Multiple biomarkers analysis for the early detection of prosthetic aseptic loosening of hip arthroplasty

Tao He · Wen Wu · Yan Huang · Xiaoling Zhang ·
Tingting Tang · Kerong Dai

Received: 3 February 2013 / Accepted: 15 February 2013 / Published online: 7 March 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract

Purpose Our aim was to define the effect of multiple biomarkers of osteolysis or bone remodelling in the early detection of aseptic loosening (AL) of total hip arthroplasty (THA).

Method One hundred subjects were recruited, including 31 candidates for revision THA (Late AL group), 15 patients who had undergone THA and had clinical and radiographic evidence of AL (early AL group), 19 patients with no sign of AL (stable group), and 40 healthy volunteers. Plasma levels of osteoprotegerin (OPG), receptor activator of nuclear factor-kappaB ligand (RANKL), cross-linked N-terminal telopeptide (NTX), procollagen I C-terminal extension peptide (PICP), tumour necrosis factor- α (TNF- α), and interleukin (IL)-1 β were measured using an immunoenzymatic method. The outcomes of biomarkers were analysed separately and synthetically using Revman software.

Results The plasma level of OPG, RANKL, NTX, TNF- α , and IL-1 β declined from late AL, early AL, stable to the healthy group, while the level of PICP inclined reversely. There was a significant difference in synthetic analysis of six biomarkers between the AL group and the stable group, and between the stable group and the healthy group (both $p=0.02$). Heterogeneity of six biomarkers in either comparison was extremely low (both $I^2=0$). Patients who had

cemented implants had significantly higher levels of TNF- α than patients with cementless varieties ($p=0.042$).

Conclusion There was significant change in the plasma level of multiple biomarkers in patients with prosthetic AL of THA, especially in the cemented arthroplasties and in patients without traditional clinical or radiographic evidence of AL.

Introduction

Aseptic loosening (AL) is the most important problem in total hip arthroplasty (THA). It is responsible for approximately 70 % of implant failures [1]. AL is a particle-induced osteolysis resulting from a foreign-body granulomatous response to wear of the implant, leading to macrophage and osteoclast-mediated bone resorption with suppression of osteoblast activity or bone formation process [2]. Many cytokines or biomarkers have been found to be relevant to AL of THA, such as the macrophage related tumour necrosis factor (TNF)- α or interleukin (IL)-1 β [3, 4], the osteoclast related receptor activator of nuclear factor-kappaB ligand (RANKL) and its suppressor osteoprotegerin (OPG) on the surface of osteoblast [5], and the bone resorption marker cross-linked N-terminal telopeptide (NTX) and bone formation marker procollagen I C-terminal extension peptide (PICP) [6]. But these results are controversial, and there is no study to compare all these important biomarkers of AL simultaneously which makes it difficult to be sure of their effects in AL of THA.

On the other hand, early detection of osteolysis or loosening in THA is difficult. The diagnosis is mainly based on clinical history, physical examination, and imaging. Current methods are unable to detect early AL, require radioactive testing, or are expensive [7]. Since there are many sensitive biomarkers related to AL, we postulate that synesthetic

T. He (✉) · W. Wu · T. Tang · K. Dai
Shanghai Key Laboratory of Orthopaedic Implants,
Department of Orthopaedics, Ninth People's Hospital,
Shanghai JiaoTong University School of Medicine,
Shanghai, China
e-mail: minglun1012@126.com

Y. Huang · X. Zhang
Institute of Health Sciences of Shanghai Institutes for Biological
Sciences affiliated with the Chinese Academy of Sciences,
Shanghai, China

biomarker analysis could more effectively detect AL than any single biomarker and even traditional X-ray examination. We therefore evaluated the systemic biomarkers of osteolysis or bone remodelling in the early detection of periprosthetic AL.

Material and methods

This study protocol was approved by the institutional Ethical Committee on human research and is in compliance with the Helsinki Declaration of 1975, as revised in 1996.

