CHANGES IN SERUM LEVELS OF TNF-A, IL-6, OPG, RANKL AND THEIR CORRELATION WITH RADIOGRAPHIC AND CLINICAL ASSESSMENT IN FRAGILITY FRACTURES AND HIGH ENERGY FRACTURES

M.G. GIGANTI[1], F. LIUNI[1], M. CELI[1], E. GASBARRA[1], R. ZENOBI[2], I. TRESOLDI[1], A. MODESTI[1], R. BEI [1] and U. TARANTINO[1]

[1]Dept. of Clinical Sciences and Translational Medicine, University of Rome "Tor Vergata", Rome, Italy; [2]Dept. of Experimental Medicine and Surgery, University of Rome "Tor Vergata", Rome, Italy

Received February 24, 2012 – Accepted June 4, 2012

Stages of bone turnover during fracture repair can be assessed employing serum markers of osteoblastic and osteoclastic activity, inflammatory cytokines, clinical evaluation and imaging instruments. Our study compare the fracture healing process in fragility fractures and high energy fractures by evaluating serum changes of interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), osteoprotegerin (OPG) and receptor activator of the nuclear factor-kB ligand (RANKL) in combination with radiographic (Radiographic Union Scale for Tibial fractures, RUST) and clinical (Lower extremity measure, LEM) assessments. We enrolled 56 patients divided into four corresponding groups: group A with high energy trauma fracture (tibial/femoral shaft); group B with low energy trauma fracture (femoral fractures); healthy (control A) and osteoporotic subjects (control B). Blood samples were collected before surgery (T0) and after 10 weeks (T10). Serum concentrations of IL-6, TNF-α, RANKL and OPG were quantified using commercial enzyme-linked immunosorbent assay (ELISA) kits. Our results show that RANKL values are significantly higher at T10 than at T0 in low energy trauma fractures (group B). OPG is significantly lower in each control group than that of the respective fractured group and its concentration at T0 and at T10 is significantly lower in high than in low energy fractures. RANKL/OPG ratio is significantly higher in both controls than in fractured groups, and significantly increases after 10 weeks. IL-6 and TNF-α concentrations significantly decrease during fracture healing and are higher in high (group A) than in low energy fractures (group B). Significant differences were also found in both RUST score and LEM between groups A and B. Changes in TNF-α and IL-6 levels correlate with RUST and LEM in fragility and high energy fractures, while RANKL/OPG ratio is associated with these clinical parameters only in fragility fractures. These findings suggest that serum levels of IL-6, TNF-α, RANKL and OPG might be used to monitor the stages of fracture repair. Further studies will be needed to confirm the role of these cytokines in fracture repair.

Elderly patients usually present with a variable degree of osteoporosis and a high number of complications has been reported following fixation of osteoporotic fractures. Several studies have supported the view that osteoporosis is associated with a delayed fracture healing process although clinical evidence

Key words: inflammatory cytokines, fragility fractures, high energy fractures, RUST, LEM

Mailing Address: Dott.ssa Maria Gabriella Giganti, Department of Clinical Sciences and Translational Medicine, University of Rome "Tor Vergata", Via Montpellier 1, 00133 Rome, Italy
Tel.: +39 06 72596563
Fax: +39 06 72596563
e-mail: giganti@med.uniroma2.it

0393-974X (2012)

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

in this regard are limited (1-4). Different stages of bone turnover during fracture repair can be assessed using serum markers of osteoblastic and osteoclastic activity, inflammatory cytokines responses, clinical evaluation and imaging instruments (5-7).

Pro-inflammatory cytokines including interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) can stimulate osteoclast development and thereby the process of bone resorption (8). IL-6 activates bone resorption pathways by inducing osteoclast differentiation and activation (9, 10) and thus contributes to early stage of fracture repair (11). TNF-α is a most commonly used inflammatory marker may participate in the induction of early inflammatory responses. TNF-α is a primary mediator of immune regulation and it is produced by a wide variety of immune and non immune cells. Moreover, TNF-α has been extensively studied in bone and implicated in the regulation of osteoclastogenesis (12).

On the other hand, IL-6 is expressed and secreted by cells of the osteoblastic lineage and osteoclasts in response to hormones, such as the parathyroid hormone (PTH) and vitamin D, that also participate in the early stages of fracture repair (13).

Recently, novel members of the TNF receptor family such as osteoprotegerin (OPG) and receptor activator of the nuclear factor-kB ligand (RANKL) were shown to be key regulators of bone mass by modulating the bone reabsorption (14). RANKL acts as a potent stimulator of bone reabsorption by binding RANK in the cell membrane of osteoclasts. On the other hand, OPG is a soluble decoy receptor for RANKL that interferes with RANKL/RANK binding and inhibits the maturation and activation of osteoclasts and their precursors. Balance of RANKL/RANK and OPG plays a key role in the regulation of bone remodelling and alteration of this balance has direct effects on bone loss and destruction in osteoporosis, chronic inflammatory arthritis and osteolitic metastasis. In addition, RANKL and OPG expression has been involved in the fracture healing process (15).

