POSTERS

P1 - MAXILLARY OSTEOPOROSIS AND GENETIC PREDISPOSITION

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Introduction: Osteoporosis is a form of dysmetabolic osteopathy of multifactorial origin, characterised by reduction of the bone matrix and mineral portion and, overall, of bone mass, leading to fragility and increased fracture risk.
AETIOPATHOGENESIS
-ENDOCRINE FACTORS: ACTH, glycocorticoids, PTH, thyroxine, oestrogen, testosterone
-GENETIC FACTORS: Major genes that regulate fundamental characteristics of bone, such as density and quality, and minor genes that regulate individual genetic background [lipoprotein receptor related protein (LRP5), TGF1, BMP, VDR, COL1A1, ER].
The DIAGNOSIS is based on history, clinical findings (vertebral or appendicular fractures), blood chemistry, conventional radiology and bone mass measurement. For the latter, it is possible to use DUAL-ENERGY X-RAY DENSITOMETRY which measures bone mineral content: according to the WHO definition, in osteoporosis bone mineral density (BMD) is more than 2.5 standard deviations below normal.
MAXILLARY OSTEOPOROSIS: because of their function as a support for teeth, which leads to the development of the alveolar process, and their role in mastication, the jawbones (maxilla and mandible) differ from all the other bones of the skeleton. This role, also involving the masticatory muscles, prompts bone trophism. In advancing age a marked reduction of the thickness of the maxillary cortical bone is observed, together with increased porosity and constant functional remodelling of the trabecular part, a phenomenon that, as it increases, leads to tooth loss. Only a mandibular area (a bucco-lingual area of cortical bone in front of the mental foramen) remains unmodified, independently of gender, age and tooth loss.
Materials and methods: Kemifar® supplies a test which can be used to study several factors (Er , VDR, COL1A1) that predispose to the development of osteoporosis. OsteoResis®Type is a simple, non-invasive test that allows the complete determination, and interpretation, of several genotypes associated with the appearance of osteoporosis. The test requires a pinprick blood sample, taken from the fingertip: from this sample, which is placed on a special paper, the genetic material is extracted for subsequent analysis. The test supplies definitive data, does have to be repeated and can be carried out at any time.
Gene polymorphism influences the appearance of osteoporotic fracture risk: individuals with an absent enzyme restriction site have a markedly reduced osteoporotic fracture risk, therefore if capital “X” indicates absence of the restriction site, the XX subject has a resistant genotype as the site is not present on either chromosome, whereas Xx and xx subjects have a susceptible genotype.
A sample of 20 subjects randomly drawn from among patients referred to our department of oral and implant surgery underwent the above test.
Discussion and conclusions: The genetic component is becoming increasingly important in the early diagnosis of osteoporosis. Even though there are many risk factors involved in the pathogenesis of the disease, the most important is still a positive family history. The single individual’s genetic makeup is important as regards bone mass peak, which is 50-60% genetically determined.
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P2 - REGENERATION TECHNIQUES IN DENTISTRY

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Grafts of autologous bone or of heterologous or homologous biomaterials are fundamental for the specific aims of oro-maxillo-facial reconstructions. They are, indeed, used in the reconstruction of defects caused by tooth loss, tumours or cysts, traumatic injuries and iatrogenic damage. Sources of autologous bone grafts are the mandibular symphysis, the mandibular ramus and the tubercle. Understanding of the surgical technique and also of correct graft management during the healing stage are crucial to subsequent prosthetic implant rehabilitation. The success of prosthetic implant rehabilitation of atrophic maxillae depends on the quality and quantity of the bone used for the three-dimensional reconstruction of the alveolar process. A careful literature review showed that, for a long time, attention was focused on the embryological nature of the donor site and of the recipient bed, affirming the biological superiority of bone of intramembranous origin.

The phase in which the graft attaches to the recipient bed is crucial in order to obtain complete healing; for this reason, our analysis focused on the various histological phases of this process. We examined the various surgical regeneration techniques and, above all, graft management, preparation of the recipient bed and graft stabilisation techniques. On the basis of current literature data we schematised the waiting times for complete healing and attachment of the graft and thus the insertion of implants.

We also present clinical cases, from the graft stage to implant insertion and subsequent prosthetic rehabilitation.

Of the various maxillary reconstruction techniques, the most reliable was found to be that involving the harvesting of intraoral autologous bone samples, but it must also be borne in mind that there exist other methods for restoring maxillary defects (heterologous bone, bone obtained from biobanking facilities and xenografts: hydroxyapatite, tricalcium phosphate, calcium sulfate, bioglass, bioapatite), and thus that it is necessary to select the surgical technique most suited to the type of defect. The advantages of intraoral harvesting are good quality of the bone, which has characteristics biologically superior to grafts obtained using other types of harvesting, given that it is compact bone that is not subject to resorption and thus allows subsequent prosthetic implant rehabilitation. Other advantages are easy surgical access and, for the patient, minimal postoperative complications. A major limitation of intraoral harvesting is the quantity of bone available. In the case of larger defects requiring extensive reconstruction, it is necessary to consider extraoral grafts, in particular from the cranial theca and iliac crest.

For intraoral harvesting, the mandibular symphysis is a site undoubtedly offering easy surgical access, but it is associated with a higher incidence of aesthetic and nervous complications. The mandibular ramus is less accessible surgically**,** but grafts harvested from this site are associated with reduced nerve impairment and do not present aesthetic problems.

For this reason, we now have at our disposal grafting methods involving heterologous or homologous biomaterials which use grids or membranes to stabilise the grafts. These approaches are certainly less invasive for the patient and do not create aesthetic or nervous problems. Biomaterial grafts are managed in much the same way as autologous bone grafts.

References: Lekhom U, et al. Oral implants in combination with bone grafts. A 3-year retrospective multicenter study using the Branemark implant system. Int J Oral Maxillofac Surg 1999;28:181-7. Isaksson S, Alberius P. Maxillary alveolar ridge augmentation with onlay bone-grafts and immediate endosseous implants. J Craniomaxillofac Surg 1992; 20:2-7. Misch CM. Use of the mandibular ramus as a donor site for onlay bone grafting. J Oral Implantology 2000;26:42-9.

P3 - HUMAN MESENCHYMAL STEM CELLS: ISOLATION AND CONCENTRATION FROM BONE MARROW USING THE REGENKIT DEVICE

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Introduction: The adult stem cells used in clinical practice are: hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). These cells, present in the bone marrow, play a key role in the physiological renewal and repair of injured tissues. MSCs can differentiate into a variety of cell types including bone cells, cartilage cells, connective cells etc., and they play an important role in the repair of tissue injured by trauma or disease. In view of all this, all the technologies for the isolation and the re-implantation, after minimal manipulation, of autologous stem cells are very interesting, also to make the use of stem cell therapy more widespread in the orthopaedic field.
The aim of this study was to verify the RegenKit method of selection and enrichment of autologous mesenchymal and hematopoietic stem cell fractions from bone marrow, used intraoperatively, in order to define a standard therapeutic application.
Materials and methods: ***Separation of MSCs using RegenKit****:* 32 ml of bone marrow aspirate is collected and divided into 4 Regen THT tubes, and then centrifuged directly in the operating room. The erythrocytes are pelletted at the bottom of the tube, while the mononuclear cells collect in the layer between the gel selector and plasma. The tubes are agitated to suspend the mononuclear cells in the plasma. The content of one of these tubes is used for a cellular efficiency and yield analysis. The sample is stratified on a lymphocyte separation medium gradient (density 1.077 g/ml, Lonza) and centrifuged at 1000xg for 30 min. The mononuclear cells are collected, washed in DPBS without Ca and Mg and seeded on a 100 mm culture plate, in growth culture medium (GM) Ham's F12, modified according to Coon, with the addition of FBS 10%, penicillin 100 IU/ml, streptomycin 100 g/ml. ***Separation of control MSCs***: A bone marrow aspirate sample not centrifuged and submitted to the same laboratory procedures is used as control. ***Cellular yield***: 48 hours after seeding, the adherent cells (MSCs) are counted directly on the growth medium. The cells present in 30 ocular fields are counted using an appropriate standard grid; the number obtained is normalised using an appropriate conversion coefficient.
Results: 10 samples from patients were prepared using the RegenKit method and 10 cell lines were obtained. The results showed that:
- A number of MSCs in the vicinity of 1 x 10^4 cells/ml initial bone marrow aspirate was obtained with both with the Regen Kit and with the control method.
- The RegenKit method made it possible to obtain almost total separation of erythrocytes with selection and enrichment of MSCs and HSCs.
- MSC yield did not show cellular loss with the RegenKit versus the control method.

*Conclusions***:** This study describes how, with the RegenKit device, it is possible to separate and concentrate the mononuclear cells derived from bone marrow aspirate, obtaining a high cellular yield. The future prospects are to verify the osteogenic ability of these cells, confirming clinical results.

P4 - IN VITRO STUDY OF THE BIOLOGICAL RESPONSE OF MONONUCLEAR CELLS IN THE PRESENCE OF METALLIC IONS

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Physiological repair following tissue damage or surgery is characterised by an initial inflammatory reaction, non-infectious, followed by a process of regeneration involving different cell elements that, through processes of differentiation and proliferation, work to restore the integrity of the tissue.
The implantation of prosthetic joints, however, can give rise to a pathological skeletal tissue response that can result in aseptic mobilisation and implant failure.
These complications are due to the chemical-physical interaction between the biomaterial and the host tissue.
The implanted material may undergo processes of electrochemical degradation as a result of contact with bodily fluids and wear-related corrosion leading to the release of metallic ions. These can be eliminated through catabolic processes, accumulate in the area of release, or spread via the vascular system and accumulate in organs such as the liver, spleen and heart. The presence of small quantities of debris is compatible with tolerance of the implant, while high concentrations can set up a persistent inflammatory process and cause local or systemic toxicity reactions.
The aim of this study was to evaluate the biological response of mononuclear cells from peripheral blood in the presence of metallic ions, evaluating both the presence, in the cytoplasm, of internalised metallic particles and the production of inflammatory cytokines.
Methods: Peripheral blood samples were taken, after obtaining the necessary informed consent, from patients with painful joint prostheses and from normal controls. The mononuclear cells were isolated from the peripheral blood by means of density gradient separation.
The cells, resuspended in culture medium (RPMI-1640 supplemented with foetal calf serum, L-glutamine, penicillin/streptomycin), were cultured in the absence and in the presence of 0.1mM of chromium, nickel, titanium, cobalt, molybdenum. The production of cytokines (IL-1, IL-8 and MIP1 α and β) in the culture supernatant was evaluated using the Luminex LabMAP system. An aliquot of the same cells was cultured on chamber slides, in the same experimental conditions, in order to evaluate, by means of confocal microscopy and laser scanning methods, the intracellular presence of metallic fragments.
The cells were washed in PBS, fixed with paraformaldehyde and stained with actin for morphological analysis of the cytoskeleton components. The nuclei were stained with 4.6-diamino-2-phenylindole, a molecule able to form fluorescent complexes with the double strand of DNA. After further washings, the slides were treated with a specific mounting medium (ProLong Gold Antifade), covered with coverslips and observed under the confocal microscope.
Results and conclusions: Analysis of the preparations using confocal microscopy and laser scanning methods revealed the presence of phagocytic vacuoles and free metallic particles in the cytoplasm of cells of the monocyte-macrophage line.
It was also possible to observe small dark areas, of different sizes, of non-biological nature, which could correspond to clusters of internalised particles.
Cell impairment was shown by the structure of the cytoskeleton, which appeared more disorganised and disgregated in the treated samples compared with the controls.
Preliminary results show the constant presence of cytokines with chemotactic activity, like IL-8 and MIP1 α and β and also the presence of pro-inflammatory cytokines produced by cells with macrophagic activity, like IL-1, in the samples treated with nickel and chromium compared with the controls.
On the basis of these observations, the presence of internalised metal fragments may be related to the release of soluble mediators of inflammation by the activated macrophages.
References: Vallés G. et al. Differential inflammatory macrophage response to rutile and titanium particles. Biomaterials 2006;27:5199-211. Ingham E and Fisher J. The role of macrophages in osteolysis of total joint replacement. Biomaterials 2005;26:1271-86. Tanaka Y et al. Osteoblasts and osteoclasts in bone remodeling and inflammation. Curr Drug Targets Inflamm Allergy 2005;4:325-8.

P5 - EVALUATION OF IMMUNOLOGICAL REACTIVITY TO METAL COMPONENTS IN PATIENTS WITH PROSTHESIS DEVICE

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In recent years, the development of innovative biomaterials and surgical techniques has led to a progressive increase in joint replacement arthroplasty procedures.
It is well known that all implant metals, in contact with biological fluids, undergo electrochemical and mechanical corrosion, releasing metallic particles that may induce toxic responses and local or systemic inflammatory reactions.
Several studies have demonstrated a possible relationship between particulate wear debris and symptoms of dermatitis and urticaria, but there is no evidence of a direct correlation between wear severity and immune response.
Published results show that the immune reaction changes with individual immunomodulatory status.
The aim of this study was to analyse the proliferative response in the presence of proper stimuli, and to identify possible modifications in the production of a wide range of cytokines, as potential biological markers for early diagnosis of aseptic loosening.
Methods: This study analyses the immune response of potentially allergic patients undergoing joint replacement arthroplasty, patients with painful prosthetic joints or joint instability, and subjects without any implants, serving as controls.
In vivo assessment of metal sensitivity includes a standard patch test for hypersensitivity reactions.
Accordingly, a standard patch test for *in vivo* assessment of metal hypersensitivity reaction, based on the level of allergic response of the skin, was performed.
Blood samples were collected after obtaining informed consent.
Activated lymphocyte proliferation was assessed by counting [^{3}H]-thymidine uptake (^{3}H-TdR).
Peripheral blood mononuclear cells, isolated from heparinised blood samples using standard density gradient centrifugation, were resuspended in RPMI1640 culture medium supplemented with foetal calf serum, L-glutamine, penicillin/streptomycin, and (cultured) incubated at 37°C in 5% CO_2, in the presence and absence of scalar concentrations (from 1 to 0.01mM) of chromium, nickel, titanium, cobalt and molybdenum.
Phytohaemagglutinin, a polyclonal mitogen which activates lymphocytes proliferation, was used as positive control.
Cells were pulsed for the last 12 hours of culture with 1 µCi of ^{3}H-TdR.
Lymphocyte proliferation, measured in CPM (counts per minutes), was assessed by scintillation counting of incorporated radioactivity; the results were expressed as stimulation index (SI).
Cytokine production in PBMC supernatants was analysed using Luminex LabMAP assay, which measures the concentrations of multiple analytes in the same sample.
Results and Conclusions: Results of metal sensitivity testing show that:
chromium and nickel at concentrations of 0.1 mM significantly enhanced proliferation of PBMCs
isolated from samples of patients submitted to joint replacements, compared with controls.
Patients with allergic reactions showed an increased proliferative response to high concentration of nickel.
No proliferative response was found in normal control subjects.
None of the patients analysed to date showed reactivity to titanium.
The analysis of lymphocyte culture supernatants showed the constant production of chemotactic cytokines, such as IL-8 and MIP1 α and β.
Chromium and nickel significantly modulated production of cytokines, such as IL-8, MIP1 α and β MCP-1, RANTES and PDGF-BB, in patients with joint implants compared with control group.
Some patients showed the presence of cytokines with regulatory activity on cell differentiation and growth, such as IL-2, and the presence of pro-inflammatory macrophage-derived cytokines, such as IL-1.
These preliminary results suggest that there is different involvement of specific cytokines and chemokines responsible for inflammatory reactions, related to different individual responses.
References: *Catelas I and Jacobs JJ. Biologic activity of wear particles. Instr Course Lect 2010;59:3-16. Hallab NJ et al. Th1 type lymphocyte reactivity to metals in patients with total hip arthroplasty. J Orthop Surg Res 2008;3:6. Thomas P et al. Hypersensitivity to titanium osteosynthesis with impaired fracture healing, eczema, and T-cell hyperresponsiveness in vitro: case report and review of the literature. Contact Dermatitis 2006;55:199-202.*

