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Stimulated whole blood cytokine/chemokine responses are associated with interstitial cystitis/bladder pain syndrome phenotypes and features of nociplastic pain: a MAPP research network study

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INTRODUCTION

Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is a debilitating urologic pain disorder that affects roughly 6% of women in the United States.[4] Care and treatment for IC/BPS represents a substantial burden on the healthcare system but these expenditures have not resulted in high patient or provider satisfaction with available treatments.[53] Difficulty treating IC/BPS stems in part from a lack of consensus amongst researchers and clinicians regarding the underlying pathophysiology of the disorder.[9] Most agree, however, that multiple overlapping mechanisms likely contribute to the disease state, and efforts are underway to improve patient phenotyping.

One such effort, driven by the NIDDK-funded Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network, has demonstrated that female IC/BPS patients with chronic overlapping pain conditions (COPCs) such as irritable bowel syndrome (IBS) and temporomandibular disorder (TMD) show heightened *ex vivo* cytokine release to stimulation with lipopolysaccharide (LPS), a classic agonist of one highly conserved component of the innate immune system, Toll-Like Receptor-4 (TLR4).[47; 49] In a study conducted at the University of Iowa as part of the MAPP Epidemiology Phenotyping Study (EPS),[10; 36] 66 female IC/BPS patients (40 with comorbid COPCs, 26 with IC/BPS only), a composite score of *ex-vivo* TLR4 stimulated cytokines (interleukins [IL] 1β and 6) from peripheral blood mononuclear cells (PBMCs) was found to be significantly elevated in those patients with comorbid COPCs. Furthermore, this composite score was associated with the spatial extent of comorbid pain measured on a comprehensive body map. In a subsample of patients who underwent experimental pain testing (n=32), greater pain sensitivity to pressure at the thumbnail was marginally associated with higher TLR4 composite scores as well.[47] Together these results were interpreted to suggest that central nervous system amplification and maintenance of pain (i.e., noicplastic pain) is associated with *ex-vivo* TLR4 stimulated cytokine/chemokine release in IC/BPS. This possibility is supported by a number of animal models indicating a role for TLR4 in pain augmentation in the central nervous system, and studies demonstrating that *the ex-vivo* peripheral and central immune responses to TLR4 stimulation are linked.[33] [15; 20]. Confirming these relationships would provide a foundation for better patient phenotyping and point to mechanistic targets for further investigation.

Following the MAPP EPS (2009–2015) a new cohort of urologic pelvic pain patients was recruited for the MAPP Research Network Symptom Phenotyping Study (SPS, 2015–2021).[8] In the MAPP SPS, a modified protocol for measuring the LPS *ex-vivo* cytokine/chemokine response was adopted across six recruiting sites, allowing for the opportunity to conduct a critical confirmatory analysis of the MAPP EPS findings.[8] Clinically, MAPP studies have shown that COPCs/widespread pain in IC/BPS patients is associated with greater psychosocial difficulties and worse quality of life, indicating an urgent need to establish underlying pain mechanisms in this subset of patients.[34] In the current study, we examined 135 female IC/BPS patients from the MAPP SPS to determine if *ex-vivo* TLR4 stimulated cytokine/chemokine release, this time measured across a larger number of cytokines/chemokines, distinguished patients with comorbid COPCs from those with IC/BPS only. We also conducted analyses to determine if the extent of widespread pain

and experimental pain sensitivity were associated with this response. Our primary purpose was to determine if we could confirm the relationship between the TLR4 *ex-vivo* cytokine/chemokine response and characteristics of nociplastic pain.

METHODS

Sample

The MAPP Research Network SPS enrolled 620 Urologic chronic Pelvic Pain Syndrome (UCPPS) patients for longitudinal follow-up of symptoms and phenotypic characteristics (ClinicalTrials.gov Identifier: NCT02514265).[8] Due to funding limitations, only a subset of the collected biomarker samples could be analyzed. A total of 155 female IC/BPS participants were selected for biomarker analysis; a power calculation was conducted prior to selecting the number of female participants based on previous data.[47] The Cohen's *d* effect size for the difference between IC/BPS only and IC/BPS + comorbid COPCs on the LPS-stimulated composite score (IL-1b+IL-6) was d= .67. Assuming two-tailed hypothesis testing, alpha=.05, and an allocation ratio of 1:2 (pelvic pain only: pelvic pain comorbid, the rough distribution in the original manuscript) 82 subjects would be required to detect the effect of interest with .80 power. We chose a larger number of samples for analysis, enough to detect an effect size of d=.50, because of the changes to the protocol (e.g., whole blood rather than PBMCs and the larger number of cytokines/chemokines tested).

The 155 individuals were selected because they provided biomarker samples at baseline, 6 months and 18 months with concurrent neuroimaging data. These longitudinal data will be analyzed as part of an ancillary R01 to the MAPP network (R01DK123164). As the primary purpose of this manuscript is to attempt a conceptual validation of MAPP EPS findings, only female patients are analyzed who also completed the full battery of COPC self-report criteria, so the final sample consisted of 135 IC/BPS participants.

