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ARTICLE

Replicating predictive serum correlates of greater translocator protein distribution volume in brain

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Greater activation of glia, a key component of neuroinflammation, is an important process to target in neuropsychiatric illnesses. However, the magnitude of gliosis varies across cases so low-cost predictors are needed to stratify subjects for clinical trials. Here, several such blood serum measures were assessed in relation to TSPO V_T, an index of translocator protein density, measured with positron emission tomography. Blood serum concentration of several products known to be synthesized by activated microglia (and to some extent astroglia) [prostaglandin E_2 (PGE_2), prostaglandin F_2 alpha ($PGF_{2\alpha}$), and tumor necrosis factor alpha (TNF_α)], controlled by an index of peripheral inflammation [C-reactive protein (CRP)] and TSPO V_T were measured in 3 cohorts: prefrontal cortex TSPO V_T of 20 subjects with major depressive episodes (MDEs) from major depressive disorder (MDD); and 56 subjects with treatment resistant MDEs from MDD; and dorsal caudate TSPO V_T of 20 subjects with obsessive-compulsive disorder. Ln(PGE_2/CRP) and ln(TNF_α/CRP) consistently correlated with TSPO V_T ($R^2 = 0.36$ to 0.11, $p = 0.0030$ to $p = 0.0076$). Assessment of threshold serum values to predict highly elevated TSPO V_T, demonstrated that a positive predictive value (PPV) of 80% was possible while retaining 40% of participant samples and that receiver operating curves (ROC) ranged from 75 to 81%. Post-hoc selection of ln(CRP) was more predictive ($R^2 = 0.23$ to 0.39, $p = 0.0058$ to $p = 0.00013$; ROC > 80%). Systematic assessment of selected peripheral inflammatory markers is promising for developing low cost predictors of TSPO V_T. Marker thresholds with high PPV will improve subject stratification for clinical trials of glial targeting therapeutics.

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INTRODUCTION

Psychiatric and neurological diseases are burdensome to society since they affect one in four people and are often treatment resistant [1]. Neuroinflammation, usually in response to neuronal damage is an important cluster of processes that occurs across many neuropsychiatric illnesses including major depressive disorder (MDD), obsessive-compulsive disorder (OCD), and neurodegenerative diseases [2–8]. Such responses typically include microglial and/or astroglial activation, which involve morphological changes of an enlarged cell body and thickened dendrites or, for microglia, possibly an ameboid shape. Increased gliosis is a promising target for immune modulating treatments to alter microglial and/or astroglial function away from potentially harmful roles like producing reactive oxygen species, prostaglandins, and proteinases. Immune modulating treatments may also alter glia cells towards more curative roles such as enhancing release of neurotrophic factors, promoting vascularization, and phagocytosing cellular debris [6, 9]. However, a critical barrier for human clinical trials of investigational treatments targeting gliosis is that there is heterogeneity in the neuroinflammatory response among individuals with neuropsychiatric diseases. Heterogeneity is attributable to multiple factors including stage of illness, comorbid disease and, most likely, multiple etiological phenotypes [2, 3]; thereby limiting optimal matching of cases to treatment.

No easily applicable, replicable, low cost measure indicative of microglial activation has been developed. Presently positron emission tomography (PET) imaging of translocator protein (TSPO) binding, is the most established *in vivo* marker although there are nuances for its interpretation: In health, binding of TSPO in brain is considered mainly attributable to binding to endothelial cells [10]. After inflammatory stimuli, brain TSPO binding is elevated and this TSPO radiotracer binding closely parallels the magnitude of greater TSPO expression in microglia, with a modest contribution from greater TSPO expression in activated astrocytes [11, 12] (for further discussion see Supplementary Information). Other PET radiotracers targeting P2X7, P2Y12 and CSF 1 receptors [13, 14] are being advanced, but all of these methods are expensive, require scarce resources, and typically need arterial blood sampling for kinetic modeling because microglia are ubiquitous throughout the brain. Another direction is cerebrospinal fluid concentration of products consequent to greater indoleamine 2,3-dioxygenase activity and/or cytokines but the necessary lumbar puncture is difficult for many patients [15, 16]. In regards to magnetic resonance imaging (MRI) methods with paramagnetic probes like Cd-bis-5-HT-DTPA that measure myeloperoxidase activity across activated microglia, neutrophils and monocytes, their sensitivity to detect microglial activation in neuropsychiatric disease is not yet established and it is possible that their application will be restricted to disease states

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with substantial blood brain barrier breakdown [17]. Moreover, to date, no blood marker has been demonstrated to consistently predict the level of microglial activation in brain.

For the present study, to design low cost blood markers, composite measures were created. Activated microglia in brain were considered an important source of inflammatory markers. Several products of activated microglia that are also actively transported out of the central nervous system into blood were measured, including serum prostaglandin E_2 (PGE_2), prostaglandin F_2 alpha ($PGF_{2\alpha}$), and tumor necrosis factor alpha (TNF_α) [18–22]. PGE_2 was given the highest priority since it is arguably the most commonly measured product of activated microglia among in vitro studies [18]. Since PGE_2, $PGF_{2\alpha}$, and TNF_α are also produced by the immune response of peripheral tissues [23] and microglial activation in brain is influenced by peripheral inflammation [9], a controlling measure of a well-accepted index of peripheral inflammation, serum C-reactive protein (CRP) concentration was applied. Hence, our primary hypothesis is that PGE_2/CRP will predict TSPO total distribution volume (V_T) in brain and $PGF_{2\alpha}$/CRP and TNF_α/CRP are our secondary candidates. While the issue could be raised that during inflammatory states astrogliosis may also contribute to the measure of TSPO V_T, to some extent this issue is mitigated because PGE_2, $PGF_{2\alpha}$ and TNF_α are not selective products of microglial activation and are also produced by astrogliosis.

In the present study, the relationship of these serum markers to brain TSPO V_T is assessed in three groups: medication free MDD; antidepressant treated, treatment resistant MDD (TRD); and medication free OCD subjects, illnesses for which TSPO V_T is elevated yet there is also substantial variability across subjects [2–8] (for further review see the Supplementary Information). In medication free MDD and TRD we prioritized the prefrontal cortex (PFC) because subregions of the PFC are often adversely affected in these illnesses [24–26], and it is a large portion of brain tissue. In OCD, the caudate nucleus was prioritized because it is the region with the most abnormally elevated TSPO V_T [2], and is strongly implicated in the pathophysiology of OCD, having the greatest convergence of neurochemical abnormalities across investigations in OCD [27–29].

MATERIALS AND METHODS

Participants

Three cohorts were recruited to assess the relationship between PET and peripheral markers. Having three cohorts provides the ability to assess the generalizability of this relationship across these disease conditions and the extent to which it replicates across these disease conditions. The cohorts included: 20 with medication free MDE [7]; 56 TRD (31 previously described [3]); and 20 medication free OCD (19 previously described [2]) were recruited from the Greater Toronto Area and the Centre for Addiction and Mental Health between July 2010 and October 2018 (Table 1). To assess the diagnosis, all participants underwent the Structured Clinical Interview for DSM-IV with confirmation by a consultation with a psychiatrist (JHM). All participants were currently experiencing significant symptoms. For MDE and TRD participants, a minimum of 17 on the 17-item Hamilton Depressive Rating Scale (17-item HDRS) [30] was required because this is a commonly applied minimum threshold to verify a current MDE. Greater TSPO V_T is commonly found during an MDE, but this may not necessarily occur during recovery [5, 8]. For OCD participants, scores on the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) [31] reflected moderate to severe illness. All participants were aged 18–72 years, non-cigarette smoking, and otherwise in good physical health (Table 1). All TRD participants were taking at least one antidepressant medication at a standard clinical dose for a minimum of 4 weeks prior to PET scanning (detailed list of medications provided in Supplementary Information).

All participants provided written informed consent after all procedures were fully explained. The study, protocol, and informed consent forms were approved by the Research Ethics Board at the Centre for Addiction and Mental Health.

Image acquisition and analysis

As previously described [2, 3, 7], each participant underwent one [^{18}F]FEPPA PET (HRRT; CPS/Siemens, Knoxville, TN, USA) and one MRI scan at the Research Imaging Centre at the Centre for Addiction and Mental Health (see the online Supplementary Information for additional detail). [^{18}F]FEPPA was administered

Table 1. Demographic characteristics

Characteristics	Medication free major depressive episode ($n = 20$)		Treatment resistant depression ($n = 56$)		Medication free obsessive-compulsive disorder ($n = 20$)		*P*-value
	N	%	N	%	N	%	
Female	12	60	36	64	11	55	0.76[a]
TSPO Genotype[b]							0.67[a]
HAB	15	75	36	64	13	65	
MAB	5	25	20	36	7	35	
Previous Antidepressant Trial	9	45	56	100	NA	NA	<0.0001[a]
	Mean	SD	Mean	SD	Mean	SD	
Age, year	34.5	11.2	34.7	11.2	27.8	7.0	0.040[c]
BMI	23.4	5.4	25.2	4.2	23.8	5.0	0.28[c]
17-Item HDRS Score[d]	20.0	3.8[e]	21.6	4.2	NA	NA	0.16[c]
Age at First Episode, Year[f]	15.7	5.2	15.9	7.0	13.1	7.4	0.26[e]
Y-BOCS Score	NA	NA	NA	NA	22.5	6.1	NA

[a]Pearson chi-squared test
[b]Single nucleotide polymorphism rs6971 of the TSPO gene known to influence [^{18}F]FEPPA binding: HAB, high affinity binders; MAB, mixed affinity binders
[c]Analysis of variance
[d]17-item Hamilton Depression Rating Scale (HDRS); scores derived on the day of scanning
[e]Missing data in one medication free MDE subject
[f]MDE for MDD; or first episode of OCD symptoms for OCD cases
BMI body mass index, *MDD* major depressive disorder, *MDE* major depressive episode, *N* number, *NA* not applicable, *OCD* obsessive compulsive disorder, *SD* standard deviation, *TSPO* translocator protein, *Y-BOCS* Yale-Brown Obsessive-Compulsive Scale, % percentage

intravenously as a bolus (mean 184.6 MBq [SD 13.0]). [^{18}F]FEPPA was of high radiochemical purity (>96%) and high specific activity (mean 83.1 TBq/mmol [SD 73.0]). Manual and automatic blood samplings (ABSS, Model #PBS-101; Veenstra Instuments, Joure, The Netherlands) were obtained to determine the unmetabolised parent radioligand in plasma which was the input function for the kinetic analysis. A brain MRI was acquired for each participant for the anatomical delineation of regions of interest (ROIs) generated using the semi-automated software (ROMI, Toronto, Ontario) [32]. A two-tissue compartment model was applied to the time–activity curves from regions of interest to measure TSPO V_T, which is the optimal model for [^{18}F]FEPPA PET [33] (see the online Supplementary Information for additional detail).

Peripheral inflammatory marker measurements

PGE_2 and $PGF_{2\alpha}$ concentrations were determined using the competitive immunoassay, Prostaglandin E_2 Assay (Parameter, R&D Systems Inc) and Prostaglandin $F_{2\alpha}$ ELISA Kit (MyBioSource), respectively. TNF_α level was analyzed using the Human Adipokine Magnetic Bead Panel 2 (Milliplex MAP, EMD Millipore Corp) and CRP was analyzed with the CRPHS Assay (Cobas C, Roche Diagnostics). PGE_2 and TNF_α levels were measured twice in the same sample and the mean value was applied, and all other markers were measured in singleton. Further details regarding sample collection are described in the online supplemental methods.

Statistical analyses

For the main analyses, TSPO V_T was the dependent variable and the natural logarithm of the blood markers (PGE_2/CRP, $PGF_{2\alpha}$/CRP, and TNF_α/CRP) were each assessed separately as predictor variables in a linear regression in each cohort. The natural logarithm was applied to produce a normal distribution of the predictor variables. To address for the effect of the rs6971 genotype on TSPO V_T, a corrective adjustment was applied to the mixed-affinity binder (MAB) TSPO V_T values based on a linear regression assessing the effect of genotype with regional TSPO V_T as the dependent variable such that the differential effect of genotype was added to the TSPO V_T of the MAB subjects (see the online Supplementary Information for further description). Since PGE_2/CRP was the primary hypothesized predictor, the threshold for significance for the linear regression was set at 0.05 for each cohort. For the other two main predictor markers being assessed ($PGF_{2\alpha}$/CRP and TNF_α/CRP), the threshold for significance of the linear regression was set at $P < 0.017$ since inclusion of these two measures resulted in a total of three high priority measures. For statistical analysis, the adjusted R^2 was assessed and reported.

Also, receiver operator characteristic (ROC) curves were generated to evaluate the performance of consistently predictive biomarkers. For this, TSPO V_T values from all subjects were treated as dichotomous variables with PFC TSPO $V_T \geq 13.5$ for MDE and TRD, and dorsal caudate TSPO $V_T \geq 9.4$ for OCD (after adjusting MAB TSPO V_T as described in the preceding paragraph). These thresholds correspond to ~30% greater than healthy controls representing ~2 standard deviation difference. All analyses were performed using IBM SPSS Statistics (version 21).

RESULTS

Linear regression assessing relationship of blood markers to TSPO V_T

Ln(PGE_2/CRP) and ln(TNF_α/CRP) were consistent and highly significant correlates of TSPO V_T for all three cohorts (Fig. 1, Table 2; ln(PGE_2/CRP): MDE, $F_{1,19} = 10.3$ to 11.8, $P = 0.0030$ to 0.0048; TRD

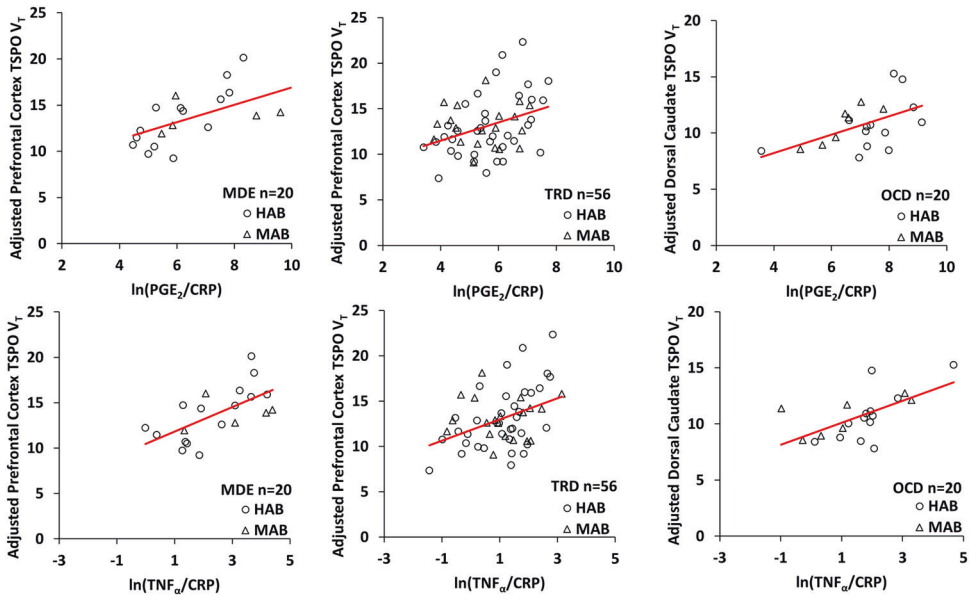

Fig. 1 Blood Serum Markers Significantly Correlated with Translocator Protein Distribution Volume in all Three Cohorts. Ln(PGE_2/CRP) and ln (TNF_α/CRP) were highly significant correlates of PFC TSPO V_T in MDE and TRD cohorts, and dorsal caudate TSPO V_T in OCD cohort. MDE cohort $n = 20$ [ln(PGE_2/CRP) $R^2 = 33.0$, $P = 0.0048$; ln(TNF_α/CRP) $R^2 = 36.2$, $P = 0.0030$]. TRD cohort $n = 56$ [ln(PGE_2/CRP) $R^2 = 10.8$, $p = 0.0076$; ln(TNF_α/CRP) $R^2 = 14.0$, $P = 0.0026$]. OCD cohort $n = 20$ [ln(PGE_2/CRP) $R^2 = 24.1$, $p = 0.016$; ln(TNF_α/CRP) $R^2 = 35.1$, $P = 0.0035$]. To address the effect of the rs6971 genotype on TSPO V_T, the differential effect of genotype in a linear regression was found for TSPO V_T. Then, the MAB TSPO V_T values were adjusted by adding the differential effect of genotype to the TSPO V_T values. (Adjusted prefrontal cortex TSPO V_T = unadjusted TSPO V_T + b1*genotype, where b1 = 4.939 in MDE cohort and b1 = 4.678 in TRD cohort. Adjusted dorsal caudate TSPO V_T = unadjusted TSPO V_T + b1*genotype, where b1 = 4.230 in OCD cohort). *CRP* c-reactive protein, *HAB* high-affinity binders, *MAB* mixed-affinity binders, *MDE* medication free major depressive episodes secondary to major depressive disorder, *OCD* obsessive compulsive disorder, PFC prefrontal cortex; PGE_2 prostaglandin E_2, TNF_α tumor necrosis factor alpha, TRD treatment resistant major depressive episodes secondary to major depressive disorder, TSPO V_T translocator protein distribution volume

Table 2. Relationship of serum predictor markers to TSPO V_T

Clinical Group	Region	Predictor marker[a]	Contribution to variance (Adjusted $R^2 \times 100\%$)	*P*-value
MDE ($n = 20$)	Prefrontal Cortex TSPO V_T	ln(PGE_2/CRP)	33.0	0.0048
		ln($PGF_{2\alpha}$/CRP)[b]	35.0	0.0093
		ln(TNF_α/CRP)	36.2	0.0030
TRD ($n = 56$)	Prefrontal Cortex TSPO V_T	ln(PGE_2/CRP)	10.8	0.0076
		ln($PGF_{2\alpha}$/CRP)	2.6	0.12
		ln(TNF_α/CRP)	14.0	0.0026
OCD ($n = 20$)	Dorsal Caudate TSPO V_T	ln(PGE_2/CRP)	24.1	0.016
		ln($PGF_{2\alpha}$/CRP)	56.3	<0.0001
		ln(TNF_α/CRP)	35.1	0.0035

[a]Linear regression with genotyped adjusted TSPO V_T as dependent variable with predictor listed as independent
[b]$PGF_{2\alpha}$ data missing from 4 medication free MDE subjects
CRP c-reactive protein, *MDE* major depressive episode, *n* number, *OCD* obsessive compulsive disorder, *PGE_2* prostaglandin E2, *$PGF_{2\alpha}$* prostaglandin F_2 alpha, *TNF_α* tumor necrosis factor alpha, *TRD* treatment resistant depression, *TSPO V_T* translocator protein distribution volume

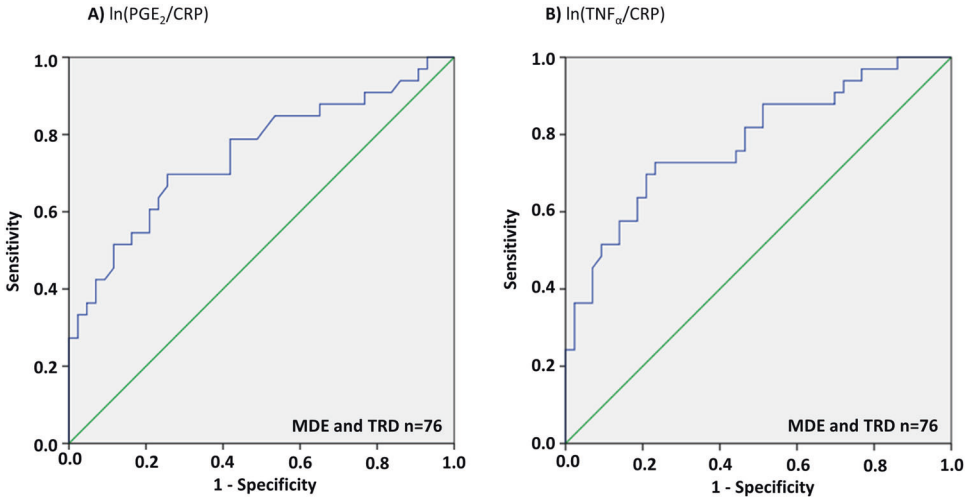

Fig. 2 Receiver Operating Characteristic Curve Analyses in Collective Depressed (MDE and TRD) Cohorts. Receiver operating characteristic (ROC) curve analyses in the collective depressed sample (MDE and TRD $n = 76$) for **a**) ln(PGE_2/CRP) and **b** ln(TNF_α/CRP). ROC curve analyses revealed accuracies of 74.9% ($P = 0.00021$, 95% confidence interval [63.5, 86.4]) and 78.1% ($p < 0.0001$, 95% confidence interval [67.5, 88.7]) in the ability of ln(PGE_2/CRP) and ln(TNF_α/CRP) biomarkers to correctly classify those with and without elevated prefrontal cortex TSPO V_T, respectively. To address the effect of the rs6971 genotype on TSPO V_T, the differential effect of genotype in a linear regression was found for TSPO V_T. Then, the MAB TSPO V_T values were first adjusted by adding the differential effect of genotype to the TSPO V_T values. (Adjusted prefrontal cortex TSPO V_T = unadjusted TSPO V_T + b1*genotype, where b1 = 4.939 in MDE cohort and b1 = 4.678 in TRD cohort). *CRP* c-reactive protein, *MDE* medication free major depressive episodes secondary to major depressive disorder, *PGE_2* prostaglandin E_2, *TNF_α* tumor necrosis factor alpha, *TRD* treatment resistant major depressive episodes secondary to major depressive disorder, *TSPO V_T* translocator protein distribution volume

$F_{1,55} = 7.7$ to 10.0, $P = 0.0026$ to 0.0076; OCD, $F_{1,19} = 7.0$ to 11.3, $p = 0.0035$ to 0.016), especially in the MDE and OCD cohorts in which they accounted for 24.1 to 36.2% of the variance. Ln($PGF_{2\alpha}$/CRP) was only significantly predictive in the MDE and OCD cohorts (Table 2; MDE, $F_{1,19} = 9.1$, $P = 0.0093$; OCD, $F_{1,19} = 25.5$, $P < 0.0001$); accounting for 35.0 and 56.3% of the variance respectively (see Figure S1 in the online Supplementary Information). Since TSPO V_T tended to be highly correlated across regions, the predictors tended to also be correlated with TSPO V_T values in other brain regions (see Table S2 in the online Supplementary Information).

Receiver operating characteristic curve analysis with A priori hypothesized blood markers

Receiver operating characteristic (ROC) curve analyses were applied for the collective depressed sample (MDE and TRD $n = 76$) for ln(PGE_2/CRP) and ln(TNF_α/CRP), assessing revealed an accuracy of 74.9% ($P = 0.00021$, 95% confidence interval [63.5, 86.4]) and 78.1% ($P < 0.0001$, 95% confidence interval [67.5, 88.7]) in the ability of the blood marker to correctly classify those with and without elevated PFC TSPO V_T (Fig. 2). ROC curve analyses were applied for the OCD cohort for ln(PGE_2/CRP), ln($PGF_{2\alpha}$/CRP), and ln(TNF_α/CRP), assessing revealed an accuracy of 80.2% ($P = 0.029$, 95% confidence interval [58.4, 100.0]), 79.1% ($P = 0.036$, 95% confidence interval [57.5, 100.0]), and 81.3% ($P = 0.024$, 95% confidence interval [60.0, 100.0]), respectively, in the ability of the blood marker to correctly classify those with and without elevated dorsal caudate TSPO V_T (see Table S3 in the online Supplementary Information). Although the a priori region is different in OCD, ROC curve analyses were applied for the whole sample ($n = 96$) to assess the ability of the blood markers to correctly classify those with and without elevated PFC TSPO V_T (see Fig. S8 in the online Supplementary Information).

Relationship of positive predictive value and sample retention
In the collective MDE and TRD sample, varying thresholds of ln(PGE_2/CRP) and ln(TNF_α/CRP) were assessed in relation to the positive predictive value and proportion of participants retained as these are key practical values for practical use of blood markers in clinical trials. With cut-offs of ln(PGE_2/CRP) = 6.9 and ln(TNF_α/CRP) = 2.1 respectively in the depressed sample, positive predictive values greater than 80% for elevated prefrontal cortex TSPO V_T were possible while retaining 40% of the participants (Fig. 3). Similarly, a threshold serum value of 7.3 for ln(PGE_2/CRP) or 1.9 for ln(TNF_α/CRP) in OCD subjects selects ~50% of cases of which 85% have dorsal caudate TSPO V_T values >9.4.

Post-Hoc Analyses
Since the ln(PGE_2/CRP) or ln(TNF_α/CRP) may be equivalently expressed as ln(PGE_2) minus ln(CRP) or ln(TNF_α) minus ln(CRP) respectively, it is feasible to test different linear combinations of ln(PGE_2) and ln(TNF_α) with ln(CRP) with linear regression (see Table S4 in the online Supplementary Information). The most optimal predictor was ln(CRP) alone which resulted in higher levels of significance in predicting PFC TSPO V_T in MDE and TRD participants and dorsal caudate TSPO V_T in OCD participants (MDE: $F_{1,19} = 13.0$, $R^2 = 0.39$, $P = 0.0020$; TRD: $F_{1,55} = 17.0$, $R^2 = 0.23$, $P = 0.00013$; OCD: $F_{1,19} = 9.8$, $R^2 = 0.32$, $P = 0.00058$). The ROC curve analysis of ln(CRP) in the OCD cohort revealed an accuracy of 81.3% ($P = 0.024$, 95% confidence interval [61.5, 100.0]) (see Table S3 in the online Supplementary Information). Interestingly, predictiveness increased further in the collective depressed sample (MDE and TRD $n = 76$) when body mass index (BMI) was included, leading to a ROC curve accuracy of 85.3% (see the online Supplementary Information for more detail and Figures S2–S7).

DISCUSSION

We found that composite blood serum measures of products previously established to be synthesized by activated microglia (and to some extent astroglia), that are also known to have strong efflux from the central nervous system, controlled for by peripheral inflammation are consistently significantly correlated with TSPO V_T. The predictiveness of two such measures created a priori, ln(PGE_2/CRP) and ln(TNF_α/CRP), replicate across three cohorts. However, it was also noted that ln($PGF_{2\alpha}$/CRP) accounted for a high proportion of variance of TSPO V_T in the OCD sample and that in post-hoc analyses that the ln(CRP) was, on average, a stronger predictor of TSPO V_T across the three cohorts. Low cost predictive markers of TSPO V_T have major implications for subject stratification for clinical trials of therapeutics targeting activated microglia.

The present study demonstrates that either serum ln(PGE_2/CRP) or ln(TNF_α/CRP) consistently identifies a subsample with a high TSPO V_T phenotype. Figure 3, indicates that a threshold serum value of 6.9 for ln(PGE_2/CRP) or 2.1 for ln(TNF_α/CRP) in the combined MDE and TRD samples selects a subsample of ~40% of cases of which 80% have PFC TSPO V_T values more than 13.5, which corresponds to 2 standard deviations above the mean of health. Similarly, a threshold serum value of 7.3 for ln(PGE_2/CRP) or 1.9 for ln(TNF_α/CRP) in OCD subjects ($n = 20$) selects ~50% of cases of which 85% have dorsal caudate TSPO V_T values >9.4. Since the heterogeneity of TSPO V_T elevation ranges from 0% to over 100% in primary regions of interest for MDE and OCD [2–4], these serum thresholds represent a practical low-cost option to stratify subjects. Moreover, there will be opportunities to assess these thresholds in clinical trials, particularly for MDD and TRD. For example, therapeutic development to target microglial activation and/or gliosis is actively occurring with P2X7 antagonists for microglial proliferation, and indolamine 2,3 dioxygenase inhibitors to promote conversion of tryptophan towards serotonin rather than the kyurenine pathway. In addition, repurposed medications like minocycline and the simvastatin that influence major histocompatibility protein II to reduce adverse functions of gliosis [34, 35] are currently being investigated.

A consistent, positive relationship between blood markers of inflammation and TSPO V_T in brain has not been previously reported. In a sample of 30 subjects (14 cases with schizophrenia and 16 healthy), no significant relationships were found between TSPO V_T and plasma IL-6, TNF_α, plasma interferon gamma (IFNγ), plasma IL-10, or cerebrospinal fluid IL-6 levels [36]. In a sample of 8 healthy subjects exposed to lipopolysaccharide, Sandiego et al. demonstrated elevations in TSPO V_T as well as TNF_α, plasma IFNγ, IL-6, IL-8, and IL-10, but there was no correlation between change among brain and peripheral blood measures [37]. Similarly no relationship between blood CRP level and TSPO V_T was reported in a sample of 10 MDD subjects some of whom were currently in a MDE, nor in our previous sample of 20 MDE subjects [7, 38]. In a sample of 48 MDD subjects with concurrent TSPO imaging and plasma samples, among 8 plasma measures including CRP, Richards et al. reported one positive correlation of plasma

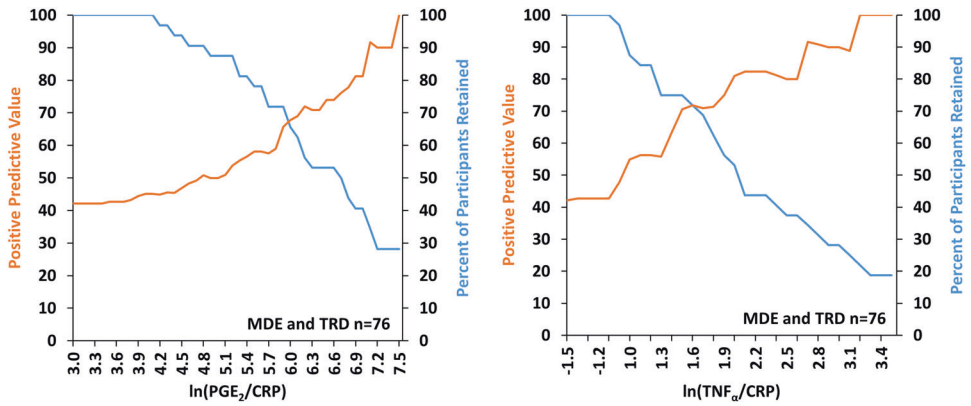

Fig. 3 Relationship of Positive Predictive Value and Proportion of Participants Retained in Collective Depressed (MDE and TRD) Cohorts. Relationship of positive predictive value and proportion of participants retained with varying thresholds of ln(PGE_2/CRP) and ln(TNF_α/CRP) in the collective depressed sample (MDE and TRD $n = 76$). Cut-offs of ln(PGE_2/CRP) = 6.9 and ln(TNF_α/CRP) = 2.1 reveal positive predictive values of ~80% for prefrontal cortex TSPO $V_T \geq 13.5$ while retaining 40% of cases. To address the effect of the rs6971 genotype on TSPO V_T, the differential effect of genotype in a linear regression was found for TSPO V_T. Then, the MAB TSPO V_T values were first adjusted by adding the differential effect of genotype to the TSPO V_T values. (Adjusted prefrontal cortex TSPO V_T = unadjusted TSPO V_T + b1*genotype, where b1 = 4.939 in MDE cohort and b1 = 4.678 in TRD cohort). *CRP* c-reactive protein, *PGE_2* prostaglandin E_2, *TNF_α* tumor necrosis factor alpha, *TSPO V_T* translocator protein distribution volume

adiponectin, at an exploratory uncorrected significance [4]. As compared to the present study, there were a number of differences in the study by Richards et al. which examined several different peripheral inflammatory markers (IL-2, IL-5, IL-6, IL-8, and INF-γ) and did not look at the ln transformation of CRP in relation to TSPO V_T.

There were several additional predictors that merit further study, due to the high level of variance in TSPO V_T accounted for by the markers. Although all our cohorts included those with neuropsychiatric disease, the diseases were not identical. Hence, ln($PGF_{2\alpha}$/CRP) which accounted for 56% of the variance in TSPO V_T in the dorsal caudate in medication free OCD but was not predictive in the TRD sample, should be reassessed in future samples of medication free OCD subjects since this finding may be specific for OCD. Similarly, since ln(CRP) and BMI collectively, albeit with most of the impact from ln(CRP), accounted for 32% of the variance and 85.3% of the area under the ROC to predict elevated TSPO V_T in the collective MDE and TRD cohorts; and that the relationship between BMI and TSPO V_T was also reported in a separate sample from ours [4] suggest that this combination should receive further study in MDE and TRD samples.

There are several limitations in the present study. First, there is some lack of selectivity for gliosis across the markers tested. For example, when elevated TSPO V_T is present, it is associated with microglial activation but TSPO overexpression is not fully selective for microglial activation since TSPO is detectable in other cells such as astroglia and endothelial cells [11, 12]. Also, some of the serum markers measured like PGE_2, $PGF_{2\alpha}$ and TNF_α, while synthesized by activated microglia are not specific to such, for example, they may also be produced by activated astrocytes [39, 40]. Second, when greater TSPO expression often occurs after exposure to inflammatory stimuli, it is associated with the morphological changes of an activated state, predominantly in microglia but there is a range of cellular functions that may occur in the activated state. Third, we did not assess the relationship of the prioritized blood markers to elevated brain TSPO V_T in healthy subjects because in health, TSPO V_T has a limited range. In addition, in neuroinflammatory states the elevation in TSPO V_T is generally viewed as reflecting variable levels of gliosis whereas the range in health is likely attributable to variation in other aspects of TSPO binding like binding to endothelial cells [10]. Fourth, it could be questioned as to which peripheral blood markers were decided a priori, a challenging issue since disclosing these prior to patenting in a public database invalidates the patent and then limits the translational impact of such markers. We can largely address this question by our emerging patenting order of peripheral blood markers which demonstrates initial selection of PGE_2/CRP and later selection of our post-hoc parameter ln(CRP), which in some examples both alone and in combination with BMI value yields even greater levels of predictiveness for TSPO V_T (see Figures S5–S7 in the online Supplementary Information).

In addition, should the serum markers of the present study be applied to other studies, it is important that the sampling protocol is highly similar to ours. One important component of our sampling protocol is that to avoid potentially rapid degradation of PGE_2 and TNF_α, blood samples taken were converted into serum over 30 min, placed in a chilled centrifuge, and stored quickly at −80 °C (see the online Supplementary Information). Also, to apply the relationship of the serum measures tested to TSPO V_T, it is also important to address whether participants sampled had recent infections since our participant sample reported no recent infections in the four weeks prior to scanning which may have lowered variability in the TSPO V_T measure.

In summary, we assessed a general strategy of testing the serum ratio of products synthesized by activated microglia that are also actively removed from the central nervous system, controlled for by markers of peripheral inflammation, identified the natural logarithm of serum PGE_2/CRP and TNF_α/CRP, as highly significant correlates of TSPO V_T in brain in three separate samples. We also noted in post-hoc analyses several linear combinations of markers that were also highly predictive including ln(CRP) itself as well as in combination with BMI. These measures may be applied at thresholds with high positive predictive value to select subjects with elevated TSPO V_T thereby addressing the heterogeneity of the TSPO V_T marker when recruiting subjects for clinical trials of therapeutics targeting gliosis. Moreover, systematically assessing collective predictors in relation to TSPO V_T has intriguing potential to be developed further towards more individualized clinical care of neuropsychiatric illnesses with elevated microglial activation and gliosis.

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AUTHOR CONTRIBUTIONS

Study concept and design: JHM, ES, AAW, SH. Acquisition, analysis, or interpretation of data: SA, ES, PMR, LM, CX, CH, SK, JHM. Drafting of the manuscript: SA, JHM. Critical revision of the manuscript for important intellectual content: SA, Setiawan, Wilson, PMR, LM, CX, CH, MIH, SK, NV, SH, JHM. Statistical analysis: SA, JHM. Obtained funding: SA, JHM, ES, AAW, SH. Administrative, technical, or material support: AAW, PMR, LM, SK, NV, SH, JHM. Study supervision: JHM.

ADDITIONAL INFORMATION

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