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ABSTRACTS

New Data Abstracts of the ECTS Congress 2019

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New Data Abstracts

POSTER

ND-P001

Age-related changes in bone cells

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Bone is remodelled throughout life. Remodelling starts with bone resorption by osteoclasts followed by bone formation by osteoblasts. Osteocytes act as mechanosensors and orchestrate the bone remodelling process. In normal bone, formation and resorption are balanced. This balance changes in ageing, leading to age-related loss of bone volume and strength. As a consequence older people have a 10-fold-increased fracture risk compared with younger individuals.

The aim of this study was to investigate changes in gene expression due to ageing in cells of the osteoblast lineage. In addition we studied whether the production of factors by osteocytes changes with age. Gene expression was studied by performing RNASeq analysis of mRNA from osteoblasts isolated from 3-month-old (young) and 14-month-old (aged) mice. Serum levels of P1NP (bone formation marker), CTX (resorption marker), sclerostin, osteocalcin and osteopontin were measured using ELISAs.

ELISA analysis showed a significant 3-4 fold reduction in circulating levels of P1NP ($p < 0.01$) and CTX ($p < 0.001$) in aged mice, indicating a reduction in bone turnover. The RNASeq analysis results showed significant changes in the expression of 40 different genes in aged osteoblasts from compared to young osteoblasts. Pathway analysis showed a cluster of genes connected to the chemokine Cxcl2 was upregulated in aged mice. Cxcl2 is known to stimulate bone resorption. A cluster of genes centred on tubulins is downregulated in aged osteoblasts. One gene in this cluster is Cdkn2a, which is involved in cellular senescence.

In conclusion, ageing is associated with a change in bone turnover, and changes in osteoblastic gene expression.

Keywords: osteoblast, osteoclast, ageing, gene expretion, CTX, P1NP

ND-P002

Synergistic effect of hyperbaric oxygen therapy with parathyroid hormone [1-34] on calvarial bone graft in irradiated rat

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Purpose: To determine the synergistic effect of parathyroid hormone [1-34] in combination with hyperbaric oxygen on bone graft in rat calvarial bone defect model under impaired osteogenic condition.

Materials and methods: Twenty four rats were divided into 3 groups. Localized radiation with a single 12 Gy dose was administered to the calvarial. 4 weeks after radiation, calvarial circular defects were created in the parietal bones. All defects were filled with biphasic calcium phosphate. After grafting, parathyroid hormone was injected subcutaneously and hyperbaric oxygen therapy was administered. At 6 weeks after the bone graft, the rats were sacrificed and specimens were harvested.

Results: Histomorphometric evaluation showed the percent new bone area was higher in the PTH and PTH/HBO groups than in the Control group. Micro computed tomographic evaluation showed bone volume of new bone volume was higher PTH group than Control group. Bone surface in new bone volume was higher PTH/HBO group than Control group. In new bone volume, bone surface density was higher in the order of Control, PTH and PTH/HBO groups; all group was significant difference ($P < 0.017$).

Conclusions: Within the limitations of this study, our data indicate that parathyroid hormone with hyperbaric oxygen may reverse the impairment of bone healing by irradiation.

Keywords: calvarial defect, bone graft, bone regeneration, parathyroid hormone, hyperbaric oxygen therapy

ND-P003

Dynamic deformation by fluid flow on hematopoietic bone marrow cells alters osteoclast differentiation

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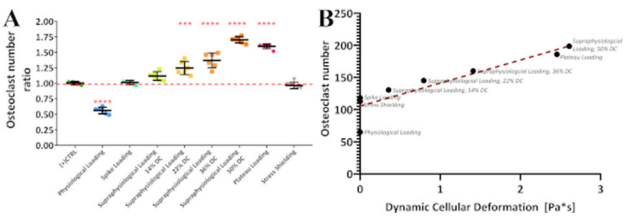
Bone undergoes constant remodeling to adapt to external mechanical loading. Under physiological conditions, mechanical loading results in bone formation to meet functional demands. In contrast, poor osseointegration of orthopedic implants changes the mechanical microenvironment in the peri-prosthetic interface leading to bone degradation. How mechanosensitive cells shift from bone formation to degradation remains elusive. The goal was to determine the influence of different fluid flow regimes on cell deformation in an in-vitro model for mechanical induced bone implant loosening.

Hematopoietic progenitor cells ($1,0 \times 10^5/cm^2$) were subjected to 2 minutes of different loading regimes by pulsating fluid flow, using a parallel-plate flow chamber. Conditioned medium from mechanically loaded cells was added to a RANKL-induced osteoclastogenesis assay.

Supraphysiological loading releases osteoclast-inducing factors, depending on the active loading duration (DC): 22% DC (1,2-fold, $p < 0.01$), 36% DC (1,4-fold, $p < 0.001$), 50% DC (1,7-fold, $p < 0.001$) and plateau loading 50% DC (1,6-fold, $p < 0.001$). Physiological loading reduced osteoclast differentiation (0,4-fold, $p < 0.001$). Spike loading, supraphysiological loading 14% DC and stress shielding did not change osteoclast differentiation (Figure 1A). The dynamic cellular deformation [Pa*s] = Plateau wall shear stress [Pa] × Plateau duration [s] and had a positive correlation with osteoclast number (Pearson $R^2 = 0.78$) (Figure 1B).

Our results suggest that the dynamic cellular deformation regulates the release of bone-modulating soluble factors by mechanosensitive cells. Understanding how mechanosensitive cells respond to changes in deformation leading to either suppression or induction of osteoclast differentiation could open up new treatment strategies to delay or stop prosthetic loosening.



[Dynamic cellular deformation regulates the release of osteoclast-modulating soluble factors.]

ND-P004

Titanium with nanotopography induces osteoblast and inhibits osteoclast differentiation

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Development of nanomaterials that are able to control osteoblast and osteoclast activities and consequently the bone remodeling process is of relevance to improve the osseointegration. This study aimed to investigate the effect of Ti with nanotopography (Ti-Nano) on osteoblast and osteoclast differentiation. Rat mesenchymal stem cells (MSCs) and RAW 264.7 cells were cultured on Ti-Nano or machined surface (Ti-Machined). The MSCs were cultured in growth medium for 7 days and the RAW 264.7 cells were cultured in osteoclast differentiation medium for 10 days. To investigate the osteogenic differentiation, the gene expression of some osteoblast markers was evaluated on days 3, 5 and 7 by real-time PCR and the protein expression of RUNX2 was evaluated on days 3 and 5 by Western blot. To investigate the osteoclast differentiation, the gene expression of *Rank* was evaluated on day 7 and the osteoclast activity was evaluated by staining for TRAP on days 3, 7 and 10. The data were analyzed by t-test ($p \leq 0.05$). Gene expression of *Runx2* was higher on days 3 and 7 on Ti-Nano. The gene expression of osterix, alkaline phosphatase and bone sialoprotein was higher on Ti-Nano in all time-points. Gene expression of osteocalcin was higher on days 5 and 7 on Ti-Nano. Gene expression of osteopontin was higher on days 3 and 5 on Ti-Nano. The protein expression of RUNX2 was higher on days 3 and 5 on Ti-Nano. Gene expression of Rank was lower on Ti-Nano on day 7. TRAP staining was lower on Ti-Nano on days 5 and 7. In conclusion, Ti with nanotopography induces osteoblast differentiation of MSCs, even in non-osteogenic conditions, concomitantly with the inhibition of osteoclast differentiation of RAW 264.7. Thus, these findings open windows for the development of smart surfaces with ability to regulate the bone remodeling process during the osseointegration of implants.

ND-P005

Effect of Nerve Growth Factor monoclonal antibody on pain behaviours in a mouse model of fracture

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Bone fractures can be very painful. In this study, we examined the effect of an Anti-Nerve Growth Factor (anti-NGF) monoclonal antibody (MEDI578) on naturalistic and evoked pain behaviours after fracture in mice.

12 week-old female mice underwent a unilateral femoral fracture maintained by an external fixator. Mice were randomised to four groups: Fracture-MEDI578, Sham-MEDI578, Fracture-NIP228, Sham-NIP228; n = 9 or 10/group. MEDI578 or a control antibody (NIP228) were administered subcutaneously before and weekly after fracture surgery at 3 mg/kg. Pain behaviour measurements were acquired from baseline and weekly throughout the study. Naturalistic behaviours were assessed at weeks 1, 3 and 6. Mice were kept for 6 weeks before sacrifice. Fracture healing and bone microstructure were assessed using micro-CT.

By week one after surgery, the Fracture groups had significant drops in mechanical thresholds (MEDI578: − 48.5%, NIP228: − 64.48%, p = 0.0001). Fracture-MEDI578 group had decreased thermal threshold (− 52.82%, p = 0.0130) compared to baseline, but not the fracture-NIP228 group. By week four after fracture, mechanical hyperalgesia had resolved in the fracture-MEDI578 group compared to baseline (p = 0.0661) but not in the Fracture–NIP228 group which had persistent mechanical hyperalgesia throughout the study. There were no significant differences in thermal hyperalgesia between the Fracture-MEDI578 and NIP228 groups from week 3 ($p = 0.1829$). Naturalistic behaviours were similar the MEDI578 and NIP228 groups, except for grooming behaviours which were significantly increased in the Fracture-MEDI578 group six weeks after surgery. Bone callus size was significantly increased at 6 weeks in the MEDI578 group compared to the NIP228 one (+ 33.55%, p = 0.0475) and cortical bone structure improved in Sham- MEDI578 group compared to the NIP228 one, suggesting that the anti-NGF has positive effects on bone.

Our study demonstrates that the anti-NGF was successful at alleviating fracture-induced mechanical pain compared to the control antibody.

ND-P006

New bone mineral density standards adjusted for biological ages in children

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Objectives: The precise age estimation is of high importance in bone mineral density (BMD) evaluation in children, since the bone structure of children is evaluated by using age and gender dependent references. Biological age estimation could help this bone structural evaluation process, since the developmental status of the skeletal system can significantly alter from the theoretical developmental status determined by chronological age in early or late maturing children. The aims were (1) to check whether volumetric BMD Z-scores estimated by considering chronological age and biological age differ significantly in children aged 7-18 years, and (2) in the case of significant inaccuracy of Z-score estimation based on only chronological age to construct new BMD standards adjusted for bone age or body developmental status.

Subjects and methods: Body structural and densitometry data of 476 healthy children aged 7-18 years were used in the analysis. pQCT measurements were performed by Stratec XCT-2000 equipment. BMD centiles were estimated by lmsChartMaker Pro2.3.

