Neutrophils are active in total joint implant loosening

Waldemar Wozniak[1], Jacek Markuszewski[1], Malgorzata Wierusz-Kozlowska[1] and Henryk Wysocki[2]

Departments of [1]Orthopedics, [2]Cardiologic Intensive Care, Poznan University of Medical Sciences, Poland
Correspondence: WW: wwozniak@sk4.am.poznan.pl
Submitted 03-10-29. Accepted 04-02-27

Background **Polymorphonuclear neutrophils (PMN) are the first cells to take part in the local foreign body reaction in aseptic loosening of endoprostheses. The aim of this study was to evaluate the systemic host reaction to total joint replacement by measuring the production of nitric oxide by neutrophils before and after total joint replacement.**

Patients and method **Blood samples were collected from 33 patients (27 hips and 6 knees) before surgery, and 2 weeks, 2 months and 2.5–3 years after surgery. The levels of nitric oxide produced by PMN were measured by the method described by Markert et al. (1994).**

Results **Patients reporting pain in the region of the implant 3 years after surgery, and also patients with radiographic signs of loosening, had higher production of NO in the early period and 3 years after the implantation than those with good clinical results.**

Interpretation **We propose that elevated levels of nitric oxide production by PMNs may serve as a marker of total joint prosthesis loosening.**

Research on periprosthetic osteolysis has focused mainly on the role of particles as the triggering factor and the role of macrophages and lymphocytes as the cells responsible for the chronic inflammation and formation of the pseudomembrane (Willert et al. 1990, Sabokbar et al. 1995, Savarino et al. 2002). Moreover, the mechanism of early osteolysis and loosening in the first years after implantation is not clear, and there have been few publications regarding the biological phenomena occurring in the early period after implantation (Hukkanen et al. 1997).

Polymorphonuclear neutrophils are known to form the first line of defense, by taking part in the nonspecific immunological response, both in septic and aseptic processes (Wierusz-Wysocka et al. 1981, Nordstrom et al. 1993, Gristina 1994). Activation of neutrophils is anticipated by a preliminary phase, characterized by an increased number of membrane receptors and higher susceptibility to the influence of stimulating factors. Stimulated neutrophils produce higher amounts of toxic oxygen derivatives, e.g. hydrogen peroxide, superoxide anions, and finally nitric oxide, which also belong to the group of reactive oxygen species (Sibelius et al. 2002).

We evaluated the production of nitric oxide by neutrophils, as an indicator of their activation in total joint replacement.

Patients and methods

We studied 33 patients with an average age of 68 (59–79) years, who underwent primary total cemented joint replacement of 27 hips and 6 knees due to idiopathic OA. We implanted 15 Johnson & Johnson and 12 Sulzer Protema THRs and 6 Zimmer TKRs. There were no postoperative complications. In the first 2 weeks postoperatively, the patients were given 75 mg indomethacin a day in 3 doses. Individuals with any symptoms of inflammatory process were excluded. The control group consisted of 14 healthy individuals aged 52–70 years, without detectable pathological changes in the musculoskeletal system.

Table 1. The mean (SD) production of nitric oxide (nmol/5x10^6/60 min) by neutrophils without stimulation in blood samples of patients before and after the implantation

	Before implantation	2 weeks after implantation	2 months after implantation	3 years after implantation
Reporting pain	0.94 (0.97)	0.53 (0.68)	1.88 (2.65)	0.38 (0.18)
Without pain	0.85 (0.72)	0.61 (0.32)	1.84 (3.07)	0.16 (0.25)

Table 2. The mean (SD) production of nitric oxide (nmol/5x10^6/60 min) by stimulated neutrophils in blood samples of patients before and after the implantation

	Before implantation	2 weeks after implantation	2 months after implantation	3 years after implantation
Reporting pain	3.53 (2.79)	4.54 (5.15)	24.4 (16.4)	9.47 (3.15)
Without pain	5.31 (5.03)	6.44 (11.4)	6.45 (5.74)	5.30 (3.92)

Blood samples were obtained 1 or 2 days before surgery, and 2 weeks, 2 months and 2.5–3 yr after surgery. Neutrophils isolated from the peripheral blood were analyzed in vitro for the production of nitric oxide using the method of Markert et al. (1994). Tests were performed in resting condition and after stimulation. Additionally, PMN peripheral blood count was assessed.

The reaction of NO with oxygen in the aqueous solution leads to the formation of nitrite which is detectable by the Griess reagent. PMN were suspended in modified Hank's solution (MSH) supplemented with Ca^{2+} and Mg^{2+} at 2 × 10^7 cells/mL. 5 × 10^6 neutrophils in suspension was incubated in a final volume of 1 mL in polypropylene tubes at 37°C for 60 min. Various stimuli such as hydroxylamine (HA, 5 mmol/L), phorbol myristate acetate (PMA, 0.3 μg/mL) were added to the cell suspensions. After incubation, the cells were centrifuged at 1200 g for 10 min and the supernatant was collected and mixed 1:1 with Griess reagent (1 part of 0.1% naphthylenediamine dihydrochloride in distilled H20 plus 1 part 1% sulfanilamine in 5% concentrated H3PO4, the two parts being mixed together within 2 h. After 10 min of incubation, absorbance was measured at 550 nm. Stable nitric oxide end-product (NOx) production was compared with a standard curve generated with known amounts of $NaNO_2$. Results are expressed as nmol NOx/5 × 10^6 PMN/h.

At the last examination, patients were separated into two groups, those reporting pain and those without pain. Additionally, a radiographic examination was performed using the criteria of loosening presented by Harris, with the modification of Bannister (1993).

Statistics

As the data were not normally distributed, we used the nonparametric Mann-Whitney U rank test and Wilcoxon matched pairs test were used for statistical analysis. A p-value < 0.05 was considered statistically significant.

Results

2.5–3 years after THR or TKR, 8 of the 33 patients examined reported pain of the operated joint on weight bearing, and in some cases even at rest. The character and intensity of the pain ranged from mild, appearing only after walking a long distance, to strong, which disrupted everyday activities.

In 5 of the 8 patients reporting pain, radiographic signs of loosening were discovered. From samples obtained at the revision surgery, the aseptic character of the loosening was proven. Preoperative ESR and CRP tests, and also histological and bacteriological analysis methods of periprosthetic samples, excluded the possibility of infection in the patients. In the other 3 patients reporting pain,

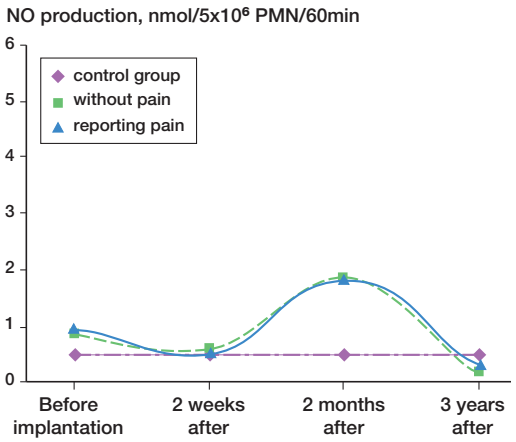

Figure 1. The production of nitric oxide by neutrophils without stimulation in blood samples of patients before and after implantation.

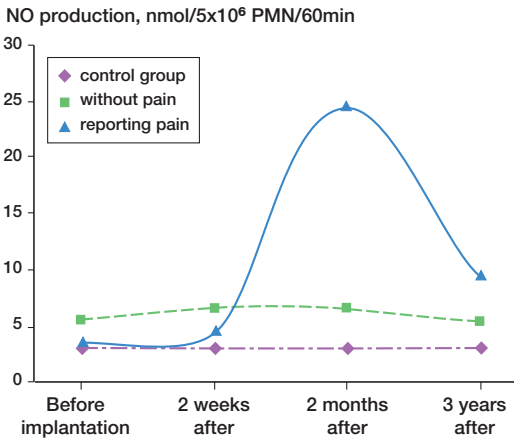

Figure 2. The production of nitric oxide by stimulated neutrophils in blood samples of patients before and after implantation.

we found no radiographic signs of loosening. In the 25 patients without pain, no signs of loosening were found on radiographs. The PMN count in the peripheral blood of all patients did not change significantly during the 2 weeks after surgery, nor after 2 months. No differences were found between the group without pain and the group with pain.

The mean production of nitric oxide by neutrophils without extrinsic stimulation in the control group was measured to be 0.57 (SD 0.54) nmol/5 × 10^6/h. These values were higher before surgery in patients reporting pain and those without pain. The production of nitric oxide without stimulation decreased slightly in the 2 weeks after surgery, but the difference was not statistically significant. After 2 months, the production of nitric oxide by neutrophils increased in all patients examined, but the difference was not statistically significant. 3 years after implantation of the endoprosthesis, in the group of patients without pain, we noticed a decrease in the production of nitric oxide ($p = 0.02$). These values were lower than the values before surgery ($p = 0.04$), and also lower than normal values ($p = 0.02$). The production of nitric oxide without stimulation in a group of patients reporting pain in the operated joint showed similar characteristics. The decrease in nitric oxide production in this group three years after the surgery was statistically significant ($p = 0.03$).

In the control group, the mean level of production of nitric oxide by stimulated neutrophils was 2.72 (SD 1.73) nmol/5 × 10^6/h. In the individuals without pain, the values before surgery were similar to the ones observed in the control group ($p = 0.2$). Throughout the follow-up period, the values varied nonsignificantly. In patients reporting pain in the operated joint, the level of production of nitric oxide by stimulated PNM increased significantly 2 months after surgery. These values were significantly higher than the ones observed in the control group ($p = 0.004$), and in the group without pain ($p = 0.01$). In the group reporting pain 3 years after total joint replacement, the level of production of nitric oxide by stimulated neutrophils was low, but it was still higher than the level observed in the control group ($p < 0.001$), or in the group without pain at the same time after the surgery ($p = 0.02$).

Discussion

Inflammatory mediators, which have the ability to modulate bone turnover, may play an important role in the pathogenesis of aseptic loosening of totally replaced joints (Sabokbar et al. 1995, Goodman 1996, Stea et al. 2000, Banit et al. 2002).

The proinflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), prostaglandins, PGE_2, and proteolytic enzymes, metalloproteinases and elastase, play an important

role in developing the aseptic inflammatory process. Elevated concentrations of these mediators have been found both in the tissues surrounding the implant and in the joint fluid (Goodman et al. 1989, 1992, Takagi et al. 1994, 1998). Increased levels of these particular inflammatory mediators were also found in patients with aseptic loosening of the endoprosthesis (Granchi et al. 1998). Since macrophages are the predominant cells in samples of periprosthetic tissues, they have been suggested to play a significant role in the process of loosening (Ishiguro et al. 1997, Granchi et al. 2000). Papatheofanis and Barmada (1991) and Goodman et al. (1998) have suggested that neutrophils are the cells that participate in the inflammatory reaction following total joint replacement. Neutrophils are the first cells involved in both septic and aseptic inflammatory processes. Although they are a very minor component of synovial-like membranes, the presence of PMN in such membranes and at the bone-cement interface suggests that the role of neutrophils might not only involve the initial phagocytic response to implanted material. Since PMN elaborate a wide variety of chemotactic agents and other mediators of inflammatory reaction, they may play a critical role in the chronic maintenance of an acute inflammatory reaction to PMMA wear debris.

The presence of neutrophils may result in a chronic reactivation of other phagocytic cells and in the persistent release of inflammatory mediators, thereby creating a local cellular environment of cyclical acute inflammatory events.

We evaluated the role of neutrophils in loosening of endoprostheses, because we thought that the priming of these cells may be a result of the increased phagocytosis of bone cement particles. The level of production of nitric oxide by neutrophils was used as a marker of their activation. Under physiological conditions, NO is produced by the endothelial cells, and exhibits vasodilatatory, anticoagulatory, antiadhesive and antiproliferative properties (Borgquist et al. 2002). The presence of inflammatory mediators in the circulating blood induces increased production of NO by cells other than endothelial cells. In particular, neutrophils may be the source of the induced form of NO. This activity may be harmful to the walls of blood vessels.

