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ORIGINAL ARTICLE

Densitometric evaluation of bone remodelling around Trabecular Metal Primary stem: a 24-month follow-up

Elena Gasbarra[1] · Riccardo Iundusi[1] · Fabio Luigi Perrone[1] · Luca Saturnino[1] · Umberto Tarantino[1]

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Abstract
Background Today, an increasing number of total hip arthroplasty (THA) procedures are being performed. Osseointegration is a physiological phenomenon that leads to the direct anchorage of an implant by the formation of bony tissue around the implant without the growth of fibrous tissue at the bone–implant interface. Several factors may affect this phenomenon: some of these depend on the patient and others may depend on implant design and materials. Variations in periprosthetic bone mineral density (BMD) can be studied through several scans by dual energy X-ray absorptiometry (DEXA) around the femoral stem.
Aims The purpose of this study is to investigate correlations between periprosthetic BMD and the factors affecting osseointegration.
Methods We retrospectively analysed patients who underwent primary THA. In all the patients, Trabecular Metal Primary (TMP), a standard uncemented tapered stem with a proximal porous tantalum coating, was implanted. Preoperatively, postoperatively, 3 and 6 months, 1 year and 2 years after implantation, DEXA scans were performed around the femoral stem. The patients were matched for diagnosis, sex, BMD of the lumbar spine and contralateral femur, Body Mass Index and age.
Results One hundred and eight patients (51 males and 57 females) with a mean age of 73 years were studied. Different BMD changing patterns were observed and a greater bone resorption was noted in all the conditions associated with poor bone quality.
Discussion The proximal coating of Trabecular Metal Primary (TMP) seemed to be effective in promoting new bone formation in the proximal femur also in the conditions associated with poor bone quality.
Conclusions At the present time, DEXA is considered the most reliable tool for evaluating bone remodelling after THA.

Keywords THA · Osseointegration · Porous tantalum · DEXA

Introduction

Total hip arthroplasty (THA) is one of the most effective orthopaedic procedures with a very high success rate as measured by pain relief, improved function and patient satisfaction [1]. An optimal fit of a cementless stem in the metaphysis of the femur is important in order to obtain a good mechanical fixation, which provides a good osseointegration of the implant.

A large number of long-term implant failures is due to aseptic loosening in which periprosthetic bone loss leads to implant instability, pain, and increased risk of periprosthetic fractures. This is particularly true in elderly people, with an altered bone metabolism [2] which is not able to provide a good mechanical and biological fixation because of reduced bone quality [3, 4].

Proximal bone resorption around femoral stems is commonly observed after cementless THA. The reasons for this phenomenon include "stress-shielding", due to the altered load transmission in the femur, and "wear-debris", an inflammatory reaction to small particles produced by friction between articulating surfaces [5].

✉ Elena Gasbarra
elenagasbarra@tiscali.it

[1] Department of Orthopaedics and Traumatology, University of Rome Tor Vergata, "Policlinico Tor Vergata" Foundation, Rome, Italy

Several authors have described molecular and cellular mechanisms that lead to the formation of new trabecular bone around an implant, a phenomenon called bone ingrowth [6–9]; others have focused their attention on the factors that can enhance or compromise this process [10, 11].

Among the factors that may affect bone remodelling after THA, age, sex, body mass index (BMI), bone mineral density (BMD) and stem design [12] play an important role. Stem design is believed to be significant in the load transfer to the femur and, consequently, in femoral remodelling [13, 14].

Dual energy X-ray absorptiometry (DEXA) is a validated method for studying BMD [15] and is currently the most effective tool to quantify bone mineral density variations around the femoral stem, which cannot be seen by conventional X-rays.

The purpose of this study is to evaluate the first step of osseointegration and bone remodelling around the proximal part of the Trabecular Metal Primary stem TMP (Zimmer, Warsaw, USA) by means of DEXA with a follow-up of 24 months.

