Title:

The immune response of the human brain to abdominal surgery

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Inflammation, surgery, [^{11}C]PBR28, brain, cognition, TSPO

Abstract

Background

Surgery launches a systemic inflammatory reaction that reaches the brain and associates with immune activation and cognitive decline. While animal and cell studies have detailed this periphery-to-brain signaling pathway, we lack information on changes in human brain immune activity after surgery and their relation to the peripheral inflammatory response. This study examines the long-term impact on the human brain immune system in relation to blood immune reactivity, plasma inflammatory biomarkers and cognitive function.

Methods

Eight males undergoing prostatectomy under general anesthesia were included. Prior to surgery, at postoperative day 3-4 and after 3 months, patients were examined using [^{11}C]PBR28 brain PET to assess immune cell activity. Concurrently, systemic inflammatory biomarkers, ex vivo blood tests on immunreactivity to LPS stimulation and cognitive function test scores were obtained.

Findings

Patients showed a global down-regulation of grey matter [^{11}C]PBR28 binding (V_T) of 26±26% (mean±SD) at 3-4 days postoperatively compared to baseline (p=0·023) with a uniform decrease in V_T within four brain regions of relevance for cognitive function. [^{11}C]PBR28 binding recovered after 3 months, with higher binding shown in four individuals compared to baseline values. Simultaneous LPS-induced release of TNF-α in whole blood displayed a marked reduction (41±39%) on the 3-4th postoperative day, corresponding to simultaneous changes in [^{11}C]PBR28 V_T. Changes in Stroop color word cognitive test

between day 3-4 and 3 months were correlated to changes in [^{11}C]PBR28 binding (p=0.027). There was no association between [^{11}C]PBR28 binding and levels of plasma inflammatory mediators.

Interpretation

This study translates animal data on changes in the brain immune system after surgery to humans, and suggests an interplay between the human brain and peripheral innate immunity to modulate the inflammatory response to surgical trauma. These processes may be related to post-surgical impairments of cognitive function

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Background

A growing body of evidence suggests that surgical trauma launches a systemic inflammatory reaction that ultimately reaches and activates the intrinsic immune system of the brain[1-4]. Triggered by surgery-induced damage-associated molecular patterns (DAMPs), an array of proinflammatory mediators and activated blood borne immune cells orchestrate a rapid spread of this systemic response, allowing for detection of immune activation in the central nervous system (CNS) within 12 hours [4,5]. In rodent models of surgery this periphery-to-brain pathway seems critically dependent on NF-κB- and pro-inflammatory cytokine signaling (e.g. interleukin-1 (IL-1)) leading to a short-lasting disruption of blood brain barrier (BBB) integrity, a process that most likely involve both inflammatory and anesthetic drug-induced impact on BBB function[2,3,6]. This process is accompanied by migration of peripheral macrophages into the CNS and potentially subsequent hippocampal neuronal dysfunction[6]. In addition to the transient syndrome of sickness behavior, including fatigue, anorexia and fever, surgery-induced immune activation is associated with prolonged impairments in learning, memory and concentration, that may correspond to the clinical syndrome postoperative cognitive dysfunction[6-8].

In patients, inflammatory molecules appear in cerebrospinal fluid within 12 hours after major surgery[4,9-11]. While such clinical observations in the human CNS are in line with a series of experimental animal studies[2,3,6] the time course pattern beyond the initial post surgery phase of immune activation within the human CNS, how it relates to the biphasic pro- and anti-inflammatory response pattern in peripheral blood[12-14], the reactivity of the innate immune system and later changes in cognitive performance is largely unknown.

The use of positron emission tomography (PET) and radioligands for the translocator protein (TSPO) provides an opportunity for translational studies exploring the brain immune activity after surgery. In brain parenchyma, TSPO is primarily expressed in microglia and the protein is thus viewed as a marker for CNS immune activity (REF här?). TSPO is typically elevated in several acute and chronic CNS disorders involving the immune system, including infection, stroke, neurodegenerative, autoimmune diseases and psychiatric disorders[15-19] as well as in animal models of acute inflammation[20] or stroke[21]. With regard to periphery-to-brain interactions, LPS-induced acute systemic inflammation is followed by a rapid and transient activation of the brain immune system as demonstrated using the TSPO radioligand [^{11}C]PBR28 in non-human primates[22] and in humans[23].

Here we examined the impact of major surgery on the human brain immune system by a series of PET examinations of TSPO binding in patients undergoing abdominal surgery and how changes in brain immune activity relates to the systemic inflammatory response and higher brain function of cognition.

Material and Methods

Patients

The study was approved by the Regional Ethics Committee on Human Research at Karolinska Institutet and the local Radiation Safety Committee, Karolinska University Hospital, Stockholm, Sweden. The protocol conformed to the standard of the Declaration of Helsinki, Finland and has been registered at the US National Institutes of Health NCT01881646 (ClinicalTrials.gov).

Eight otherwise healthy male patients, the American Society of Anesthesiologists (ASA) physical status 1-2 scheduled for a robot-assisted radical prostatectomy entered the study after oral and written informed consent. For exclusion criteria see appendix. Demographic data are presented in Table 1.

Each subject was examined on three separate occcasions, i.e. 1-3 days prior to surgery, on postoperative day 3-4 and at 3 months postoperatively. At each occasion, we obtained PET measurments on TSPO binding in brain, blood and plasma biomarkers of inflammation and neuronal injury, ex vivo blood tests on immunreactivity to LPS stimulation, and measurements of cognitive function.

On the day of surgery, anesthesia was induced between 08.00-11.00 a.m. with thiopentone and maintained with desflurane and a continuous intravenous infusion of remifentanil. Propofol was not used as this drug may affect [^{11}C]PBR28 binding[24]. For further details on perioperative procedures and postoperative care, see appendix.

