UNIVERSITATEA DE MEDICINĂ ŞI FARMACIE
"IULIU HAŢIEGANU" CLUJ-NAPOCA

CRISTIAN LORIN VOIA

CORELAŢII CLINICO-BIOLOGICE IN OSTEOPOROZĂ

TEZĂ DE DOCTORAT PENTRU OBŢINEREA TITLULUI DE DOCTOR ÎN ŞTIINTE MEDICALE, DOMENIUL MEDICINĂ, SPECIALITATEA ENDOCRINOLOGIE

REZUMAT

CONDUCĂTOR ŞTIINŢIFIC
PROF. DR. ILEANA DUNCEA

CLUJ-NAPOCA
2009

INTRODUCERE

Osteoporoza reprezinta una dintre cele mai frecvente boli ale varstnicului. Prin cresterea continua a proportiei varstnicilor in populatia generala a tarilor industrializate osteoporoza a devenit o cauza importanta de morbiditate si mortalitate. De aceea in ultimii ani acestei maladii i se acorda o atentie sporita.
Diagnosticul osteoporozei in etapa actuala cunoaste mai multe elemente si se recomanda evaluarea mai multor criterii pentru a se putea urmari evolutia, dar mai ales prevenirea complicatiilor bolii.
Prin cresterea numarului de medicamente antiosteoporotice problema esentiala ramane identificarea acelor pacienti care pot beneficia optim de pe urma terapiei.
Avand posibilitatea de a efectua un studiu biochimic si histopatologic in Centrul de Osteoporoza din Heidelberg (Germania) si cu ajutorul Departamentului de Patologie Osoasa din Hamburg-Eppendorf (Germania), sub conducerea profesorului Reinhard Ziegler (Heidelberg) in colaborare cu domnul profesor Liviu Gozariu (Cluj-Napoca) mi-am propus un studiu complex care sa compare in ce masura parametrii biochimici ai turnover-ului osos se coreleaza cu rezultatele histopatologice in vederea unei definiri cat mai exacte a ritmului de primenire osos.
De asemenea mi-am propus un studiu experimental care sa urmareasca influenta unui puternic bifosfonat (pamidronat) asupra evolutiei spontane a unei osteoporoze de tip inflamator indusa experimental prin urmarirea unor parametrii biochimici si fizici ososi.

As dori sa adresez cele mai sincere multumiri doamnei **Prof. Dr. Ileana Duncea**, conducatorul stiintific al acestei teze de doctorat, pentru indrumarea competenta si permanenta, probitatea stiintifica si increderea pe care mi-a acordat-o pe parcursul stagiului de pregatire si nu mai putin pentru asigurarea conditiilor cele mai optime in vederea realizarii lucrarii.
Fata de domnul **Prof. Dr. Liviu Gozariu**, membru al Academiei Romane ??? doresc sa imi exprim profunda recunostinta pentru ajutorul constant, solicitudinea si rigoarea stiiintifica fara de care realizarea acestei lucrari nu ar fi fost posibila.
Multumesc domnului **Dr. Pavel Orbai** pentru colaborarea in efectuarea studiilor experimentale la biobaza UMF si in laboratorul Clinicii de Endocrinologie Cluj-Napoca si pentru timpul pretios acordat indrumarii si muncii in laborator.
Domnului **Dr. S. Toader** de la biobaza UMF Cluj-Napoca ii adresez aceleasi calde multumiri penru crearea conditiilor necesare efectuarii cercetarilor experimentale.

Domnului sef de lucrari **Dr. Remus Campean** de la Catedra de Informatica Medicala a UMF "Iuliu Hatieganu" Cluj-Napoca ii multumesc pentru sprijinul acordat in realizarea acestei lucrari.
Nu in cele din urma multumirile mele se indreapta catre domnul **Prof. Dr. Reinhard Ziegler**, seful Departamentului de Endocrinologie si Boli Metabolice al Universitatii Heidelberg si catre domnul **PD Dr. Christian Kasperk**, seful Departamentului de Osteodensitometrie pentru onoarea de a ma primi in cadrul colectivului de cercetare, excelentele conditii de lucru oferite si spiritul colegial de care au dat dovada pe parcursul celor aproape doi ani petrecuti la aceasta clinica. In acest context doresc sa multumesc fundatiei **KAAD (Katholischer Akademischer Ausländerdienst)** pentru bursa acordata.

CERCETARI CLINICE

Au fost luati in studiu un numar de 132 pacienti in cadul cercetraii efectuate la Universitatea din Heidelberg, Clinica de Endocrinologie.
Nu au fost luati in studiu pacientii cu fracturi ale corpilor vertebrali precum si pacientii cu orice fel de fracura petrecuta in ultimul an; de asemenea au fost exclusi pacientii care au urmat anterior o terapie osteotropa. Tuturor pacientilor avand diagnosticul de osteoporoza anterior stabilit li s-a efectuat biopsie osoasa din creasta iliaca in vederea precizarii dinamicii osoase (tipul turnover-ului osos).
Toti bolnavii luati in studiu au fost impartiti pe baza criteriului histologic de apreciere a turnover-ului osos in:

I. lot cu turnover osos scazut
II. lot cu turnover osos crescut

Fiecare lot a fost la randul sau impartit in lot de barbati si lot de femei.
Al doilea criteriu de impartire a bolnavilor a fost varsta si anume totalitatea bolnavilor au fost impartiti in sase grupe:

I. bolnavi sub 45 ani cu turnover osos scazut
II. bolnavi intre 45-60 ani cu turnover osos scazut
III. bolnavi peste 60 ani cu turnover osos scazut
IV. bolnavi sub 45 ani cu turnover osos crescut
V. bolnavi intre 45-60 ani cu turnover osos crescut
VI. bolnavi peste 60 ani cu turnover osos crescut

Bolnavii au fost studiati pe criterii de varsta si tip de turnover osos si au fost impartiti in aceleasi grupe pentru femei si barbati, iar studiul s-a efectuat pe perioada internarii pacientilor.
Pentru precizarea diagnosticului de osteoporoza s-a efectuat osteodensitometrie utilizand metoda absorbtiometriei duale cu raze X (DXA), iar pentru aprecierea dinamicii metabolismului osos s-au determinat urmatorii parametrii biochimici: osteocalcina, fosfataza alcalina totala, deoxipiridinolina libera urinara.
Pentru evaluarea controlului homonal al metabolismului osos s-au determinat: hormonul tireotrop hipofizar (TSH), parathormonul (PTH), 25-OH vitamina D. Probele de sange au fost recoltate a jeun intre orele 8-10, au fost pregatite dupa standarde si apoi analizate.
Cercetarile au fost realizate cu acceptul neconditionat al pacientilor in conformitate cu prevederile normelor europene de deontologie profesionala si fara de care acest studiu nu putea fi realizat.

CERCETARI EXPERIMENTALE

In cadrul unui studiu realizat la Clinica de Endocrinologie Cluj-Napoca in colaborare cu Biobaza UMF Cluj-Napoca au fost selectionaţi şobolani de sex feminin (tip Bratislava) cu greutatea cuprinsă între 180-200 g, care au fost împărţiţi în 3 loturi. Toate animalele au primit zilnic hrană standard.
Pentru inducerea inflamatorie a osteopeniei s-a aplicat metoda descrisă de Minne: injectarea în 8 puncte în regiunea dorsală subcutanată a 400 mg talc (silicat de magneziu) steril dizolvat in 0,5 ml soluţie ser fiziologic.
După sacrificare au fost recoltate tibiile şobolanilor din cele 3 loturi. Tibiile au fost uscate, cantarite şi s-a determinat conţinutul de calciu şi magneziu din tibia uscată, calcinată la 600 grade Celsius. Dozările de calciu si magneziu s-au efectuat prin intermediul unui spectrofotometru cu absorbţie atomică la lungimea de undă 421 nm pentru calciu si respectiv 284 nm pentru magneziu. Densitatea osoasă s-a calculat prin raportarea masei (mg) la volum (cm^3). Măsurarea volumelor osoase s-a făcut cu un platismometru digital.
Au fost studiate trei loturi:

Lotul I (martorii)
A cuprins martorii:10 şobolani femele cu greutatea de 180-200 g fiecare, ce au fost sacrificaţi după 21 zile

Lotul II (IMO)
A fost format din 15 animale (şobolani femele) cu greutatea 180-200 g fiecare la care s-a indus IMO prin metoda descrisă. Sacrificarea s-a făcut după 21 zile.