Demographic information

Patient demographics were collected by self-report and Body Mass Index was calculated form height and weight.

Clinical pain, chronic overlapping pain conditions, and extent of widespread pain

Overall Pelvic Pain Severity (PPS) was calculated using a composite measure comprised of questions from the Genitourinary Pain Index (GUPI) and Interstitial Cystitis Symptom Index (ICSI) as described previously.[21]

Chronic overlapping pain conditions (COPCs) were assessed using the Complex Multi-Symptom Inventory and standardized diagnostic criteria.[59] Rather than administering all diagnostic criteria to all patients, patients first complete the screener which contains items that "trigger" full diagnostic criteria the administration of the full diagnostic criteria for COPCs that are relevant for that individual, which limits response burden by only administering relevant questionnaires. Possible diagnostic modules include chronic fatigue syndrome, irritable bowel syndrome, fibromyalgia, temporomandibular joint disorder, and migraine.[16; 19; 40; 60; 61]

Patients were asked to indicate whether they had pain or not and the severity of any pain on a 0–10 scale, using a 76-site body map adapted from the Collaborative Health Outcomes Information Registry project and further reduced to 12 non-pelvic regions for the assessment of widespread pain.[45] Sites with severity of pain rated at least 4 were counted.

Cytokine/chemokines under ex-vivo stimulated and unstimulated conditions

Differences between the MAPP EPS and SPS protocols—Several important differences are noted between the MAPP EPS and MAPP SPS TLR stimulation protocols. First, in the MAPP EPS, a single site (University of Iowa) collected samples for ex vivo stimulation, while in the MAPP SPS all six recruiting sites took part. Second, in the MAPP EPS PBMCs were isolated for stimulation, while in the MAPP SPS, a commercially available whole blood *ex-vivo* stimulation assay was used (TruCulture, Myriad RBM) to allow all sites to collect samples in a consistent and efficient manner. In the MAPP EPS, lipopolysaccharide (LPS) concentrations of 50ng/ml were used to stimulate samples for 72 hours, while in the MAPP SPS the concentration was 100ng/ml (the standard concentration available) for an incubation period of 24 hours. For these reasons, the current study represents a conceptual validation of the MAPP EPS findings, rather than a direct replication.

SPS protocol—The TruCulture system uses vacutainers preloaded with TLR4 agonist (LPS), or control media (unstimulated condition), which are kept frozen at −20° C until being thawed for one hour at room temperature, or overnight in a standard refrigerator prior to use. Approximately 1ml of whole blood is drawn directly into each tube and then kept in a tabletop incubator at 37° C for 24 hours. After incubation, the supernatant is isolated using a valve separator included in the kit and stored in −80° C freezer for batch analysis. All samples were sent to a central biorepository at the University of Denver Anscultz Medical Campus Biorepository Core Facility under the supervision of the Director and Co-Director of the MAPP Tissue Analysis and Technology Core. 50 μL of thawed supernatant was analyzed for seven cytokine/chemokines using Luminex® Xmap technology with R&D systems high performance assays. These were monocyte chemoattractant protein-1 (MCP-1, range of assay: 3.0–1890 pg/ml), macrophage inflammatory protein 1-α (MIP-1α, 18–9840 pg/ml), IL-1β (0.34–1500 pg/ml), IL- 6 (0.95–3400, pg/ml), IL-8 (0.78–2800 pg/ml), IL-10 (0.46–2000 pg/ml), and tumor necrosis factor -α (TNF-α; 0.78–2000 pg/ml). Unstimulated samples for IL-1β, 6, 8, 10 and TNFα were diluted 2x, while the LPS condition was diluted 20x. Unstimulated samples for MCP1 and MIP-1α were diluted 4x, and the LPS condition was diluted 10x. Values below the limit of quantification (LOQ) were set to one half of the LOQ value. This family of cytokines and chemokines was selected because they represent different aspects of the inflammatory response and are all promoted by the transcriptional factor NF-KB, whose upregulation is a well-established consequence of TLR4 stimulation.[38]

Pressure Pain Sensitivity—As in the MAPP EPS, pressure pain sensitivity was measured using the Multimodal Automated Sensory Testing (MAST) system (Arbor Medical Innovations, Ann Arbor, MI).[24] The MAST system includes a electromechanical stimulator to deliver pressure stimuli and a touchscreen-based rating scale to capture

participant responses.[22] Following a familiarization procedure to reduce testing anxiety, an ascending sequence of incremental pressure stimuli were delivered to the participants' dominant thumbnail by a 1 cm^2 rubber probe attached to the MAST stimulator. Pressure intensity started at 0.5 kgf/cm^2 and increased in 0.5 kgf/cm^2 steps at a ramp rate of 4.0 $kgf/cm^2/s$. Each pressure was held constant for 5-s in duration and was separated by a 20-s inter-stimulus rest interval. Participants rated perceived pain intensity after each stimulus using a digital 0–100 NRS displayed on the touchscreen (0 = no pain; 100 = pain as bad as you could imagine). The test was completed when the participant reached his/her pain tolerance and asked that the test be stopped, the participant reported a pain intensity of ≥ 80/100, or a maximum possible pressure intensity of 10 kgf/cm^2 was delivered. Data were automatically uploaded by the system to the MAPP Network Data Coordinating Center via a secure file transfer protocol for analysis. A three parameter logistic model was used to estimate the within-person inflection point on the stimulus-response curve between PPT and tolerance, referred to as *Pain50* [23] To ensure standardization across sites, scripted participant instructions were used and research staff completed annual in-person training.