Positron emission tomography (PET) imaging

For each subject, the three PET examinations were conducted either in the morning/before lunch or after lunch/afternoon to avoid a possible influence of diurnal differences, with one exception when this was not possible due to scheduling conflicts. [^{11}C]PBR28 was prepared and injected as described previously[25-27]. The average radioactivity administered was 435±50 MBq (mean±SD); with a specific radioactivity of 229±82 GBq/μmol; and an injected mass of 0.77±0.38 μg. PET data were acquired for 63 minutes and arterial blood was sampled throughout the examination as described in appendix. All patients were genotyped for the genetic polymorphism of rs6976 which affects binding of TSPO radioligands, including [^{11}C]PBR28, both in vitro and in vivo[25,28,29]. Six of the subjects were high-affinity binders (HAB) and one mixed-affinity binder (MAB), whereas for one subject the genotype could not be determined.

PET image analysis

Image processing and the definition of regions of interest (ROI's) definition was performed as described previously[25,26]. The primary ROI was brain grey matter (GM) and in addition a composite volume was defined for white matter (WM), we also obtained values for regional binding in hippocampus (HIP), lateral frontal cortex (LFC), lateral parietal cortex (LPC), and putamen (PUT), i.e. regions known to be relevant for cognitive functions. For each PET examination, a parametric image of the distribution volume (V_T) was generated using the stationary wavelet aided parametric imaging (WAPI) approach[30]. WAPI analysis of TSPO binding has previously shown to be sensitive to within-subject changes in V_T[31], and data based on 63 minute acquisition have shown good reliability[25]. To assess individual rate constants of k_1, k_2, k_3 and k_4, an additional analysis was performed using the two-tissue compartment model (2TCM). For additional considerations regarding quantification, see appendix.

Ex vivo LPS challenge and systemic inflammatory molecules

Immediately prior to each PET examination, five mL of blood was drawn from the arterial catheter and instantly applied for ex vivo LPS challenge. Another 10 mL of blood was sampled and directly centrifuged and plasma was frozen for later analysis. The inflammatory molecules analyzed included IL-1β, IL-6, IL-8, IL-10, TNF-α, TNF-receptor 1, C-reactive protein (CRP), and serum amyloid A (SAA). In addition two neuronal injury markers, neurofilament light chain (NFL) and Tau were measured in plasma samples using ultrasensitive single molecule array (Simoa) technology[32,33].

Cognitive testing

Cognitive function was assessed prior to each of the three PET examinations using a standardized cognitive test battery as previously described[8] (appendix). Changes in cognitive performance were calculated for each of seven test variables and corrected for practice effects and variability using data from an age-matched control group who underwent testing using the same battery and with the same intervals[8]. To quantify the change from preoperative test to the postoperative tests and between the two postoperative test occasions a Z-score was calculated for each variable[8].

Statistics

All statistical analyses relating to PET data below were performed using IBM SPSS statistics version 22. Changes of the distribution volume (V_T) for [^{11}C]PBR28 binding in grey matter, hippocampus, lateral frontal cortex, lateral parietal cortex and putamen were analysed with repeated measures ANOVA. Post-hoc analyses for individual regions of interest were performed using paired t-tests (preoperative, postoperative day 3-4 and 3 months, respectively. Due to the exploratory nature of the regional analysis and the main focus on global changes no correction for multiple comparisons was performed. Percent change of V_T

between three time points (baseline vs postop, baseline vs 3 months, and postop vs 3 months) was calculated and related to corresponding changes in cognitive test variables and blood biomarkers of inflammation using Pearson´s correlation analysis. Because the purpose of the analysis was to investigate within-subject changes, and as TSPO binding class has been demonstrated to not influence test-retest reproducibility[25] the binding class was not included in the analysis.

Statistical analyses of cytokine changes were performed using R ver. 2.9.2. The preoperative levels of leucocyte count-normalized cytokines obtained after LPS-challenge (TNF-α and IL-1β), and the systemic inflammatory markers (IL-6, IL-8, IL-10, TNF-α and HMGB1), as well as NFL and Tau were compared to levels either at 3-4 days or 3 months after surgery using paired t-test. Due to the exploratory nature of the analysis, no correction for multiple comparisons was performed.

The relative changes (%) in plasma TNF-α, IL-6 and IL-10 were related to corresponding relative change (%) in [^{11}C]PBR28 binding in grey matter. Absolute and relative differences in LPS-induced TNF-α release and the corresponding change in [^{11}C]PBR28 binding in GM were analysed using Spearman rank tests. Relative changes in global and regional [^{11}C]PBR28 binding was related to corresponding changes in cognitive test scores by comparing [^{11}C]PBR28 binding in the hippocampus to visual verbal learning tests (cumulative and delayed recall) and [^{11}C]PBR28 binding in the lateral frontal cortex to tests of executive function (Letter digit coding and Stroop color word test).

Results

PET imaging

All patients (n=8) participated in the study according to the protocol. Quantitative PET data for one subject at baseline was not available due to problems with blood sampling at that occasion. Representative parametric images for the series of three PET examinations are shown in Figure 1.

Patients showed a global down-regulation of TSPO-binding within the brain after surgery as demonstrated by a decrease in [^{11}C]PBR28 binding (V_T) to TSPO in grey matter (GM) by 26±26**%** compared to baseline ((F=5.465; p=0.023). Comparing changes in GM regions, there was a uniform decrease in V_T in all four selected brain regions having relevance for cognitive function (Fig 2A, paired t-tests).

On the third occasion 3 months after surgery, four of the seven individuals had numerically higher [^{11}C]PBR28 binding as compared to baseline values, although the group difference was not statistically significant (p>0.05) (Fig 2A-B).

There were no statistically significant differences in the free fraction of [^{11}C]PBR28 in plasma between the three time points (preoperative: 6.15±1.08, postop day 3-4: 6.19±2.29 and 3 months postoperatively: 5.06±1.73; n.s.), nor any difference in the individual rate constants as derived using the 2TCM (Supplementary Table 1, p>0.05).

