

Activation of Complement Following Total Hip Replacement

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Abstract

The aim of this study was to investigate whether complement activation, via the classical and alternative pathways, occurs following a cemented total hip replacement (THR) surgery due to osteoarthritis. Blood samples were collected systematically from 12 patients – six male and six women, with a median age of 75 (range: 59–90 years) – preoperatively, 6 h post-operatively and on the first, second and third post-operative day. Total function of classical (CH50) and alternative pathways (AH50) was evaluated, along with the determination of serum concentrations of the complement proteins C3, C4, C3d, the soluble terminal complement complex (sTCC) sC5b-9, as well as C-reactive protein (CRP) and albumin. Measurements of CRP and albumin levels elucidated a marked inflammatory response following the operation. The CH50, AH50 and C3 and C4 levels were significantly lower 6 h after the surgery compared with the preoperative levels, but elevated above the preoperative levels during the following 3 days. The complement activation product C3d levels increased continually during the whole observation period, from 13.5 AU/ml (range: 8–19 AU/ml) preoperative to 20 AU/ml (range: 12–34 AU/ml) on the third post-operative day. Furthermore, we observed an increase in the sC5b-9 levels between the preoperative and the third post-operative day. These results demonstrate a significant activation of the complement system following cemented THR. Further studies are needed to elucidate the time frame and the pathogenic role of this observed complement activation.

Introduction

To comprehend the initiation of inflammatory processes, various human models have been used that develop acute inflammation, for example, following acute infection, injury, elective surgery or other acute illness [1, 2]. In this aspect, total hip replacement (THR) due to osteoarthritis has proven to be a relevant human model [3]. Using THR as a promoter of the initiation of the inflammatory response, the timing as well as the magnitude of the trauma can be controlled.

Following THR, the body reacts with a robust post-operative inflammatory response with the rise of several cytokines that affect a wide variety of cells and may modify the inflammatory response [3, 4]. This inflammatory reaction promotes healing of the wound with an increased expression of adhesion molecules on the surface of endothelium cells and the recruitment of white blood cells, antibodies and complement to the surgery site [5]. Complement proteins participate in these inflammatory processes by attacking any unwelcome pathogens, inducing

inflammation and promoting healing of tissue by removing dead cells, cell debris and immune complexes from the surgical area [6]. However, the activation of the complement system is not entirely beneficial, because excessive activation can cause tissue damage [7]. The complement activation could possibly play an important role in the pathogenic processes underlying the aseptic loosening of the joint prosthetic parts, one of the most deleterious complications of THR. Thus, further investigation of the activation of the complement system from orthopaedic viewpoint is of importance.

Previous studies have shown an elevation of methyl methacrylate in serum, an ingredient of the cement, following cemented THR [8–10]. However, the half-life of these particles is short, only a few minutes, due to high pulmonary clearance [9]. Only few studies have addressed the question whether these methyl methacrylate particles initiate the activation of the complement system. Bengtson *et al.* demonstrated a significant activation of the complement system in 30 patients who underwent THR with a cemented component. They observed a decline in total

complement activity, a decrease in C3, C4 and C5 levels and a concomitant increase in C3a and C5a in the first 30 min of the surgery compared with 15 patients undergoing uncemented THR who showed no significant activation of the complement system [11]. However, a study conducted a few years later by Lewis and colleagues reported no significant decline in C3 and C4 levels during the first 30 min after the cement was introduced in patients undergoing a standard cemented THR procedure [12].

The object of the present study was to investigate *in vivo* the activation of the complement system in the acute inflammation reaction situation following a standardized THR with all components cemented. We followed a group of patients who underwent THR due to osteoarthritis and evaluated the complement activity at several time points. Our result demonstrated a noteworthy activation of the complement system.

Materials and methods

Patients. Twelve patients with osteoarthritis, six men and six women, participated in the study. They underwent standardized elective cemented THR performed under spinal anaesthesia. The bone cement (Refobacin®; Biomet Orthopaedics, Ried bei Kerzers, Switzerland) contained, among other ingredients, polymethyl acrylate and methyl methacrylate. Patients with any known inflammatory disorder or taking medication that affects inflammation were excluded from the study. In addition, patients who required blood transfusion following the surgery were also excluded from further participation. All participants gave their written informed consent, which was obtained by the hospital surgeon at admission. The study protocol was accepted by the Hospital Ethics Committee (31/2003/150208) and reported to The Icelandic Data Protection Authority (S1421).