Results: The total and 'cortical + subcortical' BMD changed by age in both genders. Our results confirmed when the biological age significantly differs from chronological age, BMD evaluation should be done by considering biological age in children. If the estimation of any biological age cannot be carried out, BMD references adjusted for height or other body dimensions should be used in the bone health status estimation in children.

Conclusion: Due to the increase in individual variability of rate and timing of pubertal developmental processes, the sensitivity of BMD evaluation by considering body developmental status was the lowest in the age between 12-16 years in boys and between 10-12 years in

girls. Therefore the suggested BMD adjustments for biological ages are highly recommended to use at least in children with ages outside these age intervals.

Keywords: bone mineral density, children, adjustment for biological ages

ND-P007

Inhibition of KIAA1199 (CEMIP) enhances osteoblast differentiation and in vivo bone formation

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We have identified KIAA1199 (also called CEMIP) as a secreted factor in cultured human skeletal (mesenchymal) stem cell (hMSC) and is expressed in osteoblastic progenitors and mature osteoblasts (OB) during bone remodeling in human bone biopsies as visualized by in situ hybridization (ISH). siRNA-mediated knockdown of the KIAA1199 in hMSC enhanced the alkaline phosphatase (ALP) activity and in vitro mineralization, and activated Akt, ERK and p38 MAPK signaling pathways. To determine its physiological role, we established KIAA1199 deficient mice ($KIAA1199^{-/-}$) by CRISPR technology. $KIAA1199^{-/-}$ exhibited increased trabecular and cortical bone mass: (trabecular BV/TV (+ 26%, $p = 0.01$), trabecular number (+ 11.3%, $p = 0.02$), and cordical thinkness (+ 12.2%, $p < 0.01$) compare to the corresponding wild type (WT) mice, as determined by micro-CT scanning. Moreover, the plasma P1NP levels were higher (+ 18.7%, $p = 0.04$). Cultured primary mouse MSC (mMSC) from $KIAA1199^{-/-}$ mice revealed enhanced OB differentiation evidence by increased OB gene expression and in vitro mineralization. Both bone marrow osteoclast formation and plasma CTX-1 levels (− 23.9%, $p = 0.01$) were reduced compared to matched WT mice. Furthermore, in a bone fracture model, $KIAA1199^{-/-}$ exhibited more enhanced bone healing (> 1.5 times healing vs. WT, $p < 0.01$). KIAA1199 is a novel factor expressed by MSC, and regulates the OB differentiation and bone formation in vivo and it is a possible novel therapeutic target for bone enhancing bone formation and bone regeneration.

ND-P008

PDGFRβ signaling drives the expansion, recruitment, and blood vessel affinity of skeletal stem/progenitor cells for bone repair

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Bone repair and regeneration critically depend on the activation and recruitment of osteogenesis-competent skeletal stem and progenitor cells (SSPCs). Yet, the signaling pathways and molecular mechanisms driving SSPC propagation and migration in response to trauma remain largely elusive.

Through cell fate mapping and lineage tracing using Osx-Cre:GFP;mTmG and Osx-CreERt;tdTomato mice we here show that bone trauma activates multiple cell subsets, broadly marked by Osx+ history, that altogether establish the repair tissue, thus identifying Osx+ cells as a source of reparative SSPCs. By profiling fetal Osx+ cells by bulk and single-cell RNA-sequencing, we found platelet-derived growth factor receptor (PDGFR)β standing out as strongly expressed. PDGFRβ-Cre;mTmG lineage mapping revealed that fracture callus tissues were virtually completely built by cells with active or historical PDGFRβ-expression. To investigate the in vivo functional role of PDGFRβ signaling, we generated Osx-Cre:GFP-driven PDGFRβ conditional knock out (cKO) mice. In a semi-stabilized fracture model, PDGFRβ cKO mice displayed an undersized callus (2.5-fold reduced callus volume, $p < 0.001$, n = 6-8) characterized by altered tissue composition (reduced cartilage and bone, 2-fold increased fibrotic tissue, increased marrow adiposity) and poor vascularization (2-fold decreased vessel number and size, $p < 0.05$, n = 6-8), as determined by μCT and histology. To understand how PDGFRβ signaling acts to mediate bone repair we used in vitro systems, molecular analyses, and in vivo immunofluorescence imaging by confocal/3D microscopy. These studies revealed three major mechanisms contributing to the defective callus formation in PDGFRβ cKO mice: (i) impaired proliferative responses and premature differentiation of SSPCs lacking PDGFRβ, (ii) reduced motility, and (iii) diminished vascular affinity of mutant SSPCs. In search of mechanistic mediators we further mined our Osx+ RNA-Seq databases, and identified and validated selected molecules functioning downstream of PDGF-PDGFRβ.

In conclusion, reparative SSPCs require PDGFRβ signaling to expand, migrate, and associate with blood vessels, thereby ensuring proper callus formation during bone repair.

Keywords: Skeletal stem and progenitor cells, bone regeneration, blood vessels, cell migration, PDGF receptor

The first three authors contributed equally.

ND-P009

Is targeting Wnt signalling in Osteosarcoma therapeutically relevant?

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Objective: To evaluate the therapeutic potential of targeting Wnt signalling in Osteosarcoma (OS), the most frequent primary malignant bone tumor.

Methods: Genetically-engineered H2 k-c-fos-LTR (Fos^{Tg}) mice over-expressing Fos/AP-1 were used as an experimental model for OS. An inducible bone-specific Wnt-less (Wls) loss-of-function OS model ($Wls^{\Delta OB}$-OS) was generated by combining *wls* floxed, Osx-Cre and Fos^{Tg} mice ($n \geqq 5$) and Wls was inactivated therapeutically by Dox removal. Tumors were longitudinally monitored by micro-CT, gene expression analyzed by quantitative RT-PCR (n = 5) and promoter binding by Chromatin Immunoprecipitation (n = 3).

Results: In $Wls^{\Delta OB}$-OS bones, mRNA expression of *wls* and Wnt target genes such as *axin2* was decreased upon *wls* gene inactivation. Importantly, tumor burden was decreased by 70% in $Wls^{\Delta OB}$-OS mice compared to Fos^{Tg} mice (Figure) and $Wls^{\Delta OB}$-OS tumors grew slower than Wls^{WT}-OS tumors. While the majority of OS developing in Fos^{Tg} mice appeared osteoblastic, tumors in $Wls^{\Delta OB}$-OS mice were less mineralized and enriched in fibroblastic cells surrounded by collagen fibers. Increased expression of Wnt ligands such as *Wnt7b* and *Wnt9a* was measured in bones, bone tumours and OS lines from Fos^{Tg} mice. Furthermore, c-Fos/AP-1 regulates *Wnt7b* and *Wnt9a* expression in murine OS cells through direct promoter binding.

Consistently, knockdown of c-Fos in human/mouse OS cell lines and MC3T3-E1 cells decreased *Wnt7b* and *Wnt9a in vitro*, while ectopic c-Fos expression had the opposite effect. These findings are currently being confirmed in human OS samples.
Summary and Conclusions: Wnt signalling promotes c-Fos-induced OS formation *in vivo*. Genetic inhibition of Wnt ligand secretion, results in changes in tumor burden and OS histology associated with abnormal extracellular matrix deposition. Thus, targeting Wnt signalling could be a beneficial therapeutic intervention in OS.

ND-P010

Changes of bone remodeling markers in breast cancer patients after combined treatment

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The earliest sign of decline in BMD, is the change in bone remodeling markers. However, changes in bone markers in patients with breast cancer who underwent combined treatment are not given enough attention in the literature, and in existing publications premenopausal and postmenopausal groups of patients with breast cancer are not considered separately.
Objective: To evaluate changes in bone remodeling markers in patients with breast cancer before and after combined treatment.
Materials and methods: A total of 18 patients with premenopausal breast cancer and 21 patients with postmenopausal breast cancer who underwent radical mastectomy and 4-6 courses of adjuvant polychemotherapy. The average duration of treatment was 106 days.
All patients before and after the combined treatment were studied with markers of bone resorption-β - crossLaps, bone synthesis-TP1NP and osteocalcin (OC). The study was made by Elecsys 1010 with Roche Diagnostics kits.
Results: In patients with breast cancer in postmenopausal women until the start of the combined treatment : β-crossLaps 0,691 ng/ml, TP1NP and OC 44,21 ng/ml and 34 ng/ml, respectively. After treatment : β-crossLaps, TP1NP and OC made 0,904 ng/ml, 43,15 ng/ml 40,71 ng/ml, respectively.
Premenopausal patients before the start of the combined treatment : β-crossLaps made 0,556 ng/ml, TP1NP and OC 37,98 ng/ml and of 22.45 ng/ml, respectively. β-crossLaps, TP1NP and OC 0,694 ng/ml, 42,24 ng/ml and 28.42 ng/ml, respectively.
Conclusions: The data show a statistically significant increase in bone metabolism in both groups of patients with predominance of bone lysis, especially in patients with breast cancer in postmenopause. The findings suggest the need for further research in assessing the impact of combined treatment on bone tissue in order to control the bone metabolism in this category of patients.
Keywords: Breast, cancer, bone, markers

ND-P011

MitoBoneLomics: Estradiol remodeling effect on mitochondrial activity during osteoblast differentiation

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Background: Osteoblasts differentiation and activity are crucial to maintain a balance bone remodeling. The presence of estrogen appears to be important for both processes. Mitochondria, being more than cell powerhouses, have multiple cellular roles, namely during cell differentiation. The aim of this study was to evaluate how estrogen regulates mitochondrial performance during osteoblast differentiation.
Methods: Using the MC3T3-E1 cell line, we induced cell differentiation into osteoblasts by adding 50 μg/mL of ascorbic acid and 10 mM of β-glycerophosphate to the phenol red free culture medium supplemented with charcoal-stripped FBS. Mineralization was assessed using the Alizarin Red S Staining Assay. Mitochondrial performance in the presence of 17β-estradiol (E_2) for 1, 24 and 48 h was evaluated by measuring oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) using the Seahorse XFe96 Extracellular Flux Analyzer. Statistical analysis was performed by Kruskal-Wallis non-parametric test.
Results: Alteration in mitochondrial respiration was observed when MC3T3-E1 cells were exposed to differentiation medium in the absence of E_2, showing a time dependent decrease in basal (16 and 25%), maximal (53 and 36%) and ATP-linked OCR (16 and 43%). After 48 h of treatment, 10 nM of E_2 increased basal and maximal (by 30%) and ATP-linked OCR (by 40%) in MC3T3-E1 cells.
Conclusions: Our results suggest that estrogen modulates mitochondrial activity during osteoblast differentiation. Alterations in mitochondrial performance in absence of estrogen, during osteoblast differentiation could be a regulator key in the development of menopause associated osteoporosis.
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Keywords: Osteoblast differentiation, Mitochondria, Cell Metabolism, Estradiol, Oxidative Stress