Lotul III (IMO+P)
Acest lot a cuprins 15 animale (şobolani femele) cu greutatea între 180-200 g, la care s-a injectat intravenos inainte cu 3 zile pamidronat, după care s-a indus IMO.Animalele au fost sacrificate după 21 zile.

Pamidronatul disodic (Aredia) a fost administrat intravenos în doză unică de 0,15 mg.

I. REZULTATELE CERCETARILOR CLINICE

1. Parametrii biochimici ai turnoverului osos la femeile cu osteoporoza la care examenul histologic al osului a indicat un turnover scazut si comparatia statistica a acestora este prezentata in tabelul I/1.

Nr. grupei	Grupa de varsta	Osteocalcina: media (ng/ml) ±DS	Fosfataza alcalina:media (U/l)±DS	Deoxipiridinolina libera urinara:media(nM/mM)±DS
1	Sub 45 ani	4,06± 2,8	97,8±35,57	4,3±1,54
2	Intre 45-60 ani	5,3±2,03	114,46±22,07	4,63±2,07
3	Peste 60 ani	4,88±2,34	153,08±84,15	5,52±2,2
Semnificatia statistica (p)		0,5	0,04*	0,25

$p^{*}=p<0,05$

Intre mediile osteocalcinei si intre mediile deoxipiridinolinei urinare libere nu exista diferente statistic semnificative.
Intre mediile fosfatazei alcaline totale la cele trei grupe de varsta exista diferente statistic semnificative, motiv pentru care am trecut la comparare utilizand testul „t” student.

Comparatia statistica a mediilor fosfatazei alcaline pe grupe de varsta (tabelele I/2, I/3 si I/4):
Tabel I/2:

Numarul grupei	Grupa de varsta	Fosfataza alcalina totala (U/l) ±DS
1	Sub 45 ani	97,81±35,57
3	Peste 60 ani	153,08±84,15
p: grupa 1 fata de 3		0.009*

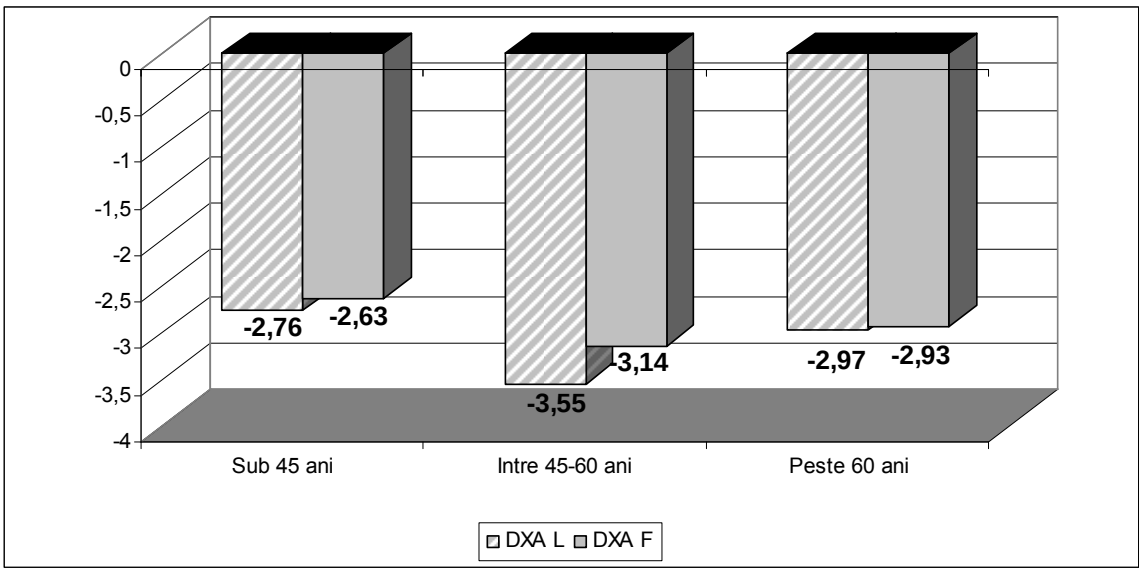
Tabel I/3:

Numarul grupei	Grupa de varsta	Fosfataza alcalina totala (U/l) ±DS
2	Intre 45-60 ani	114,46±22,07
3	Peste 60 ani	153,08±84,15
p: grupa 2 fata de 3		0,039*

Tabel I/4:

Numarul grupei	Grupa de varsta	Fosfataza alcalina totala (U/l)
1	Sub 45 ani	97,81±35,57
2	Intre 45-60 ani	114,46±22,07
p: grupa 1 fata de 2		0,19

Grafic 1: valorile comparative ale mediilor scorurilor T DXA lombare si femurale la femeile cu osteoporoza cu turnover scazut din cele trei grupe de varsta

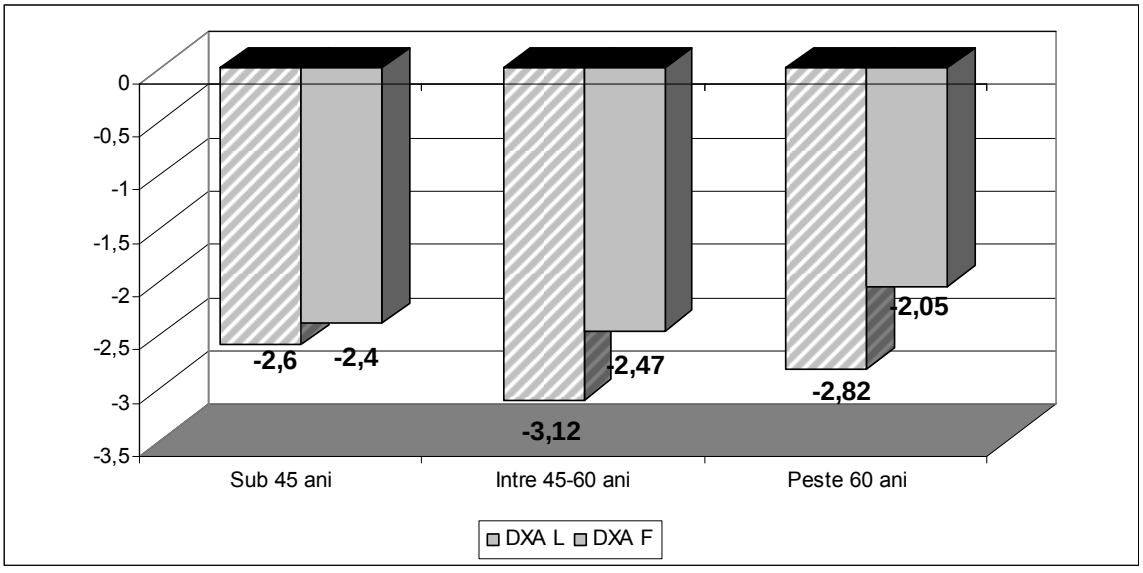


2. Comparatia statistica a mediilor osteocalcinei, fosfatazei alcaline totale si deoxipiridinolinei libere urinare pe grupe de varsta la barbatii cu osteoporoza cu turnover osos scazut sunt prezentate in tabelul II/1.
Tabel II/1:

Nr. grupei	Grupa de varsta	Osteocalcina (ng/ml):media +/-DS	Fosfataza alcalina totala (U/l):media +/-DS	Deoxipiridinolina libera urinara (nM/mM):media +/-DS
1	Sub 45 ani	5,64+/-1,40	113+/-28,33	4,61+/-1,4
2	Intre 45-60 ani	5,67+/-1,87	129,5+/-49,81	5,12+/-2,77
3	Peste 60 ani	4,85+/-2,26	123,66+/-42,22	4,05+/-1,74
Semnificatia statistica (p)		0,44	0,64	0,4

Intre mediile osteocalcinei, fosfatazei alcaline totale si deoxipiridinolinei urinare libere la barbatii cu osteoporoza cu turnover osos scazut din cele trei grupe de varsta nu exista diferente statistic semnificative (p>0,05).