Sample collection and storage. Five blood samples (plasma and serum) from each participant were collected according to the following protocol: preoperatively (preop), 6 h after the surgery (post-op) and in the early morning of the first, second and third post-operative day. After collection, the samples were put immediately on ice and centrifuged at 1200 g for 10 min within 1 h of sampling. Finally, all biosamples were then aliquoted before storage in 1-ml plastic tubes at -80°C for later analysis.

Laboratory parameters. C-reactive protein (CRP; normal range: <10 mg/l) and albumin (36–45 g/l) were measured in all blood samples by the central hospital laboratory.

Complement analysis. Total activity of the classical pathway (CH50) and the alternative pathway (AH50) was evaluated by conventional haemolysis test as previously described [13]. In brief, CH50 was measured by antibody-coated sheep red blood cells, while guinea-pig red blood cells were used for the evaluation of AH50. The activity of each pathway was proportional to the magnitude of red

blood cell lyses. The haemolysis was then compared with a standard curve to assess the total function of the classical and alternative pathways. Normal ranges for CH50 and AH50 were 60–124% and 65–198%, respectively, as determined in 100 healthy individual blood donors.

The complement activation product C3d was measured by ELISA, as previously described [14], where polyclonal rabbit anti-human C3d antibodies (DAKO, Glostrup, Denmark) were used as the coating and analysing antibody (normal range: <50 AU/ml). The concentrations of C3 (normal range: 0.74–1.38 g/l) and C4 (normal range: 0.14–0.37 g/l) in serum were measured with rate nephelometry in a Beckman nephelometer Array 360 system (Beckman Coulter®; Fullerton, CA, USA) according to the manufacturer's recommendations.

The soluble terminal complement complex (sTCC) sC5b-9 levels were measured by a commercial enzyme immunoassay according to the manufacturer's instructions (Quidel, San Diego, USA).

Statistical analyses. Data were collected in Excel (Microsoft) and analysed by GraphPad Prism 5.0 (Graphpad software, La Jolla, CA, USA). Data were presented as median with interquartile range (IQR) (25th and 75th quartile, depicted in figures) or total range (min-max, described in text). Because the results were not normally distributed, the nonparametric Friedman test was used for analysis, followed by the Dunn's multiple comparison test for further comparison of different time points. Furthermore, Wilcoxon matched-pairs signed-rank test was used for the comparison of two time points. A *P*-value of <0.05 was considered significant.

Results

Twelve patients, six men and six women, with a median age of 75 (range: 59–90), finished the 4-day study protocol.

Kinetics of the acute inflammatory markers albumin and CRP

The concentration of serum albumin declined significantly during the first 6 h post-surgery ($P < 0.001$) and stayed low throughout the rest of the study period (Fig. 1A). In contrast, serum CRP increased significantly, from 5 mg/l (range: 1–44 mg/l) preoperatively to 148 mg/l (range: 25–264 mg/l) on the third post-operative day (Fig. 1B, $P < 0.001$).

Total activity of the classical and alternative pathways (CH50 and AH50)

The total function of the classical pathway (CH50) was elevated above the normal range preoperatively (Fig. 2A, 171% (range: 125–236%)), while 6 h post-operatively, the function had decreased down towards normal upper limits (132% (range: 88–204%)). Thereafter, the activity slowly increased again and reached 202% (range: 132–320%) on