P6 - OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS: EFFECT OF STRONTIUM ION

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Introduction: Adipose tissue-derived mesenchymal stem cells (AMSCs) can differentiate into osteoblasts with analogous characteristics to bone marrow derived-mesenchymal stems, producing alkaline phophatase (ALP) and calcified nodules composed mainly of hydroxyapatite (HA), collagen and osteocalcin. These characteristics, together with the greater quantity obtainable and the lower invasiveness of the tissue sampling procedure compared with bone marrow sampling, make adipose tissue an excellent source of mesenchymal stem cells for use in bone regeneration processes. Of the drugs used to treat osteoporosis, strontium ranelate (Sr^{2+}) is one of the most versatile as it has a dual action on bone metabolism, contemporaneously reducing resorption and increasing bone tissue formation. The aim of this study was to evaluate the effects of different concentrations of Sr^{2+} on osteogenic differentiation of AMSCs.
Materials and methods: Long-term cultures of human AMSCs were prepared from adipose tissue of normal subjects by means of enzymatic digestion in collagenase type 1, followed by mechanical dispersion. The cells were cultured in Ham's F12 Coon's modification medium supplemented with 10% foetal calf serum (FCS), 1 ng/ml basic fibroblast growth factor (bFGF), and 1% antibiotics. Cells were differentiated to osteogenic phenotype through Ham's F12 Coon's modification medium supplemented with 10% FCS, 10 nM dexamethasone, 10 mM β-glycerophosphate, 50 µg/ml 2-phospho-L-ascorbic acid trisodium salt, 1% antibiotics and different $SrCl_2 \bullet 6H_2O$ concentrations, from 0.5 to 100 µg/ml, for times ranging from 4 to 28 days. Differentiation was evaluated by quantitative fluorimetric assay for ALP and HA.
Results: Primary cultures of AMSCs were prepared from five subjects. Significant increases in HA production were observed at the lower concentrations of $SrCl_2 \bullet 6H_2O$ (0.5-5 µg/ml), 7 and 14 days after induction. Production of ALP was significantly increased at the higher concentrations of $SrCl_2$ (50 and 100 µg/ml) at times ≥ 7 days after induction for two lines and at times ≥ 14 days from induction for the other three lines.
Conclusions: Our preliminary data confirm that $SrCl_2 \bullet 6H_2O$ at high concentrations (50-100 µg/ml) promotes the production of early markers of differentiation, such as ALP, and at lower concentrations (0.5-5 µg/ml) the formation of bone matrix. These findings show that the Sr^{2+} ion may play a role in the increase of osteogenic differentiation of AMSCs, promoting the formation of bone tissue from these cells and offering excellent prospects for future applications in the field of cell therapies.
References: Tognarini I et al. In vitro differentiation of human mesenchymal stem cells on Ti6Al4V surfaces. Biomaterials 2008;29:809-24. Reginster J-Y et al. Strontium ranelate in the prevention of osteoporotic fractures. Int J Clin Pract 2007; 61:324-8.

P7 - EFFECTS OF STRONTIUM ON IN VITRO PROLIFERATION OF HUMAN ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS

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Introduction: Strontium ranelate (SR) is a drug used in osteoporosis to reduce the incidence of bone fractures. It is composed of a ranelate anion, as a carrier, and two cations of strontium (Sr^{2+}), an alkaline earth metal with a high affinity for hydroxyapatite (HA) and the active component with regard to the drug's skeletal effects. Sr^{2+} acts on bone metabolism by contemporaneously stimulating bone formation and reducing bone resorption. We have previously shown that adipose tissue-derived mesenchymal stem cells (AMSCs) have the same ability to produce bone matrix as bone marrow-derived mesenchymal stem cells. However, compared to these, AMSCs are a better source of stem cells, on account of their abundance and accessibility. The aim of this study was to evaluate the effect of different concentrations of Sr^{2+} on the proliferation of AMSCs in primary culture.
Materials and methods: Long-term cultures of AMSCs were prepared from adipose tissue of normal subjects through enzymatic digestion in collagenase type 1, followed by mechanical dispersion. The cells were cultured in Ham's F12 Coon's modification medium supplemented with 10% foetal calf serum (FCS), 1 ng/ml basic fibroblast growth factor (bFGF), and 1% antibiotics. Cell proliferation was evaluated in the presence of different concentrations of $SrCl_2 \bullet 6H_2O$ (1, 10 and 25 µg/ml) in culture medium containing 1% FCS by means of cell count using phase contrast microscopy directly in the cell growth plate after 0, 3, 6, 9 and 12 days. Statistical analysis of the results was performed through linearity test and parallelism test of growth curve linear regressions, using Student's t test.
Results: Primary cultures of AMSCs were prepared from five subjects. Growth curves performed in all the five cell lines showed a significant increase in proliferation for the 25 µg/ml $SrCl_2 \bullet 6H_2O$ concentration compared with the untreated control. In one cell line, the increase was also significant for the 10 µg/ml concentration. These concentrations correspond to those observed in the serum of patients treated with 2g/day of SR. Reductions in cell doubling time (between 33% to 67% of the control) were found.
Conclusions: Our preliminary results confirm that $SrCl_2 \bullet 6H_2O$, at concentrations of between 10 and 25 µg/ml, promotes the growth of AMSCs and could thus play an important role in AMSC-based treatment of bone diseases, possibly – in accordance with the principles of tissue engineering – in combination with biomaterials.
References: Reginster J-Y et al. Strontium ranelate in the prevention of osteoporotic fractures. Int J Clin Pract 2007; 61: 324-8 . Tognarini I et al. In vitro differentiation of human mesenchymal stem cells on Ti6Al4V surfaces. Biomaterials 2008; 29: 809-24.

P8 - MEASUREMENT OF FIBROBLAST GROWTH FACTOR-23 (FGF23) IN THE SERUM OF PATIENTS AFFECTED BY JUVENILE SYSTEMIC LUPUS ERYTHEMATOSUS: A POSSIBLE MARKER OF KIDNEY DAMAGE

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The phospatonins are a new group of hormones involved in the regulation of phosphate, vitamin D and bone mineralisation. The most well-known phospatonin is fibroblast growth factor-23 (FGF23), a molecule whose action on target tissues is mediated by membrane receptors (FGFRs) together with the cofactor Klotho. There is known to be an association between FGF23 levels and chronic renal insufficiency (CRI) and, in particular, FGF23 is a factor in mortality due to cardiac complications in these subjects. Furthermore, FGF23 is involved in the pathogenesis of vascular calcifications. Scientific observations have shown that vascular and renal alterations are a negative prognostic factor in subjects affected by juvenile systemic lupus erythematosus (SLE). In this study, we measured FGF23 in a group of 53 patients (46 females, 7 males, mean age 13.3 ± 5.6) with juvenile SLE diagnosed before the age of 18 years. Twelve of the 53 patients had kidney damage. All the patients were treated with corticosteroids, hydroxychloroquine, azathioprine, cyclophosphamide, and Cellcept (MMF). Two were refractory to treatment with rituximab. At the time of recruitment 28/53 were receiving hydroxychloroquine and the others low doses of prednisone and MMF or azathioprine. One patient was on dialysis for CRI. All the patients with glomerulonephritis underwent a renal biopsy within the first 6 months: 4WHO IIA, 6 IIB, 10 III, 5 IV. We recruited 35 control patients.

We evaluated the lipid profile in all the patients (total cholesterol, LDL, HDL and triglycerides), assessed kidney function (serum creatinine, creatinine clearance, proteinuria, microalbuminuria), performed renal biospies (only on those with kidney damage) and evaluated bone mass.

FGF23 was measured using the ELISA method (Immunotopics Inc. San Clemente, CA, USA).

Serum levels of FGF23 were found to be significantly raised in the SLE patients compared with the controls (Student's t-test: 67.1±40SD vs 5±3.2SD pg/ml). Statistical analysis using the Mann-Whitney U Test showed that the patients with kidney damage had higher FGF23 levels compared with the patients without kidney damage (45.3±20 vs 13.77±9.2 SD pg/ml; p=0. 0001); finally, ANCOVA showed that the patients with severe kidney damage (WHO III-IV) had higher values compared with the patients in the WHO IIA-IIB stages (52.5±21 and 58.5±15 pg/ml respectively vs 13.7±9 and 35±10 pg/ml, p=0.004). No significant correlation emerged between FGF23 levels and lipid profile and cardiac function. Nevertheless, we did observe a trend towards a correlation between FGF23 and HDL (Pearson's correlation test r-0. 07; p=n.s.)

In conclusion, serum FGF23 was raised in patients with juvenile SLE and appeared to be correlated with kidney damage. Although further studies are needed, it appears that FGF23 could be an important marker for early identification of renal insufficiency.

P9 - A NOVEL MUTATION OF THE *PHEX* GENE IN A FAMILY WITH HYPOPHOSPHATAEMIC RICKETS

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X-linked hypophosphataemic rickets/osteomalacia (XLH) is a inherited disorder of phosphate (Pi) homeostasis characterised by renal phosphate wasting and hypophosphataemia, with inappropriately normal to low 1,25-dihydroxy vitamin D3 serum levels, normal serum concentration of calcium and bone deformity and rickets/osteomalacia. Mutations in the *PHEX* gene (Xp22.2-p22.1) are responsible for this disease. *PHEX* encodes an endopeptidase, which is a member of the M13Zn-metalloproteinase family, involved in the regulation of phosphate homeostasis. PHEX-inactivating mutations cause XLH. These mutations allow the accumulation of phosphaturic factors and/or mineralisation inhibitors.
In the present study we describe a 2-year-old boy referred to our centre exhibiting clinical features of a clear hypophosphataemia [2.3 mg/dl (n.v.: 2.7-4.5)], normocalcaemia [9.3 mg/dl (n.v.:8.6-10.3)], normal PTH circulating levels [44.8 pg/ml (n.v.:12-72)], normal vitamin D values [32 mg/ml (n.v.: 30-60)] and high levels of alkaline phosphatase [1055 mU/ml (n.v: 247-645)]. He showed asthenia, muscle pain, bowed legs and cranial deformities. Due to the family history, XLH was suspected. His mother had been diagnosed with hypophosophataemic rickets and was of short stature and developed genu varum. A cousin and the grandfather of the mother were affected by hypophosphataemic rickets with skeletal deformities mainly of the legs.
Our patient's parents were not consanguineous. The patient and his parents underwent *PHEX* mutational analysis after signing an informed consent form (in case of the patient, who is a minor, this was signed by his legal guardian). Genomic DNA was extracted from peripheral blood leukocytes. The 22 exons and the intron-exon boundaries of *PHEX* were investigated by a PCR and direct-sequencing (ABI-Prism 3100) protocol. A novel mutation of *PHEX* was identified in exon 1 at codon 2, GAA>TAA, causing a nucleotide change (Glu to STOP). This nucleotide substitution has never previously been described in the *PHEX* database (http://www.phexdb.mcgill.ca/). Finally, we are planning to use cellular models, both obtained from patients and engineered by transfection methods, in order to evaluate the functionality of the mutated gene. These approaches will be helpful to further understanding of the molecular mechanism of PHEX action and could provide a focus for future targeted therapies.

P10 - A CASE OF SUSPECTED OSTEOGENESIS IMPERFECTA DIAGNOSED IN A DELICATESSEN SHOP: WHEN THE PHYSICIAN'S EYE IS VIGILANT EVEN OUTSIDE THE OUTPATIENT CARE UNIT

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Osteogenesis imperfecta (OI) is a rare group of inherited bone diseases characterised by a connective tissue defect leading to skeletal fragility. From an early age, individuals affected by OI may present fractures after minor traumas or even fractures with no apparent cause. OI is often misdiagnosed. The clinical presentation can be variable, ranging from mild with normal stature, absent or moderate skeletal deformities and normal life expectancy (OI type I) to lethal/severe forms manifesting in the perinatal period/childhood (OI types II and III). The most striking clinical manifestations of OI type I are fractures, blue sclerae, dentinogenesis imperfecta with dental abnormalities, skeletal deformities and low bone mass density (BMD). In particular, blue sclerae are found in about 50% of all cases of OI. During a shopping trip to a delicatessen, a physician noted certain aspects of the facies and physique of the pork butcher, a 50-year-old male, namely the presence of blue sclerae, a triangular face and a posture tending to kyphosis, as seen in skeletal diseases such as OI. Prompted by clinical curiosity, questions were asked aimed at uncovering a medical history of previous fractures in prepubertal/adolescent age in the proband and his first-degree relatives; questions were also asked about his/their lifestyles and eating habits. The proband's personal and family history (father and brother) were both found to be positive for the presence of multiple fractures, even after minimal trauma, since prepubertal age, particularly involving the clavicles. These data further supported the suspicion that this may be a case of abnormal collagen-based familial osteoporosis. However, since the subject was a male, it was necessary to conduct a differential diagnosis of secondary forms of osteoporosis, which account for approximately 50% of cases of male osteoporosis. Therefore, the following clinical tests were required: bone turnover, 25OHD, hormonal tests, liver-kidney function, screening for celiac disease, lumbar and femoral DXA scans and morphometric examination of the spine. Biochemical investigations showed only the presence of hypovitaminosis D, while the DXA scans showed a condition of frank osteoporosis with high risk of fragility fractures, both at cortical and trabecular sites. Since type I OI was still strongly suspected, we decided to perform mutational analysis of collagen type I genes, *COLIA1* and *COLIA2*. This investigation is still ongoing. While awaiting the outcome of the molecular test, neridronate medication with 25 mg i.m. vials, 1 vial per month, and supplementation with cholecalciferol, 600,000 IU/year were suggested. The daily intake of calcium from food reached and still reaches 1000 mg/day.
The case here described suggests that careful observation of an individual's clinical characteristics, even outside the clinic setting, may help to identify patients at high risk of skeletal fragility fractures, as for example in those with rare conditions such as OI. Even though a physician's "curiosity", in particular when he/she is outside the outpatient care setting, could be regarded as an invasion of another's privacy, the presence of ever-present clinical "curiosity" can have positive practical advantages for a patient who, as in this case, may often be unaware of his/her own health status, particularly in the presence of "insidious" clinical forms, and particularly given that there now exist appropriate therapies able to reduce significantly the relative risk of fragility fractures.