Grafic 2: valorile comparative ale mediilor scorurilor T DXA lombare si femurale pe grupe de varsta la barbatii cu osteoporoza cu turnover scazut



3. Parametrii biochimici ai turnoverului osos la femeile cu osteoporoza la care examenul histologic al osului a indicat un turnover crescut

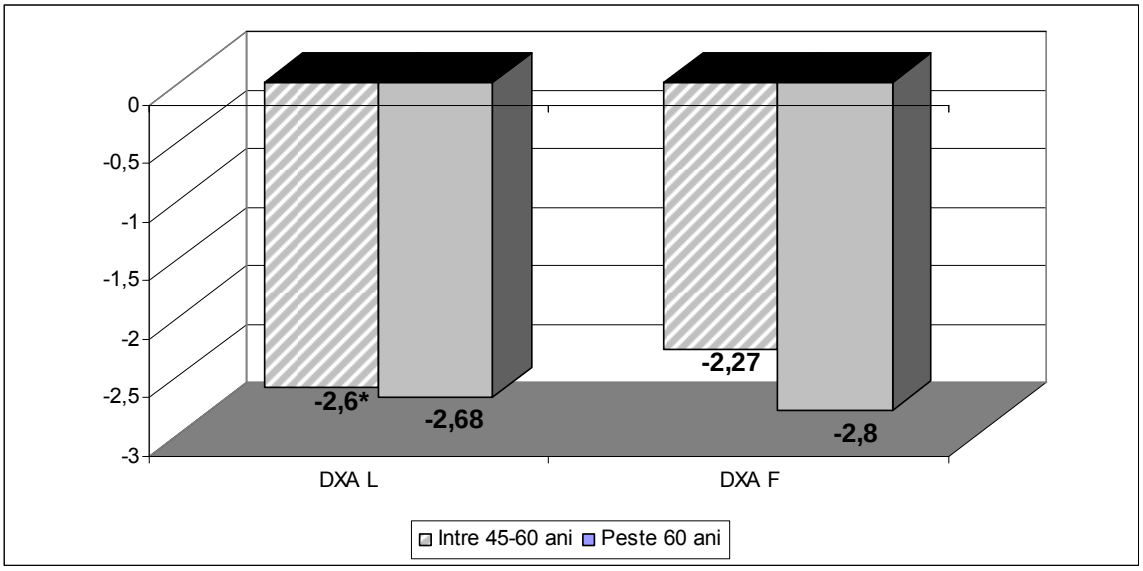
Pentru grupa de varsta sub 45 ani volumul esantionului este prea mic pentru a putea fi aplicat un test de semnificatie, de aceea aceste paciente au fost excluse din studiu.

Tabel III/1: comparatia statistica a mediilor osteocalcinei, fosfatazei alcaline totale si a deoxipiridinolinei libere urinare pe grupe de varsta la femei cu osteoporoza cu turnover osos crescut

Nr. grupei	Grupa de varsta	Osteocalcina (ng/ml):media +/-DS	Fosfataza alcalina totala (U/l):media +/-DS	Deoxipiridinolina libera urinara (nM/mM):media +/-DS
1	Intre 45-60 ani	6,86+/-3,29	124,3+/-53,12	5,27+/-2,59
2	Peste 60 ani	6,79+/-1,85	233,8+/-132,46	9,13+/-4,83
Semnificatia statisca (p)		0,95	0,03*	0,04

Intre mediile osteocalcinei si intre mediile deoxipiridinolinei libere urinare nu exista diferente statistic semnificative. Valorile medii ale osteocalcinei sunt in limite normale la ambele grupe, iar media valorilor DPD crosslinks la grupa de varsta peste 60 ani este crescuta, confirmand datele din literatura.

Grafic 3: valorile comparative ale mediilor scorurilor T DXA lombare si femurale pe grupe de varsta la femeile cu osteoporoza cu turnover crescut



Din graficul prezentat reies pierderile osoase mult mai accentuate la nivel femural odata cu inaintarea in varsta si anume la grupa de varsta peste 60 ani fata de cea de postmenopauza la femeile cu osteoporoza cu turnover osos crescut.

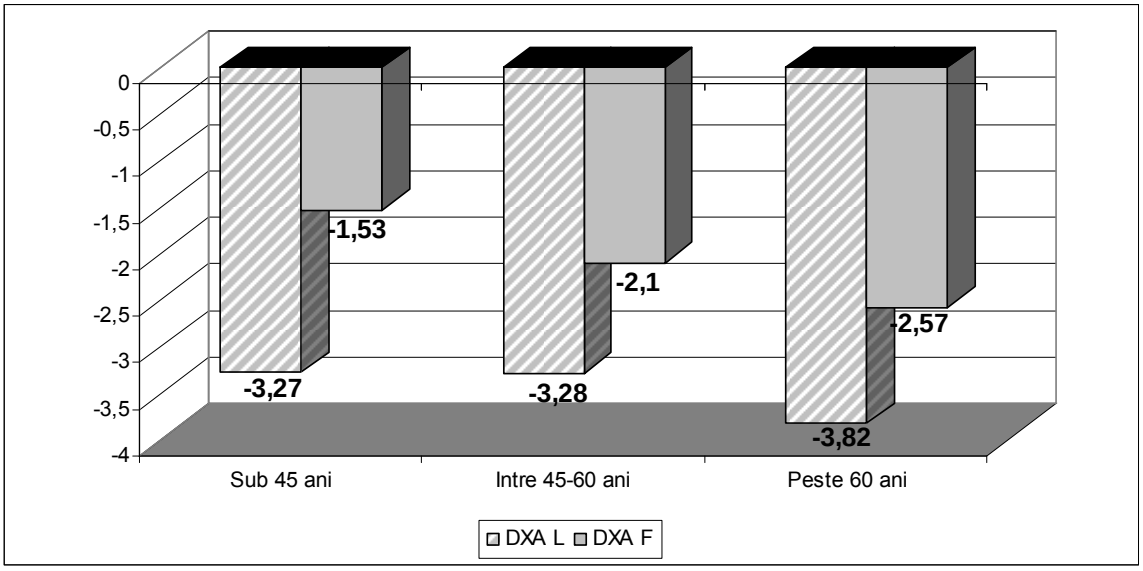
4. Parametrii biochimici ai turnoverului osos la barbatii cu osteoporoza la care examenul histologic al osului a indicat un turnover crescut

Tabel IV/1:: comparatia statistica a mediilor osteocalcinei, fosfatazei alcaline totale si a deoixipiridinolinei libere urinare pe grupe de varsta la barbati cu osteoporoza cu turnover osos crescut

Nr. grupei	Grupa de varsta	Osteocalcina serica (ng/ml):media +/-DS	Fosfataza alcalina totala serica (U/l):media+/-DS	Deoxipiridinolina urinara 9nM/mM):media +/-DS
1	Sub 45 ani	7,07+/-1,87	114,28+/-38,93	5,41+/-1,26
2	Intre 45-60 ani	7,7+/-2,61	128,28+/-28,11	4,01+/-1,77
3	Peste 60 ani	5,95+/-3,13	155+/-49,55	5,72+/-3,51
Semnificatia statistica (p)		0,45	0,18	0,38

Nu exista diferente statistic semnificative intre mediile osteocalcinei, fosfatazei alcaline totale si deoxipiridinolinei libere urinare pe grupe de varsta la barbatii cu osteoporoza la care examenul histologic al osului a aratat un turnover crescut.