Materials and methods

We retrospectively analysed patients who underwent primary THA from 2006 to 2010. All the patients who received TMP stem (Zimmer, Inc; Warsaw, IN, USA) and completed the 24-month follow-up were enrolled for the present study. We excluded patients with a pre-existing altered deambulation or previous lower-limb surgery which could affect the bone remodelling process around the implant. One hundred and eight patients (51 males and 57 females) with a mean age of 73 years (range 26–97) were studied. In 84 cases, the patients underwent surgery for osteoarthritis; in 24 cases, the patients had a medial femoral fracture.

In all the patients the TMP stem was implanted by the same surgical equipe through a direct lateral approach in 76 % of the patients and a postero-lateral approach in 24 %. TMP is a standard uncemented tapered stem, made of a Tivanium® (Ti-6al-4 V) alloy with a proximal coating of porous tantalum called Trabecular Metal™, an ultra-porous material with a structure similar to trabecular bone. It provides for a high-friction bone interface for excellent initial implant stability. In addition, its highly porous structure enables extensive bone ingrowth and strong long-term fixation (Fig. 1).

Preoperatively, postoperatively (T0), 3 months (T1) and 6 months (T2), 1 year (T3) and 2 years (T4) after implantation, the patients underwent DEXA examinations. All DEXA measurements performed were compared with the first periprosthetic scan obtained in the immediate postoperative (T0) in order to study changes in periprosthetic BMD during follow-up.

Fig. 1 Trabecular Metal Primary stem with porous tantalum proximal coating

DEXA scans were performed using i-DXA encore (GE, Lunar, USA). Preoperative scans, obtained only for patients with osteoarthritis, included lumbar spine (L1–L4) and dual-femur (neck and total) scans in order to exclude an osteoporotic condition. The postoperative scan included the operated hip; in addition, lumbar spine and contralateral hip scans were obtained for the patients with a medial femur fracture, as it was impossible to perform before surgical procedure. The BMD (g/cm2) of the operated hip was measured using the "Orthopedic Hip Analysis" scanning mode, which recognizes automatically the metal of the implant and the surrounding bone. Conventional Gruen zones were automatically adapted by software to the stem design (Fig. 2). Each patient's individual Regions Of Interest (ROI) were saved on the system and were used for all subsequent measurements to reduce bias. The patients were placed in a supine position with the affected leg

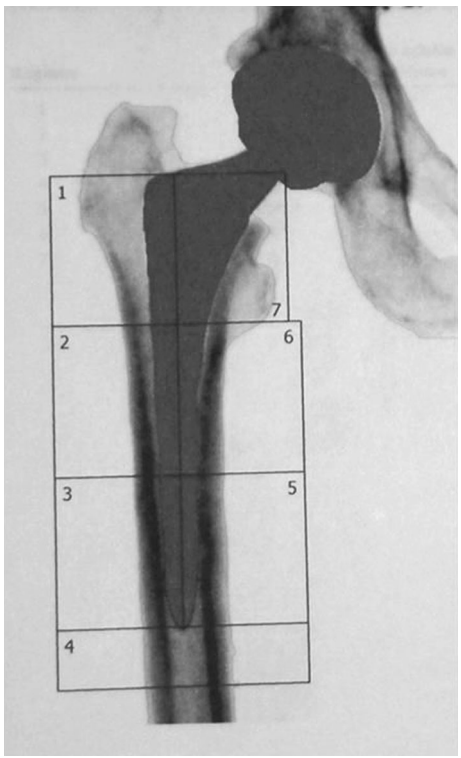

Fig. 2 Regions of Interest (ROI) automatically detected by software

secured in a proper foot positioning device to obtain reproducible rotation in all the patients and limit measurements errors, as rotation influences BMD measurement. The first postoperative scan was performed within 6 days after the operation and was considered the baseline for future examinations. Images were analysed with the use of dedicated Windows analysis software (version 12.2). Quality controls were done every morning for the DEXA equipment to verify system stability, as specified by the manufacturer's guidelines.