ND-P012

Effect of local injection of osteoblastic cells differentiated from bone marrow or adipose tissue-mesenchymal stromal cells on bone repair

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In this study, we evaluated the effect of local injection of osteoblastic cells differentiated from bone marrow or adipose tissue-mesenchymal stromal cells (BM-OB and AT-OB, respectively) on bone repair. For that, the cells were harvested from male Wistar rats (200 g), under the rules of the Committee of Ethics in Animal Research of the University of São Paulo. The BM-OB were obtained by osteoblastic differentiation of bone marrow-mesenchymal stromal cells for 10 days. The AT-OB were obtained by osteoblastic differentiation of adipose tissue-mesenchymal stromal cells for 10 days. Under general anesthesia, unilateral 5-mm defect was created in the calvaria of rats and in order to simulate preexisting defects only after 2 weeks the defects were treated. Each defect was locally injected with BM-OB or AT-OB (5×10^6 cells/defect in 50 μl PBS). PBS without cells was injected as Control. Four weeks after cell injection, the animals were euthanized, and the bone formation was analyzed by microtomography (micro-CT) and nanoindentation assay. The data were evaluated using the ANOVA test followed by the Tukey's test when appropriated ($p \leq 0.05$). The morphometric parameters generated from micro-CT images showed that bone volume, percentage of bone volume, bone surface and trabecular number were higher in defects injected with BM-OB or AT-OB compared with Control ($p = 0.001$ for all). Trabecular separation was lower in defects

injected with BM-OB or AT-OB compared with Control ($p = 0.001$). The qualitative parameters generated from nanoindentation indicated that elastic modulus and hardness of bone formed in defects injected with BM-OB or AT-OB were higher compared with Control ($p = 0.05$ for both). In conclusion, the use of local injection of osteoblastic cells differentiated from bone marrow or adipose tissue-mesenchymal stromal cells induced the same amount of bone formation opening new therapeutic possibilities for the treatment of bone defects.

ND-P013

Binding and uptake of CCL11 in preosteoclasts and osteoclasts

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Inflammatory bone resorption is dependent on the recruitment and activation of osteoclasts, a process believed to be partly mediated by local chemokine production. In patients with chronic inflammatory diseases adjacent to bone, e.g. periodontitis and rheumatoid arthritis, systemically and locally increased levels of the chemokine CC chemokine ligand 11 (CCL11) has been found. In this study, we investigate membrane binding and internalization of CCL11 in different osteoclast subpopulations.

Osteoclast precursors and mature osteoclasts were cultured from murine primary cell cultures of bone marrow macrophages supplemented with M-CSF and RANKL. After incubation with fluorescent labelled rmCCL11 (Alexa Fluor® 647), analyses of CCL11 osteoclast membrane binding and internalization were performed by using confocal imaging, scanning electron microscopy and live cell imaging. Co-localization with rmTransferrin (Alexa Fluor® 555) was used as a positive control for clathrin mediated endocytosis. Protein levels of internalized rmCCL11 was determined by ELISA.

Results showed that CCL11 was rapidly internalized and that the uptake was higher in osteoclast precursors compared to mature osteoclasts, wherein the initial CCL11 interaction mainly involved surface binding to protrusion rich sites of the cell membrane. The major internalized pool of CCL11 was detected in endosomes devoid of transferrin and co-localization between CCL11 and transferrin in endosomes could be seen only in some osteoclast precursors. This indicates that CCL11 is internalized or trafficked by an alternative route as compared to transferrin. Further studies will be performed to investigate surface binding and internalization mechanisms of CCL11 in osteoclasts and how it affects different osteoclast phenotypes.

Keywords: CCL11, osteoclasts, inflammation, bone

ND-P014

A novel fetuin-A-based fusion protein generates functional human osteoclasts in vitro

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Background: Fetuin-A (alpha$_2$-HS-glycoprotein) is a major systemic inhibitor of ectopic calcification. Fetuin-A binds calcium phosphate crystal nuclei with high affinity through negatively charged acidic amino acids clustered in its aminoterminal cystatin-like domain CY1. Thus fetuin-A prevents crystal growth and mineral precipitation from supersaturated solutions. We hypothesized that CY1 fusion should target osteoclast activators to sites of ectopic calcification.

Methods: To test this hypothesis, we designed a fusion protein of cystatin-like murine fetuin-A domain CY1 and the secreted form of murine receptor activator of nuclear factor-κβ ligand, RANKL. We expressed the fusion protein in CHO cells and tested purified recombinant murine fetuin-A CY1-RANKL in a functional osteoclast culture assay. Peripheral blood mononuclear cell (PBMCs) were isolated from whole blood collected from 3 volunteers and cultured in triplicates on bovine bone discs for 21 days under the following treatments; 1) negative control - 25 ng/ml macrophage-colony stimulating factor (MCSF)-treated cultures; 2) positive control - 25 ng/ml MCSF + 50 ng/ml recombinant human RANKL-treated cultures; 3) test culture - 25 ng/ml MCSF + 2000 ng/ml murine CY1-RANKL.

Results: Figure 1 shows microscopic views of resorption pits on bovine bone discs in both M-CSF + RANKL (Fig. 1A) and M-CSF + CY1-RANKL-treated cultures (Fig. 1B). The response to CY1-RANKL was comparable to the response to RANKL in that the percentage of resorbed area ranged from 0-74% (median 45) and 28-79 (median 43) (Fig. 2), respectively.

Conclusion: This study has shown that D1_FetuA-RANKL was able to robustly drive osteoclastic differentiation from PBMCs in vitro.

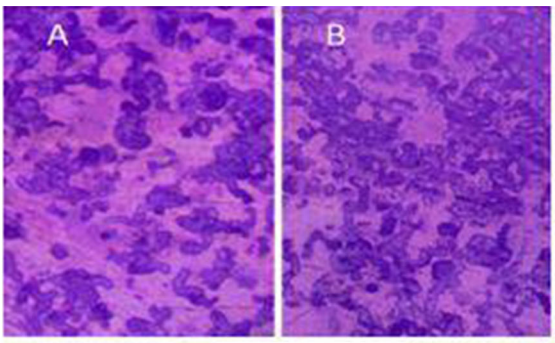

Fig. 1

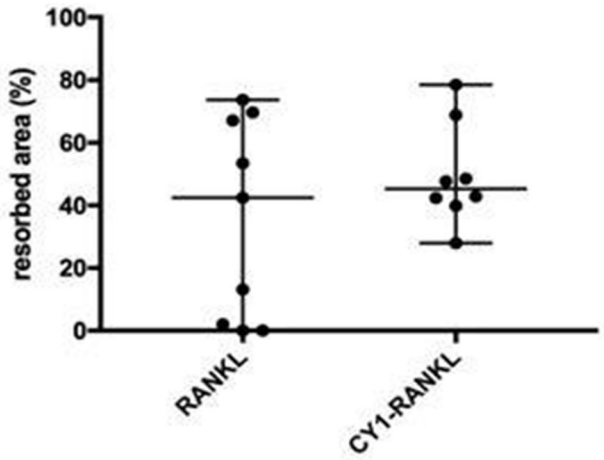

Fig. 2

[Fig 1 + 2]

The future application of this novel fusion protein as a theranostic tool to inhibit ectopic mineralization is yet to be established.

Keywords: Fetuin-A, RANKL, osteoclast, theranostics, ectopic calcification

ND-P015

CRYAB suppresses the occurrence of osteoarthritis by regulating the proliferation of chondrocytes and degradation of extracellular matrix

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Osteoarthritis (OA) is a chronic joint disease and hard to cure at present. Alpha B-crystallin (CRYAB) has been identified as a downregulated gene in OA cartilage. However, the precise roles and underlying molecular mechanisms of CRYAB in OA progression have not been elucidated. In the present study, we found that the expression of CRYAB in cartilages from patients with OA was significantly lower than that in the cartilages from patients with no prior medical history of OA. We established mouse models with OA by destabilization of the medial meniscus (DMM) surgery and found that the expression of CRYAB in OA cartilage was lower than that in the normal cartilages, too. Moreover, we demonstrated that the expression of CRYAB was increased during chondrogenic differentiation and cartilage development. Functional assays revealed that overexpression of CRYAB promoted the proliferation of chondrocytes and inhibited chondrocytes apoptosis, while knockdown of CRYAB presented opposite results. In addition, we showed that overexpression of CRYAB resulted in enhanced expression of anabolic markers, Col2a1 and ACAN, and reduced expression of catabolic markers, MMP13 and ADAMTS5. Conversely, knockdown of CRYAB blocked the expression of the anabolic markers and increased the expression of catabolic markers. Collectively, the results showed that CRYAB promoted the proliferation and extracellular matrix production of chondrocytes, and inhibited chondrocytes apoptosis and cartilage degradation simultaneously. Thus, CRYAB might be a potential therapeutic target for OA treatment.

This study was approved by the Research Ethics Committee of Nanjing Medical University, and individuals provided full written informed consent before the operative procedure.