Grafic 4: valorile comparative ale mediilor scorurilor T DXA lombare si femurale pe grupe de varsta.



Din datele prezentate in grafic reiese ca la barbatii cu osteoporoza cu turnover osos crescut pierderile osoase se accentueaza cu inaintarea in varsta atat la nivel lombar cat si femural, cele la nivel lombar fiind mai ponuntate.

5. Comparatia statistica a mediilor osteocalcinei, fosfatazei alcaline totale si deoxipiridinolinei libere urinare intre grupele cu turnover osos scazut si crescut

In ceea ce priveste mediile osteocalcinei exista o diferenta statistic semnificativa la grupa cu varsta peste 60 ani intre femeile cu osteoporoza cu turnover osos scazut si cele cu turnover osos crescut (p=0,03) si intre barbatii din grupa de varsta intre 45-60 ani (p=0,02).
Exista o diferenta statistic semnificativa a mediilor deoxipiridinolinei libere urinare la grupa de varsta peste 60 ani intre femeile cu osteoporoza cu turnover osos scazut si cele cu osteoporoza cu turnover osos crescut (p=0,04).
Compararea mediilor totale ale markerilor turnover-ului osos (osteocalcina, fosfataza alcalina totala, DPD crosslinks) intre femeile cu turnover scazut si crescut, intre barbatii cu turnover scazut si crescut si apoi mediile tuturor pacientilor luati in studiu avand turnover scazut cu cei cu turnover crescut nu a evidentiat diferente semnificative.

I. REZULTATELE CERCETARILOR EXPERIMENTALE

S-a efectuat un studiu in care s-a urmarit efectul pamidronatului asupra evolutiei osteopeniei induse inflamator la sobolani.
Animalele din cele trei loturi au fost cantarite la sfarsitul studiului iar rezultatele au fost comparate.
Rezultatele demonstrează o tendinţă de reducere a ritmului de creştere în greutate atât la animalele cu IMO cât şi la cele cu IMO tratate cu pamidronat, însă fără să apară o diferenţă semnificativă între cele 3 loturi.
S-a determinat continutul de calciu osos la animalele martor, cele cu IMO si cu IMO+P.

Tabel 1: valorile medii ale conţinutului de calciu (mg/100mg os) din oasele recoltate la animalele martor, cele cu IMO şi la cele cu IMO+P şi comparaţia lor statistică

Nr.	Loturi	Ca (mg/100 mg os)
1	Lot martor	28,37±0,78
2	IMO	22,50667±0,91
3	IMO + P	25,68±0,71
p 1 comparativ cu 2		<0,001*
p 1 comparativ cu 3		<0,001*
p 2 comparativ cu 3		<0,001*

$p^{*} = p<0.05$
Aceste rezultate comparate cu lotul martor au demonstrat ca prin administrarea de pamidronat procesul de pierdere a calciului nu a putut fi impiedicat, diferenta intre cele doua loturi fiind statistic semnificativa. In ceea ce priveste diferentele obtinute la lotul cu IMO si la cel tratat cu pamidronat valorile au fost semnificativ mai mari dupa pamidronat, ceea ce a demonstrat ca chiar in conditiile unui proces inflamator pamidronatul poate reduce pierderile de calciu fara sa se mentina insa valorile normale.
Comparatia statistica a mediilor continutului de magneziu osos la cele trei loturi este prezentata in tabelul 2.

Tabel 2: valorile medii ale conţinutului de magneziu (mg/g os) din oasele recoltate de la animalele martor, cele cu IMO şi la cele cu IMO + P şi comparaţia lor statistică

Nr.	Loturi	Mg (mg/g os)
1	Lot martor	2,23±0,15
2	IMO	1,6±0,1
3	IMO + P	1,99±0,14
p 1 comparativ cu 2		<0,001*
p 1 comparativ cu 3		<0,001*
p 2 comparativ cu 3		<0,001*

p^* = p<0.05

S-au determinat densitatile osoase la cele trei loturi de animale; in tabelul 3 este prezentata comparatia mediilor densitatilor osoase la cele trei loturi.

Tabel 3: mediile valorilor densităţilor osoase la animalele martor, cele cu IMO şi la cele cu IMO+P şi comparaţia lor statistică

Nr.	Loturi	Densitate osoasă (g/cm^3)
1	Lot martor	1.29±0.024
2	IMO	1.17±0.023
3	IMO + P	1.22±0.038
p 1 comparativ cu 2		<0,001*
p 1 comparativ cu 3		<0,001*
p 2 comparativ cu 3		<0,001*

p^* = p<0.05

Din datele prezentate rezulta ca administrarea de pamidronat la animalele la care s-a produs procesul de osteopenie indusa inflamator are o actiune semnificativa de incetinire a procesului de pierdere a calciului din oase.
Datele obtinute cu privire la pierderile de magneziu au demonstrat ca pamidronatul administrat la animalele cu osteopenie indusa inflamator reduce partial pierderile produse prin IMO, fara sa poata impiedica in totalitate pierderile produse prin inflamatie.

CONCLUZII

La femeile cu turnover osos scazut aflate in postmenopauza pierderile osoase lombare sunt mai accentuate decat cele femurale dupa care la varste peste 60 ani sunt mai putin pronuntate si tind sa se egalizeze, in timp ce la femeile cu turnover osos crescut in postmenopauza pierderile lombare si femurale au valori apropiate, iar in perioada peste 60 ani se constata pierderi mult mai pronuntate la nivel femural.
Urmarind la barbati aceleasi raporturi pe grupe de varsta spre deosebire de femei apare un scor T femural mult mai favorabil decat cel lombar; acest decalaj se mentine la toate grupele de varsta subliniind importanta hormonilor androgeni in mentinerea structurii osoase.
Studiile au demonstrat ca la pacientii cu osteoporoza diagnosticul histologic de turnover osos crescut si scazut nu poate fi sustinut prin modificari biochimice caracteristice ale markerilor metabolismului osos.
In consecinta urmarirea incadrarii evolutive a turnover-ului osos numai prin metode biochimice nu este suficienta.
Studiile experimentale pe care le-am efectuat urmarind efectul pamidronatului la animalele cu osteoporoza de tip inflamator au confirmat:
-o reducere importanta a densitatii osoase impreuna pierderea semnificativa de calciu si magneziu din oase
-administrarea de pamidronat a redus semnificativ ritmul de evolutie a osteopeniei experimentale, fara sa poata opri evolutia procesului
Tinand cont de mecanismul patogenic predominant inflamator al pierderilor de calciu si magneziu din oase produs prin inducerea osteopeniei experimentale pamidronatul s-a dovedit a avea un mecanism de actiune mai complex.
Datele obtinute le sustin pe cele clinice, care au demonstrat ca in procesele de osteoliza aparute in cadrul evolutiei unor neoplazii cu metastaze osoase pamidronatul are un puternic efect si asupra componentei patogenice inflamatorii a declansarii pierderilor de calciu din oase realizate de citokine.