Data analysis was performed exclusively for proximal ROIs, ROI1 and ROI7, first, because these are the most affected by bone resorption phenomenon according to the literature and, second, because of the presence of a porous coating only in the proximal part of the implanted stem.

The patients were matched for different variables: diagnosis, sex, age, BMD according to first DEXA evaluation and BMI.

The variation in BMD during follow-up was expressed in terms of percentage. We applied Student's t test to evaluate the hypothesis of a difference between BMD variations at different measurement time-points. We defined statistical significance as $p < 0.05$. We used the ANOVA test for statistical analysis. The statistical software package for social sciences, version 16 (SPSS Inc, Chicago, IL, USA) was used to perform all the statistical analysis.

Results

The following results are divided according to the variables analysed.

Diagnosis

For those patients operated for osteoarthritis a decrease of periprosthetic BMD has been observed, especially between the 3 and 6 months postoperative, and more pronounced in the proximal femur (ROI1 and ROI7). At 3 months postoperatively, ROI1 showed maximum resorption (-7.65 % $\pm$ 3.7 SD) with a rapid recovery at 6 months (2.96 % $\pm$ 1.5 SD), 12 months (3.70 % $\pm$ 1.6 SD) and 24 months (9.47 % $\pm$ 4.1 SD). ROI7 showed a greater amount of resorption: at 3 months, it had maximum resorption (-12.88 % $\pm$ 5 SD). Later a progressive recovery of BMD was observed (-6.65 % $\pm$ 2.1 SD at 6 months, -6.17 % $\pm$ 1.5 SD at 12 months and -0.27 % $\pm$ 0.8 SD at 24 months) although these values are lower than the results obtained for ROI1.

In the patients operated for medial femoral fracture, lower values were observed. In ROI1, a gradual resorption was observed with a good recovery at 24 months (-0.52 % $\pm$ 1.3 SD at 3 months, -7.1 % $\pm$ 2.5 SD at 6 months, -15.43 % $\pm$ 3.4 SD at 12 months and -4.21 % $\pm$ 1.8 SD at 24 months). ROI7, as already seen for patients with osteoarthritis, had lower values, with a maximum resorption at 6 months (-17.27 % $\pm$ 6.6 SD at 3 months, -21.58 % $\pm$ 3.2 SD at 6 months, -16.91 % $\pm$ 4.3 SD at 12 months and -17.32 % $\pm$ 4.5 SD at 24 months).

Comparison between the results obtained in patients affected by osteoarthritis and the results for patients with femoral neck fracture showed a statistical significance at 12 months for ROI1 ($p = 0.034$) and for ROI7 ($p = 0.018$) at 6 months (Table 1).

Sex

Analysis of periprosthetic BMD of the proximal femur (ROI1 and ROI7) gave the following results: in ROI1 of males (-1.7 % $\pm$ 1.1 SD at 3 months, 11.77 % $\pm$ 3 SD at 6 months, 6.46 % $\pm$ 2.5 SD at 12 months and 17.86 % $\pm$ 4.8 SD at 24 months) and females (-8.57 % $\pm$ 3.4 SD at 3 months, -1.97 % $\pm$ 1.2 SD at 6 months, -4.52 % $\pm$ 2.1 SD at 12 months and -8.85 % $\pm$ 3.7 SD at 24 months); in ROI7 of males (-17.09 % $\pm$ 3.2 SD at 3 months, -7.57 % $\pm$ 1.5 at 6 months, -6.58 % $\pm$ 2.7 SD at 12 months and 3.52 % $\pm$ 1.5 SD at 24 months) and females (-11.87 % $\pm$ 2.7 SD at 3 months, -15.32 % $\pm$ 2.4 SD at 6 months, -9.13 % $\pm$ 1.8 SD at 12 months and -13.06 % $\pm$ 3.8 SD at 24 months).

