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Crosslinked N-telopeptide of type I Collagen (NTx) in urine as a predictor of periprosthetic osteolysis

Running title: NTx and periprosthetic osteolysis

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Summary

Periprosthetic osteolysis is often non-symptomatic and hard to visualize by conventional radiography. Cross linked N-telopeptide of type I Collagen (NTx), a marker of osteoclast mediated bone resorption, has been suggested to evaluate local particulate-induced osteolysis in patients operated on with a total hip prosthesis.

Urine specimens were sampled after hip joint replacement in 160 patients. NTx was analyzed by a commercially available ELISA kit. Osteolysis was identified in the acetabulum and confirmed at operation.

Using analysis of covariance to correct for differences in age, gender and time after operation, NTx (mean SD) was 36-±12 BCE/nM creatinine in patients with osteolysis (n=33) and 27±13 BCE/nM creatinine in patients without osteolysis (n=127) (p=0.003). Eighteen hips of 38 (47%), demonstrating an annual wear of more than 0.2 mm and an NTx value above 29 BCE/nM creatinine, had been revised due to osteolysis. The osteolysis prevalence in this group was increased 10 times (CI 4-23, $p<0.05$). Indeed, NTx release and annual wear were both associated with increased prevalence of osteolysis, however, independently of each other.

NTx seems a feasible marker of periprosthetic osteolysis. A pre-operative baseline NTx level is likely needed for its use as a predictor of periprosthetic osteolysis in individual cases.

Introduction

The 2002 annual report from the Swedish hip arthroplasty register (http://www.jru.orthop.gu.se) showed that 12651 patients were operated on with a primary total hip arthroplasty. 1056 of these were reoperated due to osteolysis and/or loosening (revised). Today it is generally accepted that osteolysis around a cemented or uncemented hip prosthesis is driven by an inflammatory process caused by released particles, e.g. polyethylene debris (11). In the clinical situation, periprosthetic osteolysis is commonly non-

symptomatic and difficult to detect by conventional radiography. As a result, the osteolytic process may progress to an extent that severely complicates revision surgery. Computed tomography (CT) has been suggested to improve the identification of bone defects in osteolysis (14). However, besides being more expensive, CT is associated with a considerable radiation load to patients and environment. Furthermore, CT gives a static view of the condition and no information of how active the degradative processes are at present.

Markers of bone metabolism have been developed to monitor bone resorption in osteoporosis. Type I collagen degradation products in urine, particularly cross-linked telopeptide and pyridinolines, have a high specificity to bone resorption activity (7). NTx (Cross linked N-telopeptide of type I Collagen) is such a specific degradation product that is released to the urine where it can be quantified by immunoassay (http://www.ostex.com)(2). Antoniou et al. suggested that NTx might be a useful marker for the identification of patients with progressive periprosthetic osteolysis. They found increased urine NTx levels in eleven patients with osteolysis around a hybrid hip arthroplasty, as compared to eight patients without osteolysis as well as to seven non-operated controls. Furthermore, in ten of the patients with osteolysis, NTx levels decreased significantly after 6 weeks of alendronate treatment (2). In a recent study Wilkinson found a difference in NTx levels in patients with radiographic loosening as opposed to patients without osteolysis after excluding cases with pelvic loosening (18).

In the present study we aimed at extending these cross-sectional results by performing a longitudinal analysis of urine NTx values in patients operated on with hip prostheses, in order to evaluate the predictive value of NTx in the individual case. Furthermore, we examined the relationship between annual polyethylene wear, osteolysis (2) and the concentration of NTx in urine.

Materials and methods

Patients

One hundred and sixty consecutive patients, operated on with a total hip replacement because of osteoarthritis, were followed prospectively. Informed written consent was obtained from all patients. The ethical review board approved the study.

Seventy-three patients were operated on with uncemented hip prostheses (Omnifit press fit hydroxyapatite coated dual radius cup and stem Stryker Howmedica Osteonics). Eighty-seven patients were operated on with a cemented hip arthroplasty (a Zirconia ceramic head and /or a Hylamer all polyethylene cup articulation (Elite Plus® DePuy, Johnson & Johnson).

