# Correlation Between Altered Central Pain Processing and Concentration of Peritoneal Fluid Inflammatory Cytokines in Endometriosis Patients With Chronic Pelvic Pain

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**Abstract:** Translational research has not yet elucidated whether alterations in central pain processes are related to peripheral inflammatory processes in chronic pain patients. We tested the hypothesis that the concentration of cytokines in the peritoneal fluid of endometriosis patients with chronic pain correlate with parameters of hyperexcitability of the nociceptive system. The concentrations of 15 peritoneal fluid cytokines were measured in 11 patients with chronic pelvic pain and a diagnosis of endometriosis. Six parameters assessing central pain processes were recorded. Positive correlations between concentration of some cytokines in the peritoneal fluid and amplification of central pain processing were found. The results suggest that inflammatory mechanisms may be important in the pathophysiology of altered central pain processes and that cytokines produced in the environment of endometriosis could act as mediators between the peripheral lesion and changes in central nociceptive processes.

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Chronic pelvic pain in endometriosis is associated with generalized hyperexcitability of the central nervous system, leading to pain amplification.[1] Generalized hyperexcitability—considered an alteration in the processing of the nociceptive signal in the central nervous system—causes amplification of pain and is probably an important factor in the induction and maintenance of chronic pain.[2]

Endometriosis is associated with an inflammatory peritoneal environment, where multiple cytokines and growth factors are found to be elevated in the peritoneal fluid.[3] Sensitization of peripheral nociceptors by inflammatory cytokines causes neurotransmitter release from central terminals in the spinal cord and may lead to enhanced spinal nociceptive transmission and central sensitization.[4]

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The mechanisms by which deep infiltrating endometriosis lesions cause widespread hyperalgesia and the interrelationships between central hypersensitivity and inflammation of peripheral tissues are unclear. The present investigation tested the hypothesis that the concentration of inflammatory cytokines in the peritoneal fluid in endometriosis patients with chronic pain correlate with parameters of generalized hyperexcitability of the nociceptive system. The results would provide insights into the possible role of inflammatory mediators for maintaining enhanced central nociceptive processing.

## METHODS

The study was prospective and approved by the local ethics committee. All subjects gave written informed consent.

Consecutive patients undergoing laparoscopic surgery for endometriosis were recruited at the Department for Gynaecology of the University Hospital of Bern, Inselspital, between May 2007 and November 2009. The inclusion criteria were histologically confirmed diagnosis of endometriosis, pelvic pain with an intensity of at least 4 using a 10-cm visual analog scale (VAS) (0, no pain and 10, worst pain imaginable), and daily pelvic pain of at least 6 months duration. Exclusion criteria were peripheral or central neurological disorders, diabetes mellitus, pregnancy (as ruled out by pregnancy test), breastfeeding, intake of oral contraceptives or hormones, and intake of opioids during the last 2 weeks or of other analgesics during the 24 hours before testing. These patients have been analyzed in a previous study on altered central pain processing in endometriosis patients.[1] In case of previous abdominal surgery, current surgery was performed after a minimum interval of 3 months.

Age, height, weight, and body mass index were recorded. Pain intensity at the time of testing and maximum pain intensity ever experienced were determined by the VAS. The intensity of pain during menstruation, intercourse, and lower abdominal pain were measured using the numerical rating scale (NRS), whereby 0, no pain and 10, worst pain imaginable. The participants were asked to complete 4 questionnaires, namely, Beck Depression Inventory, State Trait Anxiety Inventory, Catastrophizing Scale of the Coping Strategies Questionnaire, and Short-Form 36, which were described in details in our previous investigation in patients with endometriosis.[1] All records, including self-report questionnaires, were done before surgery. Their aim was descriptive.

