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Kinga Lis[1], Agnieszka Pater[1], Wiesław Nowacki[2], Grażyna Odrowąż-Sypniewska[1]

[1]Department of Laboratory Medicine, Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz, Poland
[2]Department of Orthopaedics and Traumatology, Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz, Poland

Osteoprotegerin, sRANKL and sRANKL/ /OPG ratio in pseudosynovial fluid from patients with aseptic loosening of total hip prosthesis

Corresponding author:

Kinga Lis
Department of Laboratory Medicine,
Nicolaus Copernicus University,
Collegium Medicum
Sklodowskiej-Curie Street No. 9,
85–094 Bydgoszcz, Poland
Phone: +48 52 585 40 46
Fax: +48 52 585 36 03
e-mail: k2lis@cm.umk.pl

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ABSTRACT

Background. Total hip replacement is the final solution in advanced osteoarthritis. The survival time of the implant significantly depends on the condition of the bone in which it has been located. Upsetting the balance in the RANK/RANKL/OPG system can lead to the development of bone metabolic disorders leading to bone loss. The aim of this study was to evaluate the osteoprotegerin (OPG) and soluble RANKL (sRANKL) concentrations in the pseudosynovial fluid in women with aseptic loosening of total hip prosthesis.

Methods. OPG and sRANKL concentrations were assayed in the pseudosynovial fluid collected from 20 women during the revision total hip arthroplasty (THA) (group R), and in the synovial fluid of 13 women in the end-stage of idiopathic osteoarthritis collected during primary THA (group P). OPG and sRANKL were measured using commercially available ELISA kits.

Results. OPG concentration was significantly lower, and sRANKL concentration was significantly higher, in group R than in group P. The sRANKL/OPG ratio in group R was significantly higher than in group P. The average total joint endoprosthesis survival time was 8.6 (SD 3.9) years. The OPG concentration was slightly lower in patients with a time interval shorter than 8.6 years. The sRANKL/OPG ratio was higher in women with a shorter implant survival time.

Conclusion. Higher sRANKL/OPG ratio in the pseudosynovial fluid contributes to increased resorption of bone tissue surrounding total hip endoprosthesis, leading to its aseptic loosening.

Key words: osteoprotegerin, sRANKL, pseudosynovial fluid, aseptic prosthesis loosening

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Introduction

Osteoarthritis, a result of both biological and mechanical factors leading to a disturbance of the balance within a joint between degradation and repair processes, affects equally chondrocytes of the articular cartilage, cartilage matrix and subchondral bone. The disease is characterised by certain morphological changes in the joints, and also by visible biochemical and biomechanical changes [1–4]. The degeneration process within the joint leads to a significant limitation of motor abilities and disability. Total hip endoprosthesis implantation is usually the final solution [4, 5].

The survival times of implanted hip endoprosthesis may differ [5–7]. It has been estimated that, on average, a total hip endoprosthesis lasts in a good condition for ten years [6, 7]. The survival time of the joint implant significantly depends on the condition of the bone in which it has been located [4, 6]. Osteolysis, which occurs around the implant, has been demonstrated to be one of the numerous causes of joint endoprosthesis loosening [8].

Osteoprotegerin (OPG) is considered to be a factor which protects against osteoclastogenesis both *in vitro* and *in vivo* [9–12]. Osteoprotegerin blocks the interaction of the RANK receptor localised on the surface of osteoclasts with its specific ligand (RANKL), which inhibits signalling for preosteoclasts to undergo maturation. This interaction directly influences the process of bone remodelling [13–15]. Disturbing the balance in the RANK/RANKL/OPG system can lead to the development of numerous bone metabolic disorders with accompa-

nying bone loss [13, 15–18]. Recent results suggest that modulation of the OPG/RANKL system may be an interesting target for future osteoarthritis treatment. OPG is not only a decoy receptor for RANKL, but also acts as a modulator of RANKL half-life [13].

The composition of joint fluid reflects the local environment. Similarly, tests performed in the pseudosynovial fluid give a specific image of processes that take place in the immediate surroundings of the implanted joint endoprosthesis [19, 20]. An increased production of joint fluid may be associated with a failure of total hip or knee arthroplasty. It has been supposed that the fluids from failed arthroplasties had shown higher cytotoxicity and higher levels of wear particles, host cells, and proteins, with more severe bone loss. However, the latest data has not confirmed a toxic influence of synovial fluids from failed total hip or knee joint prostheses on *in vitro* cultured osteoblasts [21].

We aimed to evaluate the osteoprotegerin and RANKL concentrations in the pseudosynovial fluid in women with osteoarthritis, after aseptic loosening of total hip prosthesis.

Patients and methods

Osteoprotegerin and sRANKL concentrations were assayed in joint fluid collected from 33 women who underwent hip surgery. 20 women (aged 62 ± 12 years) had revision surgery because of aseptic loosening of endoprosthesis (we called this group R —Revision group). The second group (group P — Primary group) consisted of 13 women (aged 67 ± 7 years) in the end-stage of osteoarthritis in whom first total hip arthroplasty (THR) had been performed. All revision cases had originally been operated on for primary osteoarthritis. The interval between primary and revision surgery was 8.6 ± 3.9 years.