Ex vivo whole blood LPS-challenge and systemic plasma biomarkers

There was a marked reduction (41±39%) in whole blood LPS-induced release of TNF-α on the 3-4th postoperative day as compared to preoperative control levels. This reduction had returned to preoperative levels at 3 months after surgery (Fig 3A). During the study period,

changes in IL-1β release were considerably smaller and did not reach statistical significance.

The time course of changes in [^{11}C]PBR28 binding to TSPO was aligned to the time course of peripheral blood immunoactivity as assessed by LPS-induced release of TNF-α and change in [^{11}C]PBR28 binding (Table 2). At the postop time point, percent change in LPS-induced IL-1β showed a trend to a positive relationship to change in [^{11}C]PBR28 binding (V_T) (p=0.1).

Systemic plasma levels of TNF-α, IL-6, IL-10, TNF-R1, CRP, SSA and NFL were significantly increased at postop day 3-4 compared to preoperatively, while plasma-IL-1ra was reduced. There were only minor changes in plasma HMGB1 at the two postoperative time points (Fig 3B) while plasma levels of Tau remained largely unchanged in all patients at the two postoperative time points. All systemic inflammatory mediators and neuronal injury biomarkers had returned to baseline values at 3 months (Fig 3B-C).

There were trends towards negative relationships between percent change in [^{11}C]PBR28 binding (V_T) and plasma IL-6 between all-time points but none reached statistical significance (Baseline: p=0.11; postop: p=0.18; 3 months: p=0.24;) There were no evidence for relationships between changes in [^{11}C]PBR28 binding in brain and relationship to either plasma IL-10 nor TNF-α (p>0.05).

PET imaging and cognition

Only minor changes (n.s.) in the combined Z-scores for cognitive test results between the three test occasions were seen. However, changes in performance of the Stroop color word interference cognitive test from postoperative day 3-4 to 3 months correlated with changes in GM [^{11}C]PBR28 binding (p=0.027) (Table 2). No other cognitive function test showed any statistically significant relationships to regional changes in [^{11}C]PBR28 binding (p>0.05) (Table 2).

Discussion

This study uncovers a transient yet profound down-regulation of the human brain immune system in the early postoperative period after major peripheral surgical trauma. The dampening of the brain immune system coincided with a distinct, time limited reduction of immunoreactivity in peripheral blood cells. This early postoperative downregulation was followed by recovery at 3 months after surgery, and in four of seven patients, even signs of upregulation of the brain immune system were evident. In contrast to the recorded early immune suppression in blood cells, plasma levels of several inflammatory mediators were increased at the same early time point. Additionally, we found changes in cognitive function that corresponded to these late changes in brain immunoreactivity. The study is the first to translate results from surgical animal models to humans after major surgical trauma and suggests an interplay between the human brain and the systemic biphasic peripheral inflammatory response of the innate immune system to peripheral surgical trauma and with possible relation to cognitive function.

While series of experimental studies in surgical animal models have outlined the periphery-to-brain signaling pathway of the inflammatory cascade[1-3,6], the impact of acute systemic inflammation due to surgical trauma on the human brain immune system and subsequent neuroinflammation is poorly understood. The natural biphasic time course of an acute inflammatory event (e.g. infection or trauma) consists of a rapid initial systemic pro-inflammatory phase triggered by local release of damage-associated molecular patters (DAMPs). This initial phase is followed by a distinct anti-inflammatory phase characterized by a depressed immune system associated with release of anti-inflammatory molecules and

proceeds ultimately to the resolutional phase critically important for healing[12]. Upon reaching the brain, the pro-inflammatory signals interact with the resident brain immune system (e.g. microglia and astrocyte populations)[3,6,22] causing a short lasting (< 24 hours) neuroinflammatory reaction and subsequent neuronal dysfunction, including in brain regions of relevance for higher cognitive functions, as described in experimental animal models of surgery[1-4]. Using plasma tau and NFL as markers of acute neuronal injury[32,33], we tested the hypothesis that changes in brain immune response and cognition following the procedure would be associated with frank neuronal injury. The detected increase in plasma NFL concentrations could indicate such an injury but might also be the result of peripheral nerve injury during the surgical procedure. The latter interpretation is supported by the stable plasma tau concentrations over time suggesting that no or very limited central nervous system neuronal injury occurred and that the inflammatory response and changes in brain immune activity may have functional rather than structural consequences. Alternatively, tau measured in peripheral blood may be a less sensitive marker than NFL for this type of neuronal injury. Serial sampling of CSF from humans undergoing peripheral surgery have consistently shown a similar initial rapid increase in CSF biomarkers within 12 hours related to pro-inflammation and signs of impaired BBB integrity[4,10,11]. The present study extends this knowledge by uncovering simultaneous effects in brain and systemic immune system after peripheral surgery beyond the initial proinflammatory time interval.

TSPO is a mitochondrial protein expressed in immune cells in both brain and blood[34,35]. Animal studies have shown that the TSPO signal in brain is mainly derived from microglia[36,37] with a smaller contribution from astrocytes[21]. Apart from these resident immune cell populations, peripherally derived myeloid cells in the form of infiltrating or perivascular macrophages may also contribute to the signal[35,38]. In primates, systemic LPS exposure has

been shown to cause a significant increase in TSPO-binding within 1-4 hours, and post-mortem immunohistochemistry confirm a correspondence to microglia/macrophage cells whereas co-localization of TSPO and astrocyte markers was low[22]. Notably, the initial (< 4 hours) increase in global [^{11}C]PBR28 binding was followed by a profound decrease in [^{11}C]PBR28 binding at 22 hours post-injection as measured in a subset of animals. In the present study, we observed a uniform and marked decrease in [^{11}C]PBR28 binding 3-4 days after surgery which arguably corresponds to this later time point. Guided by available preclinical information, our results may indicate lower levels or lower activity of microglia and/or other myeloid cells in brain in the early postoperative period.