Immediately after laparoscopic entry into the abdomen, a minimum volume of 200 μL of peritoneal fluid was aspirated quantitatively, clarified by centrifugation, and stored at −35°C in aliquots. The concentrations of interleukin 8, tumor necrosis factor α (TNF-α), pregnancy-associated plasma protein A, glycodelin-A (PP-14), leptin, RANTES, epithelial neutrophil-activating protein 78, osteoprotegerin (OPG), midkine, macrophage colony stimulating factor-1 (MCP-1), interferon-inducible protein 10, ficolin-2 lectin, neutrophil defensins (HNP1–3), human

**TABLE 1.** Descriptive Characteristics of Included Participants and of Electrical Pain Tests

| | Mean (SD) | Median | Range |
|---|---|---|---|
| Characteristics of participants (n = 11) | | | |
| Age, y | 30 (6) | 31 | 18–37 |
| Height, cm | 162 (8) | 160 | 154–175 |
| Weight, kg | 54 (6) | 57 | 45–62 |
| Body mass index, kg/m$^2$ | 20.8 (2.6) | 20.4 | 17.0–25.8 |
| Beck Depression Inventory (score 0–63) | 7 (5) | 5 | 1–17 |
| STAI State (score 20–80) | 46 (9) | 45 | 31–61 |
| STAI Trait (score 20–80) | 39 (11) | 35 | 20–57 |
| CSQ Catastrophizing (score 0–6) | 3.4 (1.2) | 3.5 | 1.8–5.7 |
| Short-Form 36—Physical Health (0–100) | 55 (16) | 53 | 30–84 |
| Short-Form 36—Mental Health (0–100) | 64 (18) | 63 | 34–85 |
| Short-Form 36—Total (0–100) | 61 (16) | 56 | 36–85 |
| Pain rating (n = 11) | | | |
| Pain immediately before testing (VAS 0–10) | 5.3 (1.4) | 5.2 | 4.0–7.0 |
| Maximal pain (VAS) | 8.1 (1.7) | 7.9 | 5.3–10.0 |
| Menstrual pain (NRS 0–10) | 7 (3) | 7 | 3–10 |
| Pelvic pain (NRS) | 5 (1) | 5 | 4–7 |
| Intercourse pain (NRS) | 3 (2) | 3 | 0–6 |
| Pain thresholds to electrical stimulation (n = 11) | | | |
| Single stimulation, mA | 6.2 (1.7) | 6.0 | 4.0–9.0 |
| Repeated stimulation, mA | 4.9 (1.2) | 5.0 | 3.0–7.0 |
| Nociceptive withdrawal reflex (n = 11) | | | |
| Single stimulation, mA | 8.3 (1.9) | 9.0 | 5.0–12.0 |
| Repeated stimulation, mA | 5.0 (1.4) | 5.0 | 3.0–7.0 |
| Reflex receptive field (n = 11) | | | |
| Area (proportion of foot sole) | 0.45 (0.16) | 0.45 | 0.21–0.74 |
| Volume, μV * mm$^2$ | 0.40 (0.40) | 0.18 | 0.07–1.29 |

CSQ indicates Coping Strategies Questionnaire; NRS, numerical rating scale; STAI, State Trait Anxiety Inventory.

**TABLE 2.** Correlation Between Tests of Altered Central Pain Processing and Peritoneal Fluid Cytokine Concentrations (Spearman Rank-Order Correlation)

| | | Interleukin 8 | TNF-α | Pregnancy-Associated Plasma Protein A | Glycodelin (PP14) | Leptin | RANTES | Epithelial Neutrophil-Activating Protein 78 |
|---|---|---|---|---|---|---|---|---|
| Pain thresholds to electrical stimulation | | | | | | | | |
| Single electrical stimulation | Sperman ρ | −0.48 | −0.34 | −0.26 | 0.17 | −0.03 | −0.38 | −0.01 |
| | *P* | 0.124 | 0.331 | 0.433 | 0.607 | 0.903 | 0.233 | 0.968 |
| Repeated electrical stimulation | Sperman ρ | −0.36 | −0.62 | 0.11 | −0.11 | 0.12 | 0.15 | 0.36 |
| | *P* | 0.270 | 0.067 | 0.734 | 0.733 | 0.714 | 0.633 | 0.388 |
| Nociceptive withdrawal reflex | | | | | | | | |
| Single electrical stimulation | Sperman ρ | −0.45 | −0.31 | −0.09 | −0.23 | 0.20 | −0.05 | 0.21 |
| | *P* | 0.159 | 0.407 | 0.776 | 0.490 | 0.538 | 0.860 | 0.602 |
| Repeated electrical stimulation | Sperman ρ | 0.16 | **−0.81** | −0.09 | 0.02 | −0.21 | 0.37 | 0.58 |
| | *P* | 0.614 | **0.004** | 0.755 | 0.919 | 0.755 | 0.257 | 0.150 |
| ***Reflex receptive field*** | | | | | | | | |
| Area | Sperman ρ | −0.08 | 0.12 | −0.21 | **0.65** | −0.22 | 0.24 | 0.32 |
| | *P* | 0.797 | 0.948 | 0.520 | **0.049** | 0.502 | 0.467 | 0.438 |
| Volume | Sperman ρ | 0.23 | 0.03 | −0.36 | **0.77** | −0.28 | 0.07 | 0.23 |
| | *P* | 0.484 | 0.913 | 0.270 | **0.006** | 0.384 | 0.818 | 0.545 |