IL-6 promotes osteoclast activation and therefore bone reabsorption through the RANK/RANKL/OPG pathway (9). IL-6 may indirectly promote osteoclastogenesis by increasing the release of RANKL by synovial cells; it also induces differentiation of pre-osteoblasts to mature osteoblasts while it decreases the proliferation of osteoblasts at late differentiation stages. Thus, the effects of IL-6 on bone remodeling are complex and may occur in opposite directions (16).

In order to assess a potential impairment of physiological bone reparative process, the aim of this study was to compare serum levels of RANKL, OPG, IL-6 and TNF-α as well as clinical and radiological findings during the fracture healing process in fragility fractures and high energy fracture patients, using both healthy and osteoporotic volunteers as controls.

MATERIALS AND METHODS

Subjects

Twenty-eight patients of both sexes were selected, according to inclusion criteria, in the Orthopedics and Traumatology division of Tor Vergata hospital in Rome: 14 patients (26 to 48 years), with tibial/femoral shaft high energy trauma fracture, treated with endomedullary nailing or external fixation (group A); 14 patients (55 to 89 years), with pertrochanteric/subtrochanteric low energy trauma fracture, treated with endomedullary nailing (men and post-menopausal women, group B).

Twenty-eight subjects were enrolled as control. 14 healthy young volunteers (24 to 45 years, control A) and 14 osteoporotic patients, both without any history of fractures (52 to 73 years, men and post-menopausal women, control B), were recruited from the Osteoporosis Clinic of Tor Vergata hospital,

Inclusion criteria were: age, trauma mechanism (high and low energy), tibial and femoral fractures, surgical treatment with endomedullary nailing or external fixation.

Exclusion criteria were: previous or actual treatment with drugs that could lead to bone metabolism changes (Bisphosphonates, Strontium ranelate, PTH, Denosumab; chronic treatment with FANS, FAS, inhibitors of HMG-CoA reductase); pathologies that could lead to bone metabolism changes (diabetes mellitus, hyperthyroidism, osteoarthritis, rheumatoid arthritis, psoriatic arthritis, lupus); pathologic fractures in any skeletal region or bone metastasis; peripheral vascular diseases.

According to the Helsinki declaration, the informed consensus was signed by all the subjects involved in the study.

Measurement of RANKL, OPG, IL-6 and TNF-α serum levels

Ten ml blood sample was collected from the

antecubital vein in dry Vacutainer tubes (Becton Dickinson, Rutherford, NJ, USA). Sera were aliquoted after centrifugation for 15 min. at 3000 rpm for 10 min, then frozen at –80°C until assayed. All serum samples were collected from 07:00 a.m. to 09:00 a.m.

In group A and B serum samples were collected before surgery (24 hours before surgical intervention, T0) and 10 weeks after surgery (T10). In both control A and B groups serum samples were collected during outpatient visit.

Commercially available human ELISA kits were employed to determine IL-6, TNF-α (Quantikine, R&D Systems, Minneapolis, MN), RANKL (BioVendor GmbH, Heidelberg, Germany) and OPG levels (Biomedica Medizinprodukte, GmbH &Co, Austria), following the instructions of the manufacturer (17, 18). The detection limits for IL-6 and TNF-α were 3.12 and 3.96 pg/ml, respectively, for RANKL 0.4 pmol/l and for OPG 0.14 pmol/l.

Radiographic and Bone Mineral Density (BMD) assessment

X-rays in two projections of the fractured segment were performed on each patient immediately after surgery (T0) and 10 weeks after (outpatient follow-up, T10).

Images were then evaluated in chronological order by specific rating scales for evaluation of fracture healing:

The Radiographic Union Scale for Tibial fractures (RUST score) which was developed for the assessment of tibial fractures appears to be more suitable than other radiographic score in evaluating other long bones fractures, such as the femur fractures (19). The evaluation criteria take into account the presence of a fracture line and the formation of bone callus. RUST score assigns a specific score for each set of radiographs in anteroposterior and lateral projection, based on the actual healing of each of the four visible corticals (Fig. 1). Each cortical received a rating of: 1 point if the fracture line was clearly visible and if there were no signs of callus formation; 2 points if there was presence of callus, but the fracture line was still evident; 3 points in the presence of callus that unites the fracture, with inability to distinguish a clear fracture line. The scores of the four corticals were then added up to a total that could range from a minimum of 4 (definitely not healed fracture) to a maximum of 12 (completely healed fracture) (19, 20).

Osteoporotic patients belonging to groups B and control B were subjected to DEXA examination, to detect the grade of bone quality impairment. Measurements of BMD were assessed using dual-energy x-ray absorptiometry iDXA (GE, Lunar, USA). The examinations were performed with the patient in supine position to determine both the lumbar spine and one or both hips. DEXA examination was necessary for the recruitment of various subgroups of patients but also to provide informations on the BMD in relation to sex and age of the patients. In group A and control A it was not considered necessary to perform a DEXA examination for osteoporosis disease evaluation. Indeed, it has been considered sufficient the assessment of subjects age and the high-energy mechanism of trauma (in the fractured group) and the exclusion of diseases that could lead to secondary osteoporosis.