CURRICULUM VITAE

INFORMATII PERSONALE

- Nume: VOIA
- Prenume: CRISTIAN LORIN
- Adresa: Str. Iezer Nr. 2 Ap.25, Cluj-Napoca
- Telefon: 0722750406
- E-mail: cristianvoia@hotmail.com
- Nationalitate: romana
- Varsta: 35 ani
- Data naşterii: 09.04.1974
- Stare civila: casatorit

EDUCAŢIE

-1978-1981: gradinita germana
-1981-1989: Scoala generala si gimnaziul in cadrul Colegiului National "George Cosbuc" Cluj-Napoca cu limba de predare germana (limba materna)
-1989-1991: Liceul de Matematica si Fizica Nr. 1 Cluj-Napoca
-1991-1993: Liceul Teoretic Nr. 4 (Stiinte ale Naturii), sectia fizica-chimie, Cluj-Napoca
-1993-1999: student al UMF "Iuliu Hatieganu" Cluj-Napoca, Facultatea de Medicina
-2000: medic stagiar
2001-2003: beneficiar al unei burse de studiu KAAD in cadrul Clinicii Universitare Heidelberg, Germania, sectia Endocrinologie si Boli Metabolice
-noiembrie 2003: doctorand al UMF "Iuliu Hatieganu" Cluj-Napoca, sub conducerea stiintifica a doamnei Prof. Dr. Ileana Duncea, Seful Clinicii de Endocrinologie Cluj-Napoca
- incepand cu 01.01. 2004 medic rezident endocrinologie-admis in urma examenului de rezidentiat sesiunea noiembrie 2003
-incepand cu 01.01.2009 medic specialist endocrinologie , in urma examenului de medic specialist sustinut in sesiunea octombrie 2008

LUCRARI PUBLICATE

- “Korrelieren biochemische Knochenstoffwechselmarker mit einer histologisch gesicherten High-bzw. Low-Turnover-Osteoporose?”; 2002 Medizinische Klinik; 97: 588-594 (Nr.10)

- “Corelatii biochimice si histologice in stabilirea tipului de turnover in osteoporoza”; 2007 Revista Romana de Endocrinologie si Metabolism; vol.6, nr.4: 99-105.

- “Influenta osteoprotectoare a pamidronatului asupra osteopeniei induse inflamator”, 2007 Clujul Medical, vol. LXXX, nr. 3: 669-675.

LIMBI STRĂINE

- Germana: scris – f. bine, vorbit – f.bine
- Engleză: scris-f. bine, vorbit f. bine
- Franceza: scris – bine, vorbit – bine

"IULIU HAŢIEGANU" UNIVERSITY OF MEDICINE
AND PHARMACY CLUJ-NAPOCA

CRISTIAN LORIN VOIA

CLINICAL-BIOLOGICAL CORELATIONS IN OSTEOPOROSIS

DOCTORAL THESIS TO OBTAIN THE TITLE OF DOCTOR IN MEDICAL SCIENCES, DOMAIN OF MEDICINE, SPECIALTY ENDOCRINOLOGY

ABSTRACT

SCIENTIFIC COORDINATOR
PROF. ILEANA DUNCEA, PH.D.

CLUJ-NAPOCA
2009

FOREWORD

Osteoporosis represents one of the most frequent diseases of the elderly. Due to the continuous increase of the percentage of the elderly within the general population of the industrialized countries, osteoporosis has become an important cause of morbidity and mortality. This is why this disease has been paid more and more attention in the past years.
In the present stage, diagnosing osteoporosis is based on several elements and it is recommended that several criteria be assessed in order to monitor the evolution, but especially to prevent the complications of the disease.
Through the increase of the number of anti-osteoporotic medicines, the essential problem remains the identification of those patients who can most benefit from the therapy.
Having the possibility to carry out a biochemical and histopathologic study in the Centre for Osteoporosis of Heidelberg (Germany) and with the help of the Department of Bone Pathology of Hamburg-Eppendorf (Germany), under the guidance of professor Reinhard Ziegler (Heidelberg) and in collaboration with professor Liviu Gozariu (Cluj-Napoca), I have aimed at a complex study to determine how much the biochemical parameters of the bone turnover correlate with the histopathological results for as accurate a definition of the bone turnover as possible.
I have also proposed an experimental study to monitor the influence of a powerful bisphosphonate (pamidronate) over the spontaneous evolution of an induced experimentally inflammatory type of osteoporosis through the monitoring of some biochemical and bone physical parameters.

I would like to give my most sincere thanks to **Prof. Dr. Ileana Duncea**, the scientific coordinator of this doctoral thesis, for her competent and continuous guidance, for the scientific probity and for the trust she has put in me during the entire period of my training, and not in the least for the fact that she has ensured the optimum conditions for the realization of the present paper.
I would also like to express my profound gratitude to **Prof. Dr. Liviu Gozariu** for his constant help, for his solicitude and scientific rigor which made the realization of this paper possible.
I also thank **Dr. Pavel Orbai** for his collaboration in the carrying out of the experimental studies in the UMF Biobase and in the laboratory of the Clinic of Endocrinology of Cluj-Napoca and for the valuable time spent in guiding the laboratory work.
I would also like to give my warmest thanks to **Dr. S. Toader** form the UMF Cluj-Napoca Biobase, for having ensured the conditions necessary to the carrying out of the experimental research.

I also thank **Senior Lecturer Dr. Remus Campean** from the Department of Medical Informatics of the "Iuliu Hatieganu" UMF Cluj-Napoca for his support to the realization of this paper.
Last but not least, my thanks are directed to **Prof. Dr. Reinhard Ziegler**, Head of the Department of Endocrinology and Metabolic Diseases of the University of Heidelberg and to **PD Dr. Christian Kasperk**, Head of the Department of Osteodensitometry for the honour of having allowed me to be part of the research team, for the excellent working conditions and for the team spirit they showed during the nearly two years I spent in this clinic. In this context, I would also like to thank the foundation **KAAD (Katholischer Akademischer Ausländerdienst)** for the scholarship granted to me.

CLINICAL RESEARCH

132 patients have been included in the research carried out at the University of Heidelberg, The Clinic of Endocrinology.
The study excluded the patients with fractures of the vertebral bodies, as well as the patients with any kind of fracture in the past year; the study also excluded the patients who have already been under osteothrop therapy. We performed bone biopsy of the iliac crest to all patients previously diagnosed with osteoporosis in order to establish the bone dynamics (the type of bone turnover).
Patients included in the study have been divided based on the histologic criterion of bone turnover assessment into:

I. a lot with low bone turnover
II. a lot with high bone turnover

Each lot was in turn divided into men and women.
The second criterion for the division of the patients was age, that is the total of the patients have been divided into 6 groups:

I. patients under 45 with low bone turnover
II. patients between 45-60 with low bone turnover
III. patients over 60 with low bone turnover
IV. patients under 45 with high bone turnover
V. patients between 45-60 with high bone turnover
VI. patients over 60 with high bone turnover

The patients have been studied based on age and type of bone turnover criteria and have been divided in the same groups, for men and women, and the study was carried out for the period of their hospitalization.
In order to establish the diagnosis of osteoporosis, osteodensitometry has been performed using the method of dual absorbtiometry with X-rays (DXA), and in order to appreciate the dynamics of the bone metabolism the following biochemical parameters have been determined: osteocalcin, total alkaline phosphatase, the free urinary deoxipiridinolina.
To assess the hormonal control of the bone metabolism the following have been determined: the thyroid - stimulating hormone (TSH), parathormone (PTH), 25-OH vitamin D. The blood samples have been taken a jeun between 8-10, have been prepared in accordance to standards and then analysed.
The research has been carried out with the unconditional consent of the patients in compliance with the European regulations of professional deontology which were essential to this study.