Statistically significant correlations ($P < 0.05$) are bold.

epididymal protein-4, and carcinoma antigen-125 (CA-125) were determined by manual microplate ELISA in batches.

Patients were tested between the 4th and 14th day of the menstrual cycle, 12 to 18 hours before surgery. Single electrical stimulation, repeated electrical stimulation (temporal summation), and tests for reflex receptive fields were performed by a single investigator (A.Y.N.). Testing procedures were described in details in Table, Supplemental Digital Content 1, http://links.lww.com/AAP/A110. During testing, the volunteers were lying on a bed, in a quiet room. A leg rest was placed under the knees to obtain a 30-degree semiflexion during testing. Each subject underwent a training session to get familiar with the stimulation procedure before starting data collection.

The lower the thresholds to pain or reflex responses for single and repeated electrical stimulation, the higher the state of central hyperexcitability. The larger the RRF area or volume, the higher the augmentation of central nociceptive processes.

## Data Analysis

Correlations between parameters of altered central pain processes and peritoneal fluid cytokine concentrations were determined using Spearman rank-order correlation. All statistical analyses were performed in IBM SPSS Statistics 19 (IBM Software, Business Analytics, IBM Corporation, Armonk, New York). $P < 0.05$ was considered as significant.

# RESULTS

Twenty consecutive patients with histological diagnosis of endometriosis and experiencing chronic pelvic pain were included and examined. Nine of the peritoneal fluid samples collected were quantitatively insufficient to determine the cytokine concentrations, leaving 11 patients for the analyses (18–37 years old). A comparison in patient's characteristics between included (n = 11) and excluded (n = 9) patients who did not reveal signs of selection bias (see Table, Supplemental Digital Content 1, http://links.lww.com/AAP/A110). The volume of peritoneal fluid aspirated was 0.38 to 46 mL. Descriptive characteristics of these patients are shown in Table 1. Eight of the 11 patients analyzed had previous pelvic surgery. The interval between previous and current surgery was 3 months (1 patient), 4 months (4 patients), 18 months, 3 years, and 6 years (1 patient for each category). Table 1 presents also summary statistics of the pain tests.

The correlations between measurements of central pain processes and concentrations of cytokines are shown in Table 2. Pain threshold to single electrical stimulation correlated negatively with OPG (Spearman $\rho = -0.81$, $P < 0.001$); that is, the higher the OPG concentration, the higher the magnitude of central pain sensitivity, and vice versa. No correlation was observed between reflex threshold to single electrical stimulation and the concentration of any cytokine tested here.

Temporal summation reflex threshold correlated negatively with TNF-α ($\rho = -0.81$, $P = 0.004$); that is, the higher the TNF-α concentration, the higher the magnitude of central hyperexcitability, and vice versa. This correlation is illustrated in Figure 1. There was no correlation between temporal summation pain threshold and peritoneal fluid cytokines.