Statistical analyses

All analyses were performed in the R programming language, version 3.6.1.

Comparison of biomarker sample to the rest of the MAPP SPS baseline sample.—To determine if the 135 participants differed from the rest of the female IC/BPS participants in the MAPP SPS baseline sample (n=172) on variables that could plausibly influence immune parameters, we compared patient age, body mass index, depression scores, anxiety scores (Hamilton anxiety and depression scale), perceived stress (perceived stress scale), Pain50 scores, number of painful sites selected on the body map, and proportion of each sample with a COPC by t-test and X^2 tests for continuous and categorical variables, respectively.

Comparison of IC/BPS only and IC/BPS + COPC groups

Transformation of cytokine/chemokine values for analysis: To conduct parametric analyses with appropriate covariates we proceeded to transform cytokine/chemokine values with values in the detectable range using Box-Cox transformations (all TLR4 stimulated cytokines/chemokines; unstimulated TNF-α, , IL-1β, and IL-8.)[5] The Shapiro-Wilks test statistic, where values > 0.97 indicate acceptable normality, was used to evaluate these transformations and all exceeded this threshold.

Principal components analysis: We subsequently used a principal components analysis (PCA) based on the correlation matrix between the seven transformed cytokine/chemokine values under the TLR4 stimulated condition retaining components with an eigenvalue greater than one. Factor scores for the resulting components were then extracted by the regression method. PCA has previously been used as a dimension reduction technique for inflammatory variables.[29; 31]

General linear models: The primary form of analysis was a mixed-effects linear model with the TLR4 component scores as dependent variables with patient age and body mass

index included as covariates. A random intercept term was included for site of collection. The independent predictor of interest was COPC group status. We also conducted analyses with the degree of widespread pain,, and Pain50 ratings as independent predictors of the TLR4 component scores.

We recreated an inflammatory composite variable analogous to that used in the original study, which was a simple mean of the z-scores for LPS-stimulated IL-1β and IL-6 from the current study. These values were used rather than those from the MAPP EPS dataset due to the substantial differences in the protocol.

While not the focus of the current manuscript, we also repeated these analyses with the unstimulated values of TNF-α, IL-8, MCP-1, and IL-1β (transformed) as dependent variables, to determine if unstimulated values were associated with COPC status. Because a large percentage of unstimulated IL-10 (84%), IL-6 (78%), and MIP-1α (56%) values fell below the detectable limit of the assay, we did not compare these cytokines between the groups.

RESULTS

Demographic and clinical information is shown in Table 1. Values of stimulated and unstimulated cytokines/chemokines are shown in Table 2.

Comparison of biomarker sample to the rest of the baseline MAPP SPS cohort

There were no significant differences between the MAPP biomarker sample and the rest of the MAPP SPS baseline cohort on patient age, BMI, depression scores, anxiety scores, perceived stress scores, Pain50 scores, number of painful sites selected on the body map, and proportion of each sample with a COPC (all $p < .05$; data not shown).

Principal components analysis

Two components were extracted, one with high positive loadings on all 7 cytokine/chemokines that explained 63.1% of the variance, and a second component with high positive loadings for MCP1, IL-8 and IL-10, a near zero loading for MIP-1α, and negative loadings for IL-6, IL-1 and TNF-α, that explained an additional 16.1% of the variance. These findings are consistent with the first component representing a global response to LPS-stimulation, and a second component more specific to anti-inflammatory activity, the regulatory function of IL-8, and chemotactic activity (MCP1). See Figure 1 for PCA loading plot.

Comparison of IC/BPS and IC/BPS + COPC groups on TLR4 composite scores

In models controlling for patient age, body mass index, and site of collection, IC/BPS + COPC patients were found to have significantly elevated TLR4 global composite scores, ($p< .01$), but not the TLR4 anti-inflammatory/regulatory/chemotactic composite scores ($p > .05$). See Fig 2a & 2b for differences in the TLR4 global composite score and Cohen's *d* effect sizes for each of the seven stimulated cytokine/chemokines by COPC status. The basic difference in the TLR4 global composite score was apparent when stratified by site of

collection, strengthening the generalizability of the results (Supplemental Fig 1). The z-score composite of *ex-vivo* TLR4 stimulated IL-6 and IL-1β was also significantly higher in the IC/BPS + COPC group ($p < .05$). See Table 3 for model estimates.