NTx

Urine specimens were sampled from all 160 patients at regular outpatient visits between 9.30 am and 3.30 pm. No first void urine samples were used. Any medication that could affect bone metabolism, such as corticosteroids, hormones or bisphosphonate, was recorded. NTx was analyzed by a commercially available ELISA kit (Osteomark®, http://www.ostex.com/Products). Results are presented as N-telopeptide per creatinine (nM Bone Collagen Equivalents (BCE)/nM creatinine). The reference interval, according to the manufacturer, is 3-65 nM Bone Collagen Equivalents (BCE)/nM creatinine) and the intra-assay variability (C.V.) 5-19% and inter-assay precision 4-5 %.

Radiography

The radiographic examination included an anterior-posterior and a lateral view of the hip and pelvis. The films were scrutinized simultaneously by two of the authors (TvS, ÅC). Progressive and non-progressive radiolucent zones were looked for in the acetabulum according to DeLee and Charnley (4) and in the femur according to Gruen et al (10). Any change of the position of the components and/or osteolytic processes in the pelvis and the

proximal femur were registered. Bone apposition and signs of instability of the stem were evaluated according to Engh et al. (6). The radiographic examinations were made before the NTx results were available. The osteolysis was confirmed at revision.

Analysis of wear

In order to evaluate the relationship between annual polyethylene wear and osteolysis, we used wear results from radiostereometric analyses (RSA) of 127 patients and in 31 patients wear was calculated on standard radiographs. Two of 160 patients (Omnifit prostheses) were lost for wear measurements.

In brief the RSA examinations was performed using six to eight 0.8 mm ∅ tantalum beads inserted in the pelvic bone and the proximal femur at the index operation. The cup polyethylene liner was marked with nine 0.8 mm beads. RSA examinations were performed with the patient in the supine position using the uniplanar technique (15). The RSA films were measured manually with a precision of 0.15 % (13) or scanned at 16 bits/300 DPI resolution with an Umax Mirage II scanner (Umax, Inc) and measured with UmRSA Digital Measure (RSA Biomedical, Umeå, Sweden (www.rsabiomedical.com). Results were analyzed by UmRSA software.

UmRSA computed the relative motion between the exposures of the head center in relation to the geometric center of gravity of the eight markers in the polyethylene liner. The motion was registered as the projected translations along the length of the resulting vector (wear). Thus wear of the polyethylene was calculated as the displacement of the femoral head in relation to the socket. The limit for significant wear has in our institution been calculated to be 0.2 mm (CI 99%) by duplicate examinations (13). The measurement error between a micrometer “true value” and RSA using a phantom has been calculated to be ± 0.08 mm (SD 0.12-0.14) using a phantom (19). In 31 patients with an Omnifit prostheses, annual wear was calculated on

standard radiographs using a modification of the Charnley Duo method (3, 19). The measurement error for the latter method has been calculated to be ± 0,19 mm (SD 0.39) level using a phantom (19).

Statistical methods

Based on a prior cross-sectional study (2) we estimated that 46 patients were needed to detect a difference of 10 BCE/nM creatinine between groups with 80% power. Results were entered into a Statistica 99 database. Students' t-test was used for between group's comparisons and Wilcoxon´s test for dependent samples. ANCOVA was used to compensate means for covariance. Correlation analysis (Pearson r) was used. Logistic regression analysis was used to calculate the prevalence of osteolysis with 95% confidence intervals.

Results

One patient with osteolysis medicated with 10 mg methylprednisolon per day had an NTx value of 49 nM BCE/nM creatinine, another patient with osteolysis that used oral corticosteroids intermittently for 10 day periods due to bronchial asthma had an NTx value of 10 nM BCE/nM creatinine. One patient in the reference group who one day before urine sampling received an intraarticular injection of cortisone in the knee had an NTx value of 33 nM BCE/nM creatinine. A post-menopausal woman, 76 year old, without osteolysis had an NTx value of 137 nM BCE/nM creatinine without any obvious reason. All these patients were included in the analysis.

