# Circulating Cytokine Levels Compared to Pain in Patients with Fibromyalgia — A Prospective Longitudinal Study Over 6 Months

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*ABSTRACT.* ***Objective.*** This prospective study examined circulating cytokines in patients with fibromyalgia (FM) over 6 months rather than at only one timepoint, and investigated correlations between serum cytokine concentrations and pain intensity in FM patients receiving multidisciplinary pain therapy.

***Methods.*** Serum concentrations of proinflammatory cytokines interleukin 6 (IL-6), IL-8, and tumor necrosis factor-α (TNF-α) and antiinflammatory cytokines IL-4 and IL-10 were measured (Bio-Plex system) in 20 FM patients and 80 healthy subjects on admission and 10, 21, and 180 days after initiation of treatment and correlated to pain intensity.

***Results.*** On admission, serum levels of IL-8 ($p < 0.001$) and TNF-α ($p < 0.001$), but not IL-6, were elevated in patients with FM. No significant difference in IL-4 and IL-10 was found between FM patients and controls. High IL-8 levels remained consistent during the followup, but TNF-α was already reduced after 10 days and until 6 months after therapy. After 6 months' treatment with multidisciplinary pain therapy, IL-8 and TNF-α levels were significantly lower than at the beginning ($p < 0.05$ for IL-8, $p < 0.001$ for TNF-α). IL-8 but not TNF-α serum levels were correlated with pain intensity ($r = –0.782$, $p = 0.001$) in FM patients after 6 months' multidisciplinary pain therapy.

***Conclusion.*** Our results suggest that proinflammatory cytokines TNF-α and IL-8 are involved in FM, but they do not apparently provoke the pain of FM directly. Multidisciplinary pain therapy modified the cytokine profile in patients with FM during the observation period. (First Release June 1 2008; J Rheumatol 2008;35:1366–70)

*Key Indexing Terms:*
PROSPECTIVE STUDY FIBROMYALGIA CYTOKINE PROFILE SERUM LEVEL

According to the criteria of the America College of Rheumatology (ACR)[1], fibromyalgia (FM) is characterized by chronic widespread pain in all 4 quadrants of the body, of at least 3 months' duration, associated with tender points and with generalized symptoms of fatigue, aches, and nonrestorative sleep.

The etiology of FM remains elusive. Neurochemicals may have a key role in FM. In several controlled studies, spinal fluid levels of nerve growth factor and substance P were elevated[2], and spinal fluid levels of serotonin products were lowered in patients with FM[3,4].

Although FM is a noninflammatory process, many studies since 1988 have demonstrated that cytokines have a place among proposed paradigms for FM[5]. However, there have been contradictory results[6]. In Sperber's study, serum concentrations of interleukin 1 (IL-1), IL-2, and IL-6 among FM patients did not differ from the levels in healthy controls[7]. Gür and colleagues showed significantly elevated serum IL-8 and IL-2R, but not IL-1 or IL-6[8]. Recently, Üceyler and colleagues have shown lowered IL-4 and IL-10 levels in patients with chronic widespread pain[9] (Table 1).

Since cytokine production and the balance of proinflammatory/antiinflammatory cytokines are controlled by both the immune system[10] and the central nervous system[11,12] following external and/or internal stimuli, we hypothesized that patients with FM might have an imbalance of pro- and antiinflammatory cytokines in sera and that there would be a correlation between the cytokine profile and pain intensity in these patients. We investigated the circulating concentrations of both pro- and antiinflammatory cytokines and their kinetics during a study period of 6 months. We also analyzed the correlations between circulating cytokines and pain intensity in patients with FM.

## MATERIALS AND METHODS

*Study design and patients.* After giving informed consent, 20 patients who fulfilled the ACR criteria for FM (mean age 49.9 ± 6.8 yrs; 85% women, 15% men) were enrolled in this prospective longitudinal study conducted

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over 6 months. Eighty healthy subjects who had no pain disorders or infectious illness at the time of blood sampling served as the control group (mean age 45.5 ± 11.4 yrs; female 54.8%, male 45.2%). The study was approved by the local ethics committee of the University of Heidelberg.

The patients had already undergone all conventional forms of biomedical treatment and had been referred to our clinic owing to failure of standard therapy with requests for admission to a (first-time) biopsychosocial therapy. All patients were seen and examined by the director of the pain center to determine suitability for this therapy regimen. All had to meet strict inclusion and exclusion criteria (Table 2). Inclusion criteria required patients to meet ACR criteria for FM. Exclusion criteria consisted of complex regional pain syndrome, tumor illness (diagnosis from history and by radiographic/magnetic resonance imaging examination), trauma/fracture (diagnosis from history and by radiographic examination), inflammatory systemic disease or infection, other rheumatological disease, serious cardiopulmonary, vascular or other internal medical conditions, or use of medication that may influence the level of cytokines (e.g., oral or local corticosteroids; anticytokine therapy).

*Control group.* Controls comprised 80 healthy persons who did not complain of low back pain or chronic widespread pain during the previous year and did not seek medical help for pain; they were volunteers from the hospital where the study was performed. The same exclusion criteria were applied for the control group.

*Study protocol.* The study period of 6 months was divided into clinical and postclinical treatment periods. Each patient was evaluated at 4 timepoints, each investigation including analysis of blood samples, standardized questionnaires, and physical examinations. The specific timepoints were the beginning of the study, 10 days after the start of treatment, the time of discharge (21 days after the start of treatment), and at the end of 6 months of treatment.

Patients were allocated to a 3-week daily functional multidisciplinary restoration program in the clinic; this had a special emphasis on biopsychosocial factors and involved oral pain medication according to WHO recommendations, with continuous dose tapering. During the study all patients kept records of the medication they used and the pain intensity experienced in the 24 hours before each of the designated examination timepoints using a visual analog scale (VAS).

*Cytokine assays.* At the specified timepoints a standard blood sample was taken from the cubital vein between 9:00 A.M. and 12:00 noon, then centrifuged (2000 rpm) at 4°C, and stored at –80°C. Blood samples were analyzed for serum concentrations of cytokines IL-4, IL-6, IL-8, IL-10, and tumor necrosis factor-α (TNF-α) by a Bio-Plex cytokine assay (Bio-Rad Laboratories, Munich, Germany) according to the manufacturer's instructions. Data were obtained by measuring the median fluorescence intensity of each standard and each patient sample with the Bio-Plex system. Using the Bio-Plex Manager software, serum levels of cytokines were deduced from the standard curve.

*Table 1.* Summary of the literature since 1999 on specific cytokines in patients with fibromyalgia.

| Cytokine | Citation |
|---|---|
| IL-1 | Serum level normal[7,8,18,19] |
| IL-1r | Serum and production levels are increased[18,20] |
| IL-2 | Serum level normal[7] or increased[8] |
| IL-4 | Serum level decreased[9] |
| IL-6 | Serum level normal[7,8] |
| IL-8 | Increased[8,18,22] or normal[9] |
| IL-10 | Serum level decreased[9] |
| TNF-α | Serum level normal[18,19] |
| Interferon-γ | Serum level normal[18] |

IL: interleukin; TNF: tumor necrosis factor.

*Table 2.* Clinical data at time of admission.

| | Patients (n = 20) | Controls (n = 80) |
|---|---|---|
| Mean age, yrs ± SD | 49.9 ± 6.8 | 45.4 ± 11.4 |
| Women, % | 85 | 43.3 |
| Body mass index, mean (range), kg/m$^2$ | 22.1 (16.3–39.5) | 27.1 (18.7–47.8) |
| Current smoker, % | 20 | 31.5 |
| Duration of pain | 9.7 (1–36) | |
| Mean pain level within last week, visual analog scale 0–10 (SD) | 6.3 (± 2.0) | / |
| Functional disability (Roland and Morris; 0: best function; 24 lowest function), mean (SD) | 13.3 (± 5.9) | / |
| Mean duration of pain before entering study, mos ± SD | 20.4 ± 37.7 | / |

SD: standard deviation

*Statistical analysis.* SPSS 14.0 was used for all analyses. The Mann-Whitney test was used to compare groups. Pearson and Spearman correlations and receiver-operating characteristic curve analysis were calculated for the dichotomized variables to measure agreement between cytokines and pain at different timepoints.

## RESULTS

*Serum cytokine levels in FM.* Before therapy, circulating concentrations of the proinflammatory cytokines TNF-α and IL-8 were significantly higher in patients with FM than in healthy controls (both $p < 0.001$). At Day 10 after the initiation of therapy, the elevated TNF-α level in FM had normalized, but IL-8 levels remained significantly higher in FM patients than in controls. This cytokine profile was unchanged at 21 days and 180 days after the multidisciplinary pain therapy. Serum levels of IL-6 were found not to differ between patients and controls at any timepoint.

Serum levels of the antiinflammatory cytokines IL-4 and IL-10 in FM patients remained the same as those of healthy controls throughout the study (Figure 1).

*Time course of serum cytokine levels during the 6-month study period.* During the 6-month study, serum levels of the proinflammatory cytokines IL-8 and TNF-α declined significantly from Day 0 to Day 10 ($p = 0.023$ for IL-8, $p = 0.001$ for TNF-α), and stayed constant from Day 10 through Day 21 to Day 180. There were no changes in serum levels of the proinflammatory cytokine IL-6 or the antiinflammatory cytokines IL-4 and IL-10 at any point. At the end of the study circulating concentrations of IL-8 and TNF-α were significantly lower than at the beginning ($p = 0.013$ for IL-8, $p = 0.005$ for TNF-α; Figure 2).

*Correlation between serum cytokine levels and pain intensity.* The average pain intensity during the previous 24 hours was determined from recordings made by all patients on a VAS ranging from 0 (no pain) to 10 (severe pain). Correlations between pain intensity and serum cytokine levels were analyzed; no correlation was observed between

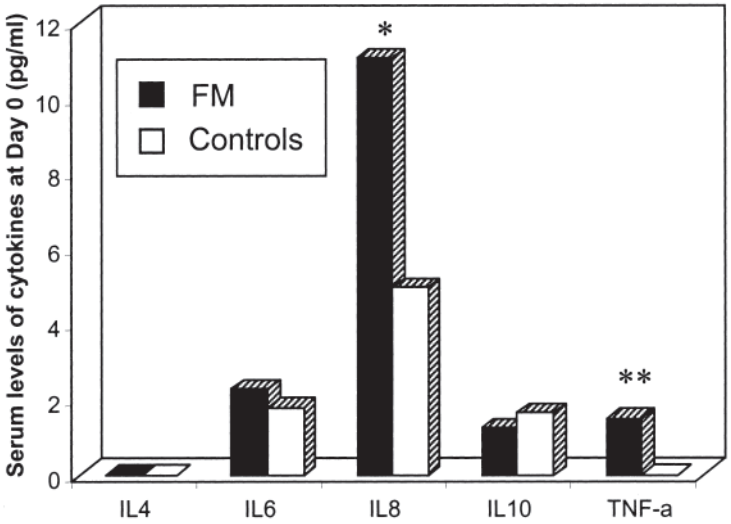


*Figure 1.* Serum levels of proinflammatory cytokines IL-6, IL-8, and TNF-α and anti-inflammatory cytokines IL-4 and IL-10 in patients with FM and controls on admission. IL-8 and TNF-α levels were significantly higher in FM than in controls. *$p < 0.05$ for IL-8; **$p < 0.001$ for TNF-α. Serum levels of IL-6, IL-10, and IL-4 did not differ significantly between patients and controls.