Study participants

Blood samples from 100 subjects were collected. Thirty-one patients had failed THA due to AL, and were candidates for revision THA (late AL group). Fifteen patients had undergone THA and had clinical and radiographic evidence of AL (early AL group). Nineteen patients had prosthetic THA that was clinically and radiographically stable (stable group). The remaining 35 individuals were clinically normal volunteers (healthy donor group) who in the last month had not taken any medication. The follow-up period was more than two years, in order to exclude changes of bone turnover associated with arthroplasty [7]. All four groups were age- and sex-matched. Patients affected by metabolic bone disease, rheumatoid arthritis, autoimmune disease, or malignant tumour around the arthroplasty were excluded from the study. Routine pre-operative blood testing, ESR, C reactive protein, and intraoperative histological testing were also used to exclude the presence of infection.

For the 34 patients in the early AL and stable groups, the indications for total hip arthroplasty were femoral aseptic necrosis (12/34, 35 %), idiopathic osteoarthritis (9/34, 26 %), developmental dysplasia of hip (6/34, 18 %), femoral neck fracture (5/34, 15 %), and osteoarthritis secondary to trauma (2/34, 6 %). The proportion of underlying diseases was similar in the two groups and laterality of arthroplasty and use of bearing material and bone cement was also matched in all three patient groups (Table 1).

All patients had a standard radiograph of the pelvis and a lateral radiograph of the hip. The prosthesis was deemed stable when neither symptoms nor radiographic finding of osteolysis were evident [6, 8]. The THA was considered to have late loosening when at least one component required replacement, and the indication for the revision surgery was the correction of pain and periprosthetic osteolysis. Computer tomography imaging was performed to confirm loosening of the prosthesis. The severity of osteolysis in the radiograph was evaluated according to the classification system of Gruen et al. [9] (dividing femur osteolysis into seven regions of interest [ROI]) and the DeLee and Charley criteria (grading periacetabular bone resorption into three ROI) [10]. Focal osteolysis was graded by assigning a score of 0 to 3 to each ROI as

follows: 0, no osteolysis; 1, osteolysis less than two millimetres; 2, osteolysis between two and ten millimetres; 3, osteolysis greater than ten millimetres [6]. The total osteolysis score (OS) was 30. The Harris hip score [11] showing the clinical results of THA and the duration of follow-up (years) of the three groups were also recorded (Table 1).

Immunoenzymatic assay

Whole peripheral blood was collected in tubes containing EDTA (K2) (Yangpu, Guangzhou, China) and centrifuged at 3,000 rpm for two minutes within two hours of sampling to prevent disruption of cells from contaminating intracellular or induced cytokines. Plasma was separated immediately and placed in another tube. Processed plasma contained approximately $0.47\text{--}45.4 \times 10^6$ leucocytes [12]. To prevent leucocyte contamination, the plasma was recentrifuged at 13,000 rpm for five minutes, transferred to plastic cuvettes, and stored at -80°C until analysis. In patients who underwent revision THA for AL, blood samples were collected the day before surgery so that any immunological changes due to anaesthesia would be avoided. All sample collection, sample preparation, and immunoenzymatic assays were performed by the same research personnel. The OPG concentration was measured using an instant human enzyme-linked immunosorbent assay (ELISA) (eBioscience, Bender MedSystems GmbH, Vienna, Austria). An anti-human OPG polyclonal coating antibody was absorbed onto microwells. Human OPG binds to the antibody before a biotin-conjugated polyclonal anti-human OPG antibody binds to the human OPG. The standard reference is between 2.5 and 1,000 pg/ml. RANKL was measured using a Milliplex map human single plex ELISA kit (Millipore Corp, MA, USA). The luminex technology uses microspheres with two fluorescent dyes, which allows more precise calculation of the RANKL concentration than routine spectrophotometry. The standard reference was between 4.8 and 5,000 pg/ml, with an intra-assay coefficient of variation (CV) less than 7 %, and inter-assay CV less than 8 %. NTX was measured using a human sandwich ELISA kit (MyBioSource, CA, USA). The results were expressed as nanoMole of bone collagen equivalents per litre (nMBCE/L). The standard reference was between 2 and 500 nMBCE/L without significant cross-reactivity or interference. PICP was measured using a quantitative human sandwich ELISA kit (BlueGene Biotech, Shanghai, China). The standard reference was between 0.1 and 25 ng/ml, with intra-assay CV less than 10 % and inter-assay CV less than 12 %. There was no significant cross-reactivity or interference. TNF- α was measured using a human sandwich ELISA kit (Quantikine, R&D Systems, MN, USA). The standard reference was between 1.6 and 1,000 pg/ml, with intra-assay CV less than 4.6 %, and inter-assay CV less than 7.4 %. IL-1 β was measured using a human sandwich ELISA kit (RayBiotech, NSW, Australia). The standard reference was