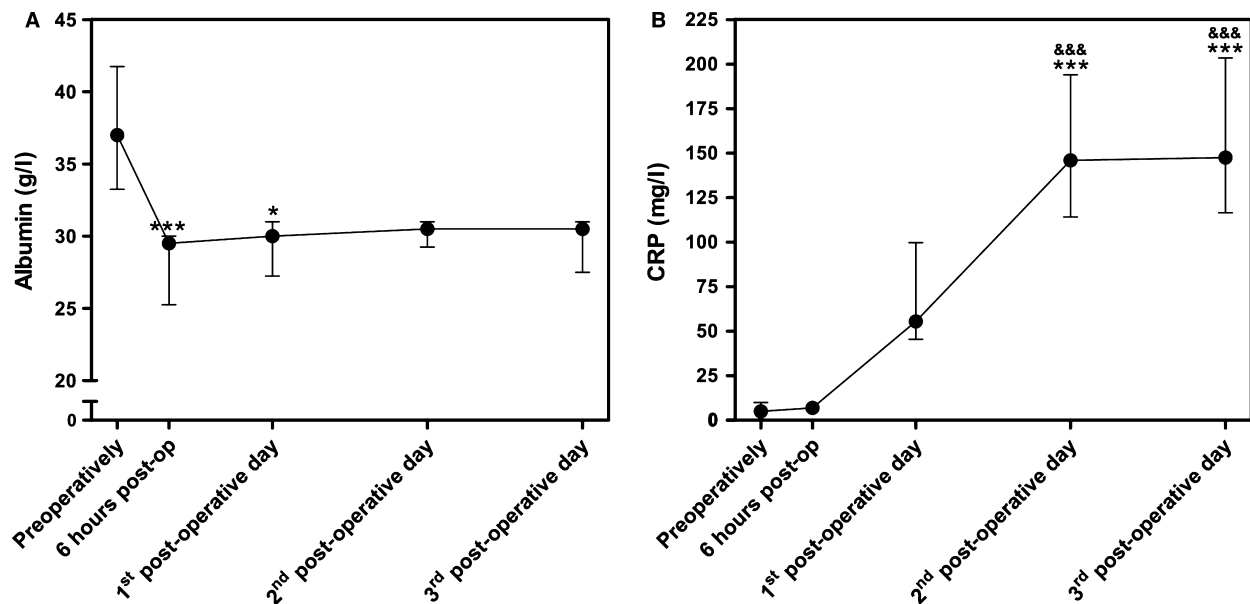


Figure 1 The median concentration of serum (A) albumin and (B) CRP in 12 patients, who underwent THR due to osteoarthritis. Data are presented as median with IQR (25th and 75th quartile). Normal value for albumin is 34–45 g/l and for CRP is <10 mg/l. Statistical analysis was performed with the nonparametric Friedman test followed by Dunn's multiple comparison test. * $P < 0.05$ and *** $P < 0.001$ compared with preoperative measurement. &&& $P < 0.001$ compared with 6 h post-operative measurement.