Informed consent was given by all participants, and the procedures were approved by the local Bioethics Committee.

Samples of synovial and pseudosynovial fluid were collected with a sterile syringe before incising the capsule. After collection, samples of synovial fluid and pseudosynovial fluid were centrifuged, treated with hyaluronidase (3,000 IU/mL, type IV S, Sigma Chemical Co., USA) in 0.15 M phosphate buffer, pH 5.29 for 10 min at 37°C as previously described by us (Sypniewska et al. 2002) to reduce viscosity and stored at –70°C for up to one month before the analyses.

All specimens were assayed for osteoprotegerin (OPG) and soluble RANKL (sRANKL) using commercially available ELISA kits (Immun Diagnostik, Biomedica Gruppe, Germany). These tests use specific animal biotinylated polyclonal anti-OPG or anti-sRANKL antibodies and streptavidin-HRP as conjugate. The detection limit for OPG was 0.14 pmol/l. The detection limit for sRANKL was 0.08 pmol/L. The intra assay CVs were < 10%.

Statistics

Statistical analysis was done with the use of Mann-Whitney's U test. Spearman correlation coefficient R was used. A p value ≤ 0.05 was considered statistically significant.

Results

The osteoprotegerin concentration in the pseudosynovial fluid taken from women during the revision surgery (group R) was significantly lower than that in the joint fluid of women in which primary total hip arthroplasty had been performed (group P). On the other hand, the concentration of sRANKL was significantly higher in group R. The sRANKL/OPG ratio in the joint fluid of group P was significantly lower than in the pseudosynovial fluid of women from group R (Tab. 1).

No significant correlation between the OPG and sRANKL concentration in the joint fluid was found in either group (OPG/sRANKL R = –0.11; R = 0.32 in groups R and P, respectively).

In both groups, there was no significant correlation of the OPG concentration in the pseudosynovial or synovial fluid with age (OPG/age: R = –0.05; R = –0.51 in groups R and P, respectively). A significant correlation of sRANKL in pseudosynovial fluid with age was observed in the revision surgery group (sRANKL/age: R = 0.49;

Table 1. OPG, sRANKL concentrations and sRANKL/OPG ratio in pseudosynovial or synovial fluid from patients with revision total hip arthroplasty (group R) and patients with primary total hip arthroplasty (group P)

	Group R [mean ± SD]	Group P [mean ± SD]	p ≤
OPG [pg/mL]	311 ± 109	587 ± 80	0.000005
sRANKL [pg/mL]	58.5 ± 69.9	19.4 ± 7.8	0.05
sRANKL/OPG ratio	0.20 ± 0.24	0.09 ± 0.17	0.02

OPG — osteoprotegerin; sRANKL — soluble RANKL

Table 2. OPG, sRANKL and sRANKL/OPG ratio in pseudosynovial fluid from patients with revision total hip arthroplasty with implant survival time shorter than 8.6 years (ST group) and with implant survival time longer than 8.6 years (LT group)

	ST group [mean±SD]	LT group [mean±SD]	p ≤
OPG [pg/mL]	275 ± 103	347 ± 106	NS
sRANKL [pg/mL]	81 ± 94	35.8± 19.7	NS
sRANKL/OPG ratio	0.28 ± 0.31	0.12± 0.10	NS

NS — not significant; OPG — osteoprotegerin; sRANKL — soluble RANKL

$p \leq 0.03$). In patients with primary hip arthroplasty, there was no correlation between synovial fluid concentration of sRANKL and age (sRANKL/age: $R = -0.36$).

The average total hip endoprosthesis survival time was 8.6 ± 3.9 years. In 50% of women from group R, the period between the first and the revision surgery was 4-7 years (ST group — Short Time). In the other half of the women, the implant survival time was longer: 9–16 years (LT group — Long Time).

Women with an implant survival time of shorter than 8.6 years were not significantly older than patients with longer prosthesis survival time (64 ± 15 years vs. 59 ± 9 years, respectively).

OPG concentration in the pseudosynovial fluid in women with revision surgery performed earlier than 8.6 years after primary THA (the ST group) was slightly lower than the OPG concentration in the fluid taken from women whose time of implant survival was longer (LT group). The sRANKL concentration in the pseudosynovial fluid in women with a shorter time of implant survival was also insignificantly lower than the sRANKL concentration in the fluid from women with a longer period of implant survival. The sRANKL/OPG ratio was higher in women with a shorter time of implant survival (Tab. 2).

There was no significant correlation between the OPG or sRANKL concentration and period of primary operation to revision surgery (OPG/time interval: $R = 0.48$; sRANKL/time interval: $R=0.26$). The implant survival time was inversely correlated with the sRANKL/OPG ratio ($R = -0.42$; $p \leq 0.05$).