Table 1 Results for patients matched for diagnosis

Follow-up period	Group	3 months	6 months	12 months	24 months
ROI 1	Osteoarthritis	−7.65 % ± 3.7 SD	2.96 % ± 1.5 SD	3.70 % ± 1.6 SD	9.47 % ± 4.1 SD
	Fracture	−0.52 % ± 1.3 SD	−7.10 % ± 2.5 SD	−15.43 % ± 3.4 SD	−4.21 % ± 1.8 SD
ROI 7	Osteoarthritis	−12.88 % ± 5 SD	−6.65 % ± 2.1 SD	−6.17 % ± 1.5 SD	−0.27 % ± 0.8 SD
	Fracture	−17.27 % ± 6.6 SD	−21.58 % ± 3.2 SD	−16.91 % ± 4.3 SD	−17.32 % ± 4.5 SD
		ROI1 $p = 0.3$	ROI1 $p = 0.2$	ROI1 $p = 0.034$	ROI1 $p = 0.3$
		ROI7 $p = 0.5$	ROI7 $p = 0.018$	ROI7 $p = 0.2$	ROI7 $p = 0.2$

Table 2 Results for patients matched for sex

Follow-up period	Group	3 months	6 months	12 months	24 months
ROI 1	Male	−1.77 % ± 1.1 SD	11.77 % ± 3 SD	6.46 % ± 2.5 SD	17.86 % ± 4.8 SD
	Female	−8.57 % ± 3.4 SD	−1.97 % ± 1.2 SD	−4.52 % ± 2.1 SD	−8.85 % ± 3.7 SD
ROI 7	Male	−17.09 % ± 3.2 SD	−7.57 % ± 1.5 SD	−6.58 % ± 2.7 SD	3.52 % ± 1.5 SD
	Female	−11.87 % ± 2.7 SD	−15.32 % ± 2.4 SD	−9.13 % ± 1.8 SD	−13.06 % ± 3.8 SD
		ROI1 $p = 0.2$	ROI1 $p = 0.1$	ROI1 $p = 0.1$	ROI1 $p = 0.013$
		ROI7 $p = 0.5$	ROI7 $p = 0.1$	ROI7 $p = 0.7$	ROI7 $p = 0.1$

Table 3 Results for patients matched for BMD of lumbar spine and contralateral femur

Follow-up period	Group	3 months	6 months	12 months	24 months
ROI 1	Normal *T* Score	−6.03 % ± 2 SD	−0.29 % ± 1.6 SD	5.98 % ± 2.7 SD	22.58 % ± 5.8 SD
	Osteopenia	−6.31 % ± 2.8 SD	0.45 % ± 3.2 SD	0.29 % ± 2.1 SD	−2.85 % ± 4.5 SD
	Osteoporosis	−2.53 % ± 5.1 SD	−8.36 % ± 4.3 SD	−3.53 % ± 4.5 SD	18.34 % ± 5.2 SD
ROI 7	Normal *T* Score	−10.78 % ± 3.8 SD	−9.97 % ± 3.4 SD	−1.42 %1.1 SD	7.59 % ± 2.7 SD
	Osteopenia	−7.29 % ± 4.2 SD	−11.25 % ± 4.4 SD	−8.99 % ± 4.4 SD	−11.21 % ± 3.7 SD
	Osteoporosis	−21.39 % ± 6.2 SD	−3.45 % ± 4.7 SD	−5.22 % ± 5.3 SD	13.63 % ± 5.6 SD
		ROI1 $p = 0.8$	ROI1 $p = 0.3$	ROI1 $p = 0.5$	ROI1 $p = 0.2$
		ROI7 $p = 0.4$	ROI7 $p = 0.4$	ROI7 $p = 0.7$	ROI7 $p = 0.2$

Comparison between the results in males and females showed a statistical significance at 24 months for ROI1 ($p = 0.013$) (Table 2).

BMD of lumbar spine and contralateral femur

Patients were divided into a first group of patients with a normal *T* Score in both lumbar and contralateral femur, a second group with a *T* Score indicating osteopenia and a third with osteoporotic values of *T* Score.

