstein and co-workers showing a negative correlation between serum levels of High mobility group box-1 (HMGB-1) and HF measures of autonomic activity (Goldstein et al., 2007). Interestingly, in the present study, we found a positive correlation between IL-10 levels in the RA group and LF/HF ($r = 0.69$; $p < 0.01$). This correlation between vagal activity and an anti-inflammatory cytokine is in line with previous data showing inverse correlation between pro-inflammatory IL-6 in serum and LF measures of autonomic activity (Kosek et al., 2015) and corroborates the concept of suppressed autonomic function in RA due to systemic inflammation effects. In order to assess if HRV measures of autonomic activity were associated with cerebral TSPO binding, we chose to assess two ROIs that had been related to vagus nerve induced activation and deactivation, respectively (Chae et al., 2003). However, as previously stated, no statistically significant associations were found between the PET [$^{11}$C]PBR28 binding in these ROIs and the assessed measures of autonomic activity.

### 4.1. Limitations

It is known that the relatively high test/retest variability of [$^{11}$C]PBR28 (Collste et al., 2016) together with the need of controlling for the genetically determined binding affinity to TSPO (Owen et al., 2011; Owen et al., 2012) requires fairly large cohorts for a study to be sufficiently powered, and it cannot be excluded that our study may have been too small to detect any significant differences between patients and control subjects. To allow for paired comparisons, and therefore increased power, we matched our controls not only according to sex and age, but also genotype. Furthermore, in order to reduce variability, ratio-based approaches such as standardized uptake ratio (SUVr) and distribution volume ratio (DVR) have been employed in clinical studies using [$^{11}$C]PBR28 (Albrecht et al., 2018c; Kreisl et al., 2013b; Loggia et al., 2015; Lyoo et al., 2015). In healthy control subjects, these approaches have shown poor reliability and low correlation to the standard outcome measure $V_T$ (Matheson et al., 2017) whereas Albrecht and colleagues were able to show increases in both DVR and $V_T$ in subjects with chronic low back pain compared to controls (Albrecht et al., 2018c) and the two methods of analysis were found to correlate in a combined group of fibromyalgia patients and healthy controls (Albrecht et al. 2018b). In our study, we found no difference using either $V_T$ or DVR values calculated with the pseudo reference region occipital cortex. Thus, our results of no increases in TSPO binding in RA patients compared to HC were confirmed using both methods of analysis.

The RA disease is based on several diagnostic criteria and associated with a potential large heterogeneity concerning numbers of, and kind of joints affected. In order to minimize heterogeneity we included only ACPA positive patients in the study. In addition, the included patients all experienced fatigue, which has earlier been considered as a symptom related to pain and CNS neuroinflammatory features (Norheim et al., 2011). Furthermore, we cannot exclude the influence of medications, given that 80% of our patients were treated with biologics. For example, TNF inhibitors, taken by 60% of our patients could have hampered glia activity, as has been shown in animal models of RA (Inglis et al., 2007). For ethical reasons we could not alter the antirheumatic treatments of our patients but we requested them to refrain from NSAIDs 48 h before PET scans and excluded patients on oral steroid medications. NSAID treatment, used by 40% of our patients was earlier shown to suppress activated microglia in animal models of Alzheimer's disease (Yan et al., 2003). The effect of NSAID usage in the present study should however be limited since only one patient was treated on a regular basis, and no NSAIDs were allowed within 48 h of the PET examination. However, as the drugs are expected to reduce, and not to increase, disease activity, our finding that lower disease activity was related to higher TSPO binding would argue against medication effects as an explanation for the lack of increased TSPO

binding in RA patients compared to controls. Also, given the negative association between DAS28 and cerebral TSPO binding, our results cannot be explained by low disease activity in our patients (7 patients had DAS ≤ 3.2, i.e., low disease activity).

## 5. Conclusion

We found no statistically significant group differences between RA patients and HC in TSPO binding as measured with [$^{11}$C]PBR28 PET. However, within the RA group, there was a strong negative association between cerebral TSPO binding and disease activity, suggesting that cerebral TSPO might relate to protective, disease modifying effects in RA. We did not find any associations between measures of systemic inflammation or autonomic activity and cerebral TSPO binding. In light of the earlier confirmed neuro-immune features of RA, these results warrant further investigations regarding neuro-immune joint-to-CNS signalling in order to open up for potentially new treatment strategies.

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