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THE ROLE OF TBS IN THE ASSESSMENT OF FRACTURE RISK IN SECONDARY ENDOCRINE OSTEOPOROSIS

PHD THESIS SUMMARY

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Table of contents

LIST OF PUBLISHED SCIENTIFIC WORKS

Articles published in specialized magazines indexed ISI:

1. **Sorohan Mădălina Cristina**, Poiană Cătălina. Vertebral Fractures in Acromegaly: A Systematic Review. Journal of Clinical Medicine. 2022 Dec 25;12(1):164. doi: 10.3390/jcm12010164. PMID: 36614962; PMCID: PMC9821150. https://www.mdpi.com/2077-0383/12/1/164 Indexing: BibCnrs, CAPlus / SciFinder, CNKI, Digital Science, DOAJ, EBSCO, Elsevier Databases, Scopus, Embase, Gale, National Library of Medicine, PubMed, PMC, OpenAIRE, OSTI (U.S. Department of Energy), PATENTSCOPE, ProQuest, PSYNDEX, SafetyLit, Web of Science, SCIE, Current Contents - Clinical Medicine. Impact factor at the moment of publishing=4.9. Current impact factor=3.9.
2. **Sorohan Mădălina Cristina**, Dusceac Roxana, Sorohan Bogdan Marian, Caragheorgheopol Andra, Poiană Cătălina. Trabecular bone score and bone mineral density in acromegalic osteopathy assessment: a cross-sectional study. Archives of Osteoporosis. 2021 Sep 16;16(1):134. doi: 10.1007/s11657-021-00986-7.PMID:34529148. https://link.springer.com/article/10.1007/s11657-021-00986-7. Indexing: AGRICOLA, Baidu, CLOCKSS, CNKI, CNPIEC, Dimensions, EBSCO Discovery Service, EMBASE, EMCare, Google Scholar, Japanese Science and Technology Agency (JST), Journal Citation Reports/Science Edition, Medline, Naver, OCLC WorldCat Discovery Service, Portico, ProQuest-ExLibris Primo, ProQuest-ExLibris Summon, Reaxys, SCImago, SCOPUS, Science Citation Index Expanded (SCIE), Semantic Scholar, TD Net Discovery Service, UGC-CARE List (India), Wanfang. Impact factor at the moment of publiching=2.9. Current impact factor=3.

Works presented at national and international scientific events:

1. „Vertebral fractures in acromegaly" **Mădălina Cristina Sorohan**, Ionela Florina Baciu , Simona Andreea Găloiu , Andra Caragheorgheopol, Carmen Nicoleta Iordăchescu, Cătălina Poiană. The 25th European Congress of Endocrinology, 13-16 May 2023, Istanbul, Turkey.
2. "*Bone mineral density, trabecular bone score and vertebral fractures in acromegalic patients".* **Mădălina Cristina Sorohan**, Ramona Dobre, Ionela Florina Baciu,

Simona Andreea Găloiu, Dan Alexandru Niculescu, Andra Caragheorgheopol, Iordăchescu Carmen Nicoleta, Cătălina Poiană. The 23rd European Congress of Endocrinology, 22-26 May 2022, Milano, Italia.

3. "*Differences in BMD, TBS and vertebral fractures between acromegalic and hypogonadal patients*". **Mădălina Cristina Sorohan**, Ramona Dobre, Ionela Florina Baciu, Simona Andreea Găloiu, Dan Alexandru Niculescu, Andra Caragheorgheopol, Iordăchescu Carmen Nicoleta, Cătălina Poiană. The 22nd Edition of the WCO-IOF-ESCEO Virtual Congress, 24-26 March 2022.
4. "*Evaluation of bone quality in acromegaly and thyrotoxicosis using trabecular bone score".* **Mădălina Cristina Sorohan**, Bogdan Sorohan, Cătălina Poiana. The 21st European Congress of Endocrinology, 18-21 May 2019, Lyon, France.
5. „Vertebral fractures in endocrine diseases" **Mădălina Cristina Sorohan**, Cristina Ana Maria Căpățână, Simona Andreea Găloiu, Iulia Florentina Burcea, Ramona Dobre, Gabriela Voicu, Cătălina Poiană. Al 31-lea Congres Național al Societății Române de Endocrinologie, 25-28 Iunie 2023, Oradea, România.
6. "Acromegaly versus hypogonadism: bone evaluation and vertebral fractures". **Mădălina Cristina Sorohan**, Ionela Florina Baciu, Simona Andreea Găloiu, Dan Alexandru Niculescu, Andra Caragheorgheopol, Carmen Nicoleta Iordăchescu, Cătălina Poiană. Forumul Tinerilor Endocrinologi, 8-10 Decembrie 2022, București.
7. "Densitate minerală osoasă sau TBS în evaluarea osteopatiei acromegalice?". **Mădălina Cristina Sorohan**, Bogdan Marian Sorohan, Andra Caragheorgheopol, Cătălina Poiană. 75 de ani de la Înființarea Institutului Național de Endocrinologie C.I. Parhon, 8 Noiembrie 2021, București.

I. GENERAL PART

1. Osteoporosis

Osteoporosis is a metabolic skeletal disease that represents a global health problem. It is a frequently encountered pathology in medical practice, there are around 22 million people with this diagnosis at European level. The clinical implications are represented by the occurrence of fragility fractures, which increases both patient morbidity and mortality. The risk of osteoporosis and fractures increases with the onset of menopause and advancing age, these two factors leading to the onset of primary osteoporosis. The prevalence of osteoporosis is 20% in women and 6%-7% in men aged between 50-84 years and the probability of a major osteoporotic fracture (spine, hip, distal forearm, humerus, pelvis) at this age category is almost 50% in women and 20% in men [1,2]. The diagnosis of osteoporosis is based on the presence of a T score≤-2.5 standard deviations (SD) on dual X-ray osteodensitometry (DXA) assessment, the presence of major osteoporotic fragility fractures even in the presence of a normal T score, or an increased risk of fracture on FRAX assessment with a T score between -1 and -2.5.