In the first group, the following BMD changes were observed: in ROI1 −6.03 % ± 2 SD at 3 months, −0.29 % ± 1.6 at 6 months, 5.98 % ± 2.7 SD at 12 months and 22.58 % ± 5.8 SD at 24 months; in ROI7 −10.78 % ± 3.8 SD at 3 months, −9.97 % ± 3.4 SD at 6 months, −1.42 % ± 1.1 SD at 12 months and 7.59 % ± 2.7 SD at 24 months.

In the second, the changes were as follows: in ROI1 −6.31 % ± 2.8 SD at 3 months, 0.45 % ± 3.2 SD at 6 months, 0.29 % ± 2.1 SD at 12 months and −2.85 % ± 4.5 SD at 24 months; in ROI7 −7.29 % ± 4.2 SD at 3 months, −11.25 % ± 4.4 SD at 6 months, −8.99 % ± 4.4 SD at 12 months and −11.21 % ± 3.7 SD at 24 months.

For the last group, the changes were as follows: in ROI1 −2.53 % ± 5.1 SD at 3 months, −8.36 % ± 4.3 SD at 6 months, −3.53 % ± 4.5 SD at 12 months and 18.34 % ± 5.2 SD at 24 months; in ROI7 −21.39 % ± 6.2 SD at 3 months, −3.45 % ± 4.7 SD at 6 months, −5.22 % ± 5.3 SD at 12 months and 13.63 % ± 5.6 SD at 24 months (Table 3).

Body mass index (BMI)

Patients were divided into a first group of non-obese patients with a BMI <30 kg/m^2 and a second group of obese patients with a BMI ≥30 kg/m^2.

Analysis of periprosthetic BMD changes in the proximal femur (ROI1 and ROI7) gave the following results: in ROI1 of non-obese patients (−5.1 % ± 2.1 SD at 3 months, 10.22 % ± 4.7 SD at 6 months, −0.31 % ± 2.2 SD at 12 months and 5.39 % ± 3.4 SD at 24 months) and obese patients (−7.24 % ± 4.1 SD at 3 months, −11.24 % ± 4.7 SD at 6 months, 4.08 % ± 2.1 SD at 12 months and 14.39 % ± 4.7 SD at 24 months); in ROI7 of non-obese patients (−15.32 % ± 4.7 SD at 3 months, −11.16 % ± 3.4 SD at 6 months, −10.20 % ± 4.1 SD at 12 months and −8.25 % ± 2.3 SD at 24 months) and obese patients (−8.82 % ± 3.6 SD at 3 months, −9.29 % ± 3.9 SD at 6 months, 1.71 % ± 3.5 SD at 12 months and 17.47 % ± 3.4 SD at 24 months).

Comparison of the results for the two groups showed a statistical significance in ROI7 ($p = 0.042$) at 24 months (Table 4).

Age

Patients were divided into a first group of patients up to and inclusive of 65 years of age and a second group of patients over 65 years.

Analysis of periprosthetic BMD changes in the proximal femur (ROI1 and ROI7) gave the following results: in ROI1 of the under-65 group (−2.90 % ± 3.9 SD at 3 months, 8.51 % ± 4.2 SD at 6 months, 3.29 % ± 3.2 SD at 12 months and 14.58 % ± 2.1 SD at 24 months) and the over-65 group (−12.94 % ± 3.7 SD at 3 months, −1.35 % ± 3.3 SD at 6 months, 5.62 % ± 2.3 SD at 12 months and −3.89 % ± 2.8 SD at 24 months); in ROI7 of the under-65 group (−16.72 % ± 5.1 SD at 3 months, −10.36 % ± 3.1 SD at 6 months, −5.31 % ± 3.5 SD at 12 months and 8.53 % ± 3.1 SD at 24 months) and the over-65 group (−7.09 % ± 3.5 SD at 3 months, −12.14 % ± 4.3 SD at 6 months, −13.79 % ± 5 SD at 12 months and −20.58 % ± 4.3 SD at 24 months).