P11 - PHARMACOGENETICS STUDY OF INDIVIDUAL PREDISPOSITION TO OSTEONECROSIS OF THE JAW IN TUMOUR PATIENTS TREATED WITH AMINOBISPHOSPHONATES

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Introduction: Aminobisphosphonates are antiresorptive drugs used for the treatment of osteoarticular disorders characterised by excessive osteoclast-mediated bone resorption. Osteonecrosis of the jaw (ONJ) is a well documented adverse reaction of long-term treatment with nitrogen-containing bisphosphonates (N-BPs). In tumour patients, affected by multiple myeloma, breast cancer or prostate cancer and treated long-term (at least 12 months) with intravenous N-BPS for bone metastases, the risk of ONJ development is 1-10 cases every 100 treated patients. To date there is no scientific evidence establishing a cause-effect correlation between ONJ development and N-BP treatment. It can be hypothesised that the reduction of bone turnover caused by N-BPs reduces the replacement of old bone with new bone and induces apoptosis and necrosis of bone cells, especially in bones with a high turnover like those of the oral cavity.
Aim of the study: The aim of our study was to analyse whether a higher interindividual response to N-BPs might be, in part, responsible for ONJ development in tumour patients treated with intravenous zoledronic acid for bone metastases from multiple myeloma, breast cancer or prostate cancer. We recruited 68 cancer patients treated with zoledronic acid and analysed the association between the *A/C rs2297480* polymorphism in the intron 1 of the farnesyl pyrophosphate synthase (*FDPS)* gene, the molecular target of the N-BPs, and the development of ONJ.
Materials and methods: We previously selected two groups, each of 34 patients, matched for sex, age, type of primary tumour and therapy duration. Both groups comprised patients treated with intravenous zoledronic acid for at least 12-18 months: one group of patients who had developed ONJ (ONJ group) and one group of patients who had not developed ONJ (control group). Genomic DNA was extracted from peripheral blood leukocytes, intron 1 of the *FDPS* gene was amplified by PCR and then digested overnight with *FauI* (*SmuI*) restriction enzyme that cuts in the presence of the C allele. Patients were characterised as homozygote AA, heterozygote AC or homozygote CC and the Fisher test was used to analyse the statistical association of the frequencies of the three different genotypes with the presence/absence of ONJ development.
Results: The AA and CC genotypes were found to be differentially distributed among the ONJ patients in comparison with the control patients, a statistically positive correlation emerging between the AA genotype and ONJ development after 12-18 months of treatment with N-BPs (p=0.033). *In silico* studies suggested that the A allele creates a binding site for the Runx1 factor, a transcription factor expressed in murine preosteoclasts and osteoclasts. The link of Runx1, on the binding site created by the A allele, seems to reduce the osteoclast activity through the inhibition of *FDPS* gene transcription. The reduction of osteoclast activity together with N-BP action could further reduce the bone turnover and increase the ONJ risk in patients bearing the A allele, particularly in the AA homozygote subjects.

FDPS Genotypes	ONJ cases n. (%)	Controls n. (%)	p
AA	21 (61.8)	12 (35.3)	0.033 ←
Others	13 (38.2)	22 (64.7)	
CC	2 (5.9)	8 (23.5)	0.045 ←
Others	32 (94.1)	22 (76.5)	

Conclusions: Recent studies have associated genetic variants of the *FDPS* gene with BMD variability and with a different response to N-BP treatment in osteoporosis patients. Therefore, we hypothesise that interindividual genetic differences in the *FDPS* genes could be responsible for a different individual sensitivity to N-BPs and could be good markers for the early identification of individuals at higher risk of developing ONJ. This study certainly needs to be extended to a higher number of individuals.

P12 - PTHC1: A CONTINUING CELL LINE EXPRESSING PTH AND GENES INVOLVED IN CALCIUM HOMEOSTASIS

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The main organs regulating serum levels of ionised calcium (Ca2+) are the parathyroids, which are composed of two different cell types: chief cells and oxyphil cells. Chief cells, through the calcium sensing receptor (CaSR), are affected by changes in calcium concentration, modifying PTH secretion in proportion to calcium levels. Current understanding of calcium regulation mechanisms connected to PTH and of the signalling pathways involved derive from *in vitro* studies carried out on primary cultures of scattered parathyroid cells, because there do not exist parathyroid cell lines able to excrete calcium-regulated PTH. Indeed, it is very difficult to obtain continuous parathyroid cell lines that conserve their functional characteristics because these cells, once cultured, quickly lose their response to calcium. PT-r cells, obtained in 1995 by subsequent clonings, constitute, to date, the only continuous parathyroid cell line described in the literature. This study describes a new cell clone able to secrete PTH, called PTHc1, obtained from hyperplastic tissue of the parathyroid rat.

Materials and methods: **Cell cultures, subcloning and karyotype analysis**: The PTHc1 epithelial cell line was cloned by dilution from primary cultures. Ham's F-12 medium, modified according to Coon and supplemented with calcium 1.1 mM, 10% foetal bovine serum, 100 IU/ml penicillin and 100 mg/ml streptomycin, was used as culture medium. For the karyotype analysis, cells were treated for 4 hours with Colcemid 10-6 M. After the hypotonic treatment for 30 minutes at 37ºC with a 0.75% sodium citrate solution cells were fixed in methanol:acetic acid (3:1). More than 300 cells were analysed in metaphase. **Analysis of cell growth**: PTHc1 cells were plated at 20 cells/cm2 density in culture medium. Growth was estimated in a Burker chamber every 24 h for 7 days. **PTH expression and analysis of genes involved in calcium homeostasis**: RT-PCR reactions of genes *PTH*, *PTHR*, *PHLP*, *Klotho*, *FGF23*, *FGF23-R1*, *FGF23-R2*, *FGF23- R3*, *FGF23-R4*, *ER* , *ER* , *GH-R*, *CDH-1*, *CDH-7*, *HRPT2*, *LRP-5*, *GCMB-1*, *GCMB-2*, *VDR*, *CaSR*, *MEN-I*, *1ALFA-IDROSSILASI* and *END-1* were conducted three-fold. **Immunocytochemistry**: PTHc1 cells were incubated for 30 min at 37ºC in culture medium with different concentrations of calcium, fixed in 4% PFA/DPBS for 20 min and permeabilised with 0.5% Triton X-100/DPBS for 10 min. Samples were stained with a primary anti-PTH antibody for 40 min followed by a secondary anti-rabbit antibody with FITC. Actin was stained with falloidine-TRITC. Nuclei were counterstained with TOTO-3 iodide for 30 min, pursuant to digestion with RNAse. Samples were assembled with a medium of polyvinyl alcohol. Images were acquired in confocal microscopy.

Results: Cell clones, obtained cultivating PTHc1 cells in 96-well plates, maintained and presented, even after 12 months of culture, a polygonal shape. A growth curve of PTHc1 clone underlines for these cells a doubling time of 25 h during the exponential phase. A quantitative analysis with RT-PCR and the following sequencing highlighted the expression of PTH, PTHR, PTHLP, ER-alpha, ER-beta, GH-R, HRPT2, LRP-5, VDR, CaSR, MEN-1 and 1-alpha hydroxylase. A confocal microscopy analysis of PTH and of actin in PTHc1 cells highlighted the presence of well-formed actin filaments at 1.2mM and 3mM calcium concentration, while the 0.5mM concentration was characterised by a low signal for actin, particularly at the apical cell pole. PTHc1 cells independently of calcium concentration presented a positive PTH staining response, albeit of varying intensity. The signal was, in fact, more intense at calcium concentrations of 1.2 mM and 3 mM compared with 0.5 mM, suggesting greater hormone secretion at lower calcium concentrations.

Conclusions: This study describes the arrangement and the characterisation of a new cell line obtained from rat hyperplastic parathyroid tissue, able to secret PTH. An extensive characterisation of proliferative and differentiative properties of PTHc1 clone is under way. We maintain that this cell model may be a useful system for understanding both the physiology and molecular basis of parathyroid gland disorders.

P13 - LOW BODY MASS INDEX CORRELATES WITH OSTEOPENIC AND/OR OSTEOPOROTIC STATUS IN POSTMENOPAUSAL WOMEN: PRELIMINARY RESULTS FROM THE PROF STUDY

P. Piscitelli[1], V. Rigliano[2], C. Neglia[1], G. Chitano[1], A. Argentiero[1], D. Paladini[1], S. Mundi[1], L. Paladini[1], M. Greco[1], C. Girasoli[1], M.E. Gianicolo[1], V. Pantile[1], D. Argentiero[1], G. De Padova[1], L. Nibio[1], L. Pansa[1], P. Di Giuseppe[2], A. Minosi[2], L. Cirasino[2], G. Laselva[2], M. Scialpi[2], M. Benvenuto[1,3], D. D'Angela[1], M.L. Brandi[4], A. Distante[1,2,5]

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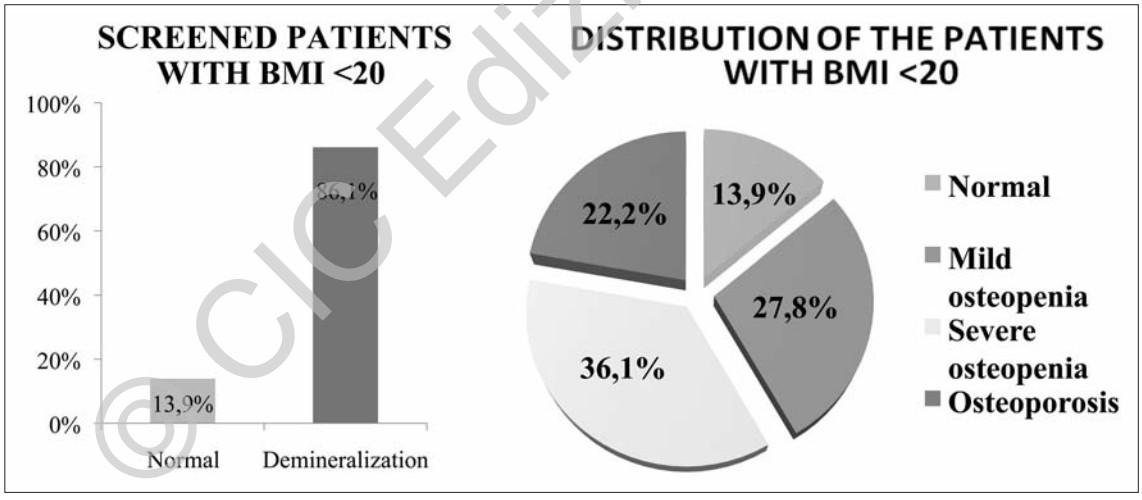
Aims: Osteoporosis affects about 4.7 million people in Italy and leads to over 300,000 bone fractures per year. In view of this, and in order to implement preventive strategies to reduce the burden of fractures in Southern Apulia, the PROF (Prevention of Osteoporotic Fractures) project was launched, based on the synergistic efforts of academic/scientific and healthcare institutions.
Within PROF, a computerised registry was set up, gathering demographic and anamnestic data on patients, such as body mass index (BMI), information about dietary habits, menopause, physical activity, previous fractures, familial fragility fractures, and other clinical/instrumental parameters deemed able to identify, early on, patients at higher risk of fractures.
The aim of the present analysis was to investigate the correlation between low BMI (<20) and osteopenic/osteoporotic status in postmenopausal women tested by bone quantitative ultrasound (QUS) examination.
Methods: 5665 postmenopausal women (mean age 55, range from 39 to 84) were screened non-invasively by QUS examination at the heel and/or phalanx. Three mineralisation categories were identified: a) Demineralisation, when any T-score <-1.0 SD was observed; b) Severe demineralisation, whenever a T-score <-2.0 was observed, corresponding to a higher risk of fracture; c) Osteoporosis, in the presence of a QUS T-score <-2.5±0.2 (for the heel) or a T-score <- 3.2±0.2 (for the phalanx).
Results: Demineralisation of various degrees was observed in 4487 cases (79%), with 1178 (21%) of all the examined subjects found to have normal parameters. Of the 4487 cases with demineralisation, 144 were postmenopausal women with a BMI <20. On QUS testing, only 20 of these (14%) showed a status around normal, while 84 had either an osteopenic or severe demineralisation status (58%) and 32 women were found to be frankly osteoporotic.
Conclusions: These data confirm that a low BMI (<20) is a clear indicator of demineralisation status in postmenopausal women, and almost doubles the risk of a frank and dangerous osteoporotic status. As a matter of fact, only 10% of postmenopausal women with low BMI had normal QUS examinations, at either heel or phalanx level. Therefore, in clinical terms, a low BMI in menopausal women signals the need to undertake pro-active measures and clinical monitoring in order to implement strategies to increase bone mineral density.