The activation of polymorphonuclear neutrophils and endothelium causes increased production of NO and cytotoxic reactive oxygen species (O_{2^-}, H_2O_2, O_1). Under these conditions, the superoxide anion reacts with NO and forms peroxynitrite (OONO-), a highly reactive molecule which damages the endothelium (Hukkanen et al. 1997, Stea et al. 1999). The result is the initiation of a local inflammatory reaction in the wall of the blood vessel. The particular features of nitric oxide allow us to consider it as a marker of oxidative stress.

The results of monitoring the PMN count in peripheral blood suggest that implantation of the endoprosthesis did not induce a strongly expressed inflammatory reaction. In addition, the levels of production of nitric oxide without stimulation also confirmed our observation. However, the increase in production of NO by stimulated neutrophils in some patients may indirectly indicate their priming. Under such conditions, neutrophils are capable of responding immediately to additional stimulation and are thus able to initiate a chronic inflammatory reaction.

It has been suggested that neutrophils are primed by the proinflammatory mediators (IL-6, IL-1, IL-8, and TNF-α) (Haynes et al. 1998). They may enter the circulating blood from the periprosthetic region. This phenomenon may also result from the activation of neutrophils by the components of complement. The factors initiating the alternative pathway of complement activation may be the particles released from the endoprosthesis or bone cement.

In our study, patients who reported pain 3 years after the implantation still had an increased level of production of NO by stimulated neutrophils 3 years after the implantation. This indicates that the presence of chronic activation of neutrophils could be responsible for the development of the chronic inflammatory process. Elevated levels of nitric oxide production by neutrophils could thus serve as an early marker of total joint prosthesis loosening.

Part of the preliminary results has been published previously: Malgorzata Wierusz-Kozlowska, Waldemar Wozniak, Jacek Markuszewski, Adam Szczepanik, Elzbieta Okon, Henryk Wysocki. “Granulocyty obojetnochlonne i produkcja wolnych rodników tlenowych po endoprotezoplastyce”

[Polymorphonuclear leucocytes and the production of reactive oxygen species after total hip and knee replacement]. Chirurgia Narzadów Ruchu i Ortopedia Polska 2002; 67 (1): 11-18.

No competing interests declared.

Banit D M, Kaufer H, Hartford J M. Intraoperative frozen section analysis in revision total joint arthroplasty. Clin Orthop 2002; (401): 230-8.

Bannister G C. Total hip replacement–which type. Curr Orthop 1993; 7: 165-70.

Borgquist J D, Quinn M T, Swain S D. Adhesion to extracellular matrix proteins modulates bovine neutrophil responses to inflammatory mediators. J Leukoc Biol 2002; 71: 764-74.

Goodman S B. Does the immune system play a role in loosening and osteolysis of total joint replacements? J Long Term Eff Med Implants 1996; 6: 91-101.

Goodman S B, Chin R C, Chiou S S, Schurman D J, Woolson S T, Masada M P. A clinical-pathologic-biochemical study of the membrane surrounding loosened and nonloosened total hip arthroplasties. Clin Orthop 1989; (244): 182-7.

Goodman S B, Chin R C, Magee F P. Prostaglandin E2 production by the membrane surrounding loose and fixated cemented tibial hemiarthroplasties in the rabbit knee. Clin Orthop 1992; (284): 283-7.

Goodman S B, Lind M, Song Y, Smith R L. In vitro, in vivo, and tissue retrieval studies on particulate debris. Clin Orthop 1998; (352): 25-34.

Granchi D, Verri E, Ciapetti G, Stea S, Savarino L, Sudanese A, Mieti M, Rotini R, Dallari D, Zinghi G, Montanaro L. Bone-resorbing cytokines in serum of patients with aseptic loosening of hip prostheses. J Bone Joint Surg (Br) 1998; 80: 912-7.

Granchi D, Ciapetti G, Filippini F, Stea S, Cenni E, Pizzoferrato A, Toni A. In vitro cytokine production by mononuclear cells exposed to bone cement extracts. Biomaterials 2000; 21: 1789-95.