Comparison between the results for the two groups showed a statistical significance at 24 months for ROI7 ($p = 0.003$) (Table 5).

Discussion

Data obtained from this study suggest that multiple variables influence osseointegration of the femoral stem, even if there is disagreement about which factors really influence periprosthetic bone remodelling [12]. We have evaluated some of the variables, such as diagnosis, sex, lumbar spine and femoral BMD, BMI and age. We have focused our findings on BMD variations in the proximal femur, ROI1 and ROI7, in which periprosthetic bone loss is higher than in other regions, as widely demonstrated in the literature [10, 16].

Table 4 Results for patients matched for BMI

Follow-up period	Group	3 months	6 months	12 months	24 months
ROI 1	BMI <30	−5.10 % ± 2.1 SD	10.22 % ± 4.7 SD	−0.31 % ± 2.2 SD	5.39 % ± 3.4 SD
	BMI ≥30	−7.24 % ± 4.1 SD	−11.24 % ± 4.7 SD	4.08 % ± 2.1 SD	14.39 % ± 4.7 SD
ROI 7	BMI <30	−15.32 % ± 4.7 SD	−11.16 % ± 3.4 SD	−10.20 % ± 4.1 SD	−8.25 % ± 2.3 SD
	BMI ≥30	−8.82 % ± 3.6 SD	−9.29 % ± 3.9 SD	1.71 % ± 3.5 SD	17.47 % ± 3.4 SD
		ROI1 $p = 0.8$	ROI1 $p = 0.05$	ROI1 $p = 0.5$	ROI1 $p = 0.5$
		ROI7 $p = 0.5$	ROI7 $p = 0.5$	ROI7 $p = 0.2$	ROI7 $p = 0.042$

Table 5 Results for patients matched for age

Follow-up period	Group	3 months	6 months	12 months	24 months
ROI 1	Under-65	−2.90 % ± 3.9 SD	8.51 % ± 4.2 SD	3.29 % ± 3.2 SD	14.58 % ± 2.1 SD
	Over-65	−12.94 % ± 3.7 SD	−1.35 % ± 3.3 SD	5.62 % ± 2.3 SD	−3.89 % ± 2.8 SD
ROI 7	Under-65	−16.72 % ± 5.1 SD	−10.36 % ± 3.1 SD	−5.31 % ± 3.5 SD	8.53 % ± 3.1 SD
	Over-65	−7.09 % ± 3.5 SD	−12.14 % ± 4.3 SD	−13.79 % ± 5 SD	−20.58 % ± 4.3 SD
		ROI1 $p = 0.1$	ROI1 $p = 0.3$	ROI1 $p = 0.2$	ROI1 $p = 0.09$
		ROI7 $p = 0.2$	ROI7 $p = 0.5$	ROI7 $p = 0.2$	ROI7 $p = 0.003$

Patients operated for osteoarthritis demonstrated a lower resorption in both proximal ROIs compared with patients who underwent THA for medial femur fracture. A great difference between these two groups is more evident in ROI1, especially at 12 months postoperatively ($p = 0.034$), where the presence of trabecular bone is significant, and consequently, bone remodelling due to metabolic changes. Also in ROI7, which is the region most affected by bone resorption, data analysis showed a clear difference between the groups ($p = 0.018$ at 6 months).

Similar differences were found when patients were matched for sex. Female patients showed higher values of bone resorption in the proximal femur, especially in ROI1. In the group of male patients, a gradual bone increase was observed while a gradual bone resorption was seen in the female group; in this region, in fact, a statistical significance between the two groups at 24 months was observed ($p = 0.013$).