P14 - UNDERTREATMENT OF HIP FRACTURES IN TUSCAN ELDERLY POPULATION

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Background: Osteoporosis affects about 4.7 million people in Italy, leading to over 70,000 hip fractures every year. However, only a minority of patients undertake any treatment and a large proportion stop therapy within 2-3 months. In this study we analysed the treatment of hip fractures in the population of Tuscany, using institutional databases.
Methods: We analysed hospital discharge and drug prescription records for the period 2005 to 2008 in order to determine the incidence of hip fractures in people aged over 65 years (both males and females) and the surgical procedures performed and medical prescriptions given to these patients within one year of the fracture. Data concerning hip fractured patients were compared to those available for all people assuming anti-fracture agents. The costs sustained by the Regional Healthcare System were estimated on the basis of data for the following DRGs: 235, 236, 209, 210, 211.
Results: Between 2005 and 2008, almost 7,000 people aged ≥65 years sustained a hip fracture each year, leading to about 6,000 surgical procedures each year. The annual hospital costs amounted to 34 million euros, with annual rehabilitation expenditure estimated at 39.5 million euros. The number of hip-fractured patients treated with a drug effective in reducing the risk of fracture declined from 13.1% to 12.0%. Persistence with treatment at 1 year was < 40%. The average medication possession rate (MPR) was found to be 27%: 77.9% of hip-fractured patients had MPRs <50% vs 55% of the general population under treatment. Only 2.0% of hip fractured patients had an MPR >90% (which is required to maximise fracture risk reduction) vs 18.6% of all people assuming anti-fracture agents. Hip-fractured patients with MPR <50% accounted for 44.3% of the daily defined dose (DDD) vs 24.1% of the overall treated population. It is to be noted that ¼ of the costs sustained to treat the general population (approx. 55 million euros/year for the whole of Italy) are wasted on the provision of very short treatment courses that are unlikely to reduce fracture risk. Therefore, the aims should be both to increase the number of hip-fractured patients under treatment and to increase adherence to therapies. There is a need for strategies aimed at increasing the number of hip-fractured patients who start treatment and remain compliant with their therapy, in order to reduce hip re-fractures and non hip fractures. Regional databases would help in the early identification of fractured patients not under treatment or showing low adherence to therapies.
Conclusions: Strategies to reduce hip re-fractures are both to increase patients on treatment and to foster better adherence to treatment. This analysis also set baseline values to measure intervention outcomes. Almost 25% of the costs sustained to treat the general population (approximately 63 million euros/year in Italy) is wasted on too-short treatment courses that are unlikely to reduce fracture risk. A good approach would be to stop short courses earlier in favour of courses lasting one year. The analysis of regional databases would help in the early identification of fractured patients showing low adherence to therapies.

P15 - REDUCED PHYSICAL ACTIVITY CORRELATES WITH OSTEOPENIC OR OSTEOPOROTIC STATUS IN POSTMENOPAUSAL WOMEN: PRELIMINARY RESULTS FROM THE PROF PROJECT

P. Piscitelli[1], G. Coli[2], A. Argentiero[1], C. Neglia[1], G. Chitano[1], D. Paladini[1], S. Mundi[1], L. Paladini[1], M. Greco[1], C. Girasoli[1], M. E. Gianicolo[1], V. Pantile[1], D. Argentiero[1], G. De Padova[1], L. Pansa[1], L. Nibio[1], P. Di Giuseppe[3], A. Minosi[3], L. Cirasino[3], G. Laselva[3], M. Scialpi[3], M. Benvenuto[1,4], D. D'Angela[1], M. L. Brandi[6], A. Distante[1,6]

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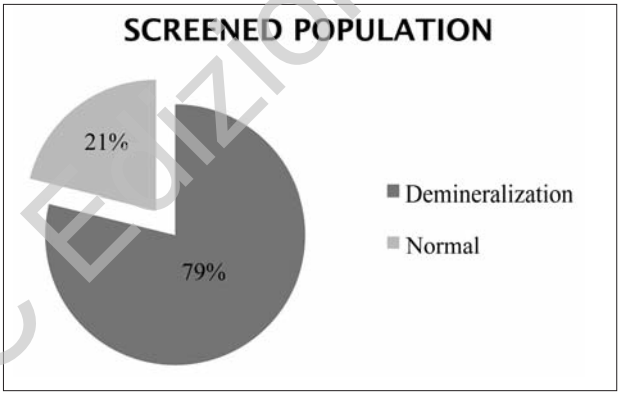
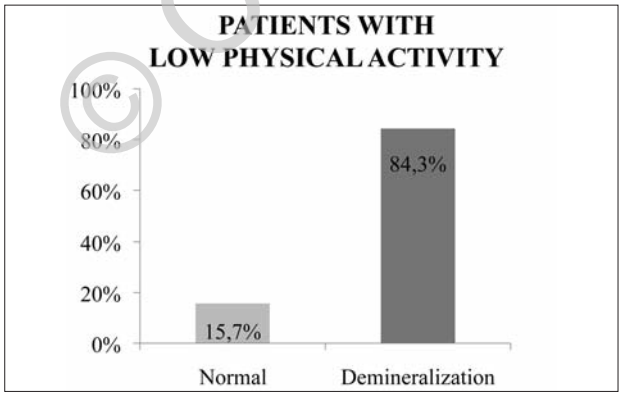
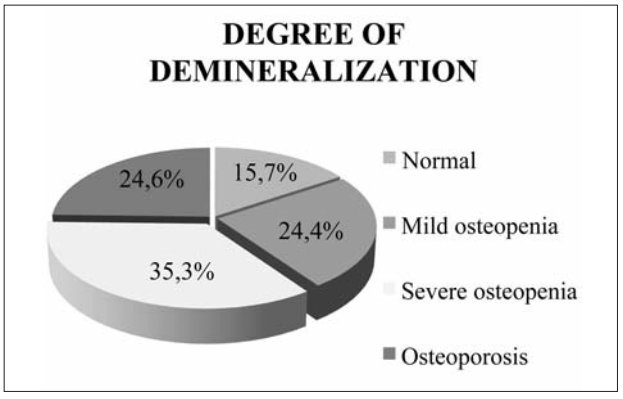
Aims: Italy has a considerable yearly incidence of osteoporotic fractures: about 300,000. In this context, preventive strategies are based mainly on early identification of people at higher risk of fractures and of clinical risk factors. Within the PROF (Prevention of Osteoporotic Fractures) project, i.e. a synergic effort of researchers and clinicians aimed at preventing osteoporotic fractures in Southern Apulia (Salento), a region with an increasing number of elderly people, we investigated the correlation between reduced physical activity and osteopenic or osteoporotic status in postmenopausal women.
Methods: During the years 2009-2010, 5665 postmenopausal women (mean age 62 years, range 39 to 86) underwent quantitative bone ultrasound (QUS) measurement at the heel and phalanx. Demographic and anamnestic data were recorded for all the patients, including BMI, nutrition, menopause, physical activity, previous fractures, familial fragility fractures. Three demineralisation categories were identified *a priori*: a) Demineralisation, when any T-score <-1.0 SD was observed; b) Severe demineralisation, whenever a T-score <-2.0 was observed, corresponding to a higher risk of fracture; c) Osteoporosis, whenever a T-score <-2.5±0.2 (for the heel) or T-score <-3.2±0.2 (for the phalanx) was observed. Descriptive statistical analyses were performed in order to assess the correlation between low physical activity (patients declaring themselves to be completely sedentary) and osteopenic or osteoporotic status.
Results: Of the 5665 women, demineralisation was observed in 4487 patients (79%), corresponding to severe osteopenia or osteoporotic status in 2823 women (50% of all the examined subjects) and frank osteoporosis in 846 patients (15%). In total, of the 1255 women with a clinical history of reduced physical activity, 1058 (84.3%) presented demineralisation corresponding at least to an osteopenic status. In addition, demineralisation typical of severe osteopenia or osteoporosis was diagnosed in 752 "sedentary" patients (60%), 309 of whom (25%) were frankly osteoporotic.
Conclusions: In the PROF dataset, a sedentary lifestyle was found to be associated with an increased occurrence of osteopenic or osteoporotic status in postmenopausal women.







P16 - FAMILIAL FRAGILITY FRACTURES CORRELATE WITH OSTEOPENIC AND/OR OSTEOPOROTIC STATUS IN POSTMENOPAUSAL WOMEN: PRELIMINARY RESULTS FROM THE PROF STUDY

P. Piscitelli[1], G. Coli[2], C. Neglia[1], G. Chitano[1], A. Argentiero[1], D. Paladini[1], S. Mundi[1], L. Paladini[1], M. Greco[1], C. Girasoli[1], M.E. Gianicolo[1], V. Pantile[1], D. Argentiero[1], G. De Padova[1], L. Pansa[1], L. Nibio[1], P. Di Giuseppe[3], A. Minosi[3], L. Cirasino[3], G. Laselva[3], M. Scialpi[3], V. Rigliano[3], M. Benvenuto[1,4], D. D'Angela[1], M.L. Brandi[5], A. Distante[1,4,6]

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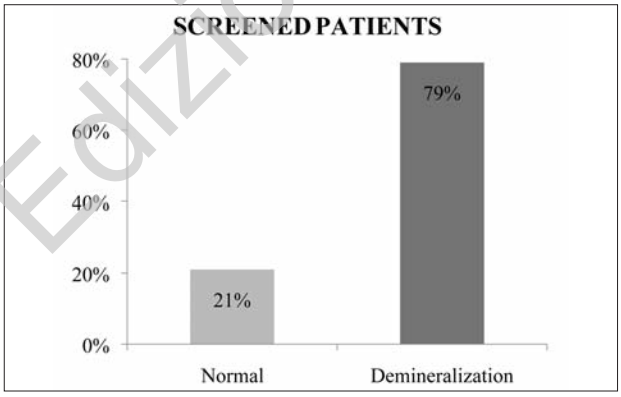
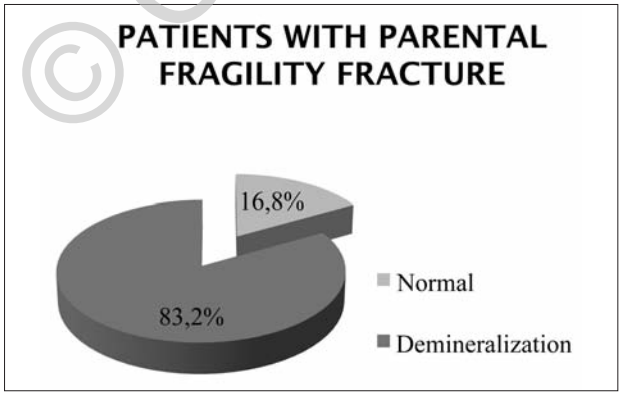
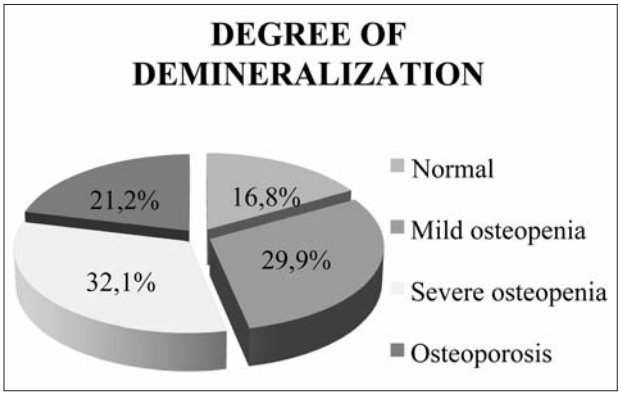
Aims: Within the PROF (Prevention of Osteoporotic Fractures) project, i.e. a synergistic effort of researchers and clinicians aimed at preventing osteoporotic fractures in Southern Apulia (Salento), we analysed the correlation between familial fragility fractures and osteopenic/osteoporotic status in postmenopausal women.
Methods: In the years 2009-2010, we screened 5665 postmenopausal women (mean age 62 years, range 39 to 86) by quantitative ultrasound testing, at the heel or phalanx. Demographic and anamnestic data were recorded for all the patients, including BMI, nutrition, menopause, physical activity, previous fractures, familial fragility fractures. Three demineralisation categories were identified *a priori*: Demineralisation, when any T-score <-1.0 SD was observed; b) Severe demineralisation, whenever a T-score <-2.0 was observed, corresponding to a higher risk of fracture; c) Osteoporosis, whenever a T-score <-2.5±0.2 (for the heel) or T-score <-3.2±0.2 (for the phalanx) was observed. Descriptive statistical analyses were performed in order to assess the correlation between familial fragility fractures (any osteoporotic fracture occurring in patients' relatives) and the osteopenic or osteoporotic status of the patients themselves.
Results: Demineralisation was observed in 4487 patients out of 5665 (79%). Demineralisation corresponding to severe osteopenia or osteoporotic status was confirmed in 2823 women (50% of all the examined subjects); 846 patients (15%) were found to be osteoporotic. A total of 358 women reported a family history of fragility fractures: of these, 298 (83%) presented demineralisation – as defined by T-score <-1 – corresponding at least to an osteopenic status. In the subgroup of women with familial fragility fractures, a status of severe osteopenia or osteoporosis was diagnosed in 191 patients (53%), with 76 of them being frankly osteoporotic (21%).
Conclusions: The PROF study dataset suggests that the presence of familial fragility fractures correlates with osteopenic and/or osteoporotic status in postmenopausal women. This finding may be regarded as a flag alerting both GPs and specialist physicians to the need for an integrated/preventive approach in the relatives of patients with fragility fractures.