Cross-sectional analysis

The demographic data are presented in Table 1. Of the 33 patients with osteolysis 30 had been operated on with the Omnifit prostheses. Mean NTx concentration was 25 (SD±10) for the 65 men and 34 (SD±16) for the 95 women. There was no relation between gender and osteolysis (p=0.6, logistic regression analysis). There was no difference in mean NTx between the two groups operated on with different types of prosthesis. Mean NTx was 30 nM BCE/nM

creatinine for those operated on with Elite and Omnifit prosthesis, respectively. In patients without osteolysis, the corresponding values were 29 (SD±16) and 27 (SD±12) nM BCE/nM creatinine for Elite and Omnifit groups, respectively (p=0.43). In the subgroup with low wear and high NTx value (>29) there were 22 patients operated on with the Elite prosthesis and 16 with the Omnifit prosthesis.

Mean NTx concentration in the 127 patients without osteolysis was 29 (SD±15) (nM BCE/nM creatinine) on average 42 months postoperatively. In the 33 patients in the osteolysis group the NTx concentration was 34 (SD±12) (nM BCE/nM creatinine) on average 73 months postoperatively (p=0,06 Student´s t-test) (Figure 1). After ANCOVA correction for difference in age, gender and time after surgery, the mean NTx value was 27±13 in patients without osteolysis (n=127) and 36 ±12 in patients with osteolysis (n=33) (nM BCE/nM creatinine) (p=0.003), respectively.

In a subgroup of patients older than 55 years, NTx was 30 (SD±16) (n=110) and 38 (SD±12) (nM BCE)/nM creatinine) (n=17,) (p=0.03 Students t-test) in the groups without and with osteolysis, respectively.

There was no correlation between the degree of polyethylene wear and the concentration of NTx in urine (r=0,04, p=0.63, Pearson r) (Figure 2). In order to further study the relationship between NTx and annual wear, and the significance of these factors for osteolysis, the patients were divided into four groups. Those with an annual wear below or above 0.2 mm (5) and /or an NTx value below or above 29 nM BCE (mean for patients without osteolysis) (Figure 2). It was found that the prevalence of osteolysis was increased 8.5 times (CI 2.5-28, $p<0.05$ logistic regression analysis) if the NTx value was above 29, whereas the prevalence of osteolysis was increased 8 times (CI 2.5-25, $p<0.05$) if annual wear was above 0.2 mm. 47% of the patients with osteolysis had an annual wear of more than 0.2 mm and an NTx value higher than 29 (nM BCE/nM creatinine) (Figure 2). In this group, the prevalence of osteolysis

was increased 10 times (CI 4-23, $p<0.05$). The prevalence of osteolysis increased by a factor of 1.055 (CI 1.021-1.090) with every unit of NTx ($p=0.001$) and by a factor of 10 (CI 1.7-59) per mm of annual wear ($p=0.011$). Interestingly, the logistic regression analysis further showed that NTx release and annual wear were both associated with increased prevalence of osteolysis independently of each other.

Longitudinal analysis

In 18 patients without osteolysis, urine samples were obtained at three and twelve months postoperatively. In five of these patients the NTx value increased during the interval, and in thirteen it decreased (Figure 2). At three and twelve months postoperatively, NTx was 47 ±23 (mean ± SD) and 37 ±19 (mean ± SD) ($p=0.07$, Wilcoxon´s test) (Figure 3).

More than one urine sample was obtained at minimum 12 months postoperatively in 12 of the 33 patients with osteolysis and 10 of the 127 patients without osteolysis. There were no individual differences with respect to NTx values between patients without osteolysis and those that developed radiographic changes or had symptoms due to osteolysis and implant loosening (Figure3).

Discussion

In the present study we show that urine NTx secretion, reflecting bone resorption, can be identified by an immunoassay and that there is a difference between groups of patients with and without osteolysis. Our results thus corroborate previous findings by Antoniou et al (2) and Wilkinson (18). However, the study design did not reveal individual differences. Our study further indicates that the NTx assay seems sensitive enough to detect an increased bone turnover during the postoperative period, likely due to local periprosthetic bone remodelling. As shown by the regression analysis, increased release of NTx and a high annual wear rate commonly revealed osteolysis, however, independently of each other (Figure 2).