In direct conjunction with [^{11}C]PBR28 PET examinations, serial ex vivo LPS stimulations were performed to assess temporal changes in immune reactivity of blood borne immune cells after surgery-induced triggering of the innate immune system. Immune activation by DAMPs is mainly mediated by receptors like TLR4 and shared between DAMPs and pathogen-associated molecular patterns (PAMPs) such as LPS. This overlapping utilization of receptors by DAMPs and PAMPs allowed us to apply the robust system of ex vivo LPS challenge in order to assess the temporal responsiveness of the leukocyte-derived immune response previously only reported for a single time point[5,13,14]. Our finding that the release of TNF-α in LPS-stimulated blood cultures were markedly reduced at day 3-4 post surgery and recovered at 3 months after surgery, corresponds to the PET data and suggests a suppressed inflammatory phenotype in the immediate postoperative period. This temporal association between the suppressed immune activity within the brain and the suppressed peripheral immune reactivity in blood is in line with recent observations of strong correlations between [^{11}C]PBR28 binding in brain and peripheral blood[26] and adds to an accumulating body of evidence indicating communication between the brain and peripheral immune cells[22,23,39-41].

Interestingly, an anti-inflammatory reflex pathway between the brain and periphery has been described and includes the vagal nerve, peripheral macrophages, acetylcholine- secreting CHAT-positive T-cells and alpha-7 subtype nicotinic acetylcholine receptor-dependent signaling within the spleen[42,43]. The purpose of this leukocyte-specific anti-inflammatory reflex would be to avoid harmful activation of adaptive immunity to avoid autoimmune reactions towards trauma-induced release of endogenous proteins, while still allowing induction of healing and subsequent return to homeostasis. Results from the present results support a regulatory role for the CNS immune system in controlling the biphasic acute inflammatory response in addition to autocrine peripheral regulation. This neuro-immunological pathway after surgery would thus act sideways with the previously described peripheral immune cell tolerance, typically triggered after endotoxin exposure by inflammatory mediators such as IL-10 and PGE_2 causing dampening of peripheral immune cell reactivity with a duration of up to five days after the pro-inflammatory triggering event[13,44-47].

The comparison of [^{11}C]PBR28 binding and cognitive data revealed an association between the increase in brain immune activity and reduction in performance of the highly sensitive cognitive Stroop color word test. This observation is in line with results from earlier animal models[2-4] and support the hypothesis that the postoperative cognitive dysfunction syndrome is related to surgery-induced activation of the brain immune system[2-4].

The lack of relationship between simultaneous changes in systemic cytokines and brain [^{11}C]PBR28 binding are in agreement with a recent human study showing no correlation between changes in TSPO and systemic cytokine levels after LPS infusion[23]. It may be argued that measured plasma levels of inflammatory mediators reflect the net balance of production and degradation during a prolonged timespan and is the combined production from multiple cell types, including stromal cells e.g. endothelial cells and hepatocytes as well as blood borne

immune cells).

Conclusions

This is the first study describing a profound downregulation of the brain immune activity in the early postoperative period with a related dampening of the immunoreactivity of peripheral blood. This downregulation is followed by a normalization or upregulation of the brain and peripheral immune systems at 3 months after surgery. Our findings suggest an interplay between the human brain and peripheral innate immunity to modulate the inflammatory response after surgical trauma. These processes may be related to post-surgical impairments of cognitive function.

Contributors

Declaration of interest

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Figures

Figure 1

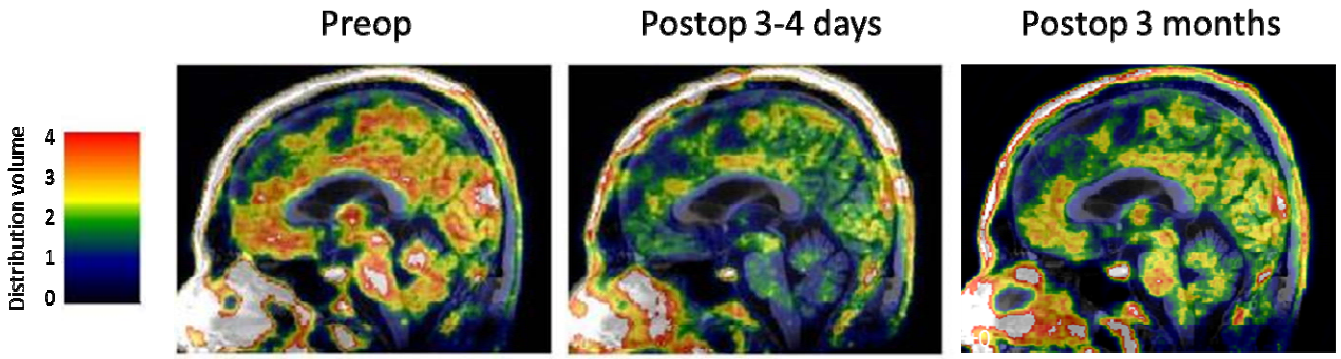


Figure 1. Representative parametric images of [^{11}C]PBR28 binding at three occasions (preoperatively, day 3-4 postoperatively and after 3 months) in a patient undergoing major abdominal surgery