P17 - EARLY MENOPAUSE INFLUENCES OSTEOPENIC OR OSTEOPOROTIC STATUS IN POSTMENOPAUSAL WOMEN: PRELIMINARY RESULTS FROM THE PROF PROJECT

P. Piscitelli [1], V. Rigliano [2], C. Neglia [1], G. Chitano [1], A. Argentiero [1], D. Paladini [1], S. Mundi [1], L. Paladini [1], M. Greco [1], C. Girasoli [1], M. E. Gianicolo [1], V. Pantile [1], D. Argentiero [1], G. De Padova [1], L. Nibio [1], L. Pansa [1], P. Di Giuseppe [2], A. Minosi [2], L. Cirasino [2], G. Laselva [2], M. Scialpi [2], D. D'Angela [1], M. Benvenuto [1,3], M. L. Brandi [4], A. Distante [1,2,5]

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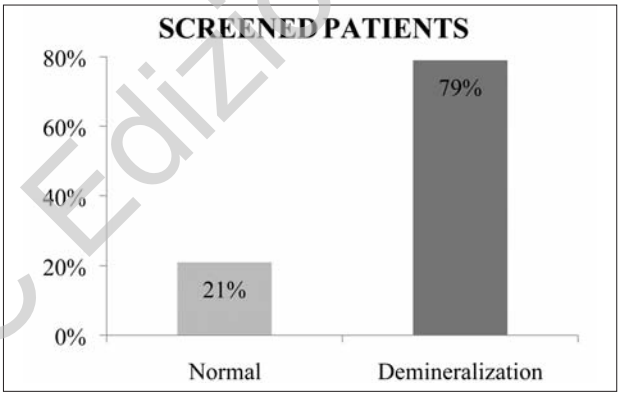
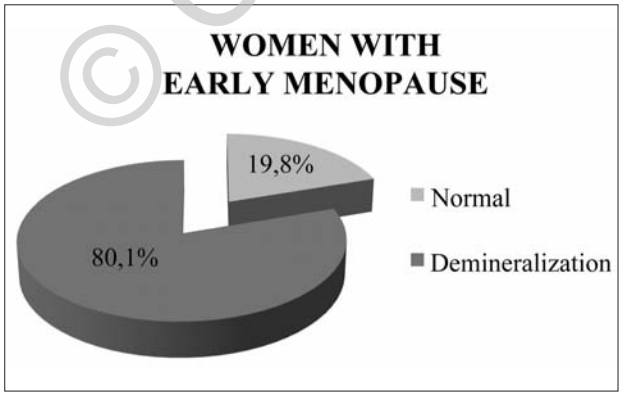
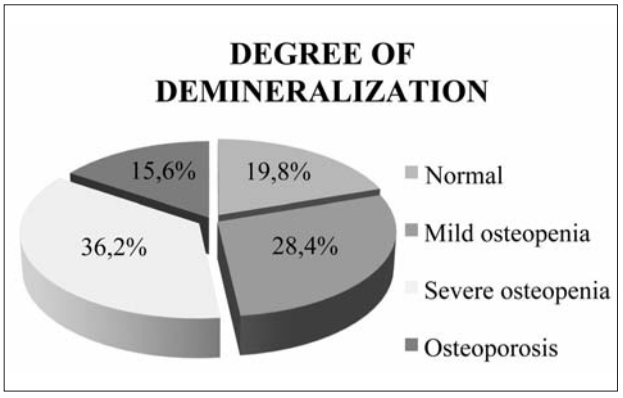
Aims: There is evidence that demographic trends in Southern Apulia are characterised by a huge proportion of elderly people relative to the general population, resulting in an ageing index which is higher than that recorded in other Southern Italian regions and/or sub-regions. Within the PROF (Prevention of Osteoporotic Fractures) project, which aims to foster synergistic efforts between researchers and clinicians, we investigated the correlation between early menopause and osteopenic or osteoporotic status in postmenopausal women by quantitative bone ultrasound evaluation (QUS).
Methods: In a period of almost six years (2004-2010), 5665 postmenopausal women (mean age 55, ranging from 39 to 84) were screened by QUS at either the heel or the phalanx. Demographic and anamnestic data were recorded for all the patients, including BMI, nutrition, menopause, physical activity, previous fractures, familial fragility fractures. Three categories of demineralisation were identified: a) Demineralisation, when any T-score <-1.0 SD was observed; b) Severe demineralisation, whenever a T-score <-2.0 was observed, corresponding to a higher risk of fracture; c) Osteoporosis, whenever a T-score <-2.5±0.2 (for the heel) or T-score <-3.2±0.2 (for the phalanx) was observed. Descriptive statistical analyses were performed in order to assess the correlation between early menopause (<45 years of age) and the osteopenic or osteoporotic status of the patients.
Results: Of the 5665 subjects examined overall, demineralisation was observed in 4487 subjects (79%), with severe osteopenia or osteoporotic status being documented in 2823 women (50%) and frank osteoporotic status in 846 (15%). In total, of 1169 women reporting an early menopause, 937 showed demineralisation corresponding to at least an osteopenic status (80%). In 605 of these patients (65%), there was a severe osteopenic or osteoporotic status, while 182 women experiencing an early menopause were found to be frankly osteoporotic (19%).
Conclusions: The PROF dataset proves that an early menopause is closely associated with osteopenic or osteoporotic status in postmenopausal women, thus suggesting the need to implement preventive and regular follow-up strategies with quantitative bone QUS testing.







P18 - THE INCIDENCE OF HIP, FOREARM, HUMERAL, ANKLE, AND VERTEBRAL FRAGILITY FRACTURES: RESULTS OF A THREE-YEAR MULTICENTRE STUDY

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Introduction: We aimed to assess the incidence and hospitalisation rate of hip fractures and "minor" fragility fractures and their incidence in the Italian population.
Methods: We conducted a three-year survey at 10 major Italian emergency departments in order to evaluate the hospitalisation rate for hip, forearm, humeral, ankle, and vertebral fragility fractures occurring in people aged ≥45 years between 2004 and 2006, both men and women. These data were compared to those recorded in the national hospitalisations database (SDO) in order to assess the overall incidence of hip fractures and minor fragility fractures, also including those events not resulting in hospital admissions.
Results: We have estimated that a total of 430,000 new hip, humeral, wrist, ankle and vertebral fragility fractures occur in Italy each year. Hospitalisation rates, referring to a total of 29,017 fractures, were the following: 92.7% for hip fractures, 36.3% for humeral fractures, 31.3% for ankle fractures, 22.6% for forearm/wrist fractures, and 27.6% for clinical vertebral fractures. According to the analyses performed on the SDO database, we estimated an annual incidence of 100,000 hip (0.40 per 100 adults), 39,000 humeral (0.15 per 100), 47,000 ankle (0.18 per 100), 73,000 wrist (0.21 per 100) and 190,000 (0.76 per 100 adults) vertebral fragility fractures in people aged >45 years. Clinical vertebral fractures were computed as 56,000 events per year (0.22 per 100).
Conclusion: A national registry of fragility fractures is needed in order adequately to assess the incidence of osteoporotic fractures in the Italian population.

P19 - MONITORING OF BONE METABOLISM IN CANDIDATES FOR PROSTHETIC IMPLANTS UNDER CHRONIC BIPHOSPHONATE TREATMENT

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Introduction: A growing number of cancer and osteoporosis patients undergoing biphosphonate treatment develop osteonecrotic lesions of the maxillary bones. For a long time, it was widely held that surgical therapy should be avoided in such patients. While the current guidelines have clarified many surgical issues, the debate on the indications for prosthetic implants remains open.
Aims: The aim of this study was to develop and document a simple, safe and effective surgical and pharmacological protocol for the prosthetic implant treatment of patients with a history of biphosphonate use.
Materials and methods: The authors retrospectively analysed a series of 15 patients under chronic treatment with biphosphonates submitted to oral implant rehabilitation. In 6 cases, clodronate was administered prior to the implant surgery, in 4 cases alendronate, and in 5 cases, risedronate. Only patients receiving significant cumulative doses of monoamine biphosphonates (at least 3 years of treatment) were analysed. Rehabilitation of the patients considered in the study was performed by means of overdentures on implants. The indication for this procedure was the presence of mandibular atrophy and total edentulism. Implants were placed in interforaminal sites in the anterior mandibular region. The patients underwent serum measurement of biochemical markers indicative of bone metabolic activity (cross-linked c telopeptide of type I collagen) in order to document their risk of ONJ. The implant fixtures were loaded 3 months after being inserted.
Results: In the intra-operative phase, we observed significantly increased single fixture screw torque; primary stability was excellent and tissue vascularisation was also normal. No morbidity was observed at 24-months follow up, and radiological follow-up examinations showed optimal osseointegration of the implants and no gaps suggesting premature deterioration. The cumulative survival curve was 100% for all implants at 24 months and mean crestal bone loss at the level of the implant-abutment junction was 0.8 mm (s.d. 1.0).
Conclusions: The chemical structure of biphosphonates together with the right implant system can influence outcome. In these patients, we observed high serum levels of biochemical bone markers, however we do not have sufficient data to correlate this finding with low surgical risk.
References: Marx RE et al. Oral biphosphonate-induced osteonecrosis: risk factors, prediction of risk using serum CTX testing, prevention and treatment. J Oral Maxillofac Surg 2007;65:2397-410. Javed F, Almas K. Osseointegration of dental implants in patients undergoing biphosphonate treatment: a literature review. J Periodontol 2010;81:479-84. Wang HL et al. Effect of long-term oral biphosphonates on implant wound healing: a literature review and a case report. J Periodontol 2007;78:584-94.

P20 - RETROSPECTIVE STUDY OF BIPHOSPHONATE-INDUCED MAXILLARY OSTEONECROSIS (2004-2009)

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Introduction: Biphosphonate-induced osteonecrosis of the jaws (ONJ) was first described in the literature in 2003. Biphosphonates, which are structural analogues of inorganic pyrophosphate, inhibit the formation, aggregation and dissolution of calcium phosphate crystals; they have a high affinity for the mineralised bone matrix and can inhibit osteoclast-mediated osseous resorption. They are divided into non-aminobiphosphonates (etidronate, clodronate, tiludronate) and aminobiphosphonates (alendronate, ibandronate, neridronate, risedronate, pamidronate, zoledronate), which have a more powerful inhibitory effect on osteoclast-mediated osseous resorption. In 2009 the AAOMS estimated the incidence of i.v. biphosphonate-induced ONJ to be 0.8-12%, and that of ONJ induced by oral biphosphonates to be 0.01-0.1%. In recent years, preventive measures have been introduced, to be adopted in all patients about to begin biphosphonate treatment, as well as behavioural measures to be adopted in patients under bisphosphonate therapy requiring dental treatment to prevent ONJ.
Aims: To establish the incidence of the disease over the years, and whether this incidence fell in 2009; to investigate correlations between ONJ and gender, age, underlying disease, type of biphosphonate taken and duration of biphosphonate treatment; to investigate treatments instituted.
Materials and methods: We collected data on all cases of ONJ diagnosed at the Careggi Hospital in Florence up to December 2009: the sample was made up of 59 patients (24 men, 35 women). We performed a statistical analysis of the data in our possession. No patient was excluded from the study.
Results: The incidence of the disease was higher in the women (59.32% versus 40.68%) and this difference was statistically significant. The mean age at diagnosis was 66 years, 8 months, with a difference emerging between the women (67 yrs 6 mths) and the men (65 yrs 3 mths). The drug responsible for the highest number of cases of ONJ was zoledronate (86.44%), followed by alendronate and pamidronate (both 5.08%), and risendronate (3.40%). The most frequent of the underlying diseases requiring the use of biphosphonates was multiple myeloma (32.20%), followed by breast cancer (25.42%) and osteoporosis (10.17%). These were followed, in order of frequency, by prostate cancer, lung cancer, lymphomas and leukaemia. The oldest diagnoses dated back to 2004. The year with the smallest number of diagnosed cases was 2009, in which 4 cases of ONJ were detected (6.78% of the total). Of these, 3 patients had taken alendronate (in 2 cases for osteoporosis) and 1 zometa. The duration of treatment varied, ranging from 3 months to 11 years (mean 30.4 months).
The appearance of the lesions was due to: tooth extractions (77.97%), trauma caused by dental prostheses (15.25%), endodontics (1.69%), implantology (1.69%), no dental intervention (1.69%). In most cases the lesions were mandibular (57.6%), in 27.1% maxillary and in 15.2% of cases present at both levels.
At diagnosis, 56 patients were at stage 2 (95%) and 3 at stage 3 (5%) of the AAOMS staging system. The stage 3 patients were treated with surgical mandibular resection and vascularised graft, while the stage 2 patients received pharmacological therapy, in some cases associated with minor surgery and hyperbaric oxygen therapy (HBOT).
Conclusions: From the data gathered in recent years on patients diagnosed with ONJ at our service, we can conclude that zoledronate caused most of the cases of ONJ. It was thus confirmed as the drug with the greatest potency and affinity, as has been shown by the literature. It can be noted that osteoporosis was found to be the third most frequent underlying disease and that the incidence of ONJ fell in 2009, presumably due to increased understanding of the condition, but that, contrary to the overall mean, in 2009 half of the cases were osteoporosis patients who had taken alendronate.
References: Lo JC et al. Prevalence of osteonecrosis of the jaw in patients with oral bisphosphonate exposure. J Oral Maxillofac Surg. 2010;68:243-53. Gliklich R, Wilson J. Epidemiology of bisphosphonate-related osteonecrosis of the jaws: the utility of a national registry. J Oral Maxillofac Surg 2009; 67(5 Suppl):71-4.

P21 - TREATMENT WITH GLUCOCORTICOIDS AND FRAGILITY FRACTURES

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Glucocorticoids, taken chronically at certain dosages (5mg/day of prednisone >3 months), interfere with bone metabolism (both the quality and quantity), making bone more fragile and increasing the risk of fractures. The osteopenic effect is most rapid during the first 6-12 months of treatment, especially at the level of the trabecular bone. The literature shows that patients chronically treated with glucocorticoids, at a minimum dose of 5 mg/day for more than 1 year, have a high risk of fragility fractures and that at least 30-50% of them sustain at least one osteoporotic fracture (vertebral body, ribs, femur). Glucocorticoids are strong inhibitors of new bone formation, an effect mediated by direct inhibition of the activity and replication of osteoblasts; at the same time, they inhibit osteoclast apoptosis and thus increase bone resorption. The aim of our study was to evaluate the frequency of use of oral glucocorticoids in a sample of subjects with hip fracture, compared with an age-matched sample of fracture-free subjects.

Materials and methods: As part of an epidemiological survey (Indaco 2 study), proposed by SIOT (the Italian Society of Orthopaedics and Traumatology), 7355 patients attending over 100 orthopaedics and traumatology clinics throughout Italy were recruited over a 6-month period. At each centre 30 patients with a femoral fracture and 30 fracture-free patients aged over 65 years were recruited. These patients were administered a questionnaire that investigated their reason for attending the clinic, various aspects of their history (walking capacity by means of the FAC scale), cognitive status, and presence/absence of chronic treatment with oral glucocorticoids.

Results: Of the 7355 questionnaires processed, 954 (12.97%) were excluded, because they referred to patients aged under 65 years or because of missing data (failure to indicate the presence/absence of treatment with glucocorticoids). Therefore, the number of questionnaires actually assessed was 6401 (87% of the recruited subjects), 2875 (44.9%) from fracture patients and 3526 (55%) from fracture-free subjects. Of the 592 (9.2%) patients found to be under chronic treatment with glucocorticoids, 11 were excluded because gender was not indicated. The population whose data we process thus comprised 581 patients: 93 males and 488 females. Of the 93 males (16%), 33 (35.5%) had fractures and 60 (64.5%) did not. Of the 488 females (84%), 185 (38%) had femoral fractures and 303 (62%) were fracture-free.