Methodologically, some issues have to be considered. The formation and subsequent identification of osteolysis takes several years and thus the patients in the osteolysis group was operated on approximately two and a half years prior to the patients without osteolysis. Accordingly, considerably fewer months had elapsed between operation and urine sampling in the two groups. However, the result of the longitudinal analysis in patients without osteolysis suggests that a possibly increased postoperative bone turnover has ceased at twelve months (Figure 2). Age and gender can be expected to influence the bone turnover/resorption and thus NTx. In the present study, the mean age in patients without osteolysis was significantly higher, and there were more women in this group. As expected, after ANOVA correction, the probability for a difference between the groups improved. Diurnal variation of crosslinked telopeptide excretion may be a concern. However, a study buy Gertz, showed no diurnal variation in crosslinked telopeptide urine excretion (9). Thus, collecting urine samples as in the present study, randomly at the outpatient department and during daytime excluding the morning void, should be a safe way to monitor urine NTx.

Another factor that could influence bone turnover is bone resorption secondary to pain and low physical activity in the postoperative period. In this study we did not study the influence of these factors with respect to NTx. According to the literature, this issue has not been thoroughly examined. In a hip fracture population, Lips (12) found an association between low mobility and increased bone resorption. On the other hand, Garnero (8) found that CTx levels above the postmenopausal threshold were associated with an increased risk of hip fracture irrespective of mobility status.

The relationship between annual polyethylene wear and osteolysis is well established (5). The most common theory is that particles generated by abrasive wear induce a macrophage activated bone resorption. Other proposed causes of osteolysis are periprosthetic hydrostatic pressure forces (17) or a combination of particles and pressure. (16). It has also been

suggested that endotoxines from bacterial cell walls cause macrophage mediated osteoclast activation (21). Indeed our results indicate that annual wear and NTx are independent factors that both reflect osteolysis.

When the present study was implemented, periprosthetic osteolysis and a high wear rate was already a clinical problem after implantation of the Omnifit uncemented and press fit hydroxyapatite coated dual radius cup and stem (20). Accordingly, we anticipated increased NTx values as a response to the observed osteolysis, but were unable to obtain urine samples before the index operation to establish baseline metabolism. Thus, the magnitude of the increase in the individual patient is not known in our material. Alvarez (1) described that several markers of bone remodeling showed considerable variability between patients with clinically stable Pagets decease, but that a resorption marker such as Cross linked T-telopeptide of type I Collagen (CTx) could be used to follow individual patients over time. The within–subject coefficient of variation for NTx was approximately 20% for a control group of postmenopausal women over a period of 9 months (9). It is thus possible that in cases with periprosthetic osteolysis, the NTx value is increased compared to the baseline level. Regarding the baseline reference value it is important to appreciate that the reference interval given by the producer of the assay (3-65 nM BCE/nM creatinine) is established to monitor the response to anti-resorptive therapy in osteoporosis patients and is therefore not feasible in patients with periprosthetic osteolysis. Another complicating issue regarding NTx release is its influence by menopause, age, and medication (9). To control for intra- and inter-individual variations in NTx in sequentially sampled urine specimens, pre- and post-operative samples should most likely be included.

Antoniou (2) showed, using the identical NTx assay, that the NTx values in 8 patients without signs of osteolysis who had a hybrid hip implant of unknown design differed from those in eleven patients with osteolysis defined on radiographs obtained not earlier than 1,5 years

postoperatively (p<0,03). In their group without osteolysis mean NTx was approximately 25 nM BCE/mM Creatinine as compared to 27 nM BCE/mM Creatinine in our study. In the group with osteolysis the mean value was approximately 70 nM BCE/mM Creatinine as compared to our 36 nM BCE/mM Creatinine. No females older than 40 years of age were included in that study in order to decrease the risk of menopause influence. Despite this fact, the mean NTx was higher than in our study. The reverse result may have been easier to explain.

A recent study, defining loosening as cavitary osteolysis and linear lesions, suggested increased NTx levels in 23 patients with radiographic loosening compared to 26 patients without loosening (18). In twelve patients both components were loose. Additionally five patients were considered having stem loosening and six patients pelvis osteolysis. However, in that study a difference was only present if patients with loosening of femoral and acetabular implants together with those having femoral implants loosening only (NTx 61 nM BCE/nM creatinine) were compared with patients without loosening and pelvic loosening only (NTx 40 nM BCE/nM creatinine) (p=0.02). To be a clinical valuable biomarker of periprosthetic osteolysis, NTx must be sensitive to both femoral and pelvic osteolysis. Linear lesions are not generally accepted as a sign of loosening with increased bone destruction. Accordingly, increased levels of NTx in the urine cannot by and large be expected using such a sign for osteolysis. We do not know why both previous studies had higher mean NTx values in patients with osteolysis than the patients in the present study. Nevertheless, our study was powered to show NTx differences due to osteolysis that was confirmed at revision surgery.