Figure 2A

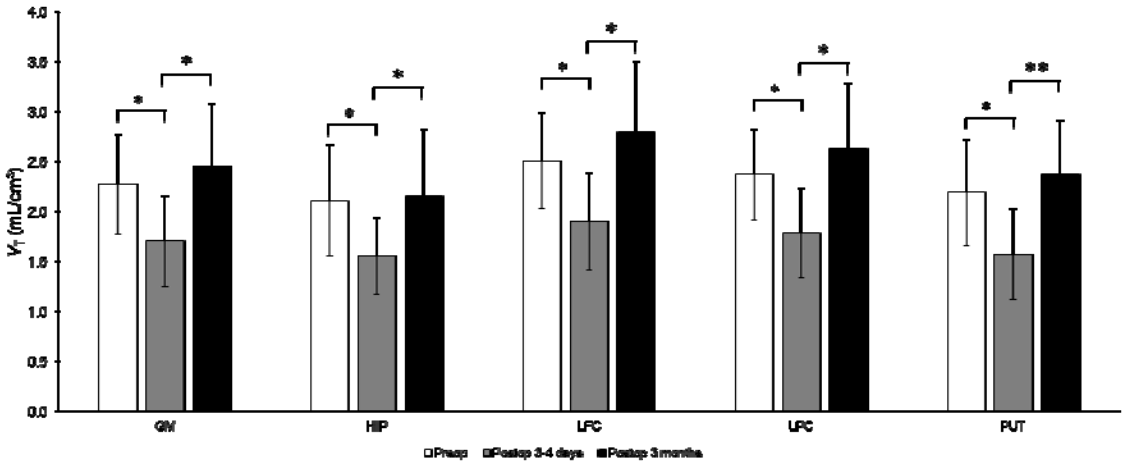


Figure 2A: Changes in V_T across regions preoperatively (white), day 3-4 postoperatively (grey) and after 3 months (black). Paired t-test, *p<0.05, **p<0.01, ***p<0.001

Figure 2B.

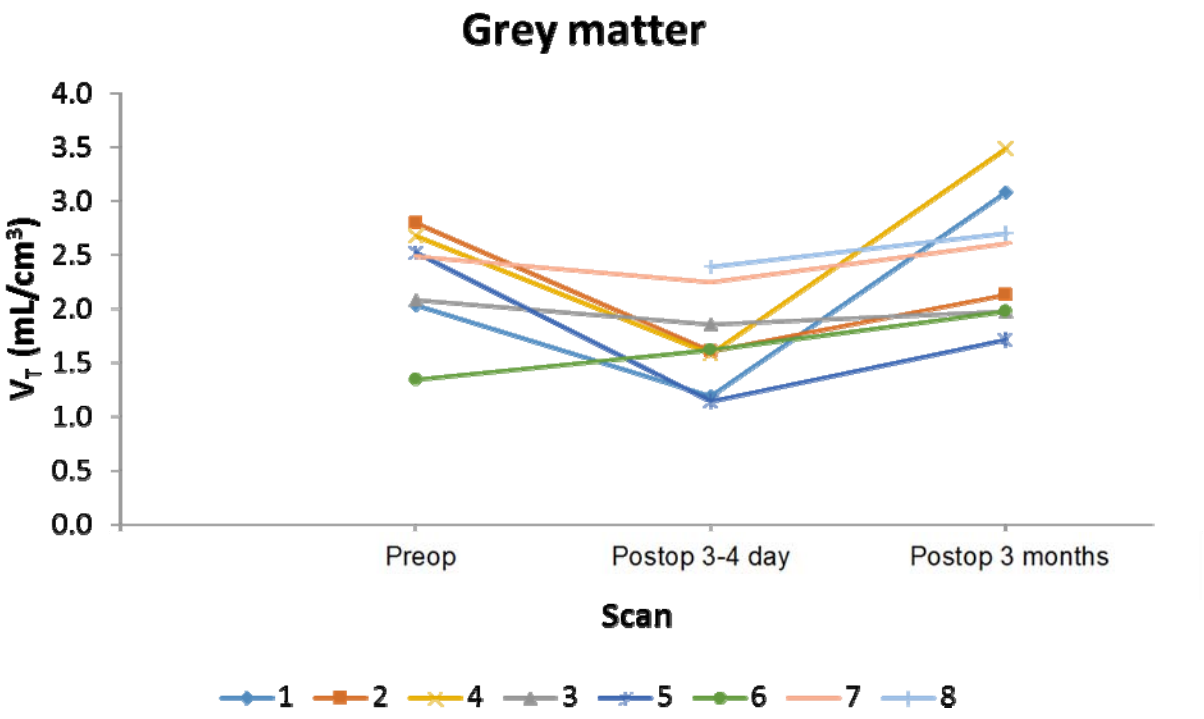


Figure 2B: Individual changes of V_T in GM across the three time points: preoperation, 3-4 days postoperatively, and 3 months.