between 0.3 and 100 pg/ml, with intra-assay CV less than 10 % and inter-assay CV less than 12 %.

The binding between protein and antibodies was detected by the following sequence of reactions: anti-human biotinylated antibody, streptavidin-peroxidase conjugates, and substrate (tetrazabenzidine) reacting with peroxidase. The reaction was stopped with sulphuric acid and optical density read at 450 nm.

Statistical analysis

Quantitative results of the clinical variables were expressed as the mean and the standard error of mean (SEM), and

tested by analysis of variance (Bonferroni test of ANOVA) using SPSS17.0 software (USA). Synthesising analysis of the continuous outcomes of six biomarkers with the total number of assigned participants as the denominator was performed using RevMan software (RevMan 5.1.2) from the Cochrane Collaboration [13]. For every comparison between different AL and control groups, the standardised mean difference (Hedges' adjusted SMD) was calculated as the effect size with a 95 % credible interval (CI) using a random-effects model to incorporate the assumption that the multiple biomarkers related to AL could estimate the intervention effects. We used visual inspection of forest plots to

Table 1 Group characteristics

Characteristics	Late loosening THA (n=31)	Early loosening THA (n=15)	Stable THA (n=19)	Healthy donors (n=35)
Gender				
Male	13	5	9	16
Female	18	10	10	19
Age (years)				
Mean± SEM	66±2.6	65±2.7	61±2.7	62±3.0
Range	39–87	51–85	37–82	30–87
Median	70	63	59	66
Duration of follow-up (years)				
Mean± SEM	16.9±0.8 ^a	5.3±0.4	5.3±0.5	
Range	8–23	3–9	2–12	
Median	18	5	5	
Indication for THA				
Femoral aseptic necrosis		4 (27 %)	8 (42 %)	
Idiopathic osteoarthritis		4 (27 %)	5 (26 %)	
Developmental dysplasia of hip		2 (13 %)	4 (21 %)	
Femoral neck fracture		3 (20 %)	2 (11 %)	
Osteoarthritis secondary to trauma		2 (13 %)	0	
Bilateral hip prostheses				
Yes	1	2	3	
No	30	13	16	
Bearing materials				
Metal-on-polyethylene	26	10	11	
Metal-on-metal		0	5	
Ceramic-on-ceramic		4	2	
Others/unknown	5	1	1	
Bone cement				
Present	9	0	2	
Absent	22	15	17	
Harris score				
Mean± SEM	63±1.4 ^a	89±1.8 ^b	93±1.1	
Range	55–75	75–95	85–100	
Median	65	91	95	
Osteolysis score	20±0.5 ^a	7±0.3 ^b	3±0.3	
Acetabular score (mean± SEM)	6±0.3 ^a	2±0.3 ^b	1±0.2	
Femoral score (mean± SEM)	14±0.4 ^a	5±0.4	2±0.3	

THA total hip arthroplasty, SEM standard error of the mean

^a The late AL group showed a significant difference compared to the early AL group (Bonferroni test of ANOVA, $p<0.01$)

^b The early AL group showed a significant difference compared to stable group (Bonferroni test of ANOVA, $p<0.01$)

Fig. 1 Synthetic analysis of plasma level of six biomarkers between the aseptic loosening (AL) and stable groups

investigate SMD with p value in 95 % CI and the weight of each biomarker in the group. A secondary analysis of the efficacy of biomarkers in diagnosing AL was performed by the outcomes of heterogeneity (shown by p value from a chi-square test), and when the value of I^2 was lower than 50 %, the heterogeneity was thought to be very low [14].