Clinical assessment

All 28 fractured patients (group A and B) underwent a standardized clinical evaluation of the lower extremity and of the disease impact on their life quality (immediately after surgery, T0 and after 10 weeks, T10) through a specific questionnaire: Lower extremity measure (LEM).

LEM is a disease specific questionnaire which evaluates the function in lower limb fractures; it has been developed in order to monitor and assess clinical changes of the patient basing on patients' reports of their function. It consists of 29 questions related to the function of the lower extremity of the subject including: getting out of bed, getting in/out of bathtub, getting on/off toilet, showering, putting on pants, food shopping, etc..

Answers are included in a range between "unable to perform" (score=1) to "no difficulties in execution" (score=5). If an activity was not part of the normal activities of the patient it was inserted as "not applicable" (N/A). The minimum score possible is 0 and the maximum was 100. Higher scores are indicative of a better function of the lower extremity (21).

Statistical analysis

The statistical analysis of serum, radiographic and clinical results were performed using SPSS 13.0 software for Windows® (SPSS, Chicago, IL, USA). Results were expressed as mean value ± standard deviation (SD). All groups were analyzed using Kolmogorov-Smirnov test for normal distribution, significant differences between control and experimental groups were assessed by ANOVA tests (Bonferroni's post-test and Dunnett's test). The correlations were performed using Spearman's correlation in each group. Differences were considered significant for $p \leq 0.05$.

RESULTS

Serum levels of OPG, RANKL, IL-6 and TNF-α

Fig. 2 shows OPG and RANKL serum concentrations in high energy fractures (group A), low energy fractures (group B), healthy and osteoporotic subjects (control A and B). RANKL values were not significantly different in group A

Table I. *RUST and LEM scores in groups A and B, values given are the means±SD.*

	RUST		LEM	
	T0	T10	T0	T10
Group A	4±0*	8.57±1.55**	20.92±7.80°/°°	50.64±12.11°°°
Group B	4±0*	7.42±1.15	6.85±4.67°	19±10.90

*Statistically significant difference RUST: * T0/T10 p<0.05, **T10A/T10B p<0.05; LEM: ° T0/T10 p<0.001, °° T0A/T0B p<0.001, °°°T10A/T10B p<0.001*

Table II. *Correlation between RUST or LEM and serum parameters in both group A and B.*

	GROUP A		GROUP B	
	RUST	LEM	RUST	LEM
IL-6	*r* = -0.815 p<0.01	*r* = -0.633 p<0.01	*r* = -0.817 p<0.01	*r* = -0.646 p<0.01
TNF-α	*r* = -0.755 p<0.01	*r* = -0.702 p<0.01	*r* = -0.591 p<0.01	*r* = -0.377 p<0.05
OPG	NS	NS	*r* = -0.427 p<0.05	NS
RANKL	NS	NS	NS	*r* = 0.470 p<0.05
RANKL/OPG ratio	NS	NS	*r* = 0.418 p<0.05	*r* = 0.479 p<0.01

NS: Not significant

as compared to control A, while in group B were significantly higher at T10 than at T0 (p<0.05).

In both group, A and B, OPG decreased at T10 as compared to T0 but not significantly, while it was significantly increased as compared to healthy (p<0.05) and osteoporotic subjects (p<0.001). In each control group the values were significantly lower than those of the respective fractured group (p<0.001). OPG levels were significantly lower in T0A than in T0B (p<0.001); similarly, these values were significantly lower in T10A than in T10B (p<0.05).-

The RANKL/OPG ratio (Fig. 3) was significantly higher in healthy control than in high energy