Figure 3A

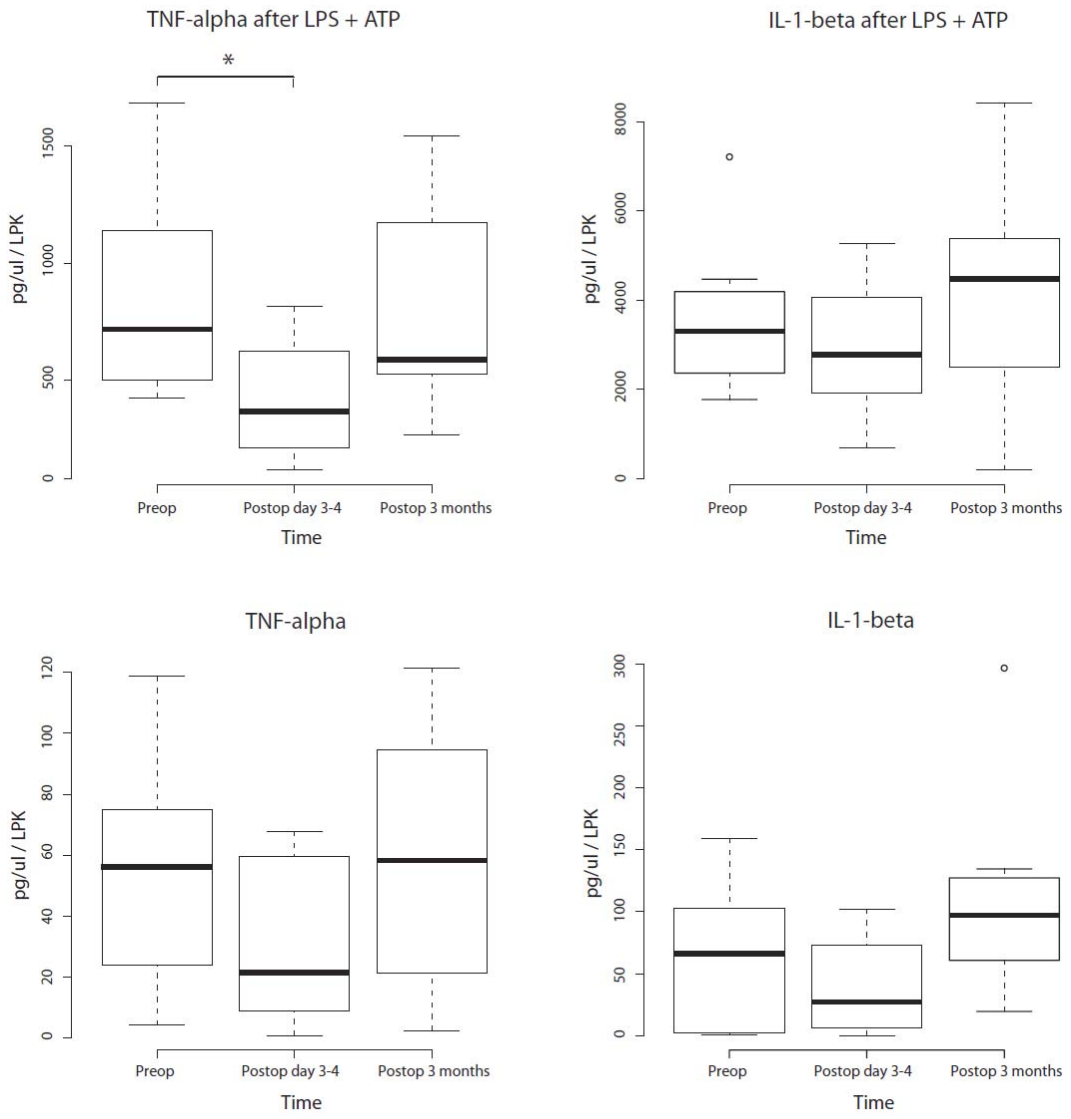
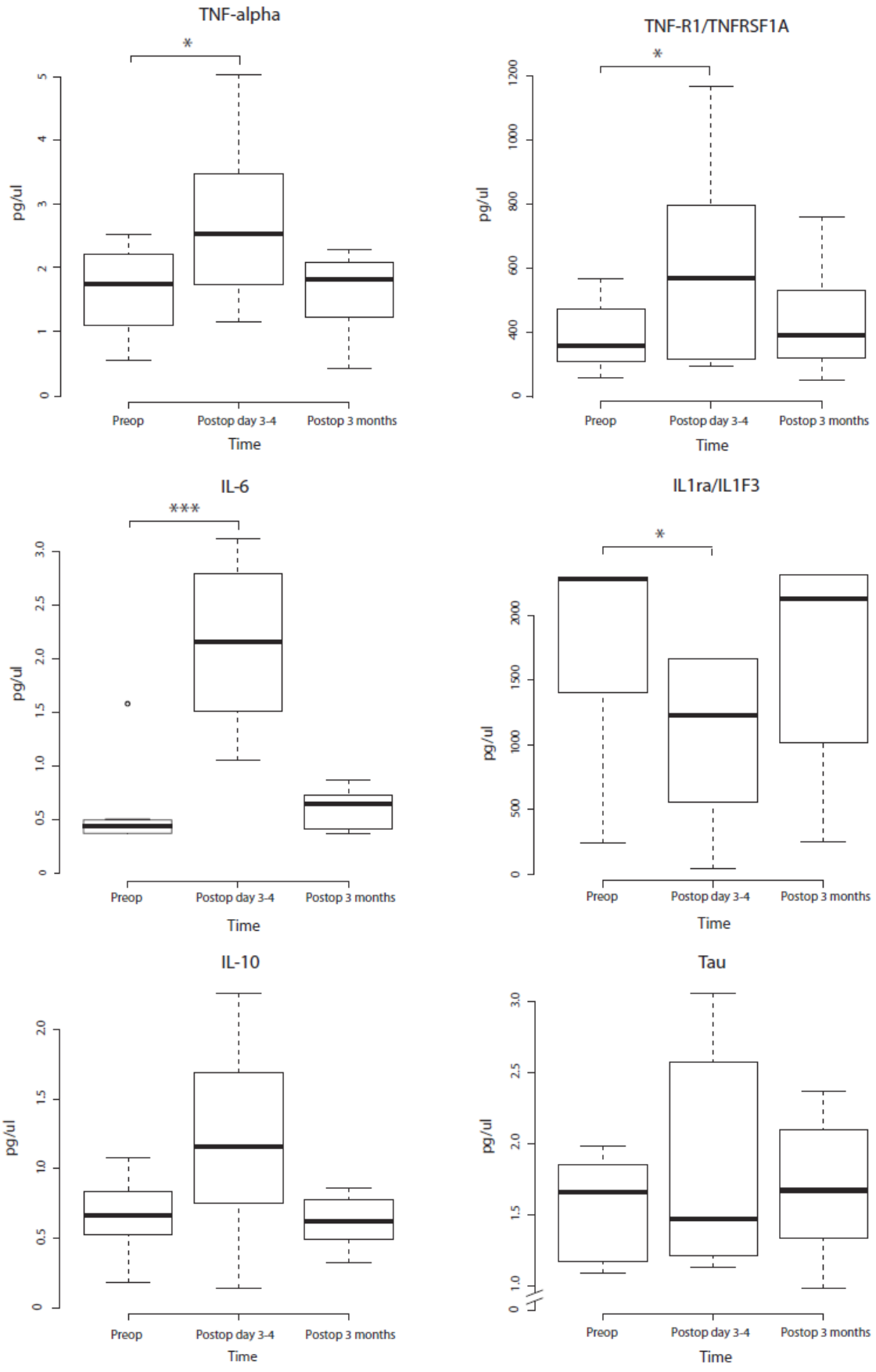