Results

In all groups, there were no differences with regard to the age, gender, indication for total hip arthroplasty, laterality of THA, prosthetic material, or use of bone cement (data not shown). However, Harris hip score was lowest and OS including femoral and acetabular OS was highest in the late AL group, followed by the early AL and stable group (Bonferroni test of AVONA, all $p < 0.01$). The follow-up years was significantly longer in the late AL than in the other two groups who were nearly the same (Bonferroni test of AVONA, all $p < 0.01$) (Table 1).

The plasma level of OPG, RANKL, NTX, TNF- α , and IL-1 β were highest in the late AL group, followed by the early

AL, stable and healthy groups. The level of PICP was highest in the healthy group, followed by the stable, early AL and late AL groups. The synesthetic analysis showed the plasma levels of six biomarkers were significantly different between the stable and healthy groups, and between the late AL and stable groups (both $p = 0.02$). The outcomes of PICP were shown by negative value because it changes reversely in relation to the other five markers. The heterogeneity of the six biomarkers is not significant ($p > 0.05$) and very low ($I^2 = 0$) in both comparisons (Figs. 1 and 2). But there was no significant difference between the early AL group and the stable (Fig. 3) or late AL groups (data not shown). The SMD (95%CI) of IL-1 β and PICP was highest in all markers between the late AL and stable (0.69 [-0.06, 1.43] and 0.71 [-0.07, 1.48], respectively) and between early AL and stable groups (0.28 [-0.64, 1.20] and 0.43 [-0.38, 1.24], respectively). The SMD (95%CI) of OPG and RANKL was highest in all markers between the stable and healthy groups (0.60 [-0.15, 1.35] and 0.52 [-0.09, 1.13], respectively).

Patients who had cemented implants had significantly longer post-operative years (ANOVA, $p = 0.031$), lower Harris hip score ($p = 0.005$), higher OS ($P = 0.013$), higher acetabular OS

Fig. 2 Synthetic analysis of plasma level of six biomarkers between the stable and healthy groups

Fig. 3 Synthetic analysis of plasma level of six biomarkers between the early aseptic loosening (AL) and stable groups

($p=0.025$), higher femur OS ($p=0.019$), and higher level of $\text{TNF-}\alpha$ ($p=0.042$) than patients with cementless hips (Table 2). But the plasma level of the other five biomarkers had no significant differences between the two groups.

Discussion

Despite the enormous success rates of hip arthroplasty, patients face a number of potential complications, including periprosthetic osteolysis, implant loosening, and joint infections. Osteolysis and subsequent aseptic joint loosening is the most common reason for joint revision [15].

Diagnostic and prognostic biomarkers have the potential to provide an early, accurate, and noninvasive diagnosis of these undesired outcomes as well as help design interventions to prevent some of these complications, especially AL.

There has been a great variability reported in the use of biomarkers to diagnose AL. The majority of these studies were case control studies with small sample sizes, and with few biomarkers investigated [3, 4, 7, 15, 16]. This type of evaluation was prone to type I error (false positive result). So it is necessary for the study to include multiple biomarkers (at least six) and simultaneously consist of at least 100 patients or healthy donors in AL of THA.