Discussion and conclusions: In the population we assessed it was found that chronic treatment with prednisone or equivalent drugs at a dose of at least 5 mg/day for more than 3 months does not further increase the risk of proximal femur fracture. Presumably, this finding depends on the very advanced mean age of the subjects examined, which probably created a “ceiling effect” for the FRAX algorithm.

References: Van Staa TP et al. The epidemiology of corticoid-induced osteoporosis: a meta-analysis. Osteoporos Int 2000;13:777-87.

P22 - COGNITIVE IMPAIRMENT IN HIP FRACTURE PATIENTS

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Introduction: Proximal femur fractures are the most frequent traumatic skeletal lesions. Despite improved understanding of the risk factors for and means of preventing these fractures, their frequency continues to increase. In Italy there are estimated to be more than 80000 new proximal femur fracture cases every year. These fractures are closely correlated with osteoporosis and are therefore more frequent in the elderly population. Indeed, other fracture risk factors are age and falls. Cognitive decline is one of the intrinsic risk factors for falling as it influences postural control and lower limb muscle strength.

Hip fracture itself is an event capable of triggering a progressive cognitive decline; the incidence of this ranges from 16% to 62% and it is associated with increased morbidity and mortality. The aim of this study was to describe the association between cognitive decline and proximal femur fractures.

Materials and methods: As part of Indaco 2, an epidemiological survey proposed by SIOT (the Italian Society of Orthopaedics and Traumatology), data were collected relating to 7355 patients attending over 100 orthopaedics and traumatology clinics throughout Italy, recruited over a 6-month period. A questionnaire was administered that, as well as covering various aspects of the patient's history, also included the Short Portable Mental Status Questionnaire (SPMSQ). This instrument is made up of 10 items that assess the patient's cognitive abilities.

Results: From the 7355 questionnaires collected, we excluded those referring to patients under the age of 65 years, this parameter being a criterion for exclusion from the study; we also excluded those with an incomplete SPMSQ. We then excluded, from the remaining 6294 questionnaires, those that failed to provide anamnestic data on the femur fracture. Therefore, the final analysis was performed on 6285 patients, who had a mean age of 77 years (±7.55).

The patients with a femur fracture totalled 2877 and their mean age was 80.3 years (±7.55). The fracture-free patients numbered 3408, and had a mean age of 74.2 years (±6.32). For the final analysis, we dichotomised the SPMSQ variable, thus forming two groups: one comprising patients with normal to mildly impaired cognitive status, and the other patients with moderately to severely impaired cognitive status.

In the group of fracture patients, the SPMSQ showed normal to mildly impaired cognitive status in 67% of the patients, who had a mean age of 78.28 years (±7.2), and moderately to severely impaired cognitive status in 33%, who had a mean age of 84.41 years (±6.49). In the fracture-free population, on the other hand, 90.75% of the patients, with a mean age of 73.64 years (±6.03), showed normal to mildly impaired cognitive status, while only 9.25%, with a mean age of 79.64 years (±6.56), showed moderately to severely impaired cognition. The difference in cognitive status between the two groups (fracture and fracture-free) was statistically significant ($p < 0.0001$), even after adjusting for the age of the patients.

Conclusions: The results of our epidemiological study confirm that cognitive status is more impaired in patients with fractures compared with fracture-free subjects, even after adjusting for age. However, the question of whether cognitive decline was the cause of, or secondary to, the fracture it remains to be established. *References: Cooper C, et al. Hip fractures in the elderly: a world-wide projection. Osteoporos Int 1992;2:285-9. Piscitelli P et al. Hip fractures in Italy: 2000-2005 extension study. Osteoporos Int 2010;21:1323-30. Pfeiffer E. A short portable mental status questionnaire for the assessment of the organic brain deficit in elderly patients. J Am Geriatr Soc 1975;23: 433-41.*

P23 - VITAMIN D AND K DEFICIENCY IN HAEMODIALYSIS PATIENTS WITH A HIGH PREVALENCE OF VERTEBRAL FRACTURES AND VASCULAR CALCIFICATIONS: A PRELIMINARY STUDY

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Introduction: Vitamin D deficiency is common in dialysis patients, whereas vitamin K status is less investigated despite its important implications for bone metabolism (bone Gla protein is vitamin K-dependent) and for vascular calcifications (matrix Gla protein is vitamin K-dependent).
Materials and methods: The aim of the study was to assess the prevalence of vitamin D and K deficiency and the presence of vertebral fractures and vascular calcifications in haemodialysis patients (compared with a healthy control group). Subjects: 68 patients, 49 males and 19 females, mean age 66.62 years (± SD 11.3), undergoing thrice-weekly haemodialysis; mean dialytic age: 68.14±56.14 months.
The presence of vertebral fractures was assessed by means of vertebral morphometry (D5-L4) using a quantitative, computerised method (MorphoXpress).
The presence of vascular calcifications was assessed by means of vertebral spinal X-ray in L-L.
We measured biohumoural bone-vascular mineral metabolism parameters: total BGP and decarboxylated BGP (ucBGP), total MGP and decarboxylated MGP (ucMGP).
The presence of vertebral fractures was taken to correspond to a >20% reduction in the height of the vertebral body; a reduction of between 15 and 20% was considered borderline (B).
*Results***:** In the patients, versus controls, there emerged: deficit of 25(OH)D (98%, 60% carenti-38% insufficienti); vitamin K1 deficiency 32.08%; increased total BGP and ucBGP, increase in total MGP and reduction of ucMGP.
The prevalence of vertebral fractures was 57.35%+B: 27.94%. Vertebral fractures were associated with: anagraphical age (p=0.028), P (p=0.0445) and total BGP (p=0.0420).
The prevalence of vascular calcifications was 84%. Vascular calcifications were associated with: anagraphical age (p=0.0205), Ca (p=0.0192) and ucMGP (0.0453).
*Conclusions***:** Marked vitamin D and K deficiency was associated with a high prevalence of vertebral fractures and vascular calcifications in haemodialysis patients with biohumoural bone mineral metabolism parameters within the KDOQI targets. Vitamin K is an important new biomarker of the bone-vascular axis in patients with chronic renal insufficiency.

P24 - GERIATRIC MEDICINE: AN INNOVATIVE CARE STRATEGY IN ORTHOPAEDICS AND TRAUMATOLOGY

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For many years, the administration of the Careggi University Hospital (CUH), in agreement with the Faculty of Medicine and Surgery of the University of Florence, has pressed for the creation of a department of general medicine within its othopaedic traumatology centre. In its decision n.243 of May 5, 2009, the administration of the CUH, along the lines of similar experiences already in place, set up a **simple departmental unit (SDU) of geriatric medicine (GM)** within the hospital's department of orthopaedics.

The aim of this unit is to guarantee continuity of care to orthopaedics inpatients, through the identification of a specific care pathway for clinically unstable patients. The clinical activity carried out, mainly in the context of the provision of continuity of care, takes the form of daily consultancy. The SDU has a series of objectives, organisational (less postponement of surgery due to medical problems, better integration of healthcare through a multidisciplinary team, provision of internal medicine and geriatric consultancy to guarantee continuity of care), clinical (reduction of peri-operative medical complications and adverse events) and strategic (improvement of the quality of geriatric and internal medicine care, better communication with patients and families). The unit strives to exploit to the full the multi-professional (doctors, rehabilitation therapists, registered nurses, social workers) and interdisciplinary (internal medicine, geriatrics, orthopaedics, physical medicine, anaesthesiology, cardiology, angiology etc.) intervention and, in the *fragile* elderly, applies a *multi-dimensional geriatric assessment* instrument.

Clinical activity: The physicians working in the GM SDU provide daily consultancy, including Saturday mornings. Constant telephone contact is available, also on Sundays and holidays.

In the period from 1/9/2009 to 31/7/2010, a total of 1867 consultancies were provided, spread over 268 days, which corresponds to a mean of 6.97 examinations/day. Of these, 652 (34.92%) were first visits and 1215 (65.08%) were follow ups. The assessments were always conducted in a spirit of multi-professional and multidisciplinary collaboration.

The assessments were carried out in the following departments: general orthopaedics II (25.98%), general orthopaedics I (21.26%), general orthopaedics III (18.26%), traumatology-orthopaedics (13.55%), orthopaedic oncology and reconstruction (11.25%) as well as, in smaller percentages, in all the other SDUs of the orthopaedics department, in the neurosurgery department, the plastic surgery department and the spinal unit.

In particular, internal and geriatric medicine consultancy for patients was requested in connection with high levels of comorbidity, polypharmacy regimens, acute confusional state, dehydration, hydro-electrolytic disorders, uncompensated type 2 diabetes mellitus, pulmonary embolism, chronic liver disease and cirrhosis, pneumonia and bronchitis causing respiratory insufficiency, decompensated congestive heart failure, targeted antibiotic therapy, chronic renal insufficiency, and management of anti-aggregant and anticoagulant therapies.

Positive aspects: the clinical assessments were made using a multidisciplinary approach, based on the fundamental collaboration of specialists in orthopaedics, anaesthesiology-resuscitation, angiology, cardiology, radiology and physical medicine; excellent collaboration with services (radiology, neuroradiology, angiology, cardiology, etc.).

Negative aspects: constant difficulties transferring clinically unstable patients to the hospital's medical specialty SDUs due to lack of beds; lack of intermediate care beds as a sort of "buffer" between the intensive care and inpatient departments; scope for improving the internal medicine skills of the nursing staff.

*Research projects***:** In synergy the hospital's other SDUs, the GM SDU takes part in projects aiming to improve care and clinical management. It currently has collaborations with the geriatrics clinic, regional centre of reference for haemostasis and thrombosis, the bone metabolism clinic, the orthopaedics clinics, the geriatrics agency, the radiology service, the continuity-of-care agency, the clinical management, and the general affairs unit. Furthermore, on the instigation of the regional health council, a working group has recently been set up on the reorganisation of the "Care pathway of elderly patients with proximal femur fracture (orthogeriatrics)".

Prospects for implementation and improvement: The aims of the "Project to reorganise and upgrade the orthopaedics and traumatology centre of the Careggi University Hospital" include: the institution of a medical geriatrics department providing medium and high intensity of care; the presence, 24 hours/day, of a specialist from the medical area in the traumatology *open space*; the involvement of the internal medicine specialist in pre-hospitalisation procedures.

References: Progetto per la riorganizzazione e riqualificazione del Polo Ortopedico e Traumatologico di Careggi. CUH, April 2010.

P25 - GROWING STRONG AND HEALTHY WITH MISTER BONE: AN EDUCATIONAL PROGRAMME TO ENSURE STRONG BONES LATER IN LIFE

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Bone mass increases steadily until the age of 20-30 years and most bone mass is acquired during the first two decades of life. Nutrition plays a critical role in the achievement of one's optimal genetically programmed peak bone mass (PBM), reducing the risk of osteoporosis later in life. PBM is the amount of bony tissue present in the skeleton at the end of skeletal maturation. Even though 90% of PBM is acquired by the end of second decade of life, skeletal mass continues to increase for up to 10-15 years after that, through the process of bone consolidation, with maximal PBM occurring at around 30 years of age. As a 10% increase in PBM corresponds to a gain of one standard deviation in bone mineral density in adulthood, osteoporotic fracture risk may be reduced by up to 50% by interventions aimed at maximising PBM in a sustainable manner in childhood and adolescence. Although genetic factors are the strongest predictors of bone mass, accounting for 50-80% of its variance, nutritional and lifestyle factors can explain an additional 20-30% of bone mass variance.

Bone is living tissue like any other, and its cells have the same kinds of nutrient needs as those of the rest of the body; it does not require only an energy supply, but also protein and micronutrients, calcium and vitamin D *in primis.* In a balanced western-style diet, about 60% of dietary calcium should come from milk and dairy products, 20% from fresh vegetables and dried fruits, and the rest from drinking water or other discrete sources.

Current research indicates that calcium intake in school-age children is below the recommended adequate level. The recommended adequate intake of calcium for children between the ages of 9 and 11 years is about 1100-1200 mg.

In response to this critical health issue it is essential to monitor children's intake of dairy products and nutrients important for bone health, such as calcium and vitamin D, in order to ensure that their nutritional needs are met and that they are receiving the nutritional intakes needed to safeguard their health later in life. The aim of our study was to monitor and promote the intake of dairy products, calcium and vitamin D in children, in order to help them achieve their optimal PBM and to safeguard their bone health later in life. Modifications in schoolchildren's nutritional behaviour were evaluated through a nutritional programme designed to increase calcium intake. The project was conducted with the support of novel instruments specifically created for this educational programme.

Our study sample comprised 180 children (48% males and 52% females) aged 9-11 years from a primary school in Florence. We evaluated the children's eating habits through a questionnaire designed to assess intake of calcium, dairy products, and total caloric energy intake at baseline and at follow up. Data were processed using nutrition software (WinFood 2.7-MediMatica) and analysed using Student's paired T-test to determine pre- versus post-intervention differences.

The results showed that total caloric intakes rose from 1690±290 before the educational intervention to 1700±330 kcal/day after the educational intervention in boys and from 1620±256 to 1640±260 kcal/day in girls. Statistical analysis of the data did not show any significant variation in pre- versus post-educational assessments ($p<0.05$), although the protein percentage increased by two points, from 14.5 to 16.5%, while both carbohydrate and lipid intake decreased by one percentage point. Student's T-test analysis of dietary intakes evaluated, through the questionnaire, before and after the educational intervention revealed a significant increase ($p<0.05$) in calcium intake, which rose from 860±190 to 1060±200 mg/day in the girls and from 890±200 to 1100±210 mg/day in the boys, and in vitamin D intake, which rose from 3.6±1.53 µg/day to 4.1±2 µg/day, without significant differences emerging between the boys and girls. Although sub-optimal, the calcium intake obtained after the educational programme was sufficient to attain the target RDI of 1100-1200 mg/day. During the educational programme the percentage of children who drank milk rose from 92 to 96%. A change in the quantity of milk intake was also detected: the results showed a significant increase from 200±35 to about 270±65 ml/day in boys and girls ($p<0.05$). The observations on hard cheese intake revealed an increase in cheese consumers, from 84% to 91% at the end of the educational period. Similarly, a positive change was recorded in the percentage of children eating fresh vegetables: an increase from 89% to 96%.