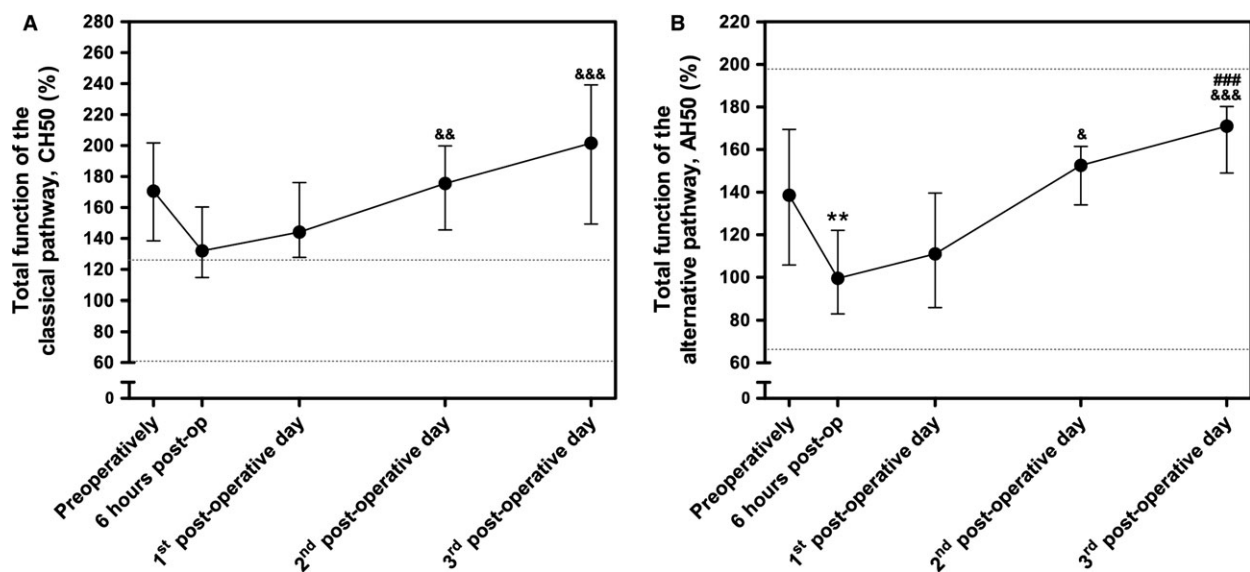


Figure 2 The total activity of the (A) classical (CH50) and (B) alternative (AH50) complement pathways in 12 patients who underwent THR due to osteoarthritis. Data are depicted as median with IQR (25th and 75th quartile). Normal upper and lower values of C3 and C4 from healthy individuals are marked by lines. Statistical analysis was performed with the nonparametric Friedman test followed by Dunn's multiple comparison test. ** $P < 0.01$ compared with preoperative measurement. & $P < 0.05$, && $P < 0.01$ and &&& $P < 0.001$ compared with 6 h post-operative measurement. ### $P < 0.001$ compared with 1st post-operative day measurement.

third post-operative day ($P < 0.001$). The total function of the alternative pathway (AH50) demonstrated a similar pattern by decreasing in function significantly in the first 6 h post-operatively, from 139% (range: 75–227%) to 100% (range: 55–140%) (Fig. 2B, $P < 0.01$), and then slowly increasing again up to 171% (range: 140–208%) on third post-operative day ($P < 0.001$).

Concentration of serum complement components

The median concentration of the C3d complement activation product was similar pre- and 6 h post-operatively, but then increased significantly during the three following observation days, from 13.5 AU/ml (range: 8–19 AU/ml) preoperatively to 20 AU/ml (range:

12–34 AU/ml) on the third post-operative morning (Fig. 3A, $P < 0.001$).

The median concentration of the sC5b-9 complement complex was comparable pre- and 6 h post-operatively, although it was slightly lower post-operatively (not significant). Then, sC5b-9 increased during the three following observation days, from 2776 ng/ml (range: 920–4227 ng/ml) preoperatively to 3625 ng/ml (range: 1876–7058 ng/ml) on the third post-operative morning; however, it did not reach significant differences (Fig. 3B). Further analyses demonstrated that eight patients increased their values from the preoperative levels to the third post-operative day, while five patients did not elevate their sC5b-9 levels post-operatively (Fig. 3C, $P = 0.0522$). Additionally, we assessed the overall correlation between the C3d and sC5b-9 values collected at the different time points and found that they correlated significantly ($r^2 = 0.1392$; $P = 0.003$).

On the other hand, the median concentrations for both the C3 and C4 decreased significantly between the preoperative and 6 h post-operative samples, from 1.165 g/l (range: 0.78–1.39 g/l) to 0.925 g/l (range: 0.59–1.2 g/l) and 0.26 g/l (range: 0.17–0.4 g/l) to 0.21 g/l (range: 0.12–0.28 g/l), respectively ($P < 0.05$), but then reached the preoperative values in the succeeding 3 days (Fig. 4A–B).

No significant differences were observed between genders in any of these measurements.

Discussion

We have previously demonstrated that patients undergoing cemented THR due to osteoarthritis develop an acute inflammatory reaction measured by an increase in CRP and

cytokine levels as well as a decrease in albumin serum levels [3]. In the present study, we investigated whether the complement system was also activated in patients undergoing THR surgery. Our results indicate that the complement system is also activated immediately following the surgery. First and foremost, we observed a significant drop in total haemolytic activity both in the classical and alternative complement pathways 6 h post-operatively, probably reflecting the consumption exceeding the production of complement components at the initiation of the acute-phase response. Consistent with these observations, we also noted a decrease in serum concentration of the C3 and C4 complement components in the same time frame. After this initial drop, we observed that both the haemolytic activity and C3 and C4 levels increased and continued to rise throughout the observation time of 3 days. Complement activation following THR was further confirmed by a steady increase in the cleavage complement fragment, C3d. This fragment is produced as a result of cleavage of the complement factor C3 following the activation of the complement pathways [15], and the terminal complement complex sC5b-9 is generated by the assembly of C5 through C9, which additionally is a sign of activation of the complement system [16].