Due to the fact that the use of DXA and FRAX have limitations in various pathologies, type 2 diabetes being the best example of this, an attempt was made to discover an alternative method of evaluating bone damage in these conditions. One such method has been proposed to be the trabecular bone score (TBS), which is a technique for determining the alteration of bone quality by indirectly assessing bone microarchitecture of trabecular bone. It involves the use of a software that evaluates the gray variations at the level of the lumbar vertebrae on the DXA image, being easily reproducible, cost-effective and not involving additional irradiation of the patient. It is currently regulated as a complementary assessment technique, the score obtained can be entered into the FRAX calculation formula, but it cannot be used independently for diagnostic purposes. Also, the reference values are not yet standardized, those proposed by the manufacturer being: normal at a value >1.350, of partially degraded bone at values between 1.200-1.350 and values ≤1.200 representing degraded bone.

2. Endocrine diseases associated with secondary osteoporosis

Around 40% of osteoporosis patients also associate secondary causes. This makes it necessary to screen for secondary causes in this pathology. The secondary causes of osteoporosis and bone fragility are numerous, including endocrine, rheumatological, hematological diseases, malignancies, diabetes, drugs or nutritional deficiencies[3]. Endocrine causes of osteoporosis represent a heterogeneous group of pathologies characterized by bone fragility, in the presence or absence of the osteodensitometric diagnosis of osteoporosis. These are represented by acromegaly, Cushing's syndrome, hypogonadism, primary hyperparathyroidism and hyperthyroidism. The hormonal excess or deficiency characteristic of each of these pathologies produces specific changes in bone metabolism and is associated with an increased risk of vertebral and/or non-vertebral fractures. In the specialized literature, the prevalence of vertebral fractures (VFs) detected radiologically in these pathologies varies from 10% to 80%, the highest figures being found in patients with Cushing's syndrome and those with acromegaly[4][5]. At the same time, non-vertebral fractures seem to have a higher prevalence in patients with hypogonadism, primary hyperparathyroidism and hyperthyroidism.

Currently, there are no specific recommendations for the diagnosis or treatment of osteopathy from endocrine pathologies, there is only a recommendation for screening for bone damage in these patients. However, not all meet osteodensitometric or FRAX criteria for osteoporosis despite having increased bone fragility and a predisposition to developing fragility fractures. This fact is due to the particular changes given by hormonal action, especially in patients with acromegaly, who rarely present an osteodensitometric diagnosis of osteoporosis, most frequently having bone mineral density (BMD) similar to that of the general population[6]. In patients with Cushing's syndrome, although they have low BMD, they frequently have vertebral fractures in association with normal BMD. Regarding patients with hyperthyroidism or those with hypogonadism, there are few studies that have analyzed the presence of morphometric vertebral fractures, despite the fact that they are common diseases and are recognized as causes of osteoporosis.

Literature data on the prevalence of vertebral fractures in acromegaly range from 6%[7] to 87%[8]. As far as osteopathy in Cushing's syndrome is concerned, the data from the specialized literature on the prevalence of vertebral fractures mainly refer to patients with exogenous Cushing's syndrome and less to the endogenous one[9]. Several studies on patients with endogenous Cushing syndrome reported prevalences of up to 75.3% of

radiologically detected vertebral fractures[10]. In the case of hypogonadism, despite being one of the most well-known causes of osteoporosis, information on the prevalence of vertebral fractures is very limited, most reports including only clinically manifest fractures, with an average value of this prevalence being 15%[11]. Results regarding vertebral fractures in primary hyperparathyroidism are varied, from a prevalence of 1.7%[12] to 47%[13]. In hyperthyroidism, the prevalence of radiologically detected vertebral fractures is around 30%[14,15].

II. ORIGINAL CONTRIBUTIONS

3. Work hypothesis and general objectives

The doctoral thesis focused on the evaluation of patients with secondary endocrinec causes of bone fragility: acromegaly, Cushing's syndrome, hypogonadism, primary hyperparathyroidism and hyperthyroidism. The objectives of the study were to evaluate these categories of patients from the point of view of bone damage with the help of bone turnover markers, DXA, TBS and the presence of vertebral fractures and to determine the risk factors for prevalent vertebral fractures. It was also aimed to evaluate the role of TBS in the evaluation of patients with endocrine diseases and its predictive value for vertebral fractures.

4. General research methodology

4.1. Type of study and study population

We carried out a prospective, observational study, in which 418 patients aged ≥18 years were included, evaluated in the Endocrinology I department (Pituitary and Neuroendocrine Pathology) of the "Constantin Ion Parhon" National Endocrinology Institute, evaluated between November 1st 2018 and April 30th 2022, having one of the following diagnoses: acromegaly, Cushing's syndrome, hypogonadism, primary hyperparathyroidism or hyperthyroidism. Of these, 209 (50%) were followed for a median of 16 months. Exclusion criteria were the presence of other causes of osteoporosis (rheumatological diseases i.e. rheumatoid arthritis, connective tissue diseases, chronic malabsorption, organ transplantation, history of rickets or osteomalacia, other chronic organ dysfunctions, osteoporosis-related drugs), and, in the case of patients with hypogonadism, the physiological onset of menopause. The confidentiality of the data of the enrolled patients was preserved, they signed the patient's informed consