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# Circulating Human Fractalkine is Decreased Post-operatively After Orthopedic and Coronary Bypass Surgery

TORBJÖRN ÅKERFELDT[1], JOHANNA HELMERSSON-KARLQVIST[1], TORSTEN GORDH[2] and ANDERS LARSSON[1]

[1]*Department of Medical Sciences, Clinical Chemistry, Uppsala University, Uppsala, Sweden;*
[2]*Department of Surgical Sciences, University Hospital, Uppsala, Sweden*

**Abstract.** *Fractalkine is an important chemokine involved in resolving normal inflammatory processes such as wound healing. Soluble fractalkine acts as a chemoattractant bringing cytotoxic and cytokine-producing cells to areas of inflammation. The aim of the present study was to investigate circulating fractalkine during inflammatory response induced by surgery. Materials and Methods: Fractalkine was analyzed in serum samples from orthopedic surgery patients (n=29) and coronary bypass patients (n=21). The samples were collected prior to surgery and 4 and 30 days after surgery, respectively. Results: Fractalkine concentrations decreased from pre-operative levels of 1,764 (1,330-2,434) pg/mL to 1,520 (1,330-2,434) pg/mL at 4 days after surgery, and to 1,285 (1,099-1,462) pg/mL 30 days after surgery in patients undergoing orthopedic procedures (p<0.01, 30 days post-operatively versus pre-operatively). Furthermore, fractalkine concentrations decreased significantly from pre-operative levels of 1,856 (1,520-2,434) pg/mL to 1,338 (964-1,650) pg/mL 4 days post-operatively and to 1,266 (1,080-1,338) pg/mL 30 days post-operatively in patients undergoing coronary bypass surgery (p<0.01, 30 days post-operative versus pre-operative values). Conclusion: A significant and persistent decrease in circulating fractalkine was observed after orthopedic and coronary bypass surgery despite a marked inflammatory response.*

Fractalkine, also named neurotactin or CX3CL, is a chemokine expressed on activated endothelial cells that mediates attachment and firm adhesion of T cells, monocytes and NK cells (1, 2). Fractalkine is found in most tissues in the body (3). The 95-100 kDa type I transmembrane form of fractalkine can be proteolytically-processed and the extracellular region be released as soluble fractalkine with a molecular weight of 60-80 kDa (4). Soluble human fractalkine is chemotactic for T-cells and monocytes and has been identified in plasma urine, cerebrospinal, amniotic and synovial fluids (5-8).

Fractalkine has been reported to be present in a large number of disorders such as rheumatoid arthritis, cancer, HIV infections, cardiovascular disease, renal disorders, allograft rejections, hypertension, chronic pancreatitis and neuropathic pain (9). There is usually a potent inflammatory response after surgery with strongly increased levels of acute-phase markers. Fractalkine synthesis is stimulated by TNF-alpha, which is a pro-inflammatory cytokine (10). At the same time fractalkine can modulate TNF-alpha secretion (11). We have earlier reported on markedly increased CRP values 4 days after orthopedic surgery and bypass surgery compared to pre-surgery, indicating a pronounced inflammatory response by the surgical procedure (12).

Surgical procedures cause tissue damage and inflammation and postoperative pain is a major concern. Apart from being a chemokine, fractalkine has also been shown to act as a modulator of central and peripheral pain (13). Intra-thecal administration of fractalkine induces allodynia and thermal hyperalgesia (14) while inhibition of cathepsin S, a protease that inhibits the release of soluble fractalkine from cell membranes, has anti-hyperalgesic effects in rats (15). Inhibition of fractalkine has, thus, been suggested as a treatment for chronic pain (16).

The aim of the present study was to investigate the effects of surgically-induced inflammation on the levels of soluble fractalkine.