EXPERIMENTAL RESEARCH

Within the study carried out at the Clinic of Endocrinology of Cluj-Napoca in collaboration with the UMF Cluj-Napoca Biobase, female rats have been selected (Bratislava type) weighing between 180-200 g, which have been divided into 3 lots. All animals were fed daily standard food.
To the inflammatory inducement of osteopenia the method described by Minne has been employed, that is: the injecting into 8 points in the dorsal subcutaneous region of 400 mg of sterile talcum (magnesium silicate) dissolved in 0,5 ml of physiological serum.
After sacrificing the tibia of the rats belonging to the three groups has been taken. The tibias were dried, weighed and then we determined the content of calcium and magnesium from the dry tibia, calcined at 600 degrees Celsius. The dosage of calcium and magnesium has been carried out by means of a spectrophotometer with atomic absorption at the wavelength of 421 nm for calcium and 284 nm for magnesium. The bone density has been calculated by relating mass (mg) to volume (cm^3). The measuring of the bone volumes has been done with a digital pletismometer.
Three lots have been studied:

Lot I (the witnesses)
included the following witnesses: 10 female rats weighing 180-200 g each, which were sacrificed after 21 days

Lot II (IMO)
included 15 animals (female rats) weighing 180-200 g each, to which IMO has been induced through the described method. They were sacrificed 21 days later.

Lot III (IMO+P)
This lot included 15 animals (female rats) weighing between 180-200 g, which were injected pamidronate intravenously three days before, after which IMO was induced. The animals were sacrificed 21 days later.

The dysodic pamidronate (Aredia) was injected intravenously in a single dose of 0,15 mg.

I. RESULTS OF THE CLINICAL RESEARCH

1. The biochemical parameters of the bone turnover in women suffering from osteoporosis, in which the histological examination of the bone indicated a low turnover and the statistical comparison of these are presented in table I/1.

Group no.	Age group	Osteocalcin: Average (ng/ml) ±DS	Alkaline phosphatase: average (U/l)±DS	Free urinary deoxypyridinoline average (nM/mM)±DS
1	under 45	4,06± 2,8	97,8±35,57	4,3±1,54
2	between 45-60	5,3±2,03	114,46±22,07	4,63±2,07
3	over 60	4,88±2,34	153,08±84,15	5,52±2,2
Statistical significance (p)		0,5	0,04*	0,25

$p^{*}=p<0,05$

There are no significant statistical differences between the average of the osteocalcin and that of the free urinary deoxypyridinoline.
There are significant statistical differences between the averages of the total alkaline phosphatase in the three age groups, which is why we proceeded at comparing them using the „t" student test.

The statistical comparison of the averages of the alkaline phosphatase on age groups (tables I/2, I/3 and I/4):
Table I/2:

Group number	Age group	Total alkaline phosphatase (U/l) ±DS
1	under 45	97,81±35,57
3	over 60	153,08±84,15
p: group 1 as compared to 3		0.009*

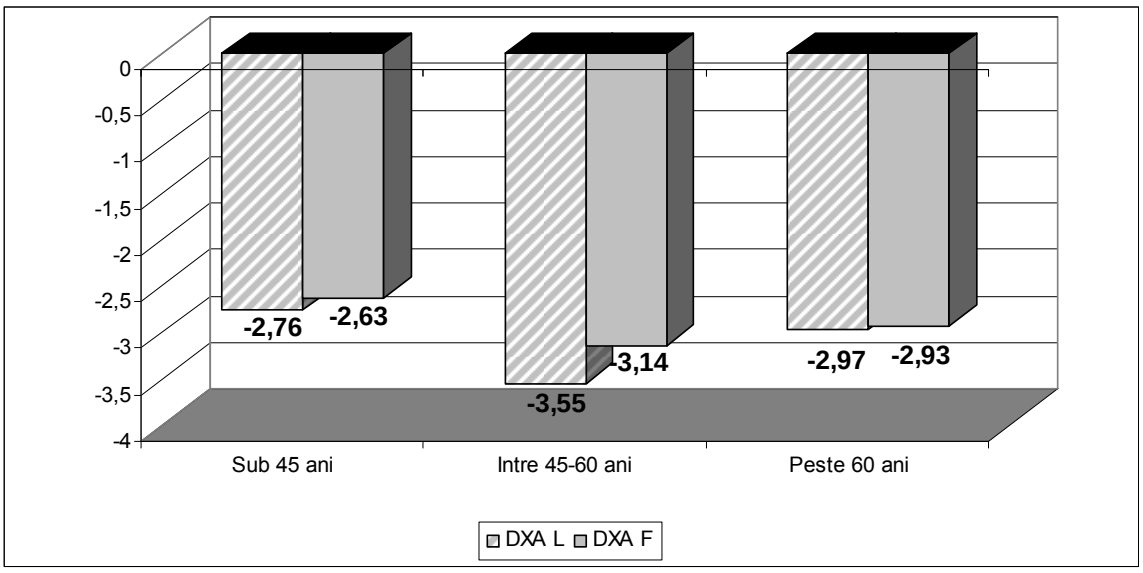
Table I/3:

Group number	Age group	Total alkaline phosphatase (U/l) ±DS
2	between 45-60	114,46±22,07
3	over 60	153,08±84,15
p: group 2 as compared to 3		0,039*

Table I/4:

Group number	Age group	Total alkaline phosphatase (U/l)
1	under 45	97,81±35,57
2	between 45-60	114,46±22,07
p: group 1 as compared to 2		0,19

Graph 1: the comparative values of the average values of lumbar and femural T DXA in women suffering from osteoporosis with a low turnover of the three age groups

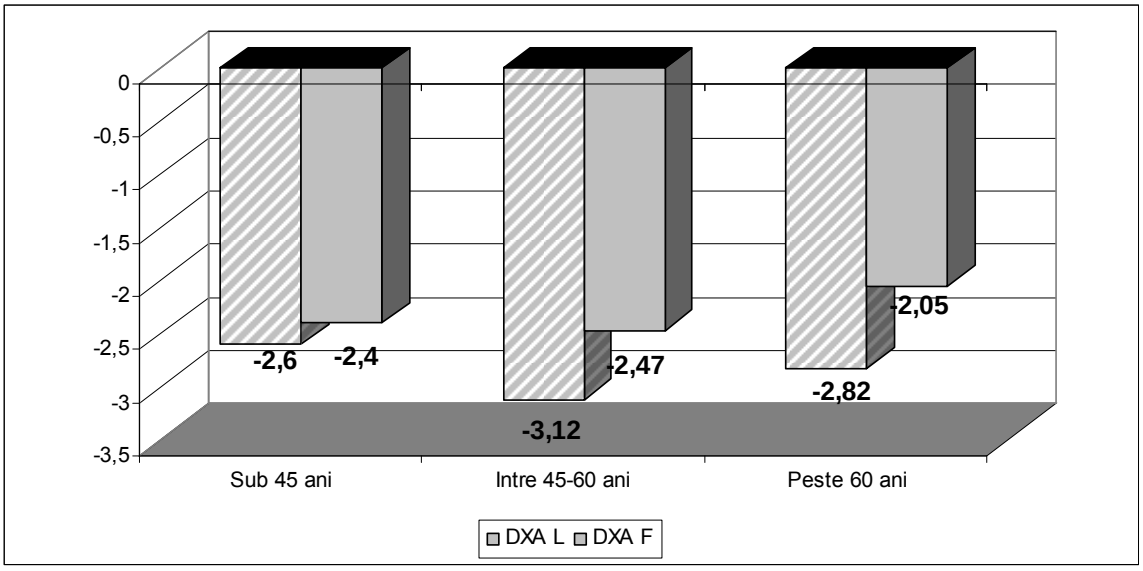


2. The statistical comparison of the averages of osteocalcin, total alkaline phosphatase and free urinary deoxypyridinoline on age groups in men suffering from osteoporosis with a low bone turnover are presented in table II/1.
Table II/1:

Group number	Age group	Osteocalcin (ng/ml):average +/-DS	Total alkaline phosphatase (U/l):average +/-DS	Free urinary deoxypyridinoline (nM/mM):average +/-DS
1	under 45	5,64+/-1,40	113+/-28,33	4,61+/-1,4
2	between 45-60	5,67+/-1,87	129,5+/-49,81	5,12+/-2,77
3	over 60	4,85+/-2,26	123,66+/-42,22	4,05+/-1,74
Statistical significance (p)		0,44	0,64	0,4

Between the averages of the osteocalcin, total alkaline phosphatase and free urinary deoxypyridinoline in men suffering from osteoporosis with a low bone turnover of the three age groups there are no significant statistical differences (p>0,05).