Keywords: osteoarthritis, CRYAB, chondrocytes, proliferation, extracellular matrix

ND-P016

Progression of histopathological OA hallmarks following mechanical joint loading

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Although much of the aetiology remains unknown the approach to understanding osteoarthritis (OA) and pain is evolving from being cartilage focussed to a multifactorial view of the disease. Here we used the mechanical joint loading (MJL) model of OA to identify how knee pathology correlates to the development of nociceptive behaviour in loaded mice. MJL was used to induce OA in the right knee of 12-week-old male C57BL/6 mice (40 cycles, 9N, 3×/week for two weeks). Age and cage matched non-loaded mice served as controls. Nociceptive behaviour was monitored by measuring mechanical sensitivity thresholds and weight-bearing ratios before loading and at weeks one, three and six post-loading. At these time points, separate groups of loaded and non-loaded mice (n = 12/group) were sacrificed, joints collected, and corticosterone levels measured in the fur. μCT analyses of subchondral bone integrity was performed before joint sections were prepared for nerve quantification, and cartilage lesion and synovitis grading (scoring system from 0-6). Loaded mice showed a progressive increase in mechanical hypersensitivity paired with altered weight-bearing from three weeks post-loading. Initial ipsilateral cartilage lesions one week post-loading (1.8 ± 0.2) had worsened at weeks three (3.0 ± 0.3, $p < 0.0001$) and six (2.8 ± 0.2, $p = 0.0005$). The increased severity of lesions correlated with the development of mechanical hypersensitivity (correlation; 0.785, $p = 0.0025$). Loaded mice displayed increased synovitis (3.6 ± 0.2) compared to non-loaded mice (1.5 ± 0.2, $p < 0.0001$) one week post-loading which returned to normal by weeks three and six. Similarly, corticosterone levels were only increased at week one post-loading (0.21 ± 0.02 ng/mg) compared to non-loaded controls (0.14 ± 0.01 ng/mg, $p = 0.0087$). Subchondral bone integrity and nerve volume were unchanged with OA progression. Our data indicates that although the loading induces an initial stress reaction and local inflammation, these processes are not directly responsible for the nociceptive phenotype observed. Instead, MJL-induced nociception is mainly associated with OA-like progression of cartilage lesions.

ND-P017

The vitamin D metabolite ratio (VMR) - a useful tool for the assessment of patients with high serum 25-hydroxy vitamin D concentrations

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Vitamin D plays a critical role in the regulation of calcium homeostasis. An increase of intestinal calcium resorption is achieved through hydroxylation of 25(OH)D, which results in the formation of $1,25(OH)_2D$, the active metabolite of vitamin D. Current guidelines recommend an optimal serum concentration of 25-hydroxyvitamin D (25(OH)D) > 100 nmol/L. However, this may not always be appropriate. In 24-hydroxylase deficient individuals, 25(OH)D concentrations > 100 nmol/L may already be associated with symptomatic hypercalcaemia and nephrocalcinosis. The simultaneous measurement of $25(OH)D_3$, $25(OH)D_2$ and $24,25(OH)_2D_3$ may provide additional information in the assessment.

Here we established a liquid-chromatography-tandem-mass-spectrometry method for determination of $25(OH)D_3$, $25(OH)D_2$ and $24,25(OH)_2D_3$. The results are used to calculate the $24,25(OH)_2D_3$/$25(OH)D_3$ ratio (VMR). The utility of this method was evaluated in 4 patients with 25(OH)D > 100 nmol/L that were referred to our laboratory.

Our method has a run time of 18 min and is based on derivatization with 4-Phenyl-1,2,4-triazole-3,5-dione (PTAD). LOD was

1.5 nmol/L for $25(OH)D_3$, 0.3 nmol/L for $25(OH)_2$ and $24,25(OH)_2D_3$, respectively. LOQ was 3.1 nmol/L for $25(OH)D_3$, 1.0 nmol/L for $25(OH)_2$ and $24,25(OH)_2D_3$, respectively. Within-day and between-day imprecision were < 15% for all three metabolites.

In all four subjects our method measured a total 25(OH)D (= $25(OH)D_3 + 25(OH)D_2$) concentration of > 100 nmol/L. One subject had a markedly low $24,25(OH)_2D_3$ concentration of 0.1%. In contrast, in the other subjects $24,25(OH)_2D_3$ and VMR ranged between 14.6 - 29.1 nmol/L and 6.5 - 11.1 %, respectively. Further assessment of the patient with low VMR revealed chronic hypercalcemia and neuromuscular symptoms. Subsequent sequencing of the 24-hydroxylase gene (CYP 24A1) confirmed an inactivating mutation.

In conclusion, the simultaneous measurement of vitamin D metabolites with our new method and calculation of the VMR is a helpful tool to assess patients high serum 25(OH)D and hypercalcemia.

Keywords: 25-hydroxyvitamin D, 24,25-dihydroxyvitamin D, 24-hydroxylase deficiency, vitamin D metabolite ratio

ND-P018

Targeting the Calcium-Sensing Receptor in an inflammatory bowel disease model

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The Calcium-Sensing Receptor (CaSR) is best known for maintaining calcium homeostasis but in many tissues its functions are still unclear. Due to its involvement in inflammatory processes in the lung, CaSR antagonists are currently being developed into a novel anti-asthma treatment. We hypothesized that the CaSR could also be a potential target for the treatment of inflammatory bowel disease (IBD).

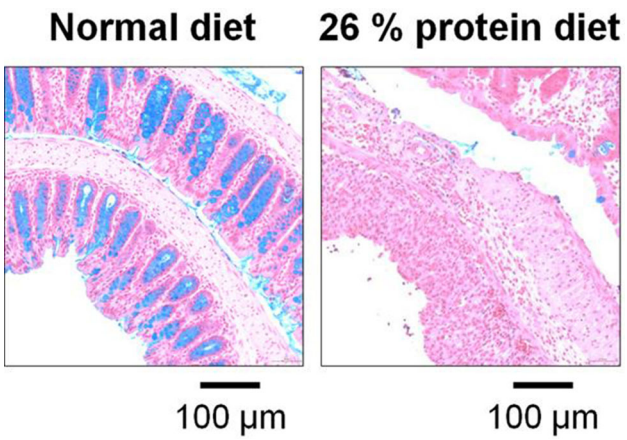
Colitis was chemically induced in female Balb/C mice by Dextran-Sulphate-Sodium. The animals were treated with dietary calcium and protein (nutritional agonists of the CaSR) or pharmacological CaSR modulators (the agonists cinacalcet and GSK30004774, and the antagonist NPS-2143; 10 mg/kg).

Compared to normal-diet (0.5% calcium), the high-calcium diet (1.5%) led only to non-significant reduction in inflammation. The high-protein diet (26%) however, had a strong pro-inflammatory effect, as measured by the length of the colon (5.3 ± 0.1 protein-diet *vs.* 6.1 ± 0.2 cm normal-diet, $p < 0.05$) and by the expression of mucin in colonocytes (Figure). The pharmacological CaSR agonists had no, or even a pro-inflammatory effect, while the CaSR antagonist led to a significant reduction in the cumulative inflammation score compared with the vehicle control (20.1 ± 14.9 *vs.* 31.0 ± 18.3 AU, $p < 0.05$). However, most of the other parameters were not affected by NPS-2143.

In conclusion, while dietary modulation of the CaSR did not yield significant beneficial effects, pharmacological inhibition of the CaSR might have the potential to be developed into a novel form of therapy for IBD.

Support: European Union (675228), FWF (29948-B28), Welsh Government (Ser Cymru II).

Keywords: CaSR, IBD, inflammation, calcium, colitis



[Mucin staining in colons of normal and high protein diet fed mice. Blue: Mucin, Pink: Tissue]

ND-P019

Red rice yeast (RRY). An alternative in Rheumatoid Arthritis (RA) and hyperlipidemia

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Introduction: Lipid profile control is essential in patients diagnosed with RA, since cardiovascular events (CV) are still the first cause of mortality. In those patients with a low or moderate CV risk index to diminish LDL cholesterol below 190 and 100 respectively, we must prescribe a statin. However, often-polymedicated patients may present intolerances or adverse side effects that limit their use. RRY, has been used in traditional Chinese medicine as a remedy to reduce the total cholesterol level (TC) in blood, LDL and TG. In addition, some studies underline its anti-inflammatory effect and its benefit in patients with inflammatory pathology.

Objective: Evaluate the efficacy of RRY in patients with elevated levels of TC and LDL in rheumatology clinic.

Methods: Prospective study that includes two cohorts of 30 patients with similar demographic characteristics. One with RA patients and the other without inflammatory disease. Both groups present high levels of TC and LDL. We study the demographic, clinical and lipid levels. A standard dose of RRY is administered to every patient and we evaluate the analytical response after 3 and 6 months.

Results: In the group of patients without RA (n = 30) the mean of baseline TC is 265.2 mg/dL ± 13.7 and LDL 176.4 ± 16. After 3 and 6 months, a significant decrease in both values was obtained (TC231 ± 19 and 209.8 ± 19 F:26.71 p 0.000 and LDL 143.9 ± 20 and 123.6 ± 19 F:22.51 p 0.000)

In the cohort of RA patients (n = 30) the mean baseline TC is 258.2 ± 14 and LDL is 176.7 ± 10. After 3 and 6 months a significant decrease of both values was obtained (TC 224 ± 24 and 196.1 ± 28 F:21.55 p 0.000 LDL 149.5 ± 12 and 122.4 ± 25 F:28.28 p 0.000)

Conclusions: The RRY significantly decreases the levels of TC and LDL in the 2 cohorts. In patients with RA and mild or moderate CV risk the use of RRY could be an effective therapeutic alternative free of adverse side effects. More studies are needed with a greater number of patients to corroborate these data.

ND-P020

The efficacy of complex kinesiotherapy in weight loss and muscle function improving in obesity patients

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Aim of the study was to estimate the affect of complex 3-week treatment with 4 kinesiotherapy methods on body weight loss and muscle function in patients with obesity.

Material and methods: 80 men and women aged 21-69 years old with alimentary obesity were enrolled in the study. The complex kinesiotherapy administered daily for 3 week and included interactive sensorimotor trainings on double unstable platform, kinesiohydrotherapy in a pool, special complex of physical exercises in a gym and ergocycle trainings.

Results: There was a significant reduction in body weight (111.3 ± 24.4 kg at baseline vs 107.9 ± 23.1 kg in 3 weeks; p = 0,000), in BMI (40.3 ± 8.1 vs 39.1 ± 7.7 kg/m^2; p = 0.000), in WC (113.4 ± 15.9 vs 109.2 ± 15.1 cm; p = 0.000) and in HC (124.1 ± 15.5 vs 119.7 ± 14.1 cm; p = 0.000) in treated obese patients. 10-meters-walk speed increased from 0.84 ± 0.15 m/sec at baseline to 0.88 ± 0.17 m/sec in 3 weeks (p = 0.000). Up-and-go test results improved from 8.4 ± 2.1 to 7.9 ± 2.09 sec (p = 0.000). We registered statistically significant elevation of the endurance to static loading in abdomen muscles from 13.1 ± 9.7 to 16.49 ± 12.8 sec (p = 0.000) and in back muscles from 14.8 ± 11.9 sec to 18.6 ± 14.9 sec (p = 0.000). The endurance to dynamic loading increased in abdomen muscles from 29.9 ± 11.2 to 34.84 ± 11.93 times (p = 0.000) and also in back muscles from 9.1 ± 7.4 to 12.2 ± 9.2 times (p = 0.000). Fall namber markably decreased from 0.14 ± 0.34 at baseline to 0.0 (95%CI: 0.02; 0.25) after completion of treatment.