Widespread pain, pressure pain sensitivity, pelvic pain severity and TLR4 composite scores

Pain50 was not associated with the TLR4 global response score ($p > .05$), however, higher pressure thresholds on this measure, representing less pain sensitivity, were significantly and positively associated with the TLR4 anti-inflammatory/regulatory/chemotactic score ($p < .01$). The number of painful regions indicated on the body map (severity ≥ 4) was positively and significantly associated with the TLR4 global composite score ($p < .05$), but there was no relationship with the TLR4 anti-inflammatory/regulatory/chemotactic score ($p > .05$). Pelvic pain severity was not associated with either composite score ($p > .05$).

Comparison of IC/BPS and IC/BPS + COPC groups on unstimulated cytokine/chemokine values.

There were no significant relationships between levels of unstimulated MCP1, IL-1β, TNFα, or IL-8 and COPC status (all $p > .05$). Model estimates are shown in Table 4.

DISCUSSION

These findings confirm a difference in immune priming between subtypes of IC/BPS patients that have localized pelvic pain versus those with comorbid pain conditions or widespread pain manifestations, as we previously demonstrated this relationship in the MAPP EPS.[47] Our findings have been expanded to include a larger number of inflammation-linked cytokines/chemokines with diverse functions, but overall the results suggest a relatively broad effect of relationship between comorbid pain and *ex-vivo* TLR4 immunoreactivity, as five of the seven tested cytokines/chemokines showed at least medium differences between the IC/BPS subtypes by conventional effect sizes (Cohen's *d*). Furthermore, experimental pain testing suggests that there may be a mechanistic link between *ex-vivo* TLR4 immunoreactivity and pain sensitivity.

Ex-vivo LPS-stimulation of PBMCs, whole blood, or isolated immune cell subsets, have previously been linked to chronic and cyclic pain in a number of patient groups: chronic fatigue syndrome, mixed chronic pain samples, chronic multisite musculoskeletal pain, dysmenorrhea, and low back pain.[7; 18; 33; 52] The study in low back pain is of particular interest because some stimulated cytokines and chemokines were also higher in *chronic* LBP patients compared to those with acute pain.[52] While not directly analogous to the current study, this supports the use of stimulated assays for subtyping within pain cohorts. Stimulation of whole blood, as conducted in the current study, tends to show similar responses for inflammation-linked cytokines/chemokines as those obtained from stimulation of isolated cells and particularly monocytes[12; 14]. Given that our current findings are similar to those obtained in the MAPP EPS with PBMC stimulation, these results further support the idea that complex forms of chronic pain are associated with immune priming of

circulating immune cells. Seen in the light of these previous studies, our results suggest that this immune priming is a common feature of what has recently been termed nociplastic pain.

We found that the second TLR4 component that was represented by positive relationships with anti-inflammatory cytokine IL-10, chemokine MCP1, and IL-8 (diverse functions), as well as negative relationships with classic pro-inflammatory cytokines IL-1β, IL-6, and TNFα, was associated with an ability to tolerate more pressure pain. It is worth noting that this component represented only a modest amount of the variance in the TLR4 cytokine/chemokine levels (19%). Nonetheless, the positive relationship with IL-10 and negative relationship with the pro-inflammatory group of cytokines suggest that this component may reflect a protective factor for pain sensitivity measured experimentally, though the diverse functions of IL-8 and chemotactic activity of MCP1 complicate the picture. For example, previous research has shown that increases in circulating IL-8 secondary to endotoxin (LPS) administration are associated with greater pain sensitivity.[27] Further studies are needed to parse different relationships under stimulated and unstimulated conditions with experimental pain sensitivity.

Animal models showing a state of hyperalgesia in response to LPS injections have been studied for more than 25 years.[57] It is clear from these studies that TLR4 expressed in the spinal cord plays a major role in the transition to chronic pain states: knockout of TLR4 or application of antisense oligodeoxynucleotides for TLR4 greatly attenuate behavioral assays of hyperalgesia.[51] These studies have led to great interest in therapeutics that target TLR4.[6] Given the high preponderance of chronic pain conditions in women, sex-dependent effects of TLR4 and TLR4 therapeutics have led to renewed focus on this pathway, particularly in visceral pain conditions.[15] Mechanistically, there is mounting evidence that peripheral immune cells and especially monocytes are capable of entering the CNS parenchyma and promoting long term behavioral changes.[43] Relevant to the current study, Kwok et al. demonstrated that the inflammatory response observed from spinal tissue stimulated with LPS is mirrored in PBMCs, suggesting that peripheral and central immune cells show similar immunoreactivity[33], a finding similar to that seen in our own animal models of IC/BPS symptoms.