Figure 3A. Ex vivo cytokine production. The cytokine responses were measured by TNF-α and IL1β protein levels after LPS + ATP stimulation of whole blood preoperatively, postoperatively day 3-4 and after 3 months. Protein levels were normalized to number of leukocytes (TNF-α or IL1β/Leucocyte particle count (top panels). The TNF-α response is dampened four days post-surgery despite an increase in leukocytes, but has returned to normal

3 months after surgery. Although similar trends were present for TNF-α and IL1β in unstimulated blood samples (bottom panels), the differences did not reach statistical significance.

Protein levels measured preop were compared to levels at postop 3-4 days and postop 3 months using paired t-test and significant differences are indicated by * and the *p*-value. Bars indicate median value and box indicates second and third quantiles.

Figure 3B


TNF-alpha
*
pg/ul
0
1
2
3
4
5
Preop
Postop day 3-4
Postop 3 months
Time
TNF-R1/TNFRSF1A
*
pg/ul
0
400
600
800
1000
1200
Preop
Postop day 3-4
Postop 3 months
Time
IL-6
***
pg/ul
0
0.5
1.0
1.5
2.0
2.5
3.0
Preop
Postop day 3-4
Postop 3 months
Time
IL1ra/IL1F3
*
pg/ul
0
500
1000
1500
2000
Preop
Postop day 3-4
Postop 3 months
Time
IL-10
pg/ul
0
0.5
1.0
1.5
2.0
Preop
Postop day 3-4
Postop 3 months
Time
Tau
pg/ul
0
1.0
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2.0
2.5
3.0
Preop
Postop day 3-4
Postop 3 months
Time

Figure 3C

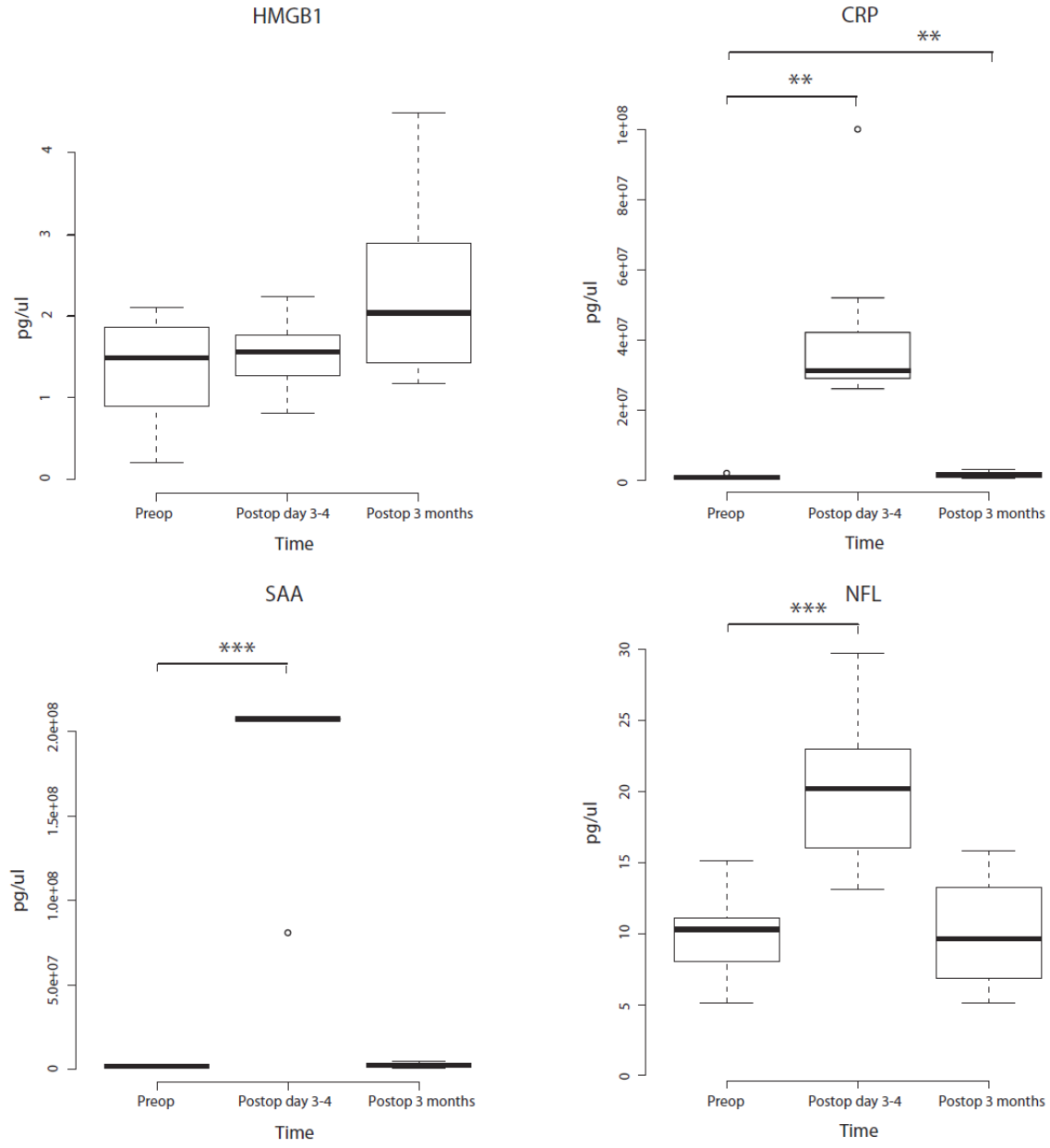

Fig 3B-C

Plasma cytokine, HMGB-1, CRP, SAA, NFL and Tau concentrations following major abdominal surgery in eight male surgical patients. Data are presented as preoperatively, postoperatively day 3-4 and after 3 months. Statistical significance are indicated by * with corresponding *p*-value (paired t-test). Bars indicate median value and box indicates second

and third quantiles.