Table 2 Results

Item	Presence of cement	N	Mean	SEM	P value of ANOVA
Age	Cemented	15	61.13	3.682	
	Cementless	50	65.16	1.768	
Postoperative years	Cemented	15	14.13	1.786	$P=0.031$
	Cementless	50	9.84	0.921	
Harris hip score	Cemented	15	68.33	4.007	$P=0.005$
	Cementless	50	80.98	2.082	
Osteolysis score (OS)	Cemented	15	16.33	1.968	$P=0.013$
	Cementless	50	10.74	1.041	
Acetabular OS	Cemented	15	4.80	0.738	$P=0.025$
	Cementless	50	3.16	0.325	
Femur OS	Cemented	15	11.40	1.316	$P=0.019$
	Cementless	50	7.58	0.774	
OPG	Cemented	13	30.256	8.238	
	Cementless	42	26.197	1.655	
$\text{TNF-}\alpha$	Cemented	14	46.175	20.358	$P=0.042$
	Cementless	35	19.202	1.624	
NTX	Cemented	14	21.390	2.065	
	Cementless	36	25.391	4.385	
PICP	Cemented	8	1.209	0.1380	
	Cementless	34	1.401	0.0534	
RANKL	Cemented	8	263.64	66.95	
	Cementless	39	167.51	23.85	
IL-1 β	Cemented	7	1.199	0.153	
	Cementless	30	1.486	0.248	

Immune inflammatory responses and macrophage release of proinflammatory cytokines IL-1 β and TNF- α [17, 18] (induced by phagocytosis of periprosthetic particle) directly induce fibroblast proliferation and tissue necrosis, and stimulate activation and proliferation of newly formed macrophages, osteoblasts (whose surface has OPG), and osteoclasts (mediated by RANKL) around the implant [19]. This causes increased bone resorption (reflected by blood NTX) or abnormal bone formation (reflected by blood PICP) [7, 20]. Periprosthetic bone resorption, increased local debris, macrophage activation, systemic proinflammatory cytokine synthesis, osteoblast activation, pain, and complete prosthetic loosening constitute the cycle of AL [4–6]. These studies showed IL-1 β , TNF- α , OPG, RANKL, PICP and NTX are important biomarkers in AL of THA. But it is not known which is best in the evaluation of AL and if they affect AL in different degrees in different stages of AL. As we know, there has been no study simultaneously comparing the six biomarkers in AL.

Although most biomarkers are sensitive in many pathological processes such as the initial stage after THA, the level of biomarkers in serum or plasma is usually very low [21] and difficult to use in the early detection of AL. So we postulated that synthesised analysis of multiple biomarkers would be more effective in reflecting AL than any single test, because multiple positive factors increase the probability of detection of disease clinical practice. In our study the plasma level of OPG, TNF- α , NTX, RANKL and IL-1 β all was from high to low in the late AL, early AL, stable and healthy groups. And PICP changed inversely in four groups. Although any single biomarkers showed no significant differences between the AL and control groups, synthetic analysis of six markers showed that there was a significant difference between the stable and healthy groups and between the late AL and stable groups. Furthermore, there was extremely low heterogeneity among different outcomes of biomarkers in our study (I^2 was zero). Our results supported the finding of the former study that OPG, TNF- α , NTX, RANKL and IL-1 β were related to AL, especially severe AL [4–6]. Furthermore, it was such that in the stable group the clinical and radiographic method could not detect the existence of AL; the synthetic analysis of multiple biomarkers showed significant difference compared to healthy donors. So the six biomarkers analysis may be better than the traditional method in diagnosing AL. But further long-term prospective or multi-centre studies are needed to further confirm our findings.

Measuring periprosthetic BMD in subjects with failed implants is technically more difficult than in subjects with fixed implants. Scan analysis can be difficult because of the destruction of bony landmarks or change in position of implanted landmarks [22, 23]. We selected the bone resorption marker NTX and bone formation marker PICP to reflect the bone turnover process. We found reduced plasma NTX

levels and increased PICP levels in patients with late loosening, early loosening, and stable prostheses, compared to healthy donors. Furthermore our results showed the SMD of PICP was higher in the AL group than in the stable group according to other markers. These findings indicate that bone formation in patients of AL quickly decreases as loosening of the arthroplasty develops, as Granchi et al. found [5].