Conclusions: Investigated complex treatment with 4 kinesiotherapy methods promotes body weight loss, WC and HC reduction in obesity. 3-week special training of obese patients is associated with increasing in gate speed and lower extremities muscle strength, and it also causes improvement in static and dynamic loading endurance of back and abdomen muscles. Those changes may probably improve balance function and decrease risk of falling in obese patients.

ND-P021

Pathogenic variants in *SGMS2* define a novel form of early-onset osteoporosis and implicate sphingomyelin metabolism in skeletal homeostasis

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Background: Mechanisms leading to osteoporosis are incompletely understood. Genetic disorders with skeletal fragility provide insight into metabolic pathways contributing to bone strength.

Methods: We evaluated six families with rare skeletal phenotypes and early-onset osteoporosis by next-generation sequencing. Functional implications of the identified gene variants were studied in cell lines and peripheral blood and by assessing patient-derived bone biopsies.

Results: In all families we identified a heterozygous variant in *SGMS2*, a gene prominently expressed in cortical bone and encoding the plasma membrane-resident sphingomyelin synthase SMS2. Four unrelated families shared the same nonsense variant c.148C>T (p.Arg50*) whereas the other families had a missense variant c.185T>G (p.Ile62Ser) or c.191T>G (p.Met64Arg). Subjects with p.Arg50* presented with childhood-onset osteoporosis with or without cranial sclerosis. Patients with p.Ile62Ser or p.Met64Arg had a more severe presentation with neonatal fractures, severe short stature, and spondylometaphyseal dysplasia. Several subjects had experienced transient peripheral facial nerve palsy or other neurological manifestations. Bone biopsies showed significantly altered bone material characteristics including defective bone mineralization. Osteoclast formation and function in vitro was normal. While the p.Arg50* mutation yielded a catalytically inactive enzyme, p.Ile62Ser and p.Met64Arg each enhanced the rate of de novo sphingomyelin production by blocking export of a functional enzyme from the endoplasmic reticulum.

Conclusions: *SGMS2* pathogenic variants underlie a spectrum of skeletal conditions ranging from isolated early-onset osteoporosis to complex skeletal dysplasia. Our findings suggest a novel and critical role for plasma membrane-bound sphingomyelin metabolism in skeletal homeostasis and in bone mineralization.

Keywords: osteoporosis, monogenic, sphingomyelin, skeletal dysplasia, qBEI

ND-P022

A new mouse model for *Osteogenesis imperfecta* reveals a link between polydenylation by TENT5A and the pathogenesis of the disease

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TENT5 is a novel family of non-canonical cytoplasmic poly(A) polymerases, class of enzymes responsible for adding adenines tails to 3' end of translationally dormant mRNAs. Using in vitro assays we proved that those proteins are indeed active enzymes able to polyadenylate RNA.

To functionally study the role of TENT5 proteins at the organismal level, we have generated a series mouse lines bearing either knock-out mutations or tag knock-ins in all genes of TENT5 family. We discovered that collectively all mutations negatively affect a wide spectrum of different aspects of mouse physiology. Although TENT5 proteins have very similar architecture, we have demonstrated that they are differentially expressed in mice tissues and their roles are very diverse.

The strongest phenotype observed is associated with TENT5A mutation. As TENT5A mutations have been found in some of *Osteogenesis imperfecta* patients, TENT5 KO may serve as a valuable model to study the pathophysiology of this disease.

We were able to show that TENT5A KO are born at expected Mendelian ratio (WT 18,7%, ± 52,5%, −/− 28,8%, n = 141) and their survival is not impaired. TENT5A KO are significantly smaller than their WT littermates (WT: 21 ± 1,2 [g], n = 20; KO: 14,5 ± 0,5 [g], n = 13; p < 0,0001). Homozygotic mutants exhibit abnormal posture with variable severity. Most commonly observed defects consist of shorter, abnormally curved limb bones and spine deformities. TENT5A KO exhibit delayed bone mineralization. Alkaline phosphatase level in TENTA KO is significantly increased comparing to WT mice (WT: 176,1 ± 21,5 [U/ml] n = 5, KO: 389,0 ± 41,5 [U/

ml] n = 4; $p < 0{,}0018$). We also observe other phenotypes such as mild anemia, low glucose level, underdeveloped mammary glands and female infertility.

Ethical approval for the procedures on animals was obtained from I Local Ethical Commission for Experiments on Animals in Warsaw (decisions no 176/2016, 781/2018, 783/2018).

ND-P023

The killifish (Nothobranchius furzeri) as unique short-lived vertebrate model to study musculoskeletal aging in a time-lapse

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The human skeleton possesses remarkable mechanical and biological characteristics to withstand extensive mechanical loads throughout life. While studies on mammalian bone have led to substantial understanding of various bone-degrading factors including physical immobilization and malnutrition, treatment options for major musculoskeletal diseases including osteoporosis and osteoarthritis are still limited. Here, we aim to establish the short-lived killifish with a life span of $\sim$ 6 months as unique short-lived vertebrate model for skeletal aging.

Male killifish of three age groups (9, 12, and 25 weeks-old) were investigated with focus on the skeletal morphology, vertebral bone (VB) structure and composition using contact X-ray radiography and micro-computed tomography.

Results indicated that vertebral bone volume (BV) increased as a function of age (BV in young, adult, aged killifish: $38 \pm 2\%$, $41 \pm 2\%$, $51 \pm 3\%$, 3 VB/group; young vs. aged: $p = 0.002$; adult vs. aged: $p = 0.01$; ANOVA with Tukey HSD). The same trends were observed for vertebral thickness and vertebral body length. In contrast, bone mineral density increased from young to adult and then decreased in aged killifish (1.37 ± 0.02 gHAP/cm^3; 1.48 ± 0.01 gHAP/cm^3; 1.36 ± 0.02 gHAP/cm^3, n = 3 VB/group, young vs. adult: $p = 0.06$; adult vs. aged: $p = 0.048$; young vs. aged: $p = 0.966$; ANOVA with Tukey HSD), which is in line with human age-dependency of bone mass, where bone mass increases during youth until reaching its peak value in adulthood and decreases again with aging.

The observed compositional changes in the killifish vertebral column potentially serve as an explanation for the spinal curvatures that were observed with radiography in these fish when aged, and that are reminiscent of the occurrence of human kyphosis in the elderly. In the scope of longitudinal studies, the killifish clearly has a remarkable potential to foster the development of preventive strategies to counteract aging-related skeletal decay and further to elucidate cellular and structural bone changes throughout skeletal aging.

ND-P024

The body composition analysis as a complementary tool in the screening of bone structural abnormalities

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Objective: The importance of early diagnosis, bone-healthy lifestyle, and medication are obligate for remaining fracture free. Dual-energy X-ray absorptiometry and ultrasound densitometry are widely used to screen osteoporosis and other bone structural diseases. Bioelectrical impedance analysis (BIA) devices can also estimate bone mineral content (BMC) but it has not been recommended for diagnostic purposes. The main aim of the present study was to analyse whether the low bone mineral content and the low levels of the body composition' components (e.g. low muscle mass) can predict low QUS parameter (BUA) in premenopausal and postmenopausal women.

Material and methods: Healthy premenopausal women (n: 130, 18-45 years) and postmenopausal women (n: 130, 46-75 years) were enrolled to the present analysis. BMC (kg) was estimated by InBody 720 analyser. Bone structure was measured by ultrasound osteometer (DTU-One Osteometer). Broadband ultrasound attenuation (BUA, dB/MHz) was used to assess bone structure. Body mass components, absolute bone mass and muscle mass (kg), were estimated by Drinkwater-Ross anthropometric method. Relative body components were expressed in the percentage of stature.

Results: The age changes of BMC, absolute and relative bone mass, muscle mass and bone structural parameters were analysed in premenopausal and postmenopausal women. BMC ($r = 0.42$, $p < 0.01$), muscle mass ($r = 0.40$, $p < 0.01$) and absolute bone mass ($r = 0.37$, $p < 0.01$) were correlated (Pearson correlation) highly with BUA in premenopausal women. In postmenopausal women weaker relationship was identified between BUA and its hypothetical predictive factors.

Conclusion: BMC and the other studied body mass components alone do not provide enough information to identify osteoporosis, but can complete and widen the screening methods for bone structural diseases. The bone mineral density of healthy premenopausal women with low BMC, low bone mass and/or low muscle mass values should be measured.

Keywords: bone mineral content, bone structure, body composition

ND-P025

Patients on dialysis have markedly abnormal cortical hip parameters by dual-energy X-ray absorptiometry

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Patients with end-stage kidney disease (ESKD) have higher fracture rates and post-fracture mortality than the general population, but bone mineral density by dual energy X-ray absorptiometry (DXA) is less predictive of fracture in this population. Data from bone biopsy and high-resolution imaging indicate that patients on dialysis have reduced cortical thickness and increased cortical porosity, which contribute to fracture risk. The aim of this study was to assess cortical parameters using DXA in patients with ESKD. Using advanced hip analysis, normal age-related ranges were determined from 752 female and 861 male femur scans, and were compared to scans of 226 dialysis patients at time of transplantation. Female dialysis patients had lower cortical thickness (mm) at the femoral neck (2.59 ± 1.42 vs. 5.23 ± 1.85), calcar (3.46 ± 1.07 vs. 5.09 ± 1.30) and femoral shaft (4.42 ± 1.21 vs. 7.44 ± 2.07); $p < 0.001$ for each site. Buckling ratios (BR), higher values indicating greater femoral neck instability, were also higher for these women (8.21 ± 4.6 vs.

3.63 ± 1.42, p < 0.001). All findings were similar for men. Prevalent fracture was documented in 29% of dialysis patients, and in adjusted models, lower femoral neck cortical thickness and a higher BR were associated respectively with a 1.73 (1.22-2.46) and 1.82 (1.49-2.86) fold increased risk of prevalent vertebral fracture per standard deviation change. Cortical parameters measured by DXA are markedly abnormal in dialysis patients and are associated with prevalent vertebral fracture. These parameters should be assessed prospectively in patients with ESKD for utility in fracture prediction and targeting treatment.

