Bone turnover markers and cytokines in joint fluid

Analyses in 10 patients with loose hip prosthesis and 39 with coxarthrosis

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Submitted 01-10-15. Accepted 02-02-20

ABSTRACT – We analyzed bone turnover markers (osteocalcin, bone ALP, β-crosslaps-CTX) and cytokines (IL-1, IL-8 and IL-10) in hip joint fluid in 10 patients before revision surgery and in 39 with idiopathic coxarthrosis. Patients with loose implants had lower concentrations of resorption marker than those with arthrosis (0.8 vs 1.3 ng/mL), but bone formation marker osteocalcin was reduced (4.2 vs 22.6 ng/mL). IL-8 and IL-10 levels were elevated in patients with implant failure (870 vs 340 pg/mL; 14.3 vs 4.0 pg/mL). We found a negative correlation between the bone resorption marker (CTX) and IL-10 in cases with prosthesis loosening and a positive correlation between IL-10 and time-to-revision.

We conclude that enhanced local production of inflammatory cytokines leading to suppressed bone formation is a part of the loosening process. The expression of anti-inflammatory mediators is not sufficient to counteract the imbalance in bone turnover.

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The interaction between an implant and bone is a complicated phenomenon, which involves the local response of the bone to mechanical stimulation and immunological reactions (Pipino 2000). An increase in fluid pressure at the interface may induce osteocyte apoptosis (Aspenberg and van der Vis 1998). Early instability of the implant increases the number of wear particles, which can initiate an inflammatory process in the interfacial membrane and a biological cascade. This results in bone resorption (Hirayama et al. 2001).

Accumulation of particles leads to a localized infiltration of macrophages and subsequent fibrous tissue formation. Activated macrophages take up small debris particles and produce inflammatory mediators, prostaglandins, soluble adhesion molecules and growth factors which stimulate bone resorption and inhibit formation of bone (Chomarat et al. 1995, Nabae et al. 1999, Takei et al. 2000). Macrophages from the interfacial tissues and osteoclasts express the same profile of cytokine and growth factor receptors, which suggests that they can stimulate the differentiation and activity of osteoclasts and modulate bone resorption (Frost et al. 1997, Neale and Athanasou 1999).

Periprosthetic bone is in direct communication with joint fluid. An increase in pro-inflammatory cytokine levels has been found in the synovial fluid of failed implants (Sabokbar and Rushton 1995). Some authors have suggested that IL-1, which is expressed more frequently at the bone-implant interface than TNF-α, may be involved in osteolysis and implant loosening (Al Saffar and Revell 1994). A higher level of TNF-α has been found in the synovial fluid of patients with a loose prosthesis and in those with no signs of loosening (Nivbrant et al. 1999). In vitro studies have shown release of TNF-α, but not of IL-1α or β, from macrophages in response to particles (Catelas et al. 1999).

Various anti-inflammatory agents, such as IL-4, IL-10, TGF-β1, IL-1 receptor, are also synthesized by macrophages and may be found at the bone-implant interface (Nabae et al. 1999, van den Berg 1999).

Recent histomorphometric data suggest that the formation of low-mineralized bone and increased resorption with macrophage-mediated inflammatory

Table 1. Characteristics of patients

	Age [a]	Sex F	Sex M	Revised [a,b] (years)	Type of prosthesis M	Type of prosthesis C
Loosened THR	68 (9)	9	1	9.2 (4.4)	7	3
OA	68 (7)	28	11	–	–	–

[a] mean (SD)
[b] Years between primary and revision THR
M metal head and polyethylene socket
C ceramic head and polyethylene socket

response in the interface membrane contribute to loosening of the prosthesis (Takagi et al. 2001). So far, no data have been reported on bone resorption markers in the joint fluid of patients with implant failure. We analyzed the concentrations of such markers and cytokines in joint fluid collected from 10 patients before revision hip surgery. 39 patients with primary coxarthrosis served as controls.

Patients and methods

Samples of joint fluid were collected from 49 patients who underwent hip surgery in our department. 10 hips were operated on because of loosening and 39 because of primary arthrosis (Table 1). Samples of synovial fluid were collected with a syringe before incising the capsule. All revision cases had originally been operated on for primary arthrosis. The interval between primary and revision surgery was 9.2 years (SD 4.4). In 7 of 10 patients with loosening, polyethylene (socket)-metal (head) implants and in 3 cases polyethylene (socket)-ceramic (head) implants were removed. Uncemented total hip arthroplasty was performed in 7 cases during the first operation.

Informed consent was given by all participants and the procedures were approved by the local bioethics committee.

Samples of joint fluid (1–2.5 mL) were centrifuged, treated with hyaluronidase (3000 IU/mL, type IV S, Sigma Chemical Co., USA) in 0.15 M phosphate buffer, pH 5.29 for 10 min at 37 °C (Millett et al. 1995) to reduce viscosity and stored at –70 °C for up to 1 month before the analyses. We could not measure the whole cytokine panel and both bone formation markers in all specimens because of the small amount of synovial fluid.