Graph 2: the comparative values of the average values of lumbar and femural T DXA on age groups in men suffering from osteoporosis with a low turnover



3. The biochemical parameters of the bone turnover in women suffering from osteoporosis in which the histological examination of the bone indicated a high turnover

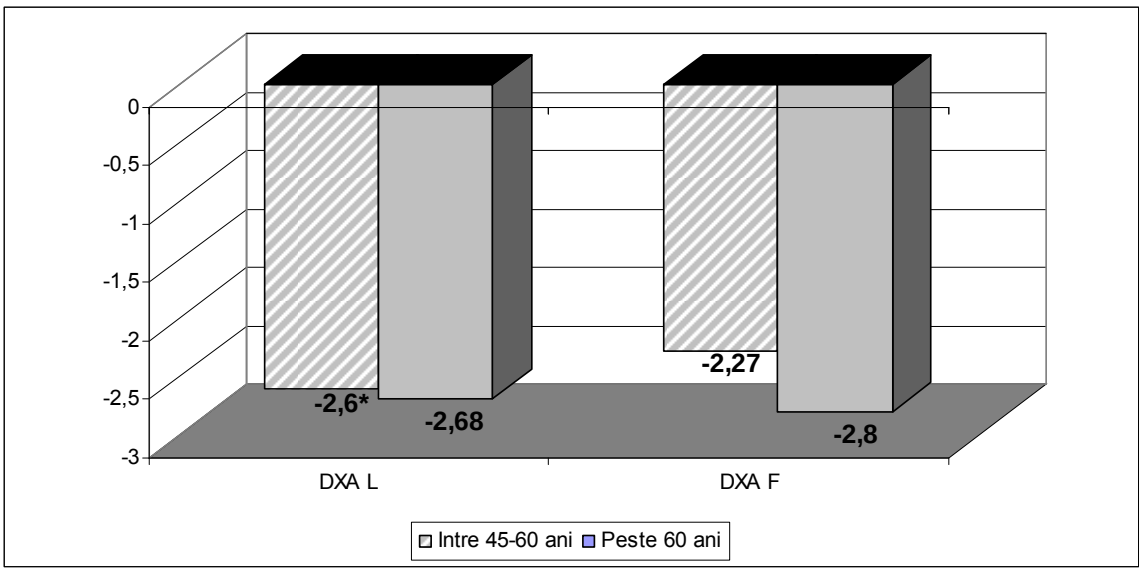
For the age group under 45 the volume of the study group is too small to allow for the application of this significance test, which is why these patients have been excluded from the study.

Table III/1: the statistical comparison of the averages of osteocalcin, total alkaline phosphatase and free urinary deoxypyridinoline on age groups in women suffering from osteoporosis with a high bone turnover

Group number	Age group	Osteocalcin (ng/ml):average+/-DS	Total alkaline phosphatase (U/l):average +/-DS	Free urinary deoxypyridinoline (nM/mM):average +/-DS
1	between 45-60	6,86+/-3,29	124,3+/-53,12	5,27+/-2,59
2	over 60	6,79+/-1,85	233,8+/-132,46	9,13+/-4,83
Statistical significance (p)		0,95	0,03*	0,04

Between the averages of osteocalcin and those of free urinary deoxypyridinoline there are no significant statistical differences. The average values of osteocalcinei are within normal limits in both groups, and the average of the DPD crosslinks in the age group over 60 is increased, confirming the existing data.

Graph 3: the comparative values of the average values of lumbar and femural T DXA on age groups in women suffering from osteoporosis with a high bone turnover



The graph presented reveals that bone losses are more accentuated at the level of the femur with age, namely at the age group over 60 as compared to that of the menopause in women suffering from osteoporosis with a high bone turnover.

4. The biochemical parameters of the bone turnover in men suffering from osteoporosis in which the histological examination indicated a high bone turnover

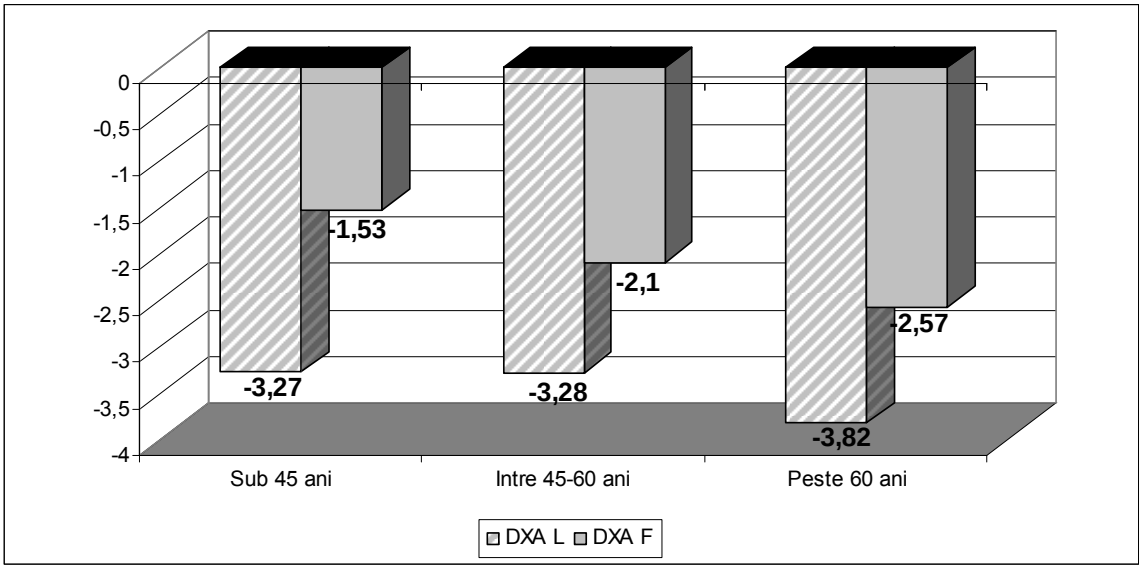
Table IV/1: the statistical comparison of the averages of osteocalcin, total alkaline phosphatase and free urinary deoxypyridinoline on age groups in men suffering from osteoporosis with a high bone turnover

Group number	Age group	Seric osteocalcin (ng/ml):average +/-DS	Total alkaline phosphatase (U/l):average+/-DS	Urinary deoxypyridinoline 9nM/mM):average +/-DS
1	Under 45	7,07+/-1,87	114,28+/-38,93	5,41+/-1,26
2	between45-60	7,7+/-2,61	128,28+/-28,11	4,01+/-1,77
3	Over 60	5,95+/-3,13	155+/-49,55	5,72+/-3,51
Statistical significance (p)		0,45	0,18	0,38

There are no significant statistical differences between the averages of osteocalcin, total alkaline phosphatase and free urinary deoxypyridinoline on

age groups in men suffering from osteoporosis in which the histological examination of the bone indicated a high turnover.