Our study results are consistent with an earlier report on activation of the complement system following a cemented THR [11]. That study revealed that concentrations of C3, C4 and C5 in plasma decreased during the surgery and the levels of C3a and C5a increased, but all levels were back to normal 24 h later. However, this study by Bengtson *et al.* covered only 24 h post-operatively, and thus, they were unable to demonstrate whether the complement system stayed active for longer period than these 24 h that they focused on in their study. On the contrary, our study

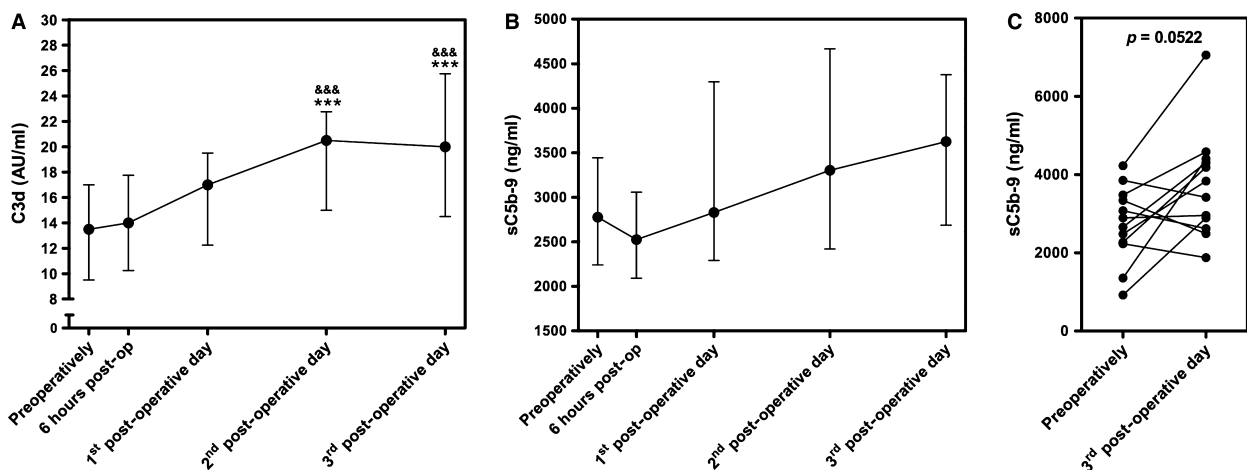


Figure 3 The concentration of (A) C3d and (B–C) sC5b-9 complement activation products in serum pre- and post-operatively in 12 patients who underwent THR due to osteoarthritis. (A–B) Data of C3d and sC5b-9 are presented as median with IQR (25th and 75th quartile). Normal value for C3d is <50 AU/ml. Statistical analysis was performed with the nonparametric Friedman test followed by Dunn's multiple comparison test. *** $P < 0.001$ compared with preoperative measurement. &&& $P < 0.001$ compared with 6 h post-operative measurement. (C) Data of sC5b-9 for the 12 individual patients preoperatively and on the 3rd post-operative day. Statistical analysis was performed with Wilcoxon matched-pairs signed-rank test.