A high mean value of NTx in the osteolysis group could be explained by a host reaction to the orthopedic implant or a host predisposition to increased bone turnover in general. Genetic factors may also predispose some individuals to react adversely to different implant related factors such as design, fixation method and material (21). Furthermore, genetic predisposition

may also influence the host response to micro motion and debris such as polyethylene. The fact that there was no difference in mean NTx between the two groups operated on with different types of prosthesis suggests that an increased mean NTx is not prosthesis related but rather a result of the osteolytic loosening. Nevertheless, a reliable marker of bone metabolism may help to identify patients that need prophylactic medication to prevent periprosthetic osteolysis.

We conclude that NTx seems a feasible marker of periprosthetic osteolysis to evaluate outcome of newly designed prosthesis. However, a pre-operative baseline NTx level is likely needed for its use as a predictor of periprosthetic osteolysis in individual cases.

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Key words: Bone turnover markers, osteolysis, Cross linked N-telopeptide of type I Collagen (NTx), total hip arthroplasty.

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Table

	Osteolysis			
All Patients	No		Yes	
Number (male/female)	127	(51/76)	33	(14/19)
Mean age - at op (range)	61	(38-75)	50	(26-70)
- at test (range)	65	(41-79)	56	(34-76)
Age matched subgroup				
Number (male/female)	110	(44/66)	17	(5/10)
Mean age - at op (range)	63	(46-75)	60	(45-75)
- at test (range)	67	(55-79)	66	(56-76)

Table 1. Demographic data of included patients.

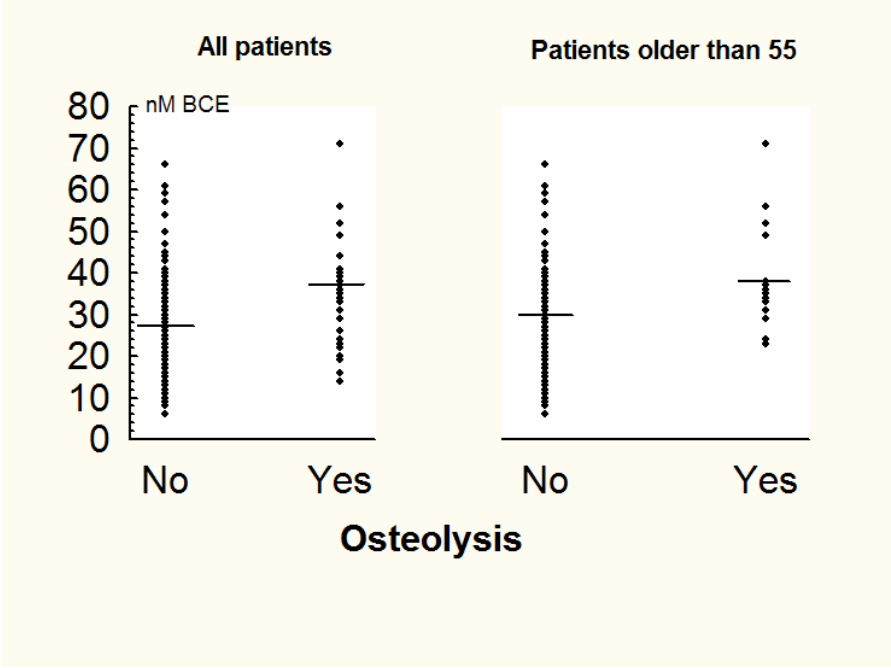

Figure 1. Scatterplot of NTx concentration at last follow up, line indicates mean values.
Left: All patients. No osteolysis n=127. Osteolysis patients n=33.
Right: Patients older than 55. No osteolysis n=110. Osteolysis patients n=17. One outlier is not shown in the graph (BCE 137 nM BCE/nM creatinine, annual wear of 0,79 mm and no osteolysis) but is included in the statistical analysis).