Our educational programme appears to be significantly effective in modifying calcium intake in children. Analysis of the questionnaire data, which showed significantly increased consumption of dairy products and vegetables, without significant changes in total caloric intakes, revealed an important change in these children's dietary habits. These behavioural modifications are the result of progressive nutritional education imparted through lessons, brochures, calendars, games, and crosswords. These findings may prompt school policy-makers to introduce educational strategies to promote students' skeletal health. *References: Rizzoli R et al. Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly. Bone 2010;46:294-305. Lombardi-Boccia G. et al. Total-diet study: dietary intakes of macro elements and trace elements in Italy. Br J Nutr 2003;90:1117-21. Livelli di assunzione giornalieri raccomandati di nutrienti per la popolazione italiana (L.A.R.N.). Società Italiana di Nutrizione Umana, revisione 1996.*

P26 - VERTEBRAL PLASTY IN THE TREATMENT OF VERTEBRAL COLLAPSE DUE TO OSTEOPOROSIS

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Vertebral compression fractures are a major cause of disability; around 1.4 million cases are estimated to occur in the world each year.

In the elderly population in particular, vertebral compression fractures cause persistent pain, kyphosis, breathing difficulties, loss of appetite, and reduced quality of life, all factors that, together, induce a negative spiral from which patients struggle to emerge. As recent clinical studies have shown, the end result of this negative spiral is an approximately 25% increase in mortality, with loss of muscle mass.

The traditional treatment for vertebral compression fractures is immobilisation of the patient for several weeks and possibly the use of a brace or orthosis. The results are poor, however; furthermore, this approach, because of the immobilisation, aggravates osteoporosis (when present), and does not correct kyphosis. It therefore fails to address the secondary consequences of the fracture, which are due to altered biomechanics.

At our hospital, S.M. Annunziata in Florence, we have developed a protocol for the treatment of osteoporotic vertebral compression fractures showing a less than 30% loss of height compared with the surrounding area. It is a non-invasive treatment involving the use of a three-point brace or a fabric brace reinforced with metal splints and mobilisation after canalisation, with follow-up X-ray at two weeks. If the patient tolerates the non-invasive treatment well, and providing there has been no further reduction in the height of the vertebral body, the treatment continues (brace and follow ups) until healing is complete. If, instead there has been a further reduction in the height of the vertebral body, an MRI scan with STIR images is requested and, depending on the results of this and the symptoms reported (pain), a vertebral plasty procedure may be suggested.

In patients who, from, the outset, present a greater than 30% reduction of the vertebral body or comorbidities that prevent or could complicate an invasive approach, an MRI scan with STIR images is requested. After identifying the vertebra or vertebrae to be treated, which will still show images consistent with oedema on the MRI sequences, fat removal is carried out followed by the vertebroplasty procedure. The methods used in this field have increased in recent years. Vertebroplasty involves the introduction of radiopaque bone cement into a vertebral body by means of a metal needle placed under amplioscopic guidance; kyphoplasty is a more recent method which consists of the introduction of radiopaque bone cement into a cavity created in the vertebral body by means of a balloon, similar to the kind used in angioplasty procedures, that is inserted percutaneously. Both methods aim to “strengthen” the fractured vertebra through the introduction, into the vertebral body, of acrylic (polymethylmetacrylate) cement, like that used to fix replacement hips to bone. We use the kyphoplasty method in cases in which we need to reduce the fracture or when there are increased risks of “linkage”, whereas in the other cases we use vertebroplasty, often with high-viscosity cement to reduce to a minimum the risk of cement leakage. All patients are offered a medical anti-osteoporosis therapy if they were not already receiving such treatment prior to the vertebral collapse.

We believe that a precise diagnosis, based on a combination of MRI STIR sequences showing bone oedema and the patient’s symptoms (pain), makes it possible, through vertebral plasty, to treat many cases of vertebral collapse due to osteoporosis, considerably reducing healing time and costs.

By combining a vertebral plasty approach aiming to restore the spine’s mechanical axis with an adequate medical therapy for osteoporosis, it is possible to reduce considerably the risk of further vertebral collapses due to osteoporosis and markedly improve the patient’s quality of life.

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P27 - BONE ULTRASONOGRAPHY IN TYPE 1 AND TYPE 2 DIABETES: CORRELATIONS WITH NEUROPATHIC AND ANGIOPATHIC COMPLICATIONS

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Altered glucose metabolism has marked harmful effects on bone metabolism, having important consequences in terms of reduced bone mass and increased fracture risk.
This study set out to explore, in patients affected by type 1 (DM1) and type 2 diabetes (DM2), possible relationships between ultrasonography parameters, indices of metabolic control and parameters indicating complications of diabetes, particularly neuropathy and angiopathy.
We studied a total of 265 patients (145 males and 120 females), affected by DM1 (51 patients, 23 males and 28 females) or DM2 (214 patients, 122 males and 92 females), consecutively referred from general medicine to a specialist diabetes centre. Anthropometric and serological parameters were evaluated in all the patients, including lipid profile and glycated haemoglobin (HbA1C).
The function of sensory nerve fibres was evaluated by determination of vibration perception threshold (VPT); this was measured at the medial malleolus and hallux by means of a biotesiometer. Autonomic function was evaluated clinically using tests that included beat-to-beat HRV, deep breathing, expiration-to-inspiration ratio, heart rate response to standing, systolic blood pressure response to standing, and the cough test. In addition, all the subjects were assessed using a device for ultrasound assessment of the heel bone (Sahara, Hologic), thereby obtaining speed of sound (SOS), broadband ultrasound attenuation (BUA) and QUI measurements. As expected, in all patients a significant inverse relation ($p<0.001$) was found between ultrasound parameters and age. QUI and BUA were positively correlated with BMI and waist circumference. In women with DM2, QUI and BUA showed a close correlation with BMI, waist circumference and % body fat (measured using the Tanita BIA technique). In all the patients we observed a significant correlation between BUA, HDL cholesterol, creatinine, uric acid and HbA1C. Still considering the whole population, SOS was found to be significantly correlated with the VPT of the malleolus ($p<0.05$), and hallux ($p<0.001$) and positively correlated with the deep breathing ($p<0.05$) and lying to standing tests ($p<0.01$). In men with DM1 there also emerged a close correlation between ultrasonography parameters, particularly QUI and SOS, and VPT, deep breathing, lying to standing and the cough test. Furthermore, in the men, QUI and SOS showed an inverse correlation with carotid intima thickness; this correlation was stronger when considering only the men with DM1.
On the basis of these results, we can conclude that the complications of diabetes, such as peripheral and autonomic neuropathy and micro and macroangiopathy, are associated with reduced ultrasound parameter values. Bone ultrasound can provide useful information for assessing the bone status of diabetic patients and may represent a further method for evaluating the extent of neurological damage in diabetic patients.

P28 - CALCIFIC TENDINOPATHY AND VITAMIN D STATUS: A POTENTIAL AETIOPATHOGENETIC FACTOR AND THERAPEUTIC APPROACH

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Calcific tendinopathy is a common pain disorder of unclear origin characterised by the deposition of hydroxyapatite crystals in a tendon, most often in tendons of the rotator cuff of the shoulder. Degenerative changes in collagen fibres have been suggested to be responsible for dystrophic calcification. A local decrease in oxygen tension, not always due to chronic strain, may lead to reduction of pH in a critical region, with fibrocartilaginous metaplasia and resultant calcification. Rotator cuff calcification occurs in 3-8% of healthy shoulders in adults, more frequently in women, during the 4^{th}-6^{th} decades of life, sometimes bilaterally.
In a group of 30 subjects (20 women, 10 men; mean age 51.1, range 27-63 years) with rotator cuff calcification, treated with two-needle US-guided percutaneous treatment, we analysed phospho-calcium metabolism markers (calcaemia, phosphorus, magnesium, 25-OH vitamin D, $1,25(OH)_2$-vitamin D, calciuria, phosphaturia and PTH). 28 patients showed a low level of 25-OH vitamin D (mean value: 16.96 ng/mL, 3-26 ng/mL), calcitriol at or above the upper limits (mean value: 62.77 pg/mL), and PTH concentration in line with the 25OHD levels, most frequently at the upper normal limits. Urinary levels of calcium and phosphorus were at or below the lower limits.
Vitamin D, as well as enhancing intestinal absorption of calcium, plays an important role in the regulation of bone mineralisation, stimulating both RANKL and osteoprotegerin expression. A transient hyperparathyroidism secondary to vitamin D deficiency may be at the origin of heterotopic calcifications in patients with rotatory cuff tendinopathy. Administration of 25OHD, by reducing transient increases of PTH and directly acting on connective tissue cells, could probably reduce the phenomenon or even prevent it.

P29 - THE MANAGEMENT OF OSTEOPOROTIC FRACTURE PATIENTS

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Osteoporotic fractures, both in men and in women, are associated with a high rate of morbidity and mortality. They are, therefore, a major problem with considerable social and economic impact. Furthermore, a significant percentage of previously fractured patients will sustain new fractures.
The orthopaedics specialist is, in many cases, the first medical professional with whom a patient comes into contact following an osteoporotic fracture. Often, treatment of the traumatic injury, with or without surgery, is not followed by a correct classification of a patient's osteoporotic disease.
In this report the authors try to draw up a diagnostic and therapeutic pathway, easy to follow and reproducible both in surgical and orthopedics and traumatology settings. In pursuit of this aim, the authors report the experience of their department in first aid, surgical and diagnostic activities related to osteoporosis.

P30 - JUVENILE OSTEOPOROSIS

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Osteoporosis is a disease of growing interest to paediatricians and other specialists. Among the readily available techniques for measuring bone mineral density (BMD), dual-energy X-ray absorptiometry (DXA) is the most widely used and has the additional benefit of precisely quantifying regional fat mass and lean tissues mass. In the context of an increasing prevalence of morbid obesity and a growing awareness of the risk factors for osteoporotic fractures, paediatric specialists are using DXA data to help identify, treat, and prevent these conditions.
This paper highlights the importance of achieving an adequate peak bone mass and evaluates the strengths and limitations of DXA as a paediatric BMD measurement method. Finally, it considers the use of this technique to identify trends and variations in lumbar and total body BMD measurements.
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P31 - LONG-TERM PROSPECTIVE STUDY OF OSTEOPOROTIC PATIENTS TREATED WITH PERCUTANEOUS VERTEBROPLASTY AFTER FRAGILITY FRACTURES

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Introduction: The purpose of this study was to evaluate factors that could increase the occurrence of new vertebral fractures (VFx) after percutaneous vertebroplasty (PVP) procedures.
Methods: In our prospective study, we included patients of both sexes with osteoporosis (OP) and at least one painful VFx. We performed a baseline biochemical evaluation (including vitamin D plasma levels) and collected demographic, BMD, and clinical data. One hundred and fifteen patients were treated with PVP and assigned to oral bisphosphonates plus Ca and vitamin D. The patients returned for follow-up visits after 1, 3, and 6 months, and every 6 months thereafter. X-rays of the dorsolumbar spine were repeated every 12 months, or in the event of pain that may indicate VFx occurrence.
Results: The mean follow-up duration was 39 +/- 16 months (range, 15-79). Thirty-two patients (27.8%) sustained new fragility VFx, all symptomatic. All the fractured patients agreed to undergo a new PVP. We compared the patients who had sustained new VFx to those who had not, and found significantly lower BMI, total hip, and femoral neck T-scores in the group with new VFx. Furthermore, baseline plasma levels of 25(OH) vitamin D (25(OH)D) were significantly lower in this group. Analysis of plasma levels of 25(OH)D 12 months after PVP showed that a significant difference still persisted: 22 +/- 12 (group with new VFx) vs 41 +/- 22 ng/ml (group with no VFx; $p < 0.01$).
Conclusions: We found that in patients with OP treated with PVP, the incidence of new VFx was 27.8% after 39 months; low BMI, BMD, and vitamin D are factors associated with increased risk of new VFx in patients treated with PVP.

P32 - A CASE OF MULTICENTRIC GIANT CELL TUMOURS WITH 25-YEAR FOLLOW UP

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Giant cell tumour of bone (GCT) is a relatively rare benign bone tumour more frequent in young people (20-40 years). Histologically, two cell types are represented, stromal cells of osteoblastic origin and a distinctive osteoclast-like population probably of monocytic origin. GCTs can be aggressive and they recur locally in up to 50% of cases; up to 5% of GCTs metastasise to the lungs and spontaneous transformation to a high-grade malignancy occurs in 1-3% of patients. The aetiology of GCT is not known, and no risk factors have been recognised, although familial clustering of both Paget's disease and GCT has been reported.
GCTs account for approximately 3-5% of primary bone tumours. GCT is rarely multicentric and usually occurs at the epiphyses of long bones, but may also affect other bones.
There are few randomised, prospective clinical trials available to guide clinical management of GCT. Recent developments have led to evaluation of newer therapeutic agents, including biphosphonates and denosumab, with encouraging results.
We report the case of a 66-year-old woman affected by GCT. In 1985 the patient, then 41 years old, presented a cystic lesion on her left tibia, which was removed surgically. This lesion relapsed two years later. Therefore the patient was hospitalised and received a diagnosis of "multicentric giant cell bone lesions" (limb-girdle, sternum, mandible, ribs), confirmed by histological examination. These lesions showed hyperactivity on bone scintigraphy. Plain radiographs demonstrated destructive lytic lesions. Blood and urinary examinations showed markedly elevated levels of bone alkaline phosphatase and urine pyridinoline and there was persistent bone pain. In 1993 normocalcaemic primary hyperparathyroidism was diagnosed and an adenoma was removed, with no relapse of the disease. Subsequently the patient started clodronate therapy, i.v., followed by alendronate-neridronate per os and clodronate i.m. for about nine years. Biphosphonate therapy caused a modest and transient decrease in bone indexes. Initial bone lesions were unchanged on computed tomography 25 years after diagnosis, but new bone lesions had appeared. MEN1 gene and CasR analyses were negative.
This is a rare case of a patient affected by multicentric giant cell tumours with a 25-year follow up. A slow progression of the lesion is documented, as well as the absence of significant effect of biphosphonate therapy.
References: Werner M. Giant cell tumour of bone: morphological, biological and histogenetical aspects. Int Orthop 2006;30:484-9. Anract P et al. Malignant giant-cell tumours of bone. Clinico-pathological types and prognosis: a review of 29 cases. Int Orthop 1998;22:19-26. Rendina D et al. Giant cell tumor and Paget's disease of bone in one family: geographic clustering. Clin Orthop Relat Res 2004,(421):218-24.

P33 - HYPOPHOSPHATASIA: POSSIBLE UNRECOGNISED CAUSE OF STRESS FRACTURES

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Stress fractures mainly affect athletes and military recruits. The bones most commonly affected are the tibia (64% of cases) and metatarsals (21%). Involvement of the femoral diaphysis ranges from 2.8% to 21%. Bilateral stress fractures are very rare: these mainly affect the tibia and metatarsals, although stress fractures of the femur have occasionally been observed.