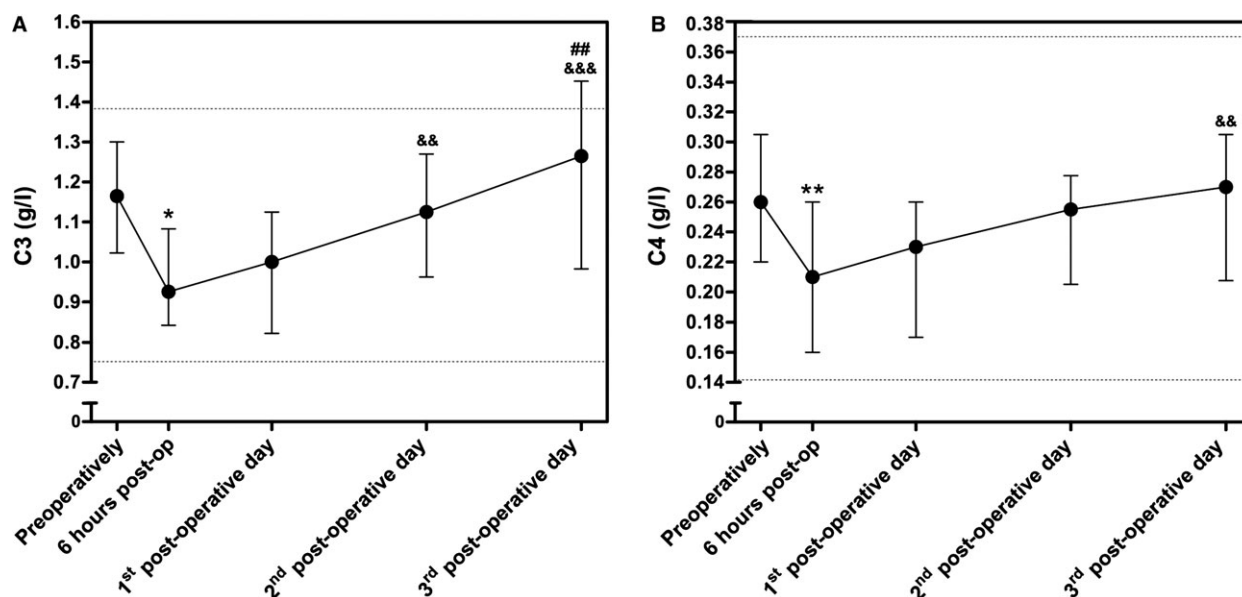


Figure 4 The median serum concentrations of (A) C3 and (B) C4 complement proteins in 12 patients who underwent THR due to osteoarthritis. Data are depicted as median with IQR (25th and 75th quartile). Normal upper and lower values of C3 and C4 from healthy individuals are marked by lines. Statistical analysis was performed with the nonparametric Friedman test followed by Dunn's multiple comparison test. * $P < 0.05$ and ** $P < 0.01$ compared with preoperative measurement. && $P < 0.01$ and &&& $P < 0.001$ compared with 6 h post-operative measurement. ## $P < 0.01$ compared with 1st post-operative day measurement.

clearly demonstrated the activation of the complement system for longer period, supported by our observation of increase in C3d and sC5b-9 levels both at the 2nd and at the 3rd post-operative days. Thus, our study also indicated the fact that the complement system was likely still activated at the end of the study period on the third post-operative day. However, another study by Lewis *et al.* did not find the activation of the complement system following THR [12].

Even though osteoarthritis is usually not considered an inflammatory disease, the joint often shows clinical signs of mild or moderate inflammation, reflected by increased white blood cell counts and elevated levels of cytokines and acute-phase proteins, although not nearly as much as in patients with rheumatoid arthritis [17]. This could explain the high presurgical haemolytic activity of the classical complement pathway. The changes in both functional complement activity and levels of C3, C4, C3d and sC5b-9 immediately after the surgery and for the following 3 days are indicative of activation of the complement system, although the concentration of these parameters was within the normal range during the whole study period. Many studies have reported a local production of complement components in various tissues [18] following general surgery [19]. If the acute-phase response after THR was mainly local, it is not surprising that we only saw mild effects in the main bloodstream. Our study did not reveal whether the increased activity of the classical and alternative complement pathways following THR was a result of independent activation of both pathways or whether the

alternative complement pathway was activated as an amplification loop for the classical complement pathway.

The cement that is used to fix the prosthesis probably contributes to the observed activation of the complement system, as an *in vitro* study has shown that methyl methacrylate, an ingredient of bone cement, can activate the complement system [20, 21]. Furthermore, and more importantly, studies have shown that a very small amount of those cement particles are found in blood for a couple of minutes after the cement has been fixed to the bone [8]. *In vitro* studies have also indicated that fibromodulin, which can be found in cartilage and endothelium, can activate the classical and alternative pathways [22]. In addition, cellular debris derived from the operative field may contribute to the activation of the complement system through the mechanisms of membrane-bound microparticles [23]. Thus, further studies with and without cement in the operative process are necessary and, under different anaesthetic methods, should be taken into account in this context. Measurements of various bone and cartilage destruction fragments (e.g. fibromodulin), tissue factors (e.g. thromboplastin) and other factors related to endothelium and coagulation are also of interest. In addition, the inflammatory process could also be explored further, for example with the measurements of adhesion molecules. Furthermore, a study model comparing circulating levels of inflammatory elements, complement components as mentioned above and cytokines with the levels of those factors at the surgical site would be enlightening. The limitation of the present study is the small group of participants. On the other hand, the

group was homogenous regarding the underlying disease and the impact of the surgery. To expand these results, it is necessary to enlarge the patient group and extend the observation time over two or 3 weeks. Such studies would increase the knowledge of acute inflammation and complement activation following THR. Knowledge such as this will improve the understanding of serious complications associated with THR, for example aseptic loosening, which is one of the most problematical complications to THR surgery besides prosthetic infection and periprosthetic fractures. All efforts should be implemented to find methods that could detect individuals at risk of serious complications following THR.

We conclude that the activation of the complement pathways may play a role in the inflammatory reaction following cemented THR. Further studies are necessary to elucidate these processes in details.

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