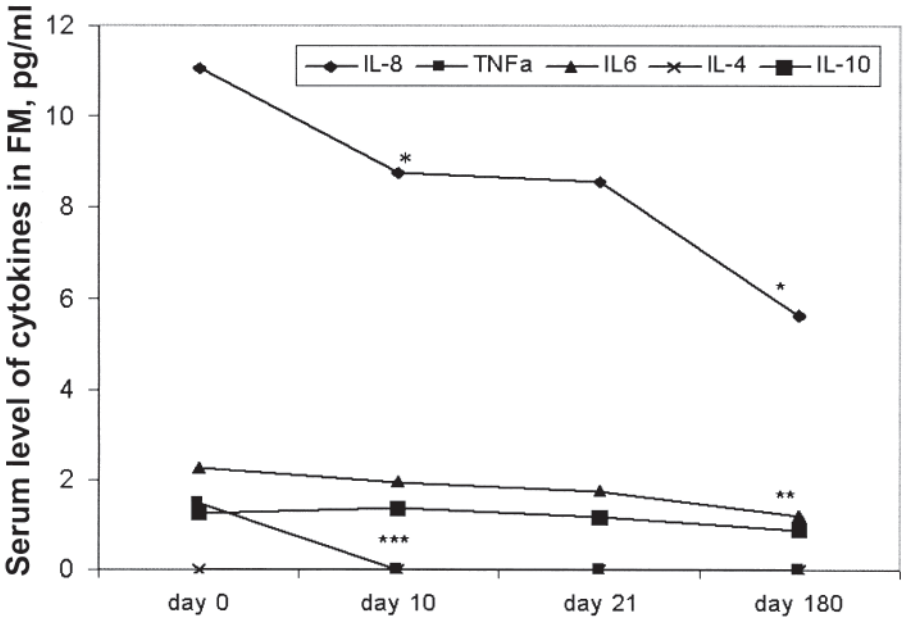


*Figure 2.* Time course of cytokines assayed at Days 0, 10, and 21 and at 6 months. Serum levels of proinflammatory cytokines IL-8 and TNF-α declined significantly from Day 0 to Day 10 ($p < 0.05$). Compared with Day 0, there were no significant differences in serum levels of IL-8 or TNF-α at Day 21. At the end of the study serum levels of both IL-8 and TNF-α were significantly reduced ($p = 0.013$ for IL-8, $p = 0.005$ for TNF-α). Serum levels of IL-4, IL-6, and IL-10 did not change over the course of the study. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

pain intensity and serum levels of IL-4, IL-6, IL-10, or TNF-α at any timepoint; only at the end of the study did the circulating IL-8 level correlate with mean pain intensity ($r = –0.784$, $p = 0.001$) in the previous 24 hours (Figure 3).

## DISCUSSION

The aim of our study was to investigate the kinetic profile of cytokines in FM with reference to a multidisciplinary pain therapy, and to determine any correlations between pain

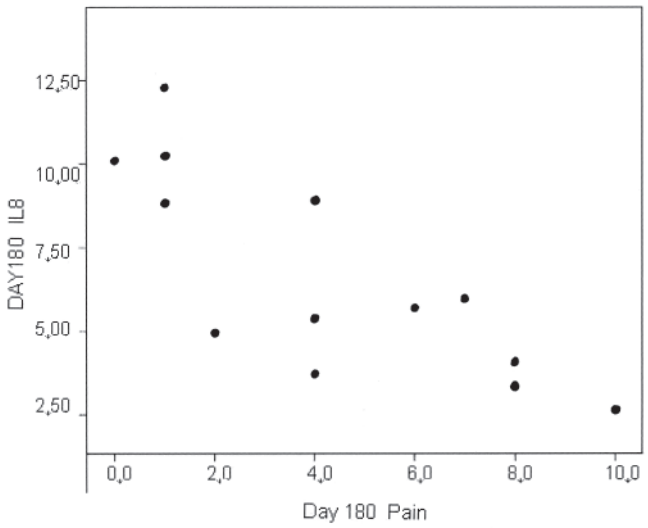


*Figure 3.* Correlation between serum IL-8 level and pain intensity. Average pain intensity of all patients during the previous 24 hours was determined from recordings ranging from 0 (no pain) to 10 (severe pain) on a VAS. At 6 months after start of multidisciplinary pain therapy a correlation was found between pain intensity and IL-8 serum levels (r = –0.784, p = 0.001).

intensity and circulating cytokines in patients with FM. For the first time, a prospective longitudinal investigation has been made of different cytokine profiles in patients with FM. We observed that serum levels of the proinflammatory cytokines TNF-α and IL-8, but not IL-6, were significantly higher in patients with FM than in healthy controls. But serum levels of the antiinflammatory cytokines IL-4 and IL-10 did not differ from those in controls. After 6 months of multidisciplinary pain therapy, serum levels of TNF-α and IL-8 were significantly reduced compared with the levels before therapy. A correlation between IL-8 and pain intensity was observed.