Stress fracture risk factors include functional overloading of the bone due to intensive training, nutritional deficiencies and endocrine disorders.

Hypophosphatasia (HPP) is a rare bone metabolism disease caused by reduced functioning of the gene encoding the tissue-nonspecific alkaline phosphatase isoenzyme. HPP causes bone tissue and dental mineralisation defects and reduced alkaline phosphatase activity in serum and bone. The bone mineralisation deficit leads to spontaneous fractures in adults.

Unlike what is seen in patients with classic stress fractures, in individuals affected by HPP, surgical stress fracture treatment with bone grafts does not result in healing.

The authors report three cases of bilateral stress fractures of the femoral diaphysis in members of the same family (2 heterozygotic twins and 1 brother). Failure of the fracture to heal in the male subject, treated with osteosynthesis, prompted blood chemistry examinations. These, revealing low levels of total and bone alkaline phosphatase, allowed a diagnosis of HPP. Further investigations of family members revealed the presence of the same type of fracture in the subject's sisters (twins), and they, too, were found to be affected by HPP.

The authors believe that the importance of HPP as a cause of stress fractures is probably underestimated and suggest that phosphatase should be routinely measured in all cases of stress fractures in adults, especially those involving the femoral diaphysis, and particularly when they are bilateral.

P34 - BONE IN CYSTIC FIBROSIS: STUDY OF 44 PATIENTS USING THE DEXA METHOD

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Bone tissue formation and maintenance, fundamental to the electrolytic homeostasis of the organism, is, like other systems, a process of constant turnover; it should therefore be studied in all forms and should not be considered an end onto itself. The metabolism of this system can, in different diseases, be altered by a range of factors. For this reason, it should be analysed, diagnosing abnormalities, evaluating their possible aetiology and, when useful, treating the condition with the means available. Between 2008 and 2010, we studied 44 patients affected by cystic fibrosis, for which they

N°	Age & Sex	Weight (Kg) & Height (m)	BMI	Femoral BMD(total) g/cm^2	Femoral T-score	Femoral Z-score	Vertebral BMD(L1-L4) g/cm^2	Vertebral T-score	Vertebral Z-score
1	20M	60-1,73	20	0,811	-1,5	-1,5	0,839	-2,3	-2,3
2	20F	51-1,68	18	0,952	0,01	0,01	0,815	-2,1	-1,9
3	20M	59-1,70	20	0,932	-0,7	-0,7	0,999	-0,8	-0,8
4	20F	52-1,64	19	0,756	-1,5	-1,5	1,013	-0,3	-0,1
5	20M	62-1,72	21	0,902	-0,9	-0,9	0,947	-1,3	-1,3
6	20M	65-1,70	22	0,858	-1,2	-1,2	0,911	-1,6	-1,6
7	21M	56-1,73	19	1,073	0,3	0,3	1,014	-0,7	-0,7
8	21M	44-1,65	16	0,772	-1,7	-1,7	0,759	-3,0	-3,0
9	21F	45-1,70	16	0,805	-1,1	-1,1	0,842	-1,9	-1,7
10	22M	65-174	21	0,873	-1,1	-1,1	0,758	-3,0	-3,0
11	23M	60-1,81	18	0,836	-1,3	-1,3	0,824	-2,4	-2,4
12	25M	67-1,75	22	0,776	-1,7	-1,7	0,859	-2,1	-2,1
13	25F	50-1,60	20	0,806	-1,1	-1,1	0,895	-1,4	-1,3
14	28F	60-1,67	22	0,698	-2	-2	0,866	-1,6	-1,6
15	29F	58-1,62	22	0,924	-0,1	-0,1	0,969	-0,7	-0,7
16	30M	56-1,65	21	0,955	-0,5	-0,5	0,889	-1,8	-1,8
17	30F	51-1,50	23	0,876	-0,5	-0,5	0,870	-1,6	-1,6
18	31F	59-1,72	20	0,872	-0,6	-0,5	1,174	1,2	1,2
19	31F	76-1,62	29	0,805	-1,1	-1,1	0,911	-1,2	-1,2
20	31F	45-1,62	17	0,788	-1,3	-1,2	0,931	-1,1	-1
21	32F	45-1,63	17	0,828	-0,9	-0,9	0,878	-1,5	-1,5
22	32M	50-1,70	17	0,581	-3	-2,9	0,653	-4	-4
23	32F	49-1,57	20	0,727	-1,8	-1,7	0,792	-2,3	-2,3
24	33F	42-1,50	19	0,811	-1,1	-1	1,031	-0,1	-0,1
25	34M	48-1,63	18	0,834	-0,9	-0,8	1,018	-0,3	-0,2
26	34F	50-1,67	18	0,669	-2,2	-2,2	0,823	-2	-2
27	34M	63-1,70	22	1,041	0,01	0,2	1,240	1,4	1,4
28	34F	35-1,53	15	0,638	-2,5	-2,4	0,856	-1,7	-1,7
29	34F	55-1,65	20	0,703	-2	-1,9	0,850	-1,8	-1,8
30	34F	54-1,53	23	0,740	-1,7	-1,6	0,828	-2	-1,9
31	36M	90-1,68	32	0,884	-1	-0,8	0,809	-2,6	-2,5
32	37F	60-1,55	25	0,672	-2,2	-2,1	0,872	-1,6	-1,5
33	38F	55-1,56	23	0,697	-2	-1,9	0,959	-1,1	-1
34	39M	51-1,65	19	0,962	-0,5	-0,3	1,040	-0,5	-0,4
35	39F	55-1,69	19	0,647	-2,4	-2,3	0,866	-1,6	-1,5
36	39F	60-1,68	21	0,735	-1,7	-1,6	0,885	-1,5	-1,3
37	39M	80-1,78	25	0,886	-1	-0,8	0,916	-1,6	-1,5
38	39M	85-1,72	29	1,129	0,6	0,8	0,914	-1,6	-1,5
39	40F	48-1,52	21	0,635	-2,5	-2,4	0,629	-3,8	-3,7
40	41F	53-1,55	22	0,707	-1,9	-1,8	0,935	-1	-0,8
41	43M	62-1,60	24	0,754	-1,8	-1,6	0,799	-2,7	-2,5
42	44M	61-1,75	20	0,750	-1,6	-1,3	0,799	-2,3	-1,9
43	44F	75-1,64	28	0,706	-1,9	-1,7	0,745	-2,7	-2,4
44	45F	58-1,62	22	0,849	-0,8	-0,5	0,959	-0,8	-0,4
Media totale			21	0,810	-1,281	-1,202	0,890	-1,577	-1,498
Media 20-31aa		20 pazienti	20	0,857	-0,963	-0,957	0,903	-1,505	-1,468
Media 32-45aa		24 pazienti	21	0,775	-1,524	-1,388	0,881	-1,632	-1,520

were receiving medical therapy: 25 females, 19 males, aged between 20 and 45 years (who had reached complete skeletal maturity).
The above patients were submitted to densitometry using dual emission X ray absorptiometry (DEXA), the gold standard method for diagnosing osteoporosis. We analysed, both at femoral and at vertebral level, each patient's BMI, BMD, T-score and Z-score; the means of these values were calculated and compared with the mean values for the healthy population.
The patients were divided into two subgroups according to age: 20-31 years and 32-45 years, in order to evaluate the effect of time on the results obtained.
The patients investigated did not have other major diseases and were following different treatment protocols. According to the results obtained, patients affected by cystic fibrosis show bone modelling that is insufficient to guarantee normal bone development, or bone development comparable to that of healthy age-matched subjects, in the context of a picture of frank and premature osteopenia. Since all the patients had a good BMI, it was not deemed useful to correlate this parameter with the ostopenia detected. In the whole study population, BMD was found to be significantly lower than the mean reference value for the Italian population. The mean BMD densities of the two patient subgroups were found to be significantly different, both when considering the lumbar and the femoral T-scores. There emerged no significant differences in BMD between males and females. The differences between the means of the values recorded in the two age groups were significant, while the correlation between longitudinal values of age and BMD was not significant (given the non-homogeneity of the sample's longitudinal values).
Patients with cystic fibrosis are particularly predisposed to osteopenia due to various factors, possibly environmental, genetic and circumstantial, and certainly iatrogenic and nutritional (deficiencies). It is known that patients with cystic fibrosis struggle to reach a normal bone mass peak and display rapid bone loss that leads to premature ostopenia. Unlike studies that assessed bone mass, but failed to consider genetic causes that may possibly be correlated with this situation of low mineralisation, recent studies have identified the presence of cystic fibrosis transmembrane regulator (CFTR) in osteoblasts, osteocytes and osteoclasts, which renders the aetiology of the ostopenia in these patients even more complex. It is worth recalling that the difficulty these patients have absorbing and storing vitamin D is a significant cofactor in the pathogenesis of their ostopenia; another contributing factor is, without doubt, the prolonged use of corticosteroids. We also wish to underline the importance of the production of inflammatory cytokines, typical of this chronic disease, which we believe to be a fundamental factor in the acceleration of the catabolic bone metabolism. There now exists evidence of excessive bone remodelling in the disease. The study we carried out showed a marked mean ossification deficit for these young subjects, in whom preventive measures could be extremely helpful. The life expectancy of these patients is increasing and in this context there emerges a need for more in-depth studies to further explore and verify the multifactorial aetiology of the condition so that, thanks to better consideration of the various aspects of the disease, interventions might guarantee, if not a prolonging of life, at least a better quality of life and a reduced risk of complications. The treatment of osteoporosis in young adults with cystic fibrosis is still in its early stages. The assessment and treatment of osteoporosis needs to become an integral part of the management of cystic fibrosis. Osteoporosis is, indeed, a complication of cystic fibrosis that requires early prevention through physical activity, proper correction of the nutritional deficiency, and vitamin-mineral supplements, possibly associated with drugs like the biphosphonates which are still in the empirical phase.
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P35 - RADIOLOGICAL ASSESSMENT OF BONE REPAIR AFTER SUPPLEMENTATION WITH HYDROXYAPATITE NANOPARTICLES

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The aim of this study was to evaluate, through the application of semi-quantitative radiological criteria, the speed of bone repair processes induced by the surgical use of materials based on hydroxyapatite nanoparticles.
We evaluated 13 patients aged between 55 and 65 years, undergoing tibial valgus osteotomy with equine bone grafts supplemented with hydroxyapatite nanoparticles.
All the patients underwent radiographic assessments post-operatively and at 30, 60, 90 and 180 after the operation. Furthermore, all were submitted to high-resolution computed tomography (HRCT) scans with thin-layer reconstructions two months after surgery; samples of these were used for bone densitometry in order to assess mineralisation in the sites of bone repair. The data were compared with those of a series of patients treated using the same technique, but without supplementation.
Compared with the findings in the control group of patients treated with non-supplemented bone grafts, all the patients receiving material supplemented with hydroxyapatite nanoparticles showed faster bone repair processes.
On the basis of these results, it can be hypothesised that the hydroxyapatite structure obtained by means of nanomolecular technology serves as a mechanism guiding the evolution of osteointegrative processes, helping to speed up the formation of regenerated bone tissue.
In conclusion, the application of nanomolecular technology in bone repair appears to give promising results, suggesting that there may be scope for its application in other bone-related fields too.

P36 - SEVERE OSTEOPOROSIS IN CUSHING'S SYNDROME

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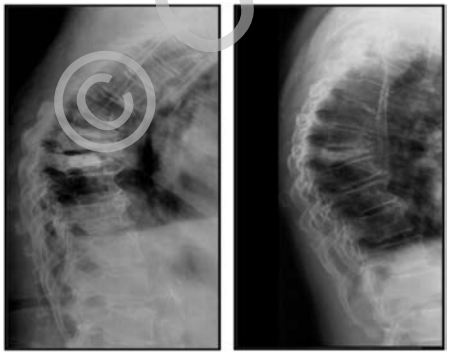
Cushing's syndrome is characterised by a series of clinical manifestations due to hypersecretion of cortisol. These include: arterial hypertension, diabetes mellitus (DM), asthenia, amenorrhea, osteoporosis and pathological fractures. We describe the case of a 70-year-old woman with Cushing's syndrome with right adrenal adenoma, vertebral compression fractures (VCFs) and severe secondary osteoporosis. This patient had been diagnosed with Cushing's syndrome in May 2008, three years after the onset of arterial hypertension and type II DM, treated with insulin; in July 2008, she underwent right adrenalectomy and replacement therapy with cortisone acetate, 37.5 mg/day, in association with a multiple drug regimen for hypertension and DM; she also had an at least 10-year history of dorso-lumbar pain with multiple disc protrusions. As part of a series of investigations for Cushing's syndrome the patient underwent femoral bone mineral densitometry, recording a T-score <-3, radiographic examination of the dorso-lumbar spine, which revealed collapse of the superior endplate of D7 and a wedge fracture of D8. At the endocrinology centre of reference for Cushing's syndrome, she began treatment with alendronate 70 mg/day without undergoing blood chemistry tests of bone metabolism and without calcium and vitamin D supplementation. At the end of August 2009, she experienced worsening spinal pain due to a new severe fracture of D9, which was confirmed on MRI as a recent fracture. At the end of December 2009 she received kyphoplasty of D9, antiresorptive therapy and a CAMP-C35 brace.



In January 2010 she was admitted to the specialist rehabilitation unit for functional recovery, in view of her comorbidities, and bone disease investigation, with collection of history relating to osteoporosis risk factors. First- and second-level blood chemistry analyses revealed the presence of iron-deficiency anaemia, mild chronic renal insufficiency, and secondary hyerparathyroidism (PTH 101ng/ml); spinal radiography revealed severe VCFs of D7, D8 and D9, treated with kyphoplasty; the patient was also assessed using the VAS for pain, the FIM to evaluate independence in activities of daily living, and the SF-36 to investigate quality of life. The alendronate treatment was suspended and the patient was given cholecalciferol 300,000 IU, administered as an oral bolus, followed by a maintenance dose of 800 IU/day. When PTH values had returned to normal, she began treatment with teriparatide 20 mcg/day s.c. (therapeutic plan in compliance with Note 79 issued by the AIFA - Italian Drug Agency).
In conclusion, this case underlines the importance of a correct diagnostic and therapeutic approach in patients with severe osteoporosis. Over time, we will evaluate the efficacy of the treatment in preventing new fractures and the whether the use of a bone anabolic agent might be the correct choice also in order to control pain and improve quality of life. There are no reports in the literature of patients with Cushing's syndrome treated with teriparatide.