Reflex receptive field area correlated positively with PP-14 ($\rho = 0.65$, $P = 0.049$) and with ficolin-2 ($\rho = 0.74$, $P = 0.028$); that is, the higher the concentration of PP-14 and ficolin-2 lectin, the higher the alteration of central pain processing. PP-14 ($\rho = 0.77$, $P = 0.006$), MCP-1 ($\rho = 0.60$, $P = 0.046$), and CA-125 ($\rho = 0.88$, $P = 0.033$) displayed a positive correlation with reflex receptive field volume, indicating again a positive association between the concentration of these cytokines and altered central pain processing.

# DISCUSSION

In the present trial in endometriosis patients with chronic pelvic pain, the concentrations of 15 peritoneal fluid inflammatory cytokines and growth factors were measured, together with

**TABLE 2.** (continued)

| OPG | Midkine | MCP-1 | Interferon-inducible Protein 10 | Ficolin-2 Lectin | Defensin | Human Epididymal Protein-4 | CA-125 |
|---|---|---|---|---|---|---|---|
| **−0.81** | −0.17 | −0.22 | −0.37 | 0.00 | −0.17 | 0.58 | −0.42 |
| **<0.001** | 0.595 | 0.512 | 0.245 | 0.977 | 0.814 | 0.242 | 0.213 |
| −0.43 | 0.09 | −0.17 | −0.30 | 0.19 | −0.09 | −0.17 | −0.27 |
| 0.178 | 0.968 | 0.595 | 0.372 | 0.619 | 0.803 | 0.740 | 0.425 |
| −0.40 | 0.10 | −0.14 | −0.31 | −0.09 | −0.17 | −0.147 | −0.49 |
| 0.210 | 0.776 | 0.681 | 0.339 | 0.794 | 0.71 | 0.714 | 0.137 |
| −0.52 | 0.22 | −0.12 | −0.35 | −0.06 | 0.25 | −0.17 | 0.01 |
| 0.088 | 0.502 | 0.707 | 0.270 | 0.839 | 0.658 | 0.714 | 0.946 |
| 0.12 | −0.10 | 0.21 | −0.11 | **0.74** | 0.09 | −0.26 | 0.21 |
| 0.693 | 0.755 | 0.535 | 0.734 | **0.028** | 0.919 | 0.658 | 0.535 |
| −0.31 | −0.05 | **0.60** | −0.17 | 0.24 | 0.60 | 0.54 | **0.88** |
| 0.324 | 0.860 | **0.046** | 0.595 | 0.537 | 0.242 | 0.297 | **0.033** |

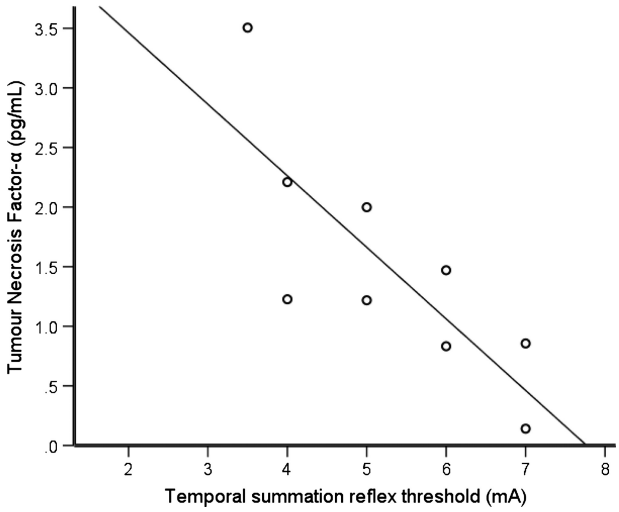


**FIGURE 1.** Correlation between temporal summation reflex threshold and concentration of TNF-α in the peritoneal fluid (Spearman ρ = –0.81, $P = 0.004$).

6 parameters assessing central pain processes. Among the markers tested, TNF-α, glycodelin (PP-14), OPG, MCP-1, ficolin-2 lectin, and CA-125 presented a statistically significant correlation with measures of altered central pain processes.