The role of *ex-vivo* TLR4 immune responses in IC/BPS symptoms has been explored in recent studies using the transgenic URO-OVA mouse model of cystitis induction to induce IC/BPS symptoms. These studies observed that URO-OVA mice, a transgenic IC/BPS-like mouse model, developed pelvic/bladder pain after cystitis induction, along with altered systemic and central TLR4 activations.[11] Splenocytes from cystitis-induced URO-OVA mice produced increased proinflammatory cytokines (IL-1β, IL-6 and TNF-α) in response to LPS stimulation *in vitro*. Consistently, spinal tissues from cystitis-induced URO-OVA mice expressed increased mRNAs for TLR4 mediated proinflammatory cytokines (IL-6 and TNF-α). Furthermore, pain sensitization following cystitis induction was attenuated in TLR4 deficient URO-OVA mice (URO-OVA$^{TLR4-/-}$) despite similar levels of bladder inflammation and voiding dysfunction. Moreover, administration of TAK-242, a TLR4 antagonist[25], in cystitis-induced URO-OVA mice reduced pain sensitization, which was associated with reduction of both splenocyte production of TLR4 mediated cytokines and spinal expression of mRNAs for TLR4 mediated cytokines. This last point is critical,

as it suggests that immune priming in peripheral and central compartments occur in tandem and point to the possibility that the immune priming we have demonstrated in association with nociplastic pain features in the MAPP EPS & SPS may mirror central TLR4 immunoreactivity.

These findings naturally raise the question of how immune priming occurs in IC/BPS patients. Genetic, psychosocial, and environmental factors may be responsible. IC/BPS is considerably more prevalent in first degree relatives of IC/BPS patients than in the general population, and twin studies suggest a moderate genetic contribution to IC/BPS.[54–56] Very large genetic studies would be needed to determine if polymorphisms related to innate immunity generally or TLRs specifically would be required to address this possibility, ideally with a focus on the IC/BPS + COPC subtype. Previous MAPP EPS analyses showed that IC/BPS patients with COPCs have elevated depression, anxiety, perceived stress, and are more likely to report childhood trauma, though they were not more likely to be using pain medication.[30; 48] There are well-established links between negative mood, childhood trauma, and immune responses, as well as glucocorticoid resistance.[1; 2; 41] Other studies have shown that childhood trauma is associated with increased NF-κB activity in PBMCs and LPS-stimulated PBMC cytokine/chemokine release.[17; 42] Finally, antecedent urinary tract infections have been associated with IC/BPS and likely implicate TLR4 signaling.[44; 62] These factors suggest psychosocial, environmental, and genetic vulnerabilities may interact to contribute to immune priming in IC/BPS.

Other studies have shown that basal circulating levels of inflammatory markers are associated with greater pain sensitivity,[26; 37; 46] and multiple of studies of endotoxin in human subjects show that increases in cytokines, including several measured in the current study (e.g., IL-6, TNFα, IL-8, IL-10) accompany enhanced pain sensitivity.[3; 13; 27; 58] In another recent manuscript examining plasma (unstimulated) inflammatory markers in the MAPP EPS, there were no relationships between IL-6, TNFα and widespread pain – this echoes the findings of our original MAPP EPS study showing that the TLR4 *ex-vivo* responses were associated with comorbid pain while plasma (unstimulated) IL-6 levels were not.[28; 47] However, exploratory analyses suggested a potential relationship between interleukin (IL)-8, Granulocyte macrophage colony-stimulating factor, and widespread pain.[28] While our findings confirm the value of TLR4 *ex-vivo* cytokine/chemokine responses in IC/BPS phenotypes, basal/unstimulated inflammation-linked cytokines/chemokines should continue to be explored in IC/BPS.

Clinical Relevance.

In addition to the immunological differences described here, IC/BPS patients with a high burden of COPCs or greater degrees of widespread pain show a number of pathophysiologic differences that suggest mechanistic divergence of the pain experience associated with central sensitization/nociplastic pain. IC/BPS patients with more widespread pain show distinct changes in brain structure of the supplementary motor area and functional connectivity between the primary somatosensory cortex and the salience network,[32] both experimental and self-reported sensory sensitivity,[24; 50] and are less likely to present with classic peripheral indications of IC/BPS like Hunner's lesions.[35] Future studies

attempting to delineate the mechanistic involvement of TLR4 in IC/BPS may wish to assess the impact of *ex-vivo* TLR4 responses on these variables. The strongest relationship between an individual cytokine under *ex-vivo* TLR4 stimulation and the TLR4 global composite score was IL-6; thus, LPS-stimulated IL-6 may be a useful proxy for more comprehensive assessment of cytokines.

Strengths and Limitations.

The current study benefits from several strengths, including the collection of data at multiple sites in geographically diverse regions of the country, deep symptom-based phenotyping of the patient population, and an *a priori* framework for pursuing the differences of interest. There are limitations as well. Only five COPCs were assessed in the current study, while up to ten are recognized by the NIH pain consortium,[39] and the study considered only female patients. Future explorations of the relationship between inflammatory markers and comorbid pain in IC/BPS will benefit from more granular explorations of inflammatory pathways using proteomic and/or transcriptomic approaches to identify cellular sources, intracellular signaling pathways, and characterization of Toll-like receptor type and density on cell surfaces. These analyses will help to delineate potential peripheral-CNS crosstalk that promotes sensitization beyond the relatively non-specific relationships encountered here.