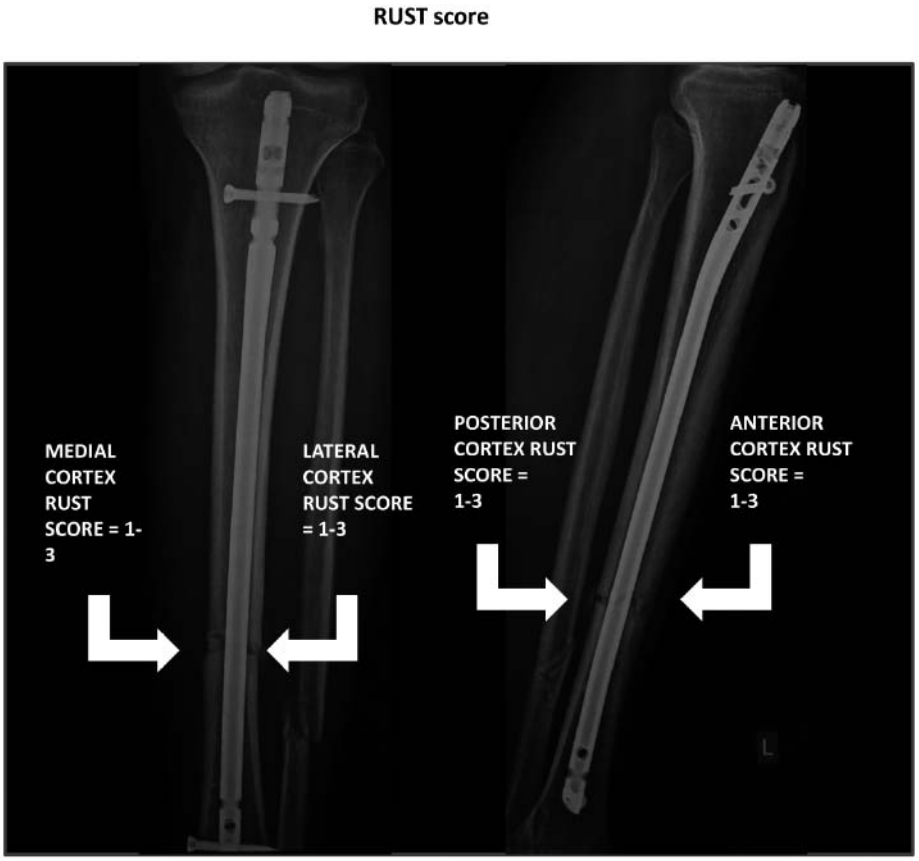

Fig. 1. *RUST score. Rust score in a tibial fracture treated with endomedullary nail at 10 weeks.*

fracture group at T0 and T10 ($p<0.001$). Also, it was significantly higher in osteoporotic subjects than in low energy fracture patients at T0 ($p<0.001$) and T10 ($p<0.01$). In both group A and B the ratio significantly increased after 10 weeks (T10A $p<0.001$, T10B $p<0.05$).

Figure 4 shows IL-6 and TNF-α values. IL-6 concentration was significantly higher in group A at T0 and T10 than in control A ($p<0.001$), in group B the values were significantly higher at T0 than at T10 and in control B ($p<0.001$). IL-6 concentrations were significantly different in between high and low energy groups: T0A levels were significantly higher than T0B ($p<0.001$) and T10A than T10B ($p<0.001$).

TNF-α values were significantly higher in T0A than in T10A and control A ($p<0.001$), while the value decrease in group B at T10 was not significant. Significant differences were also found between group A and B, T0A being significantly higher than T0B ($p<0.001$).

Radiographic assessment

In group A, the mean post-operative RUST score was 4±0 at T0 and 8.57±1.55 at T10; the difference between the stages was significant ($p<0.05$). In group B the mean post-operative RUST score was 4±0 at T0 and 7.42±1.15 at T10; difference in mean values was significant ($p<0.05$). The differences between group A and B were significant at T10 ($p<0.05$).

Clinical assessment

In high energy fracture patients the mean post-operative LEM value was 20.92±7.80 at T0, 50.64±12.11 at T10 and the difference was significant ($p<0.001$). In low energy fracture patients the mean post-operative LEM value was 6.85±4.67 at T0, 19±10.90 at T10 and the difference was significant ($p<0.001$).The differences between group A and