Previous studies showed evidence of correlation between elevated concentration of cytokines and reported daily pain in patients with endometriosis.[5] An investigation conducted on 41 patients with histologically confirmed endometriosis documented an association between the concentration of inflammatory cytokines in the peritoneal fluid and the intensity of pain during menstruation, intercourse, and lower abdominal pain. Patients with severe dysmenorrhea had increased levels of TNF-α and glycodelin, which correlated positively with reported daily pain when compared with pain-free controls, suggesting that TNF-α and glycodelin may play a role in endometriosis and the severity of menstrual pain.[5] In contrast, another study failed to detect an association between concentration of peritoneal fluid cytokines and reported pain in 43 patients with endometriosis.[3]

In a previous investigation, we observed an exaggerated reflex receptive field area and decreased pain and nociceptive withdrawal reflex thresholds in endometriosis patients, as compared with pain-free controls.[1] The findings of that study confirmed the previous observed association of chronic pelvic pain in endometriosis patients with hypersensitivity to painful stimuli,[6] but did so by using objective measurements of central nervous system hyperexcitability. An earlier study found that endometriosis was associated with elevated concentrations of multiple inflammatory cytokines and growth factors in the peritoneal fluid.[3]

It is well known that high concentrations of inflammatory cytokines may cause sensitization of peripheral nociceptors, which leads to neurotransmitter release in the spinal cord and enhanced central nociceptive transmission.[4] The present study suggests that there could be a link between peripheral production of cytokines and central hyperexcitability in the nociceptive system. It is noteworthy that measures of central hyperexcitability included both electrophysiological and psychophysical methods, and hence did not rely entirely on subjective pain reports, therefore representing objective correlates of nociceptive processes. The receptive field model was associated with most of the significant correlations, suggesting that models assessing receptive fields may at best capture the link between inflammation and central hypersensitivity. Accordingly, future studies with higher sample sizes may include this model to maximize the chances to obtain positive results.

We are not aware of previous studies that correlated parameters of central pain processes with the concentration of cytokines and growth factors in the peritoneal fluid in endometriosis patients. Hence, the present investigation provides the first evidence of a possible association between these 2 phenomena. This study was performed in consecutive patients by a single assessor according to a standardized protocol.

The main limitation of this study is the low number of analyzed samples of peritoneal fluid. Obtaining a good sample of the fluid is often difficult, as the volume may be insufficient for the required number of analyses or the sample is diluted with flush medium during the laparoscopic procedure. Moreover, hemolyzed fluids cannot be used for cytokine determinations. Cytokines are pleiotropic and produced by different tissues at the same time, with synergetic or opposing actions and varying half-lives of the biologically active form.[3] The present study analyzed the correlation between measures of central pain processes and the concentration of 15 different markers, of which fewer than half showed a significant association. Therefore, we cannot rule out that part of the positive effects was the result of chance. The analysis was not corrected for multiple comparisons, which may have produced false-positive results. Future studies with larger sample sizes may provide further insights.

In conclusion, the results of the present study suggest that inflammatory mechanisms may be important in the pathophysiology of altered central pain processes, and that cytokines produced in the environment of endometriosis could act as mediators between the peripheral lesion and changes in central nociceptive processes.

## REFERENCES

1. Neziri AY, Haesler S, Petersen-Felix S, et al. Generalized expansion of nociceptive reflex receptive fields in chronic pain patients. *Pain*. 2010;151:798–805.
2. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. *Pain*. 2011;152(suppl 3):S2–S15.
3. Overton C, Fernandez-Shaw S, Hicks B, Barlow D, Starkey P. Peritoneal fluid cytokines and the relationship with endometriosis and pain. *Hum Reprod*. 1996;11:380–386.
4. Junger H, Sorkin LS. Nociceptive and inflammatory effects of subcutaneous TNFalpha. *Pain*. 2000;85:45–51.
5. Scholl B, Bersinger NA, Kuhn A, Mueller MD. Correlation between symptoms of pain and peritoneal fluid inflammatory cytokine concentrations in endometriosis. *Gynecol Endocrinol*. 2009;25:701–706.
6. Bajaj P, Madsen H, Arendt-Nielsen L. Endometriosis is associated with central sensitization: a psychophysical controlled study. *J Pain*. 2003;4:372–380.