## Materials and methods

*Patient samples and assays*. Elective orthopedic surgery (N=29), including 13 males and 16 females and elective coronary bypass surgery (N=21), including 18 males and 3 females patients, at the Uppsala University Hospital were used in the study. The mean age was 67 years (range=45-80 years) for the orthopedic patients and 69 years (range=48-84 years) for the coronary bypass patients. Fourteen of the orthopedic patients had knee surgery and fifteen of

*Correspondence to:* Anders Larsson, Department of Medical Sciences, Uppsala University Hospital, SE 751 85 Uppsala, Sweden. Tel: +46 186114271, Fax: +46 18552562, e-mail: anders.larsson@akademiska.se

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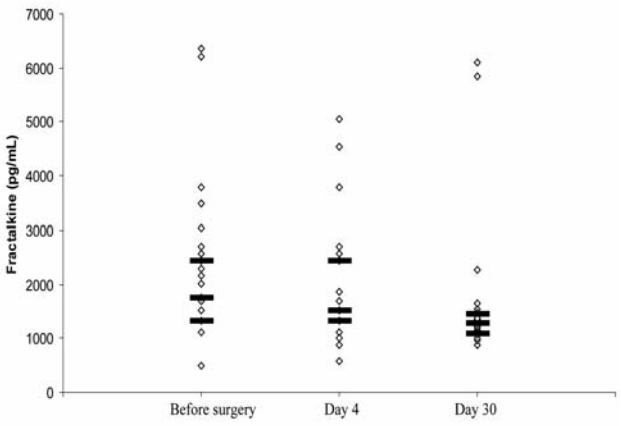


Figure 1. *Fractalkine levels in patients before (n=29), 4 days (n=29) and 30 days (n=24) after orthopedic surgery. Horizontal bars indicate median and interquartile range.*

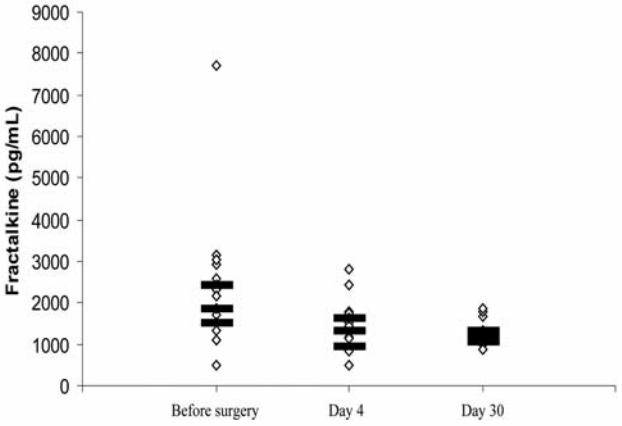


Figure 2. *Fractalkine levels in individual patients before, 4 and 30 days after bypass surgery. Horizontal bars indicate median and interquartile range.*

the patients had hip arthroplasty. The study was approved by the ethical board at the Uppsala University and all patients signed an informed consent prior to study inclusion. The study was carried out in accordance with the Helsinki declaration.

*Fractalkine and cathepsin S assays*. Serum fractalkine and cathepsin S were analyzed by commercial sandwich ELISA (DY365 and DY1183, R&D Systems, Minneapolis, MN, USA). Monoclonal antibodies specific for fractalkine or cathepsin S were used as capture antibodies. Standards and samples were pipetted into the wells of microtitre plates and fractalkine or cathepsin S was bound to the immobilized antibodies. After washing, a biotinylated antibody was added. After incubation and washing a streptavidine-HRP conjugate was added to the wells. After further incubation and washing steps a substrate solution was added. The development was subsequently stopped and the absorbance was measured in a SpectraMax 250 (Molecular Devices, Sunnyvale, CA, USA). The fractalkine and cathepsin S concentrations in the samples were determined by comparing the optical density of the sample with the standard curve. The assays were calibrated against recombinant human fractalkine and cathepsin S.

*Statistics*. Fractalkine and cathepsin S were skewed-distributed according to Shapiro-Wilks test ($W<0.95$) and were log-transformed to reach normal distribution. Values before surgery and 4 and 30 days after surgery were tested with repeated-measurement analysis of variance and paired *t*-tests. Differences between variables in the two surgery groups at baseline were tested with unpaired *t*-tests. Pearson correlation analysis was performed between fractalkine and cathepsin S at baseline and for comparing changes in fractalkine and cathepsin S, respectively, between the time points. Statistics were performed with Stata 11.0 (Stata Corporation, Collage Station, TX, USA). Descriptive statistics were reported as median and IQR (interquartile range). We regarded $p<0.05$ as statistically significant throughout the text.