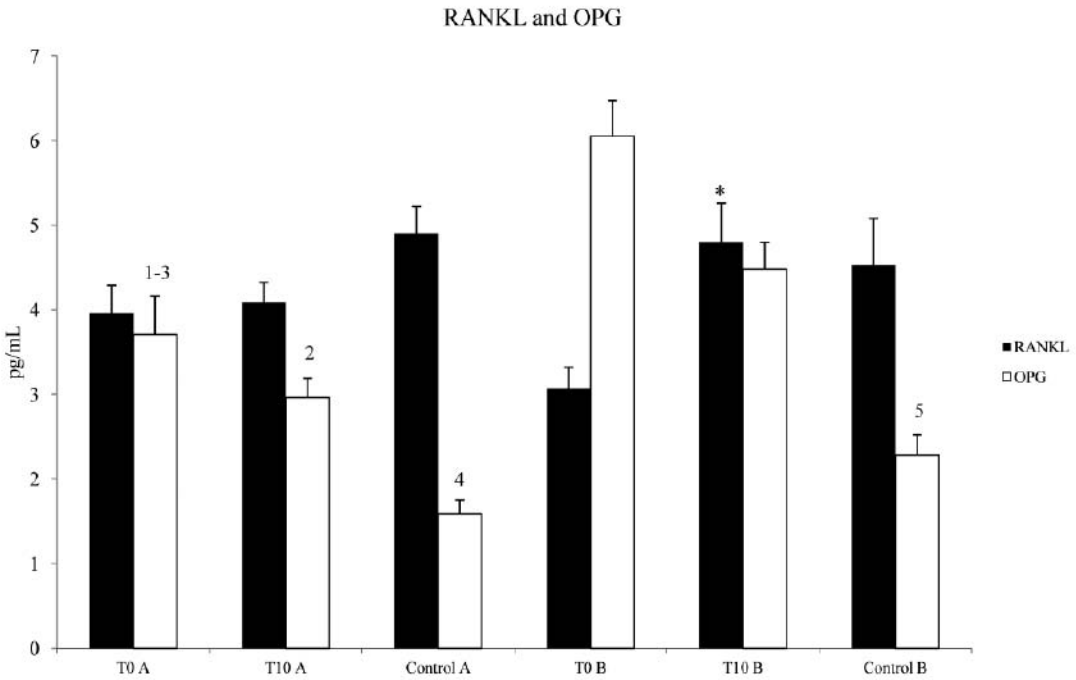

Fig. 2. *Levels of RANKL and OPG during fracture healing. In group A and control A the RANKL concentration showed no significant differences; in group B at T10 the values were significantly higher than at T0 ($p<0.05$). In each control group the OPG values were significantly lower than those of the respective fractured group ($p<0.001$). Group A compared with group B shows significantly lower values in T0A than T0B ($p<0.001$) and T10A than T10B ($p<0.05$). (Statistically significant difference. OPG: [1]T0A and T0B, [2]T10A and T10B, [3]T0A and Control A, [4]Control A and T0A and T10A, [5]Control B and T0B and T10B; RANKL: *T10B and T0B)*

group B (Table I) were significant at both times (T0 and T10, $p<0.001$).

BMD assessment

In low energy fracture patients the BMD evaluation showed osteoporotic T-score in 12 out of 14 patients and osteopenic T-score in 2 out of 14 patients, the findings being similar in the lumbar spine and hip site (-2,62 SD; -2,57 SD, respectively). In osteoporotic subjects, 13 out of 14 patients were osteoporotic, while 1 out of 14 was osteopenic. Even in this group results were similar in the two sites: lumbar spine: -2.8 SD; hip: -2.4 SD.

Correlation between RUST or LEM and serum parameters

In high energy fracture patients we observed inverse and significant correlation between RUST or LEM and inflammatory cytokines (IL-6 and TNF-α), while there was no significant correlation between RUST or LEM and OPG, RANKL or RANKL/OPG ratio.

The low energy fracture patients showed inverse and significant correlation between RUST and IL-6, while there was only a moderate inverse and significant correlation between RUST and TNF-α or OPG and between RUST and RANKL/OPG ratio. Conversely, no correlation between RUST and RANKL was found. A weak inverse and significant correlation was observed between LEM and inflammatory cytokines, as well as between LEM and RANKL or RANKL/OPG ratio, while no significant correlation between LEM and OPG was observed (Table II).

DISCUSSION

The fracture healing stages are characterized by regulatory mechanisms linked to the expression of different molecules (such as OPG and RANKL) that

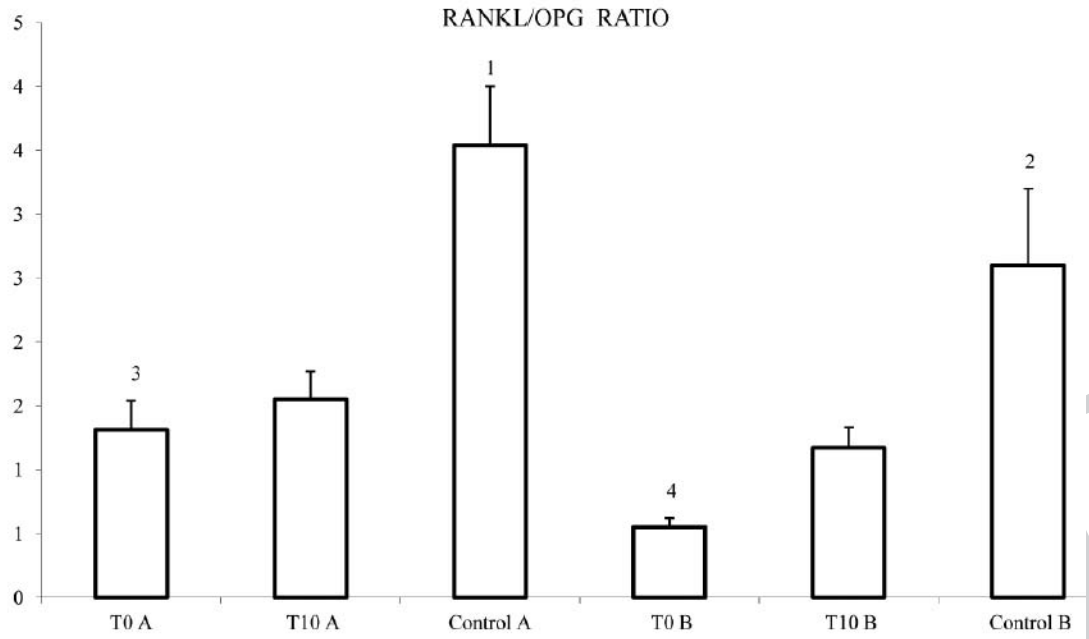

Fig. 3. *RANKL/OPG ratio during fracture healing. RANKL/OPG ratios were significantly higher in control A than in T0A and T10A ($p<0.001$) and in control B than in T0B ($p<0.001$) and T10B ($p<0.01$). In both groups increased at T10 (T10A $p<0.001$, T10B $p<0.05$). (Statistically significant difference. [1]Control A and T0A and T10A, [2]Control B and T0B and T10B, [3]T0A and T10A, [4]T0B and T10B)*

regulate proliferation and differentiation of bone tissue cells.