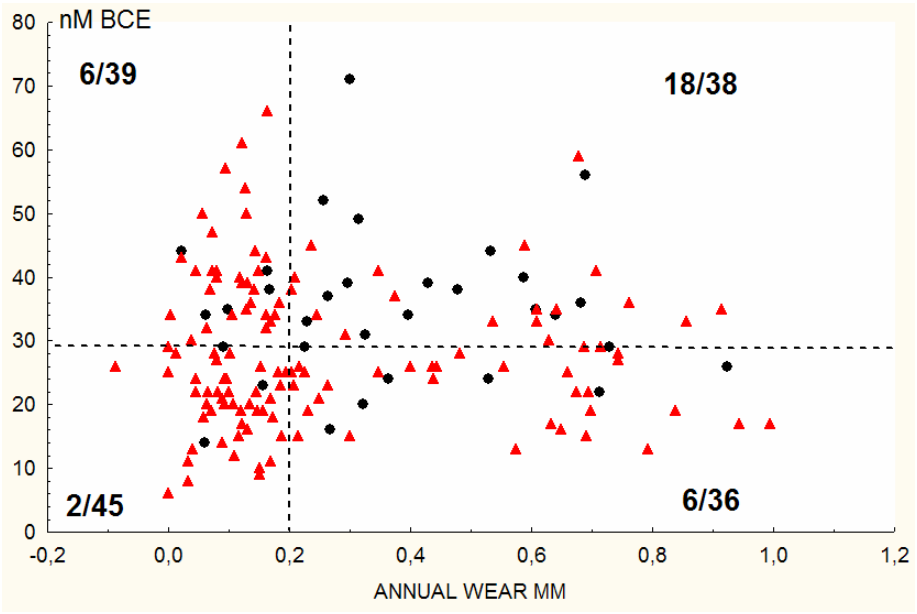

Figure 2. Scatterplot of annual wear and nM BCE/nM creatinine urine. Horizontal line indicates the mean NTx value in the control group (mean BCE = 29). Vertical line indicates mean annual wear of 0,2 mm. Numbers in each group indicates number of patients with osteolysis in each group/total number of patients in each group. Black dots represent patients with osteolysis and red triangles patients without osteolysis. One outlier is not shown in the graph (BCE 137 nM BCE/nM creatinine, annual wear of 0,79 mm and no osteolysis) but is included in the statistical analysis).

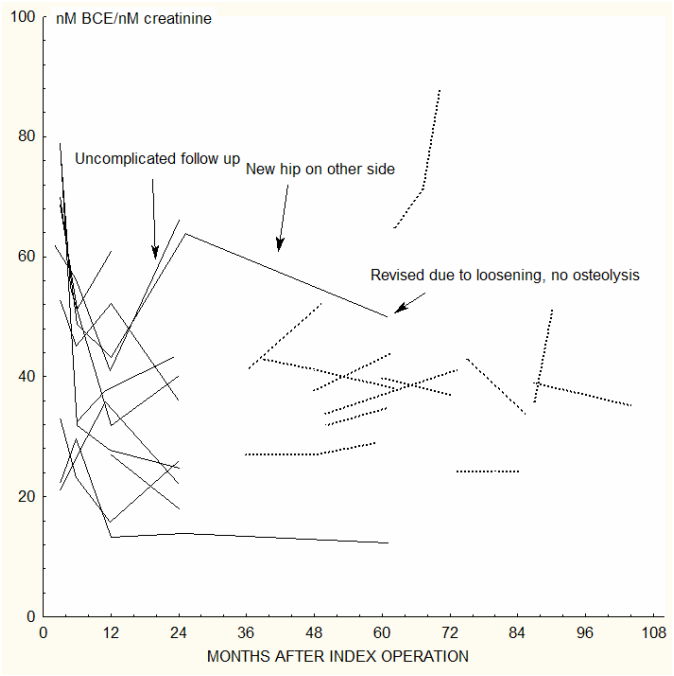

Figure 3. Scatterplot of individual longitudinal measurements. Full lines: control group (n=18), Dotted lines: Osteolysis group, all revised after last test (n=12).