Keywords: chronic kidney disease, advanced hip analysis, dual-energy X-ray absorptiometry, bone mineral density

ND-P026

Fracture-Liaison-Service in Germany: T-Score results of DXA measurements in patients suffering from a fragility fracture

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In a large number of international studies, Fracture Liaison Services (FLS) have been shown to be an effective method for secondary prevention after a fragility fracture. However, only insufficient data on FLS is available for Germany, so a research project was launched to investigate the effects of FLS in Germany.

This university hospital-based FLS was a cooperation project between the department of trauma surgery and the osteoporosis outpatient unit. Patients with fragility fracture were identified on the trauma surgery ward. The patient's individual risk factors for osteoporosis were captured by a questionnaire. If necessary, osteoporosis diagnostics and therapy were offered to the patients. Diagnostic work-up and treatment initiation were done at the Osteoporosis outpatient clinic. Bone density measurements were performed, mostly by dual-energy X-ray absorptiometry (DXA), in 81% of patients during the following year. Follow-up interviews were conducted after 3 months and 12 months, asking for state of health and treatment adherence.

Our analysis includes 241 patients (183 female and 58 male patients) with an average age of 72.5 years. Follow-up interviews were possible with 135 patients after 3 months and with 155 patients after 12 months. 39% (n = 94) of the baseline patients had ever had a bone densitometry before the FLS visit. 3 months after the intervention, 56% (n = 76) and after 12 months 81% (n = 126) had received a bone densitometry. 14% of the patients had a normal T-Score, while osteopenia was detected in 41% and in 45% osteoporosis was diagnosed. 54% of the FLS-patients would have had an indication for specific osteoporosis treatment even without their fracture.

Our data show the high prevalence of osteopenia and osteoporosis among patients who suffered a fragility fracture. Fracture Liaison Services increase the rates of osteoporosis diagnosis and treatment.

Keywords: Osteoporosis, Fracture-Liaison-Service, Bone-density-Measurement

ND-P027

The association between abdominal obesity and osteoporotic fractures among elderly Israeli women

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Objective: We aimed to investigate the association between abdominal obesity, body mass index (BMI) and osteoporotic fractures prevalence in a sample of community dwelling elderly Israeli women.

Methods: The data presented in this cross-sectional study are part of the dataset generated from a survey ('Mabat Zahav') carried out by the Israeli Ministry of Health, the Israel Center for Disease Control and The Israeli Society of Hypertension. Data on osteoporotic fractures site and circumstances were self-reported, and height, weight, waist and calf circumferences were measured.

Results: Sixty-five women reported osteoporotic fractures (14 hip fractures, 18 vertebral fractures and 39 wrist fractures). The mean age was 73. 9 ± 5.9 years, mean BMI was 29.9 ± 5.1 kg/m^2 and mean WC was 93.9 ± 12.3 cm. While BMI was not associated with osteoporotic fractures, abdominal obesity (WC > 88 cm) was positively associated with osteoporotic fractures, independently of age, smoking, physical activity (Middle and high WC tertiles [3.147(95% CI, 1.411-7.020),2.776(95%CI, 1.054-7.307), respectively].

Conclusions: Among this sample of elderly women, abdominal obesity was positively associated with fragility fractures while BMI was not. Waist circumference, an easily measured anthropometric indicator, may be useful for assessing the risk of osteoporotic fractures in elderly women.

ND-P028

Sex hormones interaction with trabecular bone score and mineral density in men

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The aim of this study was to evaluate the relationship of sex hormones with bone quality and mineral density in men.

Materials and methods: We've examined 72 men aged 40-87 years. Depending on their body mass index (BMI), all the subjects were divided into 2 groups: Group I - 19 men with obesity whose BMI was ≥ 30 kg/m^2 (mean age 60.3 ± 10.8 years), and Group II - 53 men without obesity and BMI of < 30 kg/m^2 (mean age - 60.5 ± 13.5 years). The BMD was measured by DXA (Prodigy, GEHC Lunar, Madison, WI, USA). The TBS of L1-L4 was assessed by means of TBS iNsight® software installed on our DXA machine (product of Med-Imaps, Pessac, France). Total testosterone (TT) and SHBG were measured in all the subjects using an enzyme immunoassay method.

Results: In general, we found that obese men have a significantly higher BMD at the level of lumbar spine (group I - 1.402 ± 0.232 g/cm^2, group II - 1.203 ± 0.245 g/cm^2, F = 9.08, p = 0.004) and femoral neck (I group - 1.050 ± 0.141 g/cm^2, group II - 0.925 ± 0.164 g/cm ^{2}, F = 8.80, p = 0.004) in comparison with men of no obesity. Significant differences between the groups for the TBS were not found. Obese men have a significantly lower TT (group I - 12.55 ± 3.48, group II - 17.64 ± 6.10, F = 11.74, p = 0.001) and SHBG (group I - 43.03 ± 20.27, group II - 58.15 ± 25.39, F = 5.46, p = 0.02). The level of SHBG increased with age and there was a probable negative correlation with BMD of femoral neck (r = − 0.39; p < 0.001). There was no significant correlation between total testosterone and BMD of femoral neck in men with a normal body weight (r = − 0.19, p = 0.2) and an obesity (r = 0.02, p = 0.93).

Conclusions: Men with obesity have a significantly lower TT and SHBG, but their BMD is significantly higher than the one of men with a normal weight.

ND-P029

Comparison of the efficacy of eenosumab and zoledronic acid in postmenopausal women

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Introduction: Denosumab and zoledronic acid (ZOL) currently represent the most potent antiresorptive agents for the treatment of osteoporosis. They both decrease bone turnover, increase bone mineral density (BMD), and reduce the risk of fractures. Injectable osteoporosis drugs are increasing in popularity due to their efficacy and convenient administration. .

Objective: To compare the effect of denosumab and ZOL after 1 year administration on BMD, trabecular bone score (TBS), and bone turnover markers.

Methods: A total of 305 postmenopausal women with low bone mass were included. As the denosumab group, 122 patients were recruited from 2017 to 2018, and were administered subcutaneously 60 mg every 6 months. We retrospectively reviewed 183 patients from 2015 to 2017 as the ZOL group who was treated with ZOL 5 mg intravenously yearly. BMD, TBS, and C-terminal cross-linking telopeptide of type 1 collagen (CTX) were obtained at baseline and 12 months after denosumab injection or ZOL infusion and were compared between two groups.

Results: BMD change from baseline at month 12 was significantly greater with denosumab compared with ZOL at the lumbar spine (4.45% vs. 2.41%; $p < 0.001$), total hip (2.06% vs. 0.04%; $p < 0.001$), and femoral neck (3.12% vs. 0.17%; $p < 0.001$). Denosumab group led to significantly greater reduction of CTX compared with ZOL group ($p = 0.041$). There was no statistically significant difference between the two groups in terms of fracture risk and adverse events.

Conclusions: In postmenopausal women with osteoporosis, denosumab was associated with greater BMD increase at all measured skeletal sites and greater inhibition of bone remodeling compared with ZOL.

ND-P030

A case report of pregnancy and lactation-associated osteoporosis

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Pregnancy and lactation-associated osteoporosis (PLO) is a rare form of osteoporosis. The incidence of PLO is 0.4 in 100000 women.

We report a case of a 33 year old woman who suffered from back pain and compression fracture in the vertebral column 2 months after delivery. Her medical history was not remarcable for chronic disease, drug use, smoking or alcohol use. The patient took vegetarian diet, lack of sunshine and exercise. She breastfed for 2 months and menstruation has turned to normal. Physical examination demonstrated tenderness of back, laboratory examinations including serum ALP, PTH, Ca, P were within normal ranges. MRI showed vertebral fracture of vertebra ThXII. Z-score of lumbal spine 1-4 was -3.9. The patient was diagnosed with PLO. We advised against breastfeeding, vitamin D3 3000 IU/die and citrocalcium 1000 mg medication.

With 3 month treatment of stopping breastfeeding vitamin D3 3000 IU/die and calcium 1000 mg clinical symptoms gradually improved. However 25-OH-D3 level was not increased because the patient was still lack of sunlight and activity. After increasing the dose of Vitamin D3 supplementation the 25-OH-D3 level gradually increased. 3 months later her back pain decreased significantly, bone metabolic index, BMD has improved.

ND-P031

Osteoporosis treatment effectively reduces the rate of any-cause hospitalization: data from a nation-wide analysis

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Introduction: Osteoporosis and its complications represents a significant part of healthcare budgets. Treatment of osteoporosis is cost-effective by preventing fragility fractures. There is only fewer data on the effect on hospitalization rate which is also a factor in cost-effectiveness. The aim of our investigation was to examine the effect of osteoporosis treatment on the hospitalization rate in a real-life, nation-wide population.

Methods: All citizens are registered in the database of National Health Insurance Fund of Hungary with a unique ID. Every medication redeeming and the established ICD code is also registered. We have chosen all female patients from the database who were > 55 years old, had newly diagnosed osteoporosis between 2011 and 2017 and redeemed at least one anti-osteoporotic medication. We grouped the selected patients as adherent ($> 80\%$ persistence on medication) and non-adherent ($< 20\%$). Primary outcome was the hospitalization rate per year. Secondary outcomes were time to first hospitalization, hospitalization duration and fracture incidence.

Results: We analyzed the data of 1693 adherent and 9582 non-adherent patients. Majority of the patients received bisphosphonates (97%). The rate of pre-treatment hospitalization did not differ significantly (0.13 vs. 0.11 admissions/patient-year). The osteoporosis treatment significantly decreased the risk for any-cause hospitalization (0.06 vs. 0.13 event/patient-year) and the duration of hospitalizations (5.7 vs. 7.3 days/year). The time to first hospitalization was also significantly longer in the adherent group (1348 vs. 1262 days). The good adherence rate was also proved by the decreased fracture incidence (4.8%/year vs. 6.4%/year). The fracture count was much lower than the hospitalization rate, so we can conclude that the majority of hospitalizations were not related to fragility fractures.

Conclusion: Anti-osteoporotic treatment can effectively reduce the hospitalization rate in a postmenopausal women population when prescribed for primary fracture prevention. This is also a factor of cost-effectiveness of these treatments.