Conclusions.

Heightened *ex-vivo* TLR4 immune responses are a feature of IC/BPS + COPC female patients. These responses may be mechanistically linked to nociplastic pain in IC/BPS and should be investigated further for potential clinical applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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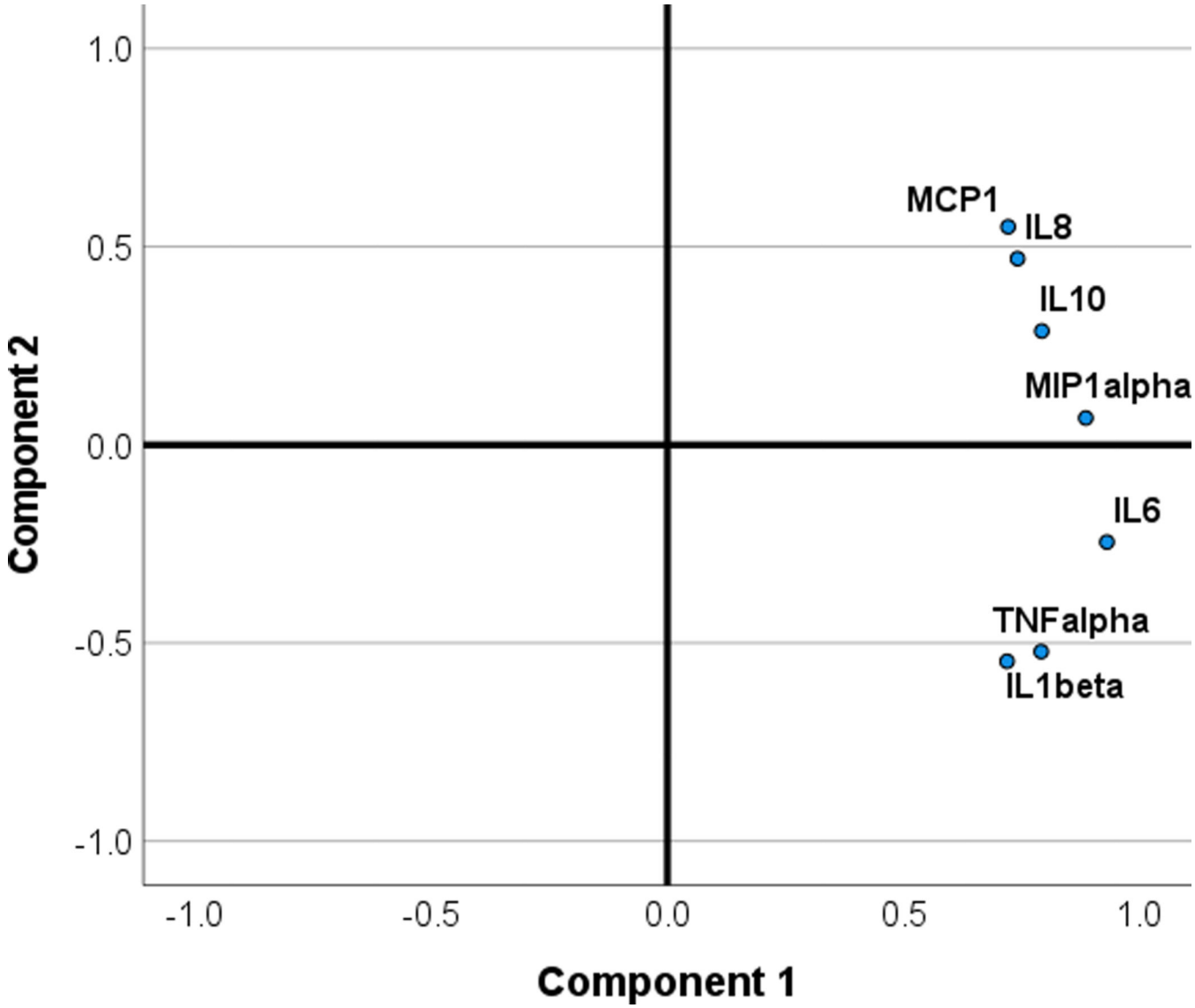

Figure 1.
Principal components analysis loading plots for the cytokines/chemokines (transformed scales) under the TLR4 stimulated condition.