Osteocalcin (OC 1-43), a marker of bone formation, was assayed on Elecsys 1010 (N-mid Osteocalcin, Roche Diagnostics, Germany) with an electrochemiluminescence method. The detection limit for OC was 0.5 ng/mL (intra-assay CV 1.4%). Another marker of bone formation, bone alkaline phosphatase (bone ALP), was assayed after precipitation with lectin by a colorimetric method on Hitachi 912 (Roche Diagnostics, Germany).

β-crosslaps (CTX), a bone resorption marker (Serum Crosslaps, Osteometer Biotech, Denmark), was assayed with ELISA. This assay had a detection limit of 0.008 ng/mL (intra-assay CV 6.5%).

IL-1α, IL-8 and IL-10 were assayed with ELISA (Endogen Human Interleukin, USA). These tests use specific animal antibodies anti-IL-1α, IL-8 or IL-10 and streptavidin-HRP as conjugate. The detection limit for IL-1α was < 1 pg/mL, for IL-8 < 2 pg/mL, for IL-10 < 2 pg/mL. The intra-assay CVs were < 10%. In some synovial fluid specimens, the concentration of IL-1α or IL-10 was below the detection limit of the conventional ELISA assays we used.

Statistics

The Mann-Whitney U-test and Spearman rank correlation coefficient were used. P-value < 0.05 was considered statistically significant.

Results

We found a lower concentration of CTX in cases with implant failure than in those with arthrosis ($p = 0.004$, Table 2). The activity of bone ALP and concentrations of osteocalcin, both markers of bone formation produced by osteoblasts, were lower in patients with loose prostheses than in those with primary arthrosis ($p = 0.01$).

We found low levels of IL-1α in both groups. There was a tendency to higher values in hips with loose implants than in those with arthrosis (Table 3). The concentrations of IL-8 and IL-10 in the failure group were higher than those seen in our control group ($p = 0.04$, $p = 0.002$). The quotient IL-8: IL-10 (calculated for median values) was 1.5-fold lower in patients with a failed prosthesis than in cases with coxarthrosis.

Table 2. Concentrations of CTX, osteocalcin and bone-ALP activity in joint fluid

	OA of the hip	Loosened THR
CTX (ng/mL)		
No. of cases	36	10
Median	1.3	0.8 [b]
Range	0.6–18.6	0.5–1.4
Osteocalcin (ng/mL)		
No. of cases	29	8
Median	22.6	4.2 [a]
Range	6.4–278	0.5–11.9
Bone-ALP (U/L)		
No. of cases	20	7
Median	98.1	68.7 [a]
Range	6.7–315	54.1–92.4

[a] p = 0.01
[b] p = 0.004

Table 3. Concentrations of IL-1α, IL-8 and IL-10 in joint fluid

	OA of the hip	Loosened THR
IL-1α (pg/mL)		
No. of cases	10	8
Median	1.4	1.9
Range	1.3–1.9	0.8–2.5
IL-8 (pg/mL)		
No. of cases	26	9
Median	340	870 [a]
Range	9.7–1095	295–1010
IL-10 (pg/mL)		
No. of cases	24	9
Median	4.0	14.3 [b]
Range	1.8–9.2	2.4–55.5

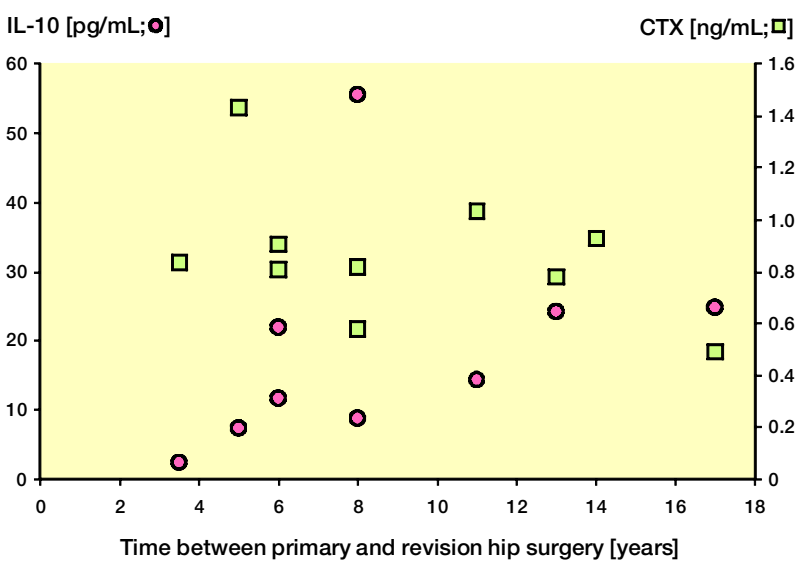
[a] p = 0.04
[b] p = 0.02

In patients with a loosened prosthesis, we found a negative association between IL-10 and CTX (r = –0.68, p = 0.04). IL-10 showed a positive correlation with time-to-failure (r = 0.74, p = 0.02).