ND-P032

Clinical recommendations for the prevention of MRONJ

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Introduction: The correlation between bisphosphonates and the osteonecrosis of the jaw was found in 2003, it has been considered to be an individual disease since 2005. Upon the recommendation of the AOOMS the BRONJ was modified to MRONJ in 2014 as it has become obvious that besides taking bisphosphonates antiresorptive

and antiangiogenic medicines may also lead to the osteonecrosis of the jaw. The recommendations of the different nations differ considerably as far as the possibilities of prevention of MRONJ are concerned.
Materials and methods: We have studied 15 different recommendations for prevention and treatment of MRONJ, the latest available ones from between 2006 and 2018. The recommendations were from the following countries: Australia, Spain, France, Japan, Ireland, USA (AAE, AOOMS), Hungary, Scotland, Germany, Romania, Canada, Austria, Korea and Italy. We have studied the following elements of the guidelines: risk groups, drug holiday, antibiotic prophylaxis, dental treatment before the commencing antiresorptive therapy.
Results: 12 recommendations out of 15 emphasize the significance of achieving good oral health and hygiene before starting the treatment, 7 of them recommend some kind of antibiotic prophylaxis, 7 of them mention the possibility of the drug holiday as a factor with possibly positive effect and 9 of them use risk groups.
Discussion: There are few researches concerning the topic and they are not evidence based enough or not evidence based at all. It is clear from these data that judgement of the medical activities to prevent MRONJ is not uniform. It seems to be sure that good communication between the doctor supervising the therapy and the dentist, achieving good oral health and hygiene and informing the patients that the therapy used may have effects on the dental treatment are of great importance.
Keywords: BRONJ, MRONJ

ND-P033

Romanian teriparatide therapeutic protocol- relationship to the patient's profile and adherence to treatment

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Background: Starting with august 2014, teriparatide has been available as fully reimbursed treatment under a special national osteoporosis therapeutic protocol targeting osteoporotic patients with prevalent fractures, glucocorticoid exposure, long term bisphosphonate (BP) treatment or specifically defined non responders to BP.
Subjects were 44 patients admitted in our department who received teriparatide treatment and had more than 2 years since the initiation moment.
Materials and methods: Retrospective analysis of medical records: inclusion criteria, densitometric and fracture risk related, adherence to treatment and after treatment options data was also collected.
Results: 44 patients (42 women and 2 men with mean age of 67 yo) were included in the free of charge treatment protocol more the 2 years before our analysis began: out of 44 patients, 7 as first antiosteoporotic treatment and 37 after BP. 34 out of 44 had prevalent fractures, 12 had glucocorticoid exposure, 5 had more than 5 yrs of BP. 16 out of 44 patients completed the 2 years treatment, 4 completed only one year due to lack of minimum efficiency required by the protocol, 19 were lost of follow up and considered non adherent and 5 quit treatment due to adverse effects. Persistence to treatment was 20,5 months and the rate of good persistence was 59 %. Of those who completed treatment received antiresorbtive treatment after, 8 zoledronic acid, 3 denosumab, and 5 other kind of BP
Conclusion: The persistence to teriparatide treatment was 59%, most frequent documented lost of adherence to treatment being around 15 months; yet, the increased number of patients lost of follow up between 12 and 24 months could be due to protocol's gaps rather that non adherence. The profile of the patients who lost adherence was not different from the adherent ones regarding densitometry age, medical history, previous treatment.
Keywords: teriparatide, adherence

ND-P034

Manifestations of left ventricle function and arrhythmia in patients with long-standing hypoparathyroidism and pseudohypoparathyroidism

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Background: Hypocalcemia and hyperphosphatemia have adverse effects on cardiovascular system. Cardiac damage due to severe hypocalcemia has been reported. However, data about the affection of long-standing hypoparathyroidism (HP) and pseudohypoparathyroidism (PHP) on heart are still limited.
Objective: Investigate the effect of long-standing HP and PHP on cardiac structure, heart function and conductive system in patients receiving regular treatment.
Methods: 18 HP and 8 PHP patients with regular follow-up aged 45.4 ± 15.4 and 22.1 ± 6.4 years, and 26 age- and gender-matched healthy controls were included. General characteristics, biochemical indexes were recorded. Cardiac function and structure were assessed by creatine kinase (CK), creatine kinase-myocardial isoenzyme (CKMB), cardiac troponin I (cTnI), B-type natriuretic peptide (BNP) and echocardiography. The 12-lead electrocardiogram and 24 hour Holter electrocardiography were performed to evaluate the conductive function.
Results: Levels of serum calcium in HP and PHP were 2.05 ± 0.16 mmol/L and 2.25 ± 0.19 mmol/L, respectively. CK, CKMB, cTnI and BNP were within normal range. Adjusting for age of heart evaluation and body mass index, all M-mode measurements, left ventricular mass (LVM), LVM index and relative wall thickness were comparable between patients and controls. The mean LVEF were $68.4 \pm 6.4\%$ and $66.5 \pm 7.4\%$ in HP and PHP patients, respectively. The cardiac geometry was normal in all patients. Prolongation of corrected QT (QTc) intervals occurred in 52.6% (10/19) of patients; and 6.7% (1/15) of patients had more than 100 episodes of supraventricular and ventricular extrasystoles as well as supraventricular tachycardia. None of above arrhythmia was related to any severe clinical events.
Conclusions: Hypoparathyroidism and pseudohypoparathyroidism patients with well controlled serum calcium presented normal heart morphology and ventricular function, as well as prolonged QTc intervals and a small percentage of mild arrhythmias of little clinical significance.
Keywords: hypoparathyroidism, pseudohypoparathyroidism, heart, arrhythmia

ND-P035

Gender features of bone mineral density in the Ukrainian patients with neurological disorders

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The **aim of** this research was to study the gender features of bone mineral density and body composition indexes in patients with stroke and Parkinson´s disease.

We've preformed a cross-sectional case-control research design and examined 295 patients aged 50-80 years old, who were divided into three groups: the first group - patients without PD and any other illnesses and conditions which may influence the bone state and turnover (control group, n = 126 (66 women and 60 men)), the second group - patients with PD (n = 85 (47 women and 38 men)), the third group - patients after stroke (n = 84, 40 men and 44 women).

Bone mineral density was measured using the dual-energy X-ray absorptiometry (DXA) method (Prodigy, GEHC Lunar, Madison, WI, USA).

Result: Our study showed an increased incidence of osteoporosis in patients with neurological disorders in comparison with the control group. Males after stroke had a significantly lower total body, upper extremity and trunk BMDs compared to indexes of control group without any differences in lower extremity BMDs in contrast to the women after stroke who had lower BMDs of total body, trunk and upper/lower extremities in comparison with parameters of the control group. The results of our study indicated significant difference between the BMD values in PD patients compared to controls in women at lumbar spine, femoral neck and proximal femur, distal radius and total body in contrast to men, who demonstrated lower BMD indexes of femoral neck, distal radius and total body compared with control group.

Conclusion: BMDs in patients with neurological disorders have their own national and gender particularities that should be taken into account during their assessment and development of rehabilitation programs.

ND-P036

Improvement of bone microarchitecture after 30 months of testosterone substitution therapy in young patients with Klinefelter syndrome

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Background: Klinefelter Syndrome (KS) patients, defined by a 47 XXY karyotype, suffer from osteoporosis, which characteristics remain unknown.

Patients and methods: In KS patients naïve from testosterone substitution therapy (TST), we assessed bone microarchitecture at distal radius and tibia by HR-pQCT, and body composition by DXA, before and after 30 months of TST. Sex steroids (total testosterone [tT], bioavailable testosterone [bio-T], and total 17β-estradiol [17βE2]) were measured at baseline.

Findings: Twenty-four KS patients (mean ± SD age: 23.7 ± 7.8 years) were paired with 72 age-matched controls. Low levels of tT (≤ 10·4 nmol/L), bio-T (≤ 2·25 nmol/L), and 17βE2 (≤ 66·0 pmol/L) were found in respectively 10 (42%), 15 (62%) and 17 (71%) KS patients. Relative appendicular lean mass (RALM) was significatively lower in KS patients (7.53 ± 1.28 vs 8.65 ± 1.05 kg/m^2, $p < 0.001$). They presented impaired cortical and trabecular compartments, particularly at the tibia, for example for cortical area at the tibia: 122.1 ± 28.4 vs. 161.8 ± 29.1 mm^2, $p < 0.001$. Those alterations were found mostly in KS patients with baseline low levels of sex steroids or low RALM. After a median 30·4 [29·7-31·0] months of TST, 16 (67%) of KS patients were reassessed. We observed an increase of cortical volumetric BMD and cortical thickness, respectively at the radius 787 ± 81 to 838 ± 46 mg/cm^3; $p < 0.01$ and 0.75 ± 0.25 to 0.86 ± 0.18 mm; $p < 0.05$ and at the tibia 847 ± 49 to 862 ± 44 mg/cm^3; $p < 0.01$ and 1.15 ± 0.25 to 1.26 ± 0.24 mm; $p < 0.05$. Trabecular areas decreased at both radius and tibia ($p < 0.05$).

Conclusion: Young TST naïve KS patients have early bone impairment at both radius and tibia, which improve after 30 months of THT. KS patients should benefit from TST before the achievement of peak bone mass especially if they are hypogonadic.

Keywords: HR-pCT, hypogonadism, osteoporosis, sex steroids, Klinefelter Syndrome

ND-P037

Bone mineral density and fractures after lung transplantation - a long-term observational study

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Objectives: Lung transplantation is commonly followed by bone loss leading to osteoporosis and fragility fractures, increased risk of mortality and reduced quality of life. Some patients have decreased bone density even before the transplantation due to the original lung disease, and many factors further contribute to the progression of osteoporosis after transplantation, like the immunosuppressive medications and nutritional and lifestyle status. This study aimed to assess bone mineral density and incidence of fractures in patients living with transplanted lung.

Patients and Methods: This observational study analysed the medical records of 19 patients (9 females/10 males, mean age 42.8 ± 15.5 ys) transplanted between 2005 and 2015. The original pulmonary diseases leading to transplantation were as follows: Chronic Obstructive Pulmonary Diseases (COPD, 42.1%), primary and secondary Pulmonary Hypertension (21.1%) and Cystic Fibrosis (36.8%). All patients have long-term been treated by oral glucocorticoids. BMD were measured by DXA. Demographics, clinical variables of lung disease, laboratory data and osteoporosis risk factors were analysed, and fracture occurrence was detected.