## Results

*Baseline concentrations of fractalkine*. The changes in fractalkine concentrations in the surgery groups are graphically presented in Figure 1. Baseline concentrations of fractalkine did not differ between the orthopaedic-surgery patient group and the coronary-bypass surgery patient group ($p=0.67$).

*Fractalkine and orthopaedic surgery*. Fractalkine concentrations decreased significantly from median 1,764 (1,330-2,434) pg/mL prior to surgery to 1,520 (1,330-2,434) pg/mL 4 days after orthopaedic surgery and to 1,285 (1,099-1,462) pg/mL 30 days after orthopaedic surgery, ($p<0.01$, 30 days post-operative *versus* pre-operative values) (Figure 1).

*Fractalkine and coronary bypass surgery*. Fractalkine concentrations decreased significantly from median 1,856 (1,520-2,434) pg/mL prior to surgery to 1,338 (964-1,650) pg/mL 4 days after coronary by pass surgery ($p<0.01$). The fractalkine values remained significantly decreased 30 days after coronary bypass surgery, 1,266 (1,080-1,338) pg/mL, ($p<0.01$, 30 days post-operative *versus* pre-operative values (Figure 2).

*Correlations between fractalkine and cathepsin S in both surgery groups*. Fractalkine concentrations decreased from median 1,856 (1,330-2,434) pg/mL prior surgery to 1,444 (1,120-1,782) pg/mL 4 days after surgery ($p<0.01$) and remained significantly decreased 30 days after surgery, 1,266 (1,080-1,444) pg/mL, ($p<0.01$, 30 days post-operative *versus* pre-operative values). Serum Cathepsin S concentrations decreased from median 18.4 (15.7-20.5) ng/mL prior to

surgery to 14.4 (12.1-16.3) ng/mL 4 days after surgery ($p<0.001$) and than increased again 30 days after surgery to 19.7 (18.5-23.6) ng/mL, ($p<0.001$, 30 days postoperative *versus* preoperative and 4 days after surgery, respectively). Fractalkine and cathepsin S showed a borderline significant positive linear correlation at baseline (R=0.26, $p=0.07$). The changes in fractalkine and cathepsin S between preoperative values and 4 days postoperative and between 4 and 30 days postoperatively did not show linear correlations (R=0.01, $p=0.96$ and R=0.13, $p=0.45$, respectively).

## Discussion

Surgical procedures cause tissue damage and subsequent wound healing on affected areas. The repair of damage and restoration of normal tissue is an interactive process that involves many mediators and the process can be divided in three sequential phases: inflammation, proliferation and maturation (17). Chemokines are not only chemoattractants that induce leukocyte infiltration in the surgical wound, but may also modulate the function of non-hematopoietic cells. Fractalkine and its receptor, CX3CR1 mediated wound healing in a mouse skin model by promoting macrophage and fibroblast accumulation and function (18). The authors found increased expression of fractalkine in the wound area three to six days after surgery. There are also reports that fractalkine is induced by TNF-alpha and that fractalkine is increased in inflammatory diseases.

It has been shown that astrocytes and microglia become activated in the spinal cord following peripheral nerve injury, always occurring at surgery in various degrees, and also that they modulate the neuronal mechanisms of chronic pain in spinal cord and probably also in the brain (13). One of these emerging signaling pathways involves fractalkine and its receptor CX3CR1 and it has been postulated that fractalkine contributes to the development and maintenance of chronic pain. Fractalkine is considered a promising target for therapeutic interventions in patients with sustained pain. The development of such drugs is presently on-going and experimental data indicate their potential use in reducing pain (16). Fractalkine may, thus, have several functions during the postoperative phase.

Despite previous reports showing that fractalkine is increased in patients with inflammatory disorders the soluble fractalkine levels decreased significantly postoperatively. Similar findings in both orthopedic and cardiopulmonary bypass patients strengthen the finding and strongly suggest that circulating fractalkine indeed decreases after surgery. Cathepsin S has previously been reported to decrease at day 4 post-operatively in comparison to pre-surgical values, but increased again at day 30 (12). The lack of correlation between cathepsin S and fractalkine indicates that cathepsin S mediated release of soluble fractalkine do not have a major impact on circulating fractalkine levels in this setting.

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