In patients with arthrosis, a positive correlation was noted between IL-8 and CTX (r = 0.65, p = 0.004). An increase in IL-10 was associated with an increase in bone ALP activity (r = 0.65, p = 0.04). Both bone ALP and OC correlated with CTX values (r = 0.85, p = 0.001; r = 0.63, p = 0.003). Similarly, IL-8 correlated positively with IL-10 (r = 0.75, p = 0.001).

Discussion

The cytokines like IL-1α, IL-1β have been found at the bone-implant interface from failed prostheses (Millett et al. 1995, Goodman et al. 1996, Stea et al. 2000). Moreover, the production of IL-1α in



Concentrations of CTX and IL-10 in relation to time between primary and revision hip surgery. Circles and squares represent individual IL-10 and CTX values, respectively.

the periprosthetic tissue of loose implants has been reported to be positively related to the severity of osteolysis (Stea et al. 2000).

In both groups of our patients, synovial fluid concentrations of IL-1α were similar. The inability to find a difference between patients with implant failure and those with arthrosis could be due to the paucity of observations. Higher IL-1β levels in joint fluid have been reported in cases with loosened implants than in those with arthrosis (Nivbrant et al. 1999). However, the levels of inflammatory cytokines in patients with loose and stable prostheses did not differ.

We found low levels of IL-1α in synovial fluid, possibly an effect of fewer IL-1α than IL-1β secreting cells (Westacott et al. 1992), lower production of IL-1α by macrophages (March et al. 1985) or presence of autoantibodies against IL-1α. Such antibodies have been reported in normal subjects and in patients with various inflammatory disorders, who have higher levels (Bendtzen 1998). These autoantibodies could interfere in the cytokine assay.

Previous studies have indicated that IL-6 may play an important role in the process of hip implant loosening (Sabokbar and Rushton 1995, Goodman et al. 1996, 1998). Recent data have shown similar levels of IL-6 in the joint fluid of loose and arthritic hips (Nivbrant et al. 1999). The increase in the level of another inflammatory cytokine, IL-8, has been observed in the synovial tissues and fluid of patients undergoing revision hip surgery (Sabokbar and Rushton 1995, Lassus et al. 2000). IL-8 contributes to the release of proteinases, prostaglandin E2, adhesion molecules and superoxide molecules into joint fluid that may play an essential role in prosthesis loosening (Sabokbar and Rushton 1995, Lassus et al. 2000). It has been reported that the level of IL-8 is markedly increased or similar in the joint fluid of patients with loosening and other pathological conditions, associated with bone loss, especially rheumatoid arthritis and arthrosis (Sabokbar and Rushton 1995, Kaneko et al. 2000).

In our study, the concentration of IL-8 in hip joints with loose prostheses was higher than in primary arthrosis, but similar to that observed in rheumatoid arthritis (authors' unpublished data).

Elevated IL-8 and IL-10 concentrations in patients before revision hip surgery have been related to reduced levels of bone formation markers and less marked reduction in the resorption marker. Takagi et al. (2001) made similar observations in a morphological study with histomorphometric analysis, in which they showed a high bone turnover with formation of poorly mineralized, fragile periprosthetic bone.

The CTX value in patients with implant failure was almost twice as high in synovial fluid as in the serum of a healthy age-matched population (Sypniewska and Chodakowska-Akolinska 2000).

Since there are no reference values for CTX in "normal" joint fluid, it is difficult to speculate whether the concentration of bone resorption marker was increased in the joint fluid of loose hips.

Kim et al. (1998) reported higher levels of bone-resorbing cytokines in joint fluid from patients with implant failure than in those who had arthrosis. They found higher levels of IL-6 and tartrate-resistant acid phosphatase, an osteoclast-derived resorption marker, in patients with loosen. Surprisingly, we observed a lower level of CTX in patients with loose prostheses. It is clear from our study that bone formation markers were considerably lower in these patients than in those with primary coxarthrosis. This may have been caused by an increase in the local production of pro-inflammatory cytokines resulting in uncoupling of bone turnover. This hypothesis is based on observations in two studies. Konttinen et al. (1997) reported an imbalance between bone resorption and formation in aseptic loosening. Andersson et al. (2000) found a lower proliferation of human osteoblasts in primary cultures after adding synovial fluid from patients who underwent revision hip surgery because of aseptic loosening. We suggest that a marked increase in IL-10 and a lower ratio of pro- to anti-inflammatory cytokines in our patients with a loose prosthesis may explain this difference. The correlations between IL-10 and CTX, IL-10 and time-to-implant failure (Figure) suggest that enhanced production of anti-inflammatory mediators may affect periprosthetic bone turnover. The production of IL-10, however, is not sufficient to counteract the imbalance in bone metabolism. Improved understanding of factors which contribute to aseptic loosening of hip implants is important before anti-inflamma-

tory therapy aiming to reduce bone resorption and enhance bone formation is introduced to increase the longevity of reconstructed joints.

This study was supported by the Medical University of Bydgoszcz, grant BW 49/ 98 and Bellco Biomedica Poland.

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