Patients were also divided by taking into consideration their pre-existing bone density on the basis of *T* Scores of the lumbar spine and contralateral femur. Some authors have suggested that systemic BMD can predict periprosthetic bone loss [17, 18]. In this study the non-osteoporotic population showed a slight bone resorption at 3 and 6 months and a rapid bone enhancement in both proximal ROIs, with positive values al 24 months. A different pattern of periprosthetic BMD changes was observed in patients affected by osteopenia, with higher values of bone resorption observed, mainly in ROI7. This surely denotes an altered bone metabolism in this second group of patients. Surprisingly, a good recovery of periprosthetic bone stock was seen within 24 months in the group of patients affected by osteoporosis. This could be due to the fact that this is a retrospective study and a pharmacological therapy for osteoporosis was not taken into account. In our department, each patient with femoral fracture or affected by osteoporosis is routinely treated pharmacologically with antiosteoporotic therapy.

Differences in terms of BMI were also investigated. From the analysis of our data, higher BMI seems to act protectively against bone resorption in the proximal femur. In fact early bone resorption, within 6 months was observed in ROI1 and ROI7 in patients with BMI >30 and following bone apposition starting at 12 months postoperatively. On the contrary, patients with BMI <30 showed a different BMD pattern: in ROI1, early bone resorption with a following slight recovery was recorded; in ROI7, pronounced bone resorption within 6 months did not show any considerable new bone formation around the implant. However, ROI7 is known to be the region most affected by bone resorption caused by altered load transmission, especially when a standard femoral stem is implanted [19]. In this region, a statistical significance was observed at 24 months ($p = 0.042$), while in ROI1, a statistical significance was observed at 6 months ($p = 0.05$).

Finally, interesting results were obtained when patients were matched by age. Under-65-year olds demonstrated in both proximal regions, ROI1 and ROI7, early bone resorption and a consecutive rapid bone formation around the femoral stem. Instead, over-65-year olds showed bone resorption in both proximal ROIs; a slight recovery was observed in ROI1, while progressive bone resorption was noted in ROI7. The over-65's, independently of the diagnosis, were characterized by worse bone quality and, compared with the under-65's, showed higher bone resorption and more time to set up bone ingrowth around the femoral stem ($p = 0.003$ in ROI7 at 24 months).

Our data analysis shows that all the conditions in which an altered bone quality, such as osteopenia and osteoporosis, low BMI, female gender and advanced age, are affected by pronounced bone resorption. Altered bone quality particularly affects trabecular bone, significant in the proximal femur, which is the region most affected by periprosthetic bone resorption.

Conclusions

Proximal coating of TMP, thanks to the osteoconductive and osteoinductive features of porous tantalum, seems to be effective in promoting new bone formation in the proximal femur. Periprosthetic bone resorption is a physiological reaction to the new biomechanical configuration and is strongly influenced by preoperative bone quality [19]; in all the groups studied, we observed a bone enhancement, which is recognizable also in patients with altered bone quality.

At the present time, DEXA is considered the most reliable tool for evaluating pre-existing bone quality in order to plan correctly surgical procedure and study bone remodelling after THA as it is more sensitive and precise than conventional X-rays [20, 21]. Analysis of seven periprosthetic Gruen zones is the most commonly used protocol for evaluating bone remodelling after the implantation of conventional femoral stems. However, we have to consider that the lumbar spine and femoral neck BMD, measured by DEXA scan, does not reproduce global bone resistance; the literature shows how fragility fractures also occur in patients with a normal *T* Score [15, 22–24].

In our experience, one patient, not enrolled for this study, in whom a TMP stem was implanted for osteoarthritis presented a femoral intraoperative crack during femoral broaching. This patient showed a normal *T* Score in the preoperative DEXA scan, although intraoperatively a cortical thinning was detected.

Our study has some limitations. First of all, data were collected retrospectively. Moreover, all the patients affected by osteoporosis were treated with antiosteoporotic drugs, so periprosthetic BMD values can be conditioned during follow-up.

Compliance with ethical standards

Conflict of interest On behalf of all the authors, the corresponding author states that there is no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent For this type of study formal consenti is not required.

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