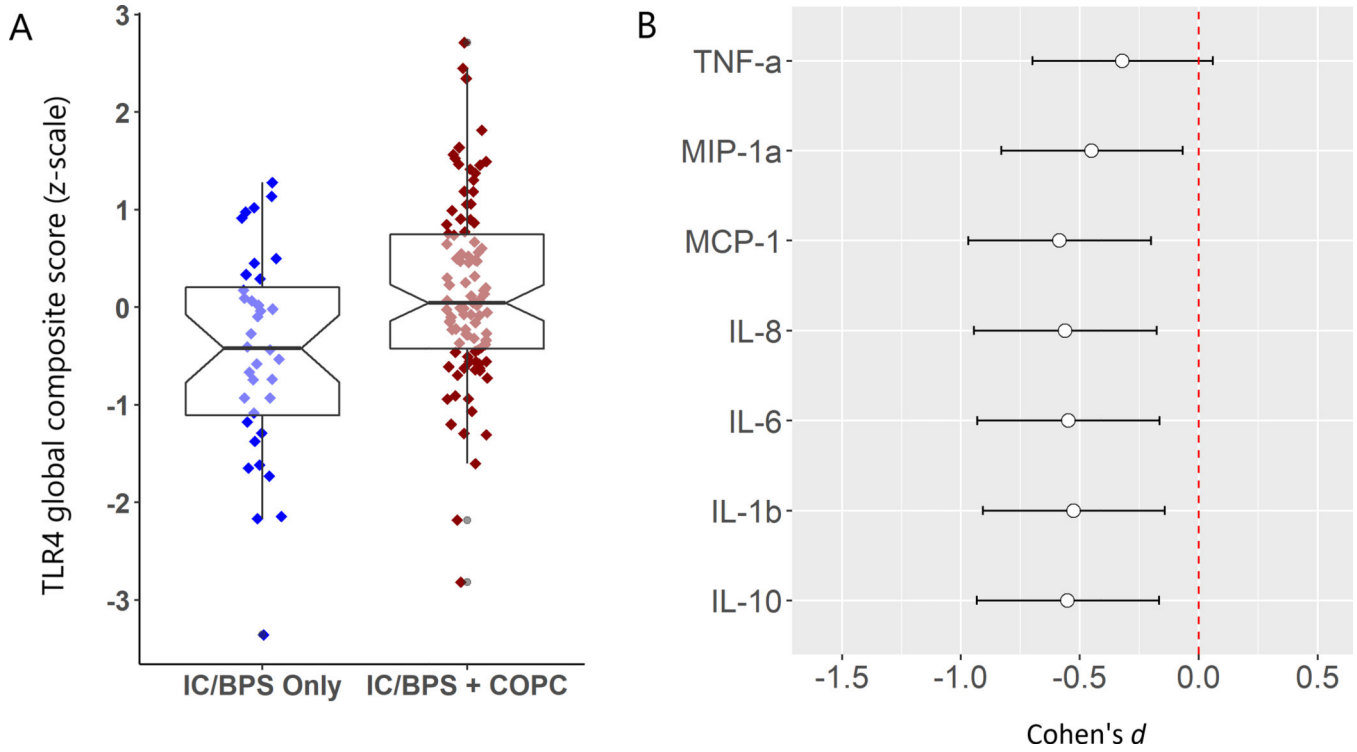

Figure 2.

A. ex-vivo TLR4 global composite score for female IC/BPS patients stratified by presence (n=99) or absence (n=36) of COPCs. B. Cohen's *d* effect sizes for individual cytokines/ chemokines on the transformed scale associated with IC/BPS only status.

Table 1.

UCPPS symptoms and distribution of COPC types by COPC status.

	IC/BPS only(n=36)		IC/BPS+ COPC(n=99)		All(n=135)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
age	47.21	16.32	40.97	14.17	42.63	14.97
Body mass index	25.64	5.31	26.96	5.46	26.61	5.43
Age of symptom onset	32.03	15.86	27.64	14.66	28.82	15.06
Genitourinary pain severity	9.26	5.16	13.48	6.15	12.35	6.17
Urinary symptom severity	11.68	3.87	16.45	5.18	15.18	5.29
	n	%	n	%		
Chronic Overlapping Pain Conditions						
Fibromyalgia	0	0	10	10.1		
Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome	0	0	25	25.3		
Irritable Bowel Syndrome	0	0	57	57.6		
Temporomandibular Disorder	0	0	46	46.5		
Migraine	0	0	53	53.5		

Table 2.

Unstimulated and LPS-stimulated cytokine values by COPC status.