Tables

Table 1. Demographic data for eight male patients undergoing robot-assisted prostatectomy under general anesthesia.

Age	yrs	61 ± 7
Height	cm	176 ±5
Weight	kg	82 ± 5
Body mass index		26 ± 2
Duration of surgery	hrs	3.1 ± 0.8
Blood loss	mL	105 ± 77
Length of stay	days	2.4 ± 1.0

Table 2. Correlations of % change [^{11}C]PBR28 V_T vs Cognitive Z-scores

	Test	Region		
		GM	HIP	LFC
Baseline vs Postop (n=7)	Visual verbal learning, cummulated	-0.534	-0.54	-
	Visual verbal learning, delayed recall	0.150	0.211	-
	Letter digit coding	-0.147	-	-0.283
	Stroop test, part 3, time	-0.216	-	-0.342
Baseline vs 3 months (n=7)	Visual verbal learning, cummulated	0.154	-0.012	-
	Visual verbal learning, delayed recall	-0.018	0.226	-
	Letter digit coding	0.385	-	0.334
	Stroop test, part 3, time	0.582	-	0.531
Postop vs 3 months (n=8)	Visual verbal learning, cummulated	-0.051	-0.114	-
	Visual verbal learning, delayed recall	-0.221	-0.017	-
	Letter digit coding	0.208	-	0.186
	Stroop test, part 3, time	0.650	-	0.736*

Showing Pearson Correlation with significance levels at *p<0.05 (2-tailed)

Supplementary Table 1. Individual rate constants from two-tissue compartment model.

Rate constant	Time point		
	Preop	4 days postop	3 months postop
	Mean± SD	Mean± SD	Mean± SD
K_1	0.10±0.02	0.08±0.02	0.10±0.02
k_2	0.14±0.03	0.16±0.03	0.13±0.05
k_3	0.13±0.04	0.14±0.06	0.12±0.07
k_4	0.06±0.02	0.05±0.01	0.05±0.01

Showing mean±SD. Paired t-test with significance level at *p<0.05 (2-tailed).

Supplementary Table 2.

Panel	Protein	Time point				
		Preop	Postop 3-4 dya		Postop 3 months	
		Mean±SD	Mean± SD	p	Mean± SD	p
LPK		6.6±1.7	10.4±3.2	0.01	6.8±1.5	0.4
Unstim.	TNF	54.1±37	31.1±27.4	0.09	59.1±46	0.9

WB						
	IL1b	62.8±60.7	39.4±38.9	0.3	112.8±90.6	0.4
LPS + ATP WB	TNF	852±465	415±259	0.04	828±468	0.9
	IL1b	3587±1735	2936±1547	0.4	4123±2733	0.7
Plasma	TNF	1.6±0.7	2.7±1.3	0.04	1.6±0.6	0.6
	TNF R1	389±116	603±321	0.02	437±170	0.1
	IL-6	0.6±0.4	2.1±0.8	0.0009	0.6±0.2	0.8
	IL-1ra	1804±771	1076±628	0.02	1684±820	0.4
	IL-10	0.7±0.3	1.2±0.7	0.1	0.6±0.2	0.7
	HMGB1	1.3±0.7	1.5±0.4	0.1	2.3±1.1	0.052
	CRP	898731±483890	41338910±25013850	0.002	1673016±891551	0.008
	SAA	1692109±822395	191662200±4479615	0.00006	2230488±1379959	0.07
	NFL	9.9±3	20.1±5.4	0.0001	10.1±3.9	0.8

Chemok.	Eotaxin	176±34	151±17	0.04	170±28	0.5
	IP-10	278±82	310±142	0.5	330±98	0.09
	MCP-1	51±17	51±16	0.99	58±22	0.4
	MCP-4	82±24	75±15	0.3	88±30	0.4
	MDC	985±257	1119±309	0.2	971±144	0.9
	MIP-1β	62±15	87±51	0.1	66±19	0.4
	TARC	102±60	121±130	0.7	116±44	0.5
Cytokines	IL-12/23p40	71±25	83±59	0.5	82±26	0.3
	IL-15	2±0.6	2.7±1.3	0.1	2.4±2.1	0.5
	IL-16	141±41	140±44	0.9	156±37	0.2
	IL-7	3.9±2.1	3.4±1.2	0.5	4.6±2.1	0.4
	IFN-γ	6±2.3	10.9±15.1	0.4	7.9±4.4	0.2
	IL-8	4.5±1.1	5.1±1.3	0.3	4.9±1.3	0.4
	sICAM	238313±55248	304369±104058	0.054	260272±59277	0.09
	sVCAM	364864±47446	404741±86753	0.1	388947±58941	0.1
Angiogen.	bFGF	33±29	26±25	0.4	46±33	0.2
	PlGF	25±4	30±3	0.003	22±5	0.1

Flt-1	58±20	60±17	0.6	59±18	0.5
Tie-2	5979±745	4651±333	0.0004	5802±698	0.3
VEGF	87±51	70±19	0.3	99±54	0.5
VEGF-C	76±56	56±26	0.2	83±30	0.6
VEGF-D	609±209	640±174	0.4	635±203	0.07