Results: Osteoporosis (T-score < -2.5) was found in 20% of the study population, and osteopenia (T-score from -1.0 to -2.5) in approximately 60%, while only 20% had normal bone density (T-score > -1.0). 3 patients suffered low-trauma fracture after transplantation. COPD patients had higher prevalence of osteoporosis. Patients with osteopenia/osteoporosis were significantly older (45.2 vs. 32,7 ys, $p = 0.032$) and had a lower body mass index (20.9 vs 24.1 kg/m^2, $p = 0.041$) than patients without osteopenia/osteoporosis.

Summary: Even in a small group of patients, a high prevalence of osteopenia and osteoporosis has been demonstrated in this study. COPD seems to make a significant contribution to the development of bone loss. The measurement of bone mass and usage of bone-preserving therapy is suggested both in the pre-transplant and in the post-transplant period.

Keywords: bone mineral density, fracture, lung transplantation

ND-P038

Bone structural examinations in new subgroups of Turner-syndrome patients

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Objective: Turner-syndrome is one of the most common genetic disorder which is characterized by the absence of the entire or the part of second X sex chromosome. The presence of the X chromosome is necessary for the development of ovaries, which produce estrogen. Estrogen plays an important role in the development of bone mineral content and the formation of peak bone mass. The main aim of the presentation is to introduce the results of a body and bone structural study of Turner-syndrome patients by highlighting the most important relationships between body structure, karyotype and treatment type in the syndrome.

Subjects and Methods: Twenty girls and women with Turner-syndrome, who received or not received hormone replacements, were studied. Bone mass was estimated by the Drinkwater-Ross four component model. Bone structure was measured by ultrasound DTU-One osteometer. Bone mineral density was measured by XCT 2000 peripherial Quantitative Computed Tomography. Muscle mass, fat mass and visceral fat area were estimated by bioelectrical impedance analysis (InBody 720 device). The pattern of body and bone structure parameters were analyzed by cluster analysis.

Results: The Turner patients' body structure was very inhomogeneous. Based on the results of the cluster analysis of bone and body structural parameters, we could separate the subgroups of the syndrome thus bone density and the relative length of the limb segments were the most important separators. The subgroups were: subgroup A - a group of patients who have 45,X0 karyotypes; subgroup B: patients with 46,XX/45,X0 mosaic karyotypes; subgroup C - patients with isochromosomes (subgroup D - patients who could not be classified into the other three groups). The bone structure and the bone density parameters significant differences between the subgroups.

Conclusion: Regular examinations of body and bone structure are of high importance for patients with Turner-syndrome.

Keywords: Turner-syndrome, bone density, bone structure

ND-P039

Pro-osteogenic PEDF-PPARγ axis is antagonized by TGF-β pathway in type VI osteogenesis imperfecta (OI)

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Background: Loss-of-function mutations in *SERPINF1*, which encodes pigment epithelium-derive factor (PEDF), cause type VI OI, a severe progressive bone dysplasia. The distinctive bone phenotype of type VI OI includes accumulation of unmineralized osteoid due to delayed mineralization and disorganization of bone matrix with "fish-scale" appearance. To investigate the underlying mechanisms of mineralization defect in type VI OI, osteoblasts from *Serpinf1* knockout mice, a mouse model of type VI OI, were utilized.

Results: *Serpinf1*-deficiency decreased PPARγ expression in differentiating osteoblasts. Consistently, *PPARG* expression was reduced in cells from type VI OI patients. *PPARG* down-regulation was also observed in atypical type VI OI, which is caused by a point mutation (p.Ser40Leu) in *IFITM5* and reduces *SERPINF1* expression. PPARγ plays a role in the formation of lipid droplets (LDs), which are lipid storage sites and broken down in times of need to generate ATP. PPARγ down-regulation in *Serpinf1*-deficient cells was accompanied by reduced expression of ATGL more than 2-fold ($P < 0.05$) and other LD-associated genes. Being also known as a PEDF-Receptor, ATGL catalyzes the first step of intracellular triacylglycerol hydrolysis. ATGL-deficient mouse osteoblasts showed massive accumulation of LDs due to the defect in LD break-down. Accordingly, ATGL-deficiency remarkably increased expression of PPARγ and LD-associated genes and also accelerated extracellular matrix mineralization. Previous studies using OI mouse models proposed that excessive activation of TGF-β signaling is a pathogenic mechanism of OI. We found that *Serpinf1*-deficiency increased TGF-β signaling pathway and that stimulation of TGF-β pathway inhibited expression of PEDF and PPARγ in osteoblasts by more than 70% ($P < 0.05$). This was accompanied by down-regulation of osteocalcin and PPARγ coactivator genes.

Conclusions: Our findings suggest that PEDF-PPARγ axis regulates osteogenesis via lipogenesis and that TGF-β pathway antagonizes the pro-osteogenic activity of PEDF-PPARγ axis.

Keywords: PEDF, PPARγ, TGF-β, lipogenesis, OI

ND-P040

A case of differential diagnosis of hypophosphatemic rickets in Raine syndrome

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Introduction: Raine syndrome [RS, osteosclerotic bone dysplasia] is a rare autosomal recessive lethal bone disease, affecting $1/10^6$ individuals, due to mutations in *FAM20C*, a 10-exons gene on chromosome 7p22.3 encoding a Golgi casein kinase that phosphorylates serine residues of many proteins, including fibroblast growth factor 23 [FGF23] and the SIBLINGs proteins involved in mineralization processes.

A milder form of RS, due to less severe mutations, allowing survival into adulthood and characterized by facial dysmorphism, depressed nasal bridge, short nose, exophthalmos, generalized osteosclerosis, mild hypophosphatemia and increased serum FGF23 levels, has been described.

Patient: An 11-year old female, firstborn to consanguineous parents (without evidence of bone diseases), with hypophosphatemia, taking cholecalciferol supplementation for vitamin D deficiency, was sent to our attention to confirm/exclude a diagnosis of hypophosphatemic rickets. She had short stature (without GH deficiency), visual and hearing defects, premature tooth loss, severe cognitive delay, craniostenosis, brachycephaly.

Methods: Biochemical markers and bone mineral density (BMD) were evaluated. Molecular analysis of *PHEX* gene was performed.

Results: Upon repeated measures, serum calcium, 25-OH-vitamin D, alkaline phosphatase, CTx and NTx were normal for age. Parathyroid hormone was higher than normal for age (104.5 ± 25.5 ng/L). Low serum phosphate (2.5 ± 0.2 mg/dL) was always found.

Normal BMD (with DXA): lumbar spine Z-score + 1; total body less head Z-score − 1.4.

X-rays: one vertebral fracture (D10); pre-metaphysis femoral-tibial bilateral *striae*.

PHEX gene analysis: no pathogenic mutations.

Treatment with phosphate salts (500 mg twice daily) was prescribed.

Recently, RS (*FAM20C* mutation) was diagnosed in a 10-month-old brother with a similar phenotype, and on this basis, genetic analysis of *FAM20C* and measurement of serum FGF23 are being carried out in our patient.

Conclusion: This experience suggests that the non-lethal form of RS should be suspected in unexplained cases of hypophosphatemia, in the presence of suggestive phenotypic features.

ND-P041

X-linked hypophosphataemia and hypoparathyroidism: amelioration of renal phosphate wasting and bone disease

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Introduction: Parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) regulate renal phosphate excretion through the same sodium-phosphate cotransporters. We reported previously two cases of XLH with hypoparathyroidism (HOPT), who normalization of renal tubular maximum reabsorption rate of phosphate (TmP/GFR) despite manifold elevation in FGF23, but both patients had chronic kidney disease (CKD). We sought to explore the interaction effect of FGF23 and PTH on TmP/GFR in XLH with HOPT.

Methods: The following 3 groups were compared with respect to FGF23, TmP/GFR, PTH, 1,25-dihydroxyvitamin D (1,25D), and bone remodelling markers: FGF23-dependent hypophosphataemia (FDH) (n = 28) including congenital hypophosphataemia (n = 25) and tumour induced osteomalacia (n = 3); CKD (n = 30); HOPT (n = 17). One case of XLH-HOPT self-administered daily 20 μg PTH1-34 (teriparatide) for 28 days in order to assess the response of TmP/GFR, FGF23, soluble Klotho, and nephrogenous cyclic adenosine monophosphate (NcAMP).

Results: FGF23 was highest in CKD ($p = 0.031$). TmP/GFR was lowest in FDH, intermediate in CKD, and highest in HOPT ($p < 0.001$). PTH was lowest in HOPT, intermediate in FDH, and highest in CKD ($p < 0.001$). 1,25D was similar in FDH and HOPT, but lower in CKD ($p < 0.001$). Bone remodelling markers were consistently lower in HOPT than FDH ($p < 0.001$). For the 2 cases of XLH-HOPT, TmP/GFR was higher than in all the cases of HBD, and TmP/GFR overlapped with the lower end of HOPT and with the upper end of CKD. Bone remodelling markers in the 2 cases approximated normality. After 28 days of teriparatide therapy in one case of XLH-HOPT, TmP/GFR lowered from 1.10 mmol/L to 0.48 mmol/L, without any change in FGF23 or soluble Klotho, but there was a marked increase in NcAMP.

Conclusion: In cases of FGF23 excess rendered PTH deficient, there is amelioration of renal phosphate wasting and consequent improvement in the associated bone disease.

Keywords: X-linked hypophosphataemia, hypoparathyroidism

ND-P042

Difficulties in the diagnosis of periapical translucencies of cemento-osseous dysplasia

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Cemento-osseous dysplasia (COD) is a rare non-neoplastic, fibro-osseous lesion of the jaws, associated with vital teeth usually without any clincal symptoms. It often causes a differential diagnostic problem in the early, osteolytic stage because it could imitate the image of a periapical lesion. According to the literature, it is most common in middle-aged African and Asian females, but in the past three years we have also reconized the disease in our own Caucasian patients. We present a rare familial and another case of COD in middle-aged Caucasian women, both confirmed by histological diagnosis. Our cases demostrate well the difficulties of differential diagnosis of cemento-osseous dysplasia and other periapical pathologies and highlight the risk of lacking the right diagnosis of COD, what can lead to unnecessary root canal treatment. In contrast to the severe, advanced cases described by Noffke (1) we have found mild, early stages of COD in our patients, what can be explained by the fact that Hungarians visit the dentist more often than Africans. Since we have found further cases similar to COD by checking imaging modalities, it raises the question whether COD is really so rare among Caucasian people or it is possible that many cases remain unrecognized or induce unnecessary root canal treatment by diagnosing periapical inflammation.

Keywords: cemento-osseous dysplasia, periapical translucencies, differential diagnosis, fibro-osseous lesions of the jaws