Graph 4: the comparative values of the average values of lumbar and femural T DXA on age groups.



The graph presented reveals that in men suffering from osteoporosis with a high bone turnover, the bone losses are accentuated with age both at lumbar and femural level, those at lumbar level being more pronounced.

5. The statistical comparison of the averages of osteocalcin, total alkaline phosphatase and free urinary deoxypyridinoline between the groups with a low and high bone turnover.

In as far as the averages of osteocalcin are concerned, there is a significant difference from a statistical point of view in the age group over 60 between the women suffering from osteoporosis with a low bone turnover and those with a high bone turnover (p=0,03) and the men of the age group between 45-60 (p=0,02).
There is a statistically significant difference of the averages of the free urinary deoxypyridinoline in the age group over 60 between women suffering from osteoporosis with a low bone turnover and those with a high bone turnover. (p=0,04).
The comparison of the total averages of the markers of the bone turnover (osteocalcin, total alkaline phosphatase, DPD crosslinks) between the women with a high or low turnover, between the men with a high and low turnover and then the averages of all the patients included in the study with a low turnover and those with a high turnover did not reveal significant differences.

I. RESULTS OF THE EXPERIMENTAL RESEARCH

A study has been carried out focussing on the effect of pamidronate on the evolution of inflammatory induced osteopenia in rats.
The animals of the three lots were weighed at the end of the study and the results were compared.
The results demonstrate a tendency to reduce the rhythm of weight gaining in both animals with IMO and those with IMO treated with pamidronate, but without there being a significant difference between the two lots.
The content of bone calcium was determined in the witness animals, those with IMO and those with IMO+P.

Table 1: the average values of the content of calcium (mg/100mg bone) of the bones taken from the witness animals, those with IMO and those with IMO+P and their statistical comparison

No.	Lots	Ca (mg/100 mg bone)
1	Witness Lot	28,37±0,78
2	IMO	22,50667±0,91
3	IMO + P	25,68±0,71
p 1 as compared with 2		<0,001*
p 1 as compared with 3		<0,001*
p 2 as compared with 3		<0,001*

$p^{*} = p<0.05$
These results that have been compared with the witness lot, have demonstrated that through the treatment with pamidronate, the process of calcium loss could not be prevented, the difference between the two lots being statistically significant. In as far as the differences obtained at the lot with IMO and that treated with pamidronate are concerned, the values have been significantly higher after pamidronate, which demonstrated that even under the conditions of an inflammatory process pamidronate can reduce the calcium loss despite the fact that the normal values could not be maintained.
The statistical comparison of the averages of the bone content of magnesium in the three lots is presented in table 2.

Table 2: the average values of the content of magnesium (mg/g os) in the bones taken from the witness animals, from those with IMO and those with IMO + P and their statistical comparison

No.	Lots	Mg (mg/g bone)
1	Witness lot	2,23±0,15
2	IMO	1,6±0,1
3	IMO + P	1,99±0,14
p 1 as compared to 2		<0,001*
p 1 as compared to 3		<0,001*
p 2 as compared to 3		<0,001*

p^* = p<0.05

The bone density in the three lots of animals has been determined; the comparison between the averages of the bone densities in the three lots is presented in table 3.

Table 3: the averages of the values of the bone densities in the witness animals, those with IMO and those with IMO+P and their statistical comparison

No.	Lots	Bone density (g/cm^3)
1	Witness lot	1.29±0.024
2	IMO	1.17±0.023
3	IMO + P	1.22±0.038
p 1 as compared to 2		<0,001*
p 1 as compared to 3		<0,001*
p 2 as compared to 3		<0,001*

p^* = p<0.05

The data presented reveal that the treatment with pamidronate of the animals in which the process of inflammatorily induced osteopenia has been triggered, has a significant action of slowing down the process of bone calcium loss.
The data obtained regarding the loss of magnesium have revealed that pamidronate given to the animals with inflammatorily induced osteopenia partially reduces the losses produced through IMO, without completely stopping the losses produced through the inflammation.

CONCLUSIONS

In women with a low bone turnover in menopause, the lumbar bone losses are more accentuated than the femoral ones, which after the age of 60 are less pronounced and tend to become equal, while in women with a high bone turnover in menopause the lumbar and femoral losses have close values, and in the period over 60 one can notice more pronounced losses at the femoral level.
Monitoring men with the same age groups, differently from women, there is a T femoral score that is more favourable than the lumbar, this difference being maintained in all age groups underlining the importance of the androgen hormones in the maintenance of the bone structure.
Studies have demonstrated that in patients with osteoporosis the histological diagnosis of high and low bone turnover cannot be supported through the characteristic biochemical modifications of the markers of the bone metabolism.
Consequently, the monitoring of the evolution of the bone turnover only through biochemical methods is insufficient.
Experimental studies carried out monitoring the effect of pamidronate on animals with ospteoporosis of the inflammatory type have confirmed:
-a significant reduction of the bone density together with the significant loss of calcium and magnesium from the bones.
-the treatment with pamidronate has significantly reduced the rhythm of evolution of experimental osteopenia, but could not stop the evolution of the process
Taking into consideration the predominantly inflammatory pathogenic mechanism of the calcium and magnesium loss from bones produced through the induction of experimental osteopenia, pamidronate has proved to have a more complex action mechanism.
The data obtained support the clinical data, which have demonstrated that in the processes of osteolyse, which appeared within the evolution of some neoplasia with bone metastasis, pamidronate also has a powerful effect on the inflammatory pathogenic composition of the triggering of the losses of calcium from the bones, caused by cytokines.

CURRICULUM VITAE

PERSONAL INFORMATION

- Surname: VOIA
- Name: CRISTIAN LORIN
- Address: 2 Iezer Str. Ap.25, Cluj-Napoca
- Telephone: 0722750406
- E-mail: cristianvoia@hotmail.com
- Nationality: Romanian
- Age: 35
- Date of birth: 09.04.1974
- Marital status: married

EDUCATION

-1978-1981: German kindergarten
-1981-1989: Primary school and Gymnasium : “George Cosbuc” School, Cluj-Napoca, the German line of study (mother tongue)
-1989-1991: Nr. 1Mathematics and Physics High-school, Cluj-Napoca
-1991-1993: Nr. 4 Theoretical High-School (Natural Sciences), Department of Physics-Chemistry, Cluj-Napoca
-1993-1999: “Iuliu Hatieganu” Univerisity of Medicine and Pharmacy, Cluj-Napoca, The Medical School
-2000: intern doctor
2001-2003: KAAD scholarship at the University Clinic of Heidelberg, Germany, Department of Endocrinology and Metabolic Diseases
-November 2003: A doctoral candidate at “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca, coordinated by Prof. Dr. Ileana Duncea, Head of the Clinic of Endocrinology of Cluj-Napoca
- 01.01. 2004 – 01.01.2009: a resident doctor in Endocrinology
- 01.01.2009 – present: specialist medical practitioner in Endocrinology

PUBLISHED PAPERS

- “Korrelieren biochemische Knochenstoffwechselmarker mit einer histologisch gesicherten High-bzw. Low-Turnover-Osteoporose?”; 2002 Medizinische Klinik; 97: 588-594 (Nr.10)

- “Biochemical and Histologic Correlations in Determining the Turnover Type in Osteoporosis”; 2007 Revista Romana de Endocrinologie si Metabolism; vol.6, nr.4: 99-105.

- “The Osteoprotective Influence of Pamidronat on the Inflammatory Induced Osteopoenia ”, 2007 Clujul Medical, vol. LXXX, nr. 3: 669-675.

FOREIGN LANGUAGES SPOKEN

- German: written – proficiency, speaking – proficiency
- English: written-advanced, speaking - advanced
- French: written – intermediary, speaking – intermediary