Gristina A G. Biomaterial-centered infection: microbial adhesion versus tissue integration. Science 1987; 237: 1588-95.

Gristina A G. Implant failure and the immuno-incompetent fibro-inflammatory zone. Clin Orthop 1994; (298): 106-18.

Haynes D R, Boyle S J, Rogers S D, Howie D W, Vernon-Roberts B. Variation in cytokines induced by particles from different prosthetic materials. Clin Orthop 1998; (352): 223-30.

Hukkanen M, Corbett S A, Batten J, Konttinen Y T, McCarthy I D, Maclouf J, Santavirta S, Hughes S P, Polak J M. Aseptic loosening of total hip replacement. Macrophage expression of inducible nitric oxide synthase and cyclooxygenase-2, together with peroxynitrite formation, as a possible mechanism for early prosthesis failure. J Bone Joint Surg (Br) 1997; 79: 467-74.

Ishiguro N, Kojima T, Ito T, Saga S, Anma H, Kurokouchi K, Iwahori Y, Iwase T, Iwata H. Macrophage activation and migration in interface tissue around loosening total hip arthroplasty components. J Biomed Mater Res 1997; 35: 399-406.

Markert M, Carnal B, Mauel J. Nitric oxide production by activated human neutrophils exposed to sodium azide and hydroxylamine: the role of oxygen radicals. Biochem Biophys Res Commun 1994; 199: 1245-9.

Nordstrom D, Santavirta S, Gristina A, Konttinen Y T. Immune-inflammatory response in the totally replaced hip: a review of biocompatibility aspects. Eur J Med 1993; 2: 296-300.

Papatheofanis F J, Barmada R. Polymorphonuclear leukocyte degranulation with exposure to polymethylmethacrylate nanoparticles. J Biomed Mater Res 1991; 25: 761-71.

Sabokbar A, Rushton N. Role of inflammatory mediators and adhesion molecules in the pathogenesis of aseptic loosening in total hip arthroplasties. J Arthroplasty 1995; 10: 810-6.

Savarino L, Granchi D, Ciapetti G, Cenni E, Nardi P A, Rotini R, Veronesi C A, Baldini N, Giunti A. Ion release in patients with metal-on-metal hip bearings in total joint replacement: a comparison with metal-on-polyethylene bearings. J Biomed Mater Res 2002; 63: 467-74.

Sibelius U, Hattar K, Hoffmann S, Mayer K, Grandel U, Schenkel A, Seeger W, Grimminger F. Distinct pathways of lipopolysaccharide priming of human neutrophil respiratory burst: role of lipid mediator synthesis and sensitivity to interleukin-10. Crit Care Med 2002; 30: 2306-12.

Stea S, Visentin M, Granchi D, Melchiorri C, Soldati S, Sudanese A, Toni A, Montanaro L, Pizzoferrato A. Wear debris and cytokine production in the interface membrane of loosened prostheses. J Biomater Sci Polym Ed 1999; 10: 247-57.

Stea S, Visentin M, Granchi D, Ciapetti G, Donati M E, Sudanese A, Zanotti C, Toni A. Cytokines and osteolysis around total hip prostheses. Cytokine 2000; 12: 1575-9.

Takagi M, Konttinen Y T, Santavirta S, Sorsa T, Eisen A Z, Nordsletten L, Suda A. Extracellular matrix metalloproteinases around loose total hip prostheses. Acta Orthop Scand 1994; 65: 281-6.

Takagi M, Santavirta S, Ida H, Ishii M, Mandelin J, Konttinen Y T. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in loose artificial hip joints. Clin Orthop 1998; (352): 35-45.

Wierusz-Wysocka B, Wysocki H. Participation of neutrophilic granulocytes in a local inflammatory focus. Pol Arch Med Wewn 1981; 66: 393-9.

Willert H G, Bertram H, Buchhorn G H. Osteolysis in alloarthroplasty of the hip. The role of ultra-high molecular weight polyethylene wear particles. Clin Orthop 1990; (258): 95-107.