The receptor activator of nuclear factor- κ B ligand (RANKL) is a member of the TNF superfamily. It appears to play an important role in both immune responses and bone morphogenesis [24, 25]. The ratio of the expression of RANKL to OPG is critical to the outcome of bone resorption [26]. Kim et al. [27] demonstrated decreased levels of OPG in failed THA. We found the plasma level of OPG increased in AL. OPG level may reflect a protective mechanism of the skeleton to increased bone resorption. This defence inhibits osteoclast formation and bone resorbing activity in AL [6, 28]. In confirmation, the SMD of biomarkers in our study showed OPG including RANKL acted mainly in the early stage of AL compared to other proinflammatory or bone turnover biomarkers. However, the systemic proinflammatory cytokine IL-1 β was more pronounced in the severe stage of AL of THA than the two key meditative factors of AL. These results may explain why there was much variability about the same biomarker in a similar study.

In our study, OS including acetabular and femur OS were all significantly higher and yet Harris hip scores were significantly lower in cemented THA than in cementless THA. At the same time, plasma levels of TNF- α were significantly higher in patients with cemented prostheses than those with cementless varieties. These results suggest that cement promoted the release of proinflammatory cytokines such as TNF- α both locally and systematically, leading to prosthetic osteolysis in total hip arthroplasty [29]. But the postoperative years of cemented THA was significantly longer than that of cementless hips in this study, so further study including more samples to confirm the conclusion is needed. A problem with using biomarkers is the high level of intra-individual variability [30]. And the Elisa kits we used came from different manufacturers, which facilitated the collection of kits, but made the stability and coherence of test methods difficult to guarantee. This study emphasises synthetic biomarkers analysis is superior to the conventional test methods in early diagnosis of AL of THA, (because of the development of high throughput protein chip [21, 31] techniques containing multiple markers and giving prompt results), are good for the combination analysis of multiple biomarkers.

References

1. Malchau H, Herberts P, Ahnfelt L (1993) Prognosis of total hip replacement in Sweden: follow-up of 92,675 operations performed 1978–1990. *Acta Orthop Scand* 64:497–506