Discussion

The survival times of joint endoprostheses differ, and it has been estimated that on average 30% of total hip endoprostheses undergo aseptic loosening within 10–14 years [7]. The reason for hip endoprosthesis loosening has not been defined yet. Osteolysis, which occurs around the implant, has been mentioned among numerous possible causes [6–8, 22, 23].

Osteoprotegerin is considered to be a cytokine protecting the bone from excessive resorption [24, 25]. A proper balance in the RANKL/OPG system is essential for the osteoclast's activity, and its uncoupling leads to significant disturbances in bone turnover [13, 24, 26, 27]. As has been suggested by Malik et al., genetically determined disturbances in the RANK/RANKL/OPG system may have the essential influence on joint endoprosthesis survival [28]. Interface tissue fibroblasts are able to produce RANK ligand. RANKL, which is present in pseudosynovial fluid, can induce osteoclastogenesis during the loosening process [29, 30].

Others have observed that the OPG concentration in the synovial fluid of those who suffer from osteoarthritis increases with the progress of degeneration changes [31]. In patients with a total hip endoprosthesis, the bone is in direct contact with the pseudosynovial fluid filling the joint space [21]. According to Gehrke et al. [32], macrophages and osteoclasts present in the immediate surroundings of the prosthesis play the essential role in the activation of bone remodelling and its destruction. However, osteoprotegerin could be a locally efficient preventative factor [32]. Failure of implantation leads to an increase of its wear products, something that will initiate inflammation in the interface membrane. Inflammatory changes cause a whole cascade of biological phenomena that enhance bone resorption in the surroundings of a prosthesis [6, 20, 22]. As has been shown in joint disorders with intensified inflammation, decreased expression of osteoprotegerin in joint tissues was observed [33]. We found that OPG concentration in the pseudosynovial fluid from patients with a loosened endoprosthesis was almost two fold lower than in the synovial fluid taken during primary THA. This probably results from inflammation developing in the immediate surroundings of the implanted endoprosthesis [20]. Lower OPG concentration in the pseudosynovial fluid from patients with aseptically failed total joint prostheses has also been observed by Gallo et al. [21]. However, Wang et al. did not find significant differences in the OPG levels in the synovial fluid between patients with loosened total hip arthroplasty (THA) and primary THA patients [34].

A higher concentration of sRANKL in patients with revision accompanies the decreased osteoprotegerin concentration in the pseudosynovial fluid. According to Goater et al., the inflammation may be caused by wear debris which may stimulate the expression of RANKL receptor, something that intensifies osteolysis causing the implant to loosen [35]. Increased RANKL levels in the pseudosynovial fluid were found in our study in pa-

tients who underwent the revision surgery. Similar data were recently reported by Wang et al. [34] in patients with loosened THA and by Gallo et al. [21] in patients with aseptic prosthesis loosening.

The excessive loss of bone mass is connected to an increase of the sRANKL/OPG coefficient [24, 26, 27]. Essentially, a higher sRANKL/OPG ratio in women with aseptic loosening of total hip endoprosthesis suggests a disturbed balance in the RANK/RANKL/OPG system, which may cause excessive resorption of the bone surrounding the endoprosthesis. Similarly to our results, a higher sRANKL/OPG ratio has been reported by others [21, 34].

Implanted endoprostheses have only a limited so called 'survival time' [6, 7]. In the discussed work, the average time between the primary and revision surgery was 8.6 years (4–16 years). According to Granchi et al., high serum OPG concentration means a good prognosis concerning the survival of the implanted endoprosthesis, whereas increased RANKL concentration correlates with an increased level of bone resorption in the surroundings of the prosthesis [36]. Testing the fluid taken from the immediate surroundings of the implanted endoprosthesis allows more accurate evaluation of local processes in the joint [20]. The balance between RANKL and OPG concentrations seems to be more important than individual OPG or RANKL value. We observed that the shorter the interval that elapses between the primary and revision THA, the higher the sRANKL/OPG ratio. The same was suggested earlier by Granchi et al. [36].

Osteolysis is associated with aseptic loosening of total hip endoprosthesis [6, 20, 22]. The results of Wang et al. suggest that ultra-high molecular weight polyethylene particles (UHMWPE) may induce the over-expression of RANKL, inflammatory cytokines and chemokines in the periprosthetic microenvironment [34]. These molecules by independent or synergistic actions result in periprosthetic osteolysis and loosening of THA. Low concentration of bone protecting factor osteoprotegerin, with simultaneously high RANKL, seems to be an important reason for premature loosening of total hip endoprosthesis and shortening of its survival [37, 38]. High RANKL levels and low OPG concentrations are also in agreement with the theory of aseptic loosening and periprosthetic osteolysis [21].

From the practical point of view, it would be of interest as to whether serum OPG and RANKL could be a measure of risk of bone osteolysis after endoprosthesis implantation.

Conclusions

A higher sRANKL vs OPG ratio in the pseudosynovial fluid contributes to increased bone resorption in the periprosthetic microenvironment, leading to aseptic prosthesis loosening.

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