	IC/BPS only(n=36)		IC/BPS+ COPC(n=99)		All(n=135)	
	median	**25th – 75thpercentile**	**median**	**25th – 75thpercentile**	**median**	**25th – 75thpercentile**
Unstimulated (pg/ml)						
monocyte chemoattractant protein-1	118	78–156	103	78–148	109	78–151
macrophage inflammatory protein 1-alpha[a]	58	36–136	36	36–108	36	36–112
Interleukin-1β	1	1–2	1	<1–3	1	<1–3
Interleukin-6[a]	1	1–1	1	1–1	1	1–1
Interleukin-8	37	23–68	37	37–79	37	37–77
Interleukin-10[a]	<1	<1−<1	<1	<1−<1	<1	<1−<1
Tumor necrosis factor – α	4	3–5	5	5–6	4	4–6
Stimulated (pg/ml) (						
monocyte chemoattractant protein-1	1182	896–1658	1715	1033–2542	1526	1000–2310
macrophage inflammatory protein 1-alpha	38497	23818–58503	48184	37793–75389	45923	33951–72730
Interleukin-1β	5427	4195–9035	8982	4686–14479	8309	4316–8309
Interleukin-6	14605	10209–21923	21218	15739–25878	20244	14374–25815
Interleukin-8	11793	7197–15349	14812	9316–20675	12550	9004–19368
Interleukin-10	53	24–83	68	41–107	65	37–98
Tumor necrosis factor – α	3807	2294–5648	4448	2899–5591	4123	2715–5591

[a] > than 25% of values below limit of quantification

Table 3.

General linear model estimates for relationship between pain variables and TLR4 stimulated composite scores.

TLR4 global composite score					
	estimate	**S.E.**	**df**	**t value**	**p**
(Intercept)	−0.670	0.481	110.343	−1.393	0.167
Age (years)	0.003	0.006	130.289	0.451	0.653
BMI	0.003	0.015	129.671	0.177	0.860
COPC (yes)	0.608	0.188	128.939	3.232	0.002
(Intercept)	−0.319	0.479	97.096	−0.667	0.506
Age (years)	−0.001	0.006	129.277	−0.160	0.873
BMI	0.005	0.016	128.691	0.341	0.734
Body map sites	0.071	0.032	125.837	2.258	0.026
(Intercept)	−0.070	0.553	113.500	−0.127	0.899
Age (years)	−0.001	0.006	130.400	−0.112	0.911
BMI	0.008	0.016	129.700	0.516	0.607
Pain50	−0.048	0.069	129.600	−0.695	0.489
(Intercept)	−0.574	0.518	111.100	−1.107	0.270
Age (years)	0.000	0.006	130.400	0.054	0.957
BMI	0.004	0.016	129.600	0.268	0.789
Pelvic Pain Severity	0.026	0.016	127.900	1.613	0.109
TLR4 anti-inflammatory, regulatory, chemotactic composite score					
(Intercept)	−1.089	0.494	114.403	−2.205	0.030
Age (years)	0.006	0.006	130.623	1.024	0.308
BMI	0.025	0.016	129.622	1.599	0.112
COPC (yes)	0.263	0.195	128.789	1.348	0.180
(Intercept)	0.962	0.474	114.361	−2.031	0.045
Age (years)	0.004	0.006	129.988	0.734	0.464
BMI	0.026	0.016	129.194	1.637	0.104
Body map sites	0.054	0.032	125.527	1.686	0.094
(Intercept)	−1.845	0.520	124.280	−3.549	< 0.001
Age (years)	0.004	0.006	128.286	0.792	0.430
BMI	0.030	0.015	130.722	2.007	0.047
Pain50	0.233	0.066	130.970	3.507	< 0.001
(Intercept)	−0.897	0.517	121.466	−1.734	0.085
Age (years)	0.005	0.006	130.977	0.784	0.434
BMI	0.028	0.016	129.973	1.747	0.083
Pelvic Pain Severity	−0.001	0.016	127.865	−0.080	0.936

TLR4 global composite score					
	estimate	**S.E.**	**df**	**t value**	**p**
TLR4 z-score (IL-6+IL-1β)					
(Intercept)	−0.171	0.491	93.193	−0.349	0.728
Age (years)	−0.001	0.006	129.598	−0.183	0.855
BMI	−0.008	0.015	128.996	−0.533	0.595
COPC (yes)	0.491	0.188	128.121	2.606	0.010

Table 4.

General linear model estimates for COPC status and unstimulated cytokines/chemokines.

unstimulated MCP1					
(Intercept)	2.272	0.069	124.194	32.945	<0.001
Age (years)	0.002	0.001	130.995	2.483	0.014
BMI	−0.001	0.002	130.323	−0.509	0.611
COPC (yes)	0.017	0.028	129.987	0.605	0.546
unstimulated IL-8					
(Intercept)	1.967	0.118	131.000	16.648	<0.001
Age (years)	0.000	0.001	131.000	0.252	0.801
BMI	0.002	0.004	131.000	0.575	0.567
COPC (yes)	−0.013	0.048	131.000	−0.276	0.783
unstimulated IL-1β					
(Intercept)	−0.402	0.490	131.000	−0.821	0.413
Age (years)	0.002	0.006	131.000	0.308	0.759
BMI	0.015	0.016	131.000	0.930	0.354
COPC (yes)	−0.017	0.198	131.000	−0.084	0.933
unstimulated TNFα					
(Intercept)	1.093	0.198	110.610	5.511	0.000
Age (years)	0.003	0.002	130.295	1.099	0.274
BMI	−0.003	0.006	129.807	−0.406	0.685
COPC (yes)	0.047	0.077	129.180	0.611	0.542