2. Purdue PE, Koulouvaris P, Potter HG (2007) The cellular and molecular biology of periprosthetic osteolysis. *Clin Orthop Relat Res* 454(1):251–261
3. Hernigou P, Intrator L, Bahrami T et al (1999) Interleukin-6 in the blood of patients with total hip arthroplasty without loosening. *Clin Orthop Relat Res* 366:147–154
4. Hundrić-Haspl Z, Pecina M, Haspl M et al (2006) Plasma cytokines as markers of aseptic prosthesis loosening. *Clin Orthop Relat Res* 453(12):299–304
5. Granchi D, Pellacani A, Spina M et al (2006) Serum levels of osteoprotegerin and receptor activator of nuclear factor-kappaB ligand as markers of periprosthetic osteolysis. *J Bone Joint Surg Am* 88(7):1501–1509
6. Savarino L, Granchi D, Cenni E et al (2005) Systemic cross-linked N-terminal telopeptide and procollagen I C-terminal extension peptide as markers of bone turnover after total hip arthroplasty. *J Bone Joint Surg Br* 87-B:571–576
7. Li MG, Thorsen K, Nilsson KG (2004) Increased bone turnover as reflected by biochemical markers in patients with potentially unstable fixation of the tibial component. *Arch Orthop Trauma Surg* 124:404–409
8. Lavernia CJ, Alcerro JC (2011) Quality of life and cost-effectiveness 1 year after total hip arthroplasty. *J Arthroplasty* 26(5):705–709
9. Gruen TA, McNeice GM, Amstutz HC (1979) “Modest of failure” of cemented stem-type femoral components: a radiographic analysis of loosening. *Clin Orthop* 141:17–27
10. Delee JG, Charnley J (1976) Radiological demarcation of cemented sockets in total hip replacement. *Clin Orthop* 121:20–32
11. Harris WH (1969) Traumatic arthritis of the hip after dislocation and acetabular fractures: treatment by mold arthroplasty: an end result study using a new method of evaluation. *J Bone Joint Surg Am* 51-A:737–755
12. TNC Centre (2011) Review manager (RevMan), 5.1 for Windows edn. The Cochrane Collaboration, Copenhagen
13. Schmidt-Rohlfing B, Thomsen M, Niedhart C et al (2002) Correlation of bone and cartilage markers in the synovial fluid with the degree of osteoarthritis. *Rheumatol Int* 21:193–199
14. Li YL, Wang L, Li J, Huang Y et al (2011) The correlation between uric acid and the incidence and prognosis of kidney diseases: a systematic review and meta-analysis of cohort studies. *Chin J Intern Med* 50:555–561
15. Mertens MT, Singh JA (2011) Biomarkers in arthroplasty: A systematic review. *Open Orthop J* 5:92–105
16. Savarino L, Avnet S, Greco M (2010) Potential role of tartrate-resistant acid phosphatase 5b (TRACP 5b) as a surrogate marker of late loosening in patients with total hip arthroplasty: a cohort study. *J Orthop Res* 28(7):887–892
17. Schwarz EM, Lu AP, Goater JJ et al (2000) Tumor necrosis factor alpha/nuclear transcription factor-kappa B signaling in periprosthetic osteolysis. *J Orthop Res* 18:472–480
18. Sipniewska G, Lis K, Bilinski PJ (2002) Bone turnover markers and cytokines in joint fluid: analyses in 10 patients with loose hip prosthesis and 39 with coxarthrosis. *Acta Orthop Scand* 73:518–522
19. Urban RL, Shanbhag AS, Lee SH et al (1996) Particulate-induced, prostaglandin and cytokine mediated bone resorption in an experimental system and failed joint replacements. *Am J Ther* 3:27–41
20. Lassus J, Salo J, Jiranek WA et al (1998) Macrophage activation results in bone resorption. *Clin Orthop Relat Res* 352:7–15
21. van der Heide HJ, van der Kraan PM, Rijnberg WJ et al (2010) Elevated levels of numerous cytokines in drainage fluid after primary total hip arthroplasty. *Int Orthop* 34:1099–1102
22. Takagi M, Santavirta S, Ida H et al (2001) High-turnover periprosthetic bone remodeling and immature bone formation around loose cemented total hip joints. *J Bone Miner Res* 16:79–88
23. Arabmotlagh M, Sabljic R, Rittmeister M et al (2006) Changes of the biochemical markers of bone turnover and periprosthetic bone remodeling after cemented hip arthroplasty. *J Arthroplasty* 21:129–134
24. Anderson DM, Maraskovsky E, Billingsley WL et al (1997) A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. *Nature* 390:175–179
25. Lacey DL, Timms E, Tan HL, Kelley MJ et al (1998) Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 93:165–176
26. Masui T, Sakano S, Hasegawa Y et al (2005) Expression of inflammatory cytokines, RANKL and OPG induced by titanium, cobalt-chromium and polyethylene particles. *Biomaterials* 26:1695–1702
27. Kim KJ, Kotake S, Udagawa N et al (2001) Osteoprotegerin inhibits in vitro mouse osteoclast formation induced by joint fluid from failed total hip arthroplasty. *J Biomed Mater Res* 58:393–400
28. Zhou X, Zhang P, Zhang C et al (2010) Promotion of bone formation by naringin in a titanium particle-induced diabetic murine calvarial osteolysis model. *J Orthop Res* 28(4):451–456
29. Kovacic MW, Gradisar IA, Haprian JJ et al (2000) Osteolytic indicators found in total knee arthroplasty synovial fluid aspirates. *Clin Orthop Relat Res* 379:186–194
30. Woitge HW, Petchersdorfer M, Li Y et al (1999) Novel serum markers of bone resorption: clinical assessment and comparison of established urinary indices. *J Bone Miner Res* 14:782–801
31. Shanbhag AS, Kaufman AM, Hayata K et al (2007) Assessing osteolysis with use of high-throughput protein chips. *J Bone Joint Surg Am* 89:1081–1089