It has been proposed that FM is due to neurogenic inflammation induced by an inflammatory response to allergens, infectious agents, irritants, chemical exposures, or emotional stress[13]. There has been much research into the pathogenesis of FM, and especially into the role of cytokines in FM, in the last 20 years. The results have been highly contradictory. Several studies have shown elevated levels of the inflammatory transmitter substance P (SP) and of calcitonin gene-related peptide (CGRP) in the spinal fluid of patients with FM[14-16]. The levels of platelet serotonin were also abnormal in FM[17]. Wallace, *et al* postulated increased serum levels of IL-1R and IL-8, but not IL-1ß, IL-2, IL-10, sIL-2R, or interferon-r, in FM[18]. Gür, *et al* showed higher serum levels of IL-8 and IL2R, but not IL-1 or IL-6, in FM[8]. Üceyler, *et al*, in contrast, showed that while proinflammatory cytokines IL-2, IL-8, TNF-α, and transforming growth factor-ß1 did not increase, the antiinflammatory cytokines IL-4 and IL-10 were lower in FM patients than in controls[9]. An investigation by Sarchielli, *et al* into glial cell line-derived neurotrophic factor (GDNF) and somatostatin levels in cerebrospinal fluid (CSF) of patients with FM showed a significant positive correlation between CSF values of GDNF and those of somatostatin ($r = 0.68$, $p < 0.008$). However, no previous study has tracked the changes in cytokine concentrations in patients with FM over a given time period.

We found elevated IL-8 and TNF-α levels in the serum of patients with FM. Thus, we confirmed a high circulating level of IL-8 in FM, as in previous studies[8,18]. We also found that serum levels of IL-6 in FM patients did not differ from those in controls, consistent with results reported in other studies. The discrepancy between our results on IL-4 and the findings recorded in Üceyler's study may be due to the small number of patients in our study.

In addition, we observed the kinetic course of these cytokines prospectively over 6 months. While the pain intensity declined from Day 0 through Day 21 to Day 180, the circulating concentration of IL-8 remained constant from Day 0 to Day 21 during the pain therapy, with no development in parallel with that of pain. This suggests that IL-8 is not the direct cause of the pain in FM — there was no correlation between an elevated IL-8 level and pain intensity at the beginning of the study before the multidisciplinary pain therapy was started; only at the end of the study did the IL-8 level show a correlation with pain intensity. Why there was no correlation between IL-8 and pain intensity at the beginning of therapy but there was at the end is not clear. We surmise that this may be an epiphenomenon, or that elevation of IL-8 may be, not causative in FM, but instead, secondary to other factors such as medication; and further studies should be devoted to possible mechanisms for this. Serum TNF-α levels were elevated on admission and had normalized after 6 months of multidisciplinary pain therapy, paralleling the decline in pain intensity, yet there was no correlation between serum TNF-α and pain intensity, indicating that TNF-α also was not the cause of the pain in FM. Apart from the lack of any correlations between any pairs of IL-6, IL-10, and pain intensity, we assume that these cytokines do not directly cause pain in FM. There must be another means or mechanism triggering the pain in FM; or pain medication may interfere with the cytokine profile. Further large-scale studies are needed on this subject.

The clinical relevance of IL-8 and/or TNF-α in FM remains uncertain, especially in evaluation of a treatment strategy based on the cytokine results of our study and in others.

The origin of FM is multifactorial; psychosocial, psychosomatic, and psychological patterns combine and have important roles in FM. A multidisciplinary pain therapy seems to be more effective than a conventional medical therapy alone for these patients (unpublished data).

In the interpretation of our results, a number of possible confounding factors have to be considered. First, the patient population was too small; our results should be confirmed in

a larger patient population. Second, concomitant diseases may influence cytokine expression profiles. Data in one of our unpublished studies show that depression stimulates the production of some cytokines. Third, the duration of pain, which was not considered here, may play a part in the expression profile of cytokines. Finally, we did not investigate the mRNA expression of cytokines in the skin of FM patients. This would yield information about the cytokine profile in both sera and skin in FM patients.

We assume that the combination of elevated serum levels of TNF-α and IL-8 contributes to the pathogenesis of FM. There is a dysregulation of proinflammatory cytokines in FM. However, the serum levels of these cytokines seemed not to influence the pain intensity of FM patients directly. Thus, TNF-α and IL-8 cannot be used as predictors of pain intensity in patients with FM.

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