The recent discovery of RANK, RANKL and OPG members of the TNF and TNF-receptor superfamily enabled to explain the mechanisms of interaction between the osteclastic and osteoblastic lineage. In fact, the RANKL-RANK/OPG signaling pathway was shown to play a key role in the formation and activation of osteoclasts in conjunction with various cytokines and calciotropic hormones (22, 23).

This study aimed at determining the serum levels of TNF-α, IL-6, OPG, RANKL in fragility fractures and high energy fractures and evaluating whether changes in serum levels of these molecules correlate with radiographic and clinical assessment of the patients.

Our results show that OPG values were higher in both group B and control B. This result may be misleading regarding the inhibitory role played by this molecule on osteoclast activity and its production by osteoblastic cells. However, increases in OPG may occur as a consequence of increased bone loss as a part of the mechanism minimizing bone turnover and thereby reducing bone loss in osteoporotic patients (24). Indeed, Szulc et al. (25) showed an increase in OPG values in aging (in non-fractured patients), highlighting a potential correlation between this molecule and increased levels of bone turnover, as a compensatory response to an increased bone reabsorption in osteoporotic patients.

Low serum RANKL levels observed in osteoporotic patients at T0B might be associated with a high risk of low energy fractures, suggesting that low serum RANKL may reflect suppressed bone turnover with accumulation of micro damages and reduced bone quality.

A recent study reported that physiological fracture healing elicits a cytokine cascade that involves up-regulation of IL-6 and RANKL, in a hierarchical manner, and down-regulation of OPG, resulting in

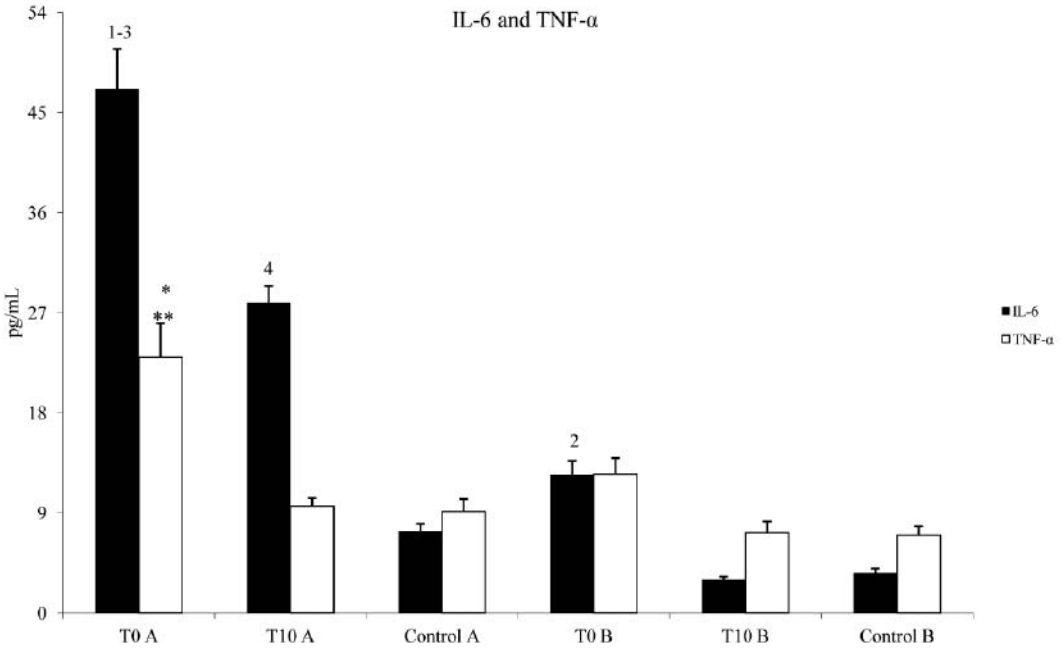

Fig. 4. *Levels of IL-6 and TNF-α during fracture healing. IL-6 levels were higher in T0A than T10A and control A ($p<0.001$); in group B the levels at T0 were higher than at T10 and in control B ($p<0.001$). Significant differences were also found between group A and B: T0A was higher than T0B ($p<0.001$) and T10A higher than T10B ($p<0.001$). The TNF-α concentration was higher in T0A than T10A and control A ($p<0.001$). Significant differences were also found between group A and B: T0A was significantly higher than T0B ($p<0.001$). (Statistically significant difference. IL-6: [1]T0A and T10A and control A, [2]T0B and T10B and control B, [3]T0A and T0B, [4]T10A and T10B; TNF-α: *T0A and T10A and control A, **T0A and T0B)*

the initiation of direct bone reabsorption of the stress fracture (26).

In our study, the RANKL/OPG ratio showed higher values in control A than in control B; considering this ratio as an index of metabolic activity (27) our result may indicate a stronger metabolic activity in younger patients than in osteoporotic ones.

Higher IL-6 and TNF-α levels in young patients at T0A and T10A may reflect a more effective inflammatory activity than an increased osteoclastic activity. As a matter of fact, the main activity of IL-6 on bone is its effect on osteoclastogenesis and bone reabsorption (28, 29).

TNF-α is one of the most potent osteoclastogenic cytokines produced during inflammation. Defined as a potent bone-resorbing factor, TNF-α is responsible for stimulating osteoclastic bone reabsorption (30, 31). The higher TNF-α values at T0A, as compared to control A, might confirm the involvement of this molecule in the early stages of inflammation. Since TNF-α and IL-6 stimulate osteoclast differentiation in a synergic manner, the increase in IL-6 production might be closely related to osteoclastogenesis and bone reabsorption. The radiographic evaluation of fracture healing showed that in both groups there is a progressive significant increase in the RUST score during fracture repair. In addition we observed that at T10, group A achieved an higher score than group B (19).

Regarding the clinical evaluation of patients carried out by the "Lower Extremity measure" questionnaire, a clear difference was noted in group A scoring as compared with group B; the former had significantly higher scores after surgery and at T10 (21).

Overall, our results showed that the healing process is significantly compromised in osteoporotic patients as compared to younger fractured patients. Indeed, these patients showed an early inflammatory response, an increased osteoblastic cellular activity,

as indirectly measured by cytokines release, and higher clinical and radiographic test scores.

Changes in the profiles of RANKL, OPG, IL-6 and TNF-α levels, RANKL/OPG ratios and altered repair of a fracture can occur in osteoporotic patients. Our results showed the early upregulation of IL-6 and confirmed a central role for these factors in the initiation of cartilage and periosteal woven bone formation (32).

It should be considered that the reparative response in patients with osteoporotic conditions has been only marginally addressed by Nikolau VS et al. (2) who compared the two different conditions in femur fracture healing, but using only clinical and radiographic parameters.

Another finding of this study was a correlation between biological, clinical and radiographic parameters during fracture healing. In particular the strong inverse correlation observed between RUST and IL-6 in both group emphasizes the key role of this inflammatory cytokine in fracture healing. Overall, our results suggest that changes in TNF-α and IL-6 levels correlate with radiographic and clinical assessment in fragility fractures and high energy fractures, while RANKL/OPG ratio is associated only with radiographic and clinical assessment in fragility fractures. Accordingly, these serum parameters might be used to assess the stages of fracture healing and to monitor the efficacy of different treatment in patients with poor bone quality (33, 34). Still, further studies in a larger cohort of patients are required to confirm our data and clarify the complex fracture healing process.

REFERENCES

1. Gruber R, Koch H, Doll BA, Tegtmeier F, Einhorn TA, Hollinger JO. Fracture healing in the elderly patient. Exp Gerontol 2006; 41:1080-93.
2. Nikolaou VS, Efstathopoulos N, Kontakis G, Kanakaris NK, Giannoudis PV. The influence of osteoporosis in femoral fracture healing time. Injury 2009; 40:663-68.
3. Tarantino U, Cerocchi I, Scialdoni A, et all. Bone healing and osteoporosis. Aging Clin Exp Res 2011; 23(S):62-4.
4. Tarantino U, Iundusi R, Lecce D, Tempesta V, Perrone FL, Rao C, Cerocchi I, Gasbarra E. Osteosynthesis of fragility fractures. Aging Clin Exp Res 2011; 23(2):54-6.
5. Civitelli R, Armamento-Villareal R, Napoli N. Bone turnover markers: understanding their value in clinical trials and clinical practice. Osteoporosis Int 2009; 20:843-51.
6. Andreoli A, Scalzo G, Masala S, Tarantino U, Guglielmi G. Body composition assessment by dual-energy X-ray absorptiometry (DXA). Radiol Med 2009; 114(2):286-300.
7. Signore A, Chianelli M, Bei R, Oyen W, Modesti A. Targeting cytokine/chemokine receptors: a challenge for molecular nuclear medicine. Eur J Nucl Med Mol Imaging 2003; 30(1):149-56.
8. Manolagas SC. Role of cytokines in bone resorption. Bone 1995; 17(2):63-7.
9. Kwan TS, Padrines M, Theoleyre S, Heymann D, Fortun Y. IL-6, RANKL, TNF-alpha/IL-l: inter relations in bone resorption pathophysiology. Cytokine Growth Factor Rev 2004; 15:49-60.
10. Kousteni S, Bellido T, Plotkin LI, et al. Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity. Cell 2001; 104(5):719-30.
11. Beeton CA, Chatfield D, Brooks RA, Rushton N J Bone Joint Surg Br. Circulating levels of interleukin-6 and its soluble receptor in patients with head injury and fracture 2004; 86(6):912-7.
12. Pacifici R. Cytokines, estrogen, and postmenopausal osteoporosis--the second decade. Endocrinology 1998; 139(6):2659-61.
13. Feyen JH, Elford P, Di Padova FE, Trechsel U. Interleukin-6 is produced by bone and modulated by parathyroid hormone. J Bone Miner Res 1989; 4(4):633-8.
14. Lacey DL, Timms E, Tan HL, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998; 93:165-76.
15. Kon T, Cho TJ, Aizawa T, Yamazaki M, Nooh N, Graves D, Gerstenfeld LC, Einhorn TA. Expression of osteoprotegerin, receptor activator of NF-kappaB ligand (osteoprotegerin ligand) and related proinflammatory cytokines during fracture healing. J Bone Miner Res 2001; 16(6):1004-14.
16. Le Goff B, Blanchard F, Berthelot JM, Heymann D, Maugars Y. Role for interleukin-6 in structural

joint damage and systemic bone loss in rheumatoid arthritis. Jt Bone Spine 2010; 77(3):201-5.
17. Visco V, Bei R, Moriconi E, Gianni W, Kraus MH, Muraro R. ErbB2 immune response in breast cancer patients with soluble receptor ectodomain. Am J Pathol 2000; 156(4):1417-24.
18. Masuelli L, Focaccetti C, Cereda V, et al. Gene-specific inhibition of breast carcinoma in BALB-neuT mice by active immunization with rat Neu or human ErbB receptors. Int J Oncol 2007; 30(2):381-92.
19. Kooistra BW, Dijkman BG, Busse JW, Sprague S, Schemitsch EH, Bhandari M. The Radiographic Union Scale in Tibial Fractures: Reliability and Validity. J Orthop Trauma 2010; 24:S81-6.
20. Whelan DB, Bhandari M, Stephen D, Kreder H, McKee MD, Zdero R, Schemitsch EH. Development of the Radiographic Union Score for Tibial Fractures for the Assessment of Tibial Fracture Healing After Intramedullary Fixation. J Trauma 2010; 68: 629-32.
21. Jaglal S, Lakhani Z, Schatzker J. Reliability, Validity, and Responsiveness of the Lower Extremity Measure for Patients with a Hip Fracture. J Bone Joint Surg Am 2000; 82:955-62.
22. Mysliwiec J, Zbucki R, Nikolajuk A, Mysliwiec P, Taranta A, Kaminski K, Bondyra Z, Dadan J, Gorska M, Winnicka MM. Role of interleukin-6 on RANKL-RANK/osteoprotegerin system in hypothyroid ovariectomized mice. Folia Histochem Cytobiol 2010; 48(4):549-54.
23. Trouvin AP, Goëb V. Receptor activator of nuclear factor-κB ligand and osteoprotegerin: maintaining the balance to prevent bone loss. Clin Interv Aging 2010; 19(5):345-54.
24. Jabbar S, Drury J, Fordhma JN, Datta HK, Francis RM, Tuck SP. Osteoprotegerin, RANKL and bone turnover in postmenopausal osteoporosis. J Clin Pathol 2011; 64:354-7.
25. Szulc P, Hofbauer LC, Heufelder AE, Roth S, Delmas PD. Osteoprotegerin Serum Levels in Men: Correlation with Age, Estrogen, and Testosterone Status. J Clin Endocrinol Metab 2001; 86:3162-5.
26. Kidd LJ, Stephens AS, Kuliwaba JS, Fazzalari NL, Wu ACK, Forwood MR. Temporal pattern of gene expression and histology of stress fracture healing. Bone 2010; 46:369-78.
27. Rogers A, Eastell R. Circulating Osteoprotegerin and Receptor Activator for Nuclear Factor {kappa} B Ligand: Clinical Utility in Metabolic Bone Disease Assessment. J Clin Endocr Metab 2005; 90(11):6323-31.
28. Koteke S, Sato K, Kim KJ Takahashi N, Udagawa N, Nakamura I, Yamaguchi A, Kishimoto T, Suda T, Kashiwazaki S. Interleukin-6 and soluble Interleukin-6 receptors in the synovial fluids rheumatoid arthritis patients are responsible for osteoclast-like cell formation. J Bone Miner Res 1996; 11:88-95.
29. Sedlár M, Kudrnová Z, Erhart D, Trca S, Kvasnicka J, Krska Z, Mazoch J, Malíková I, Zeman M, Linhart A. Older age and type of surgery predict the early inflammatory response to hip trauma mediated by interleukin-6 (IL-6). Arch Gerontol Geriatr 2010; 51(1):1-6.
30. Thomson BM, Mundy GR, Chambers Tj. Tumor necrosis factors alpha and beta induce osteoclastic cells to stimulate osteoclastic bone resorption. J Immunol 1987; 138:775-9.
31. Konig A, Muhlbauer RC, Fleisch H. Tumor necrosis factors alpha and Interleukin-1 stimulate bone resorption *in vivo* as measured by urinary [3h] tetracycline excretion from prelabeled mice. J Bone Miner Res 1988; 3:621-7.
32. Fazzalari NL. Bone fracture and bone fracture repair. Osteoporos Int 2011; 22:2003-6.
33. Iolascon G, Resmini G, Tarantino U. Inhibition of RANK ligand: a new option for preventing fragility fractures. Aging Clin Exp Res 2011; 23(2):28-9.
34. Tarantino U, Iundusi R, Cerocchi I, Liuni FM, Feola M, Celi M, Baldi J, Gasbarra E. Role of the orthopaedic in fragility fracture and in the prevention of a new fracture: SIOT 2009 recommendations. Aging Clin Exp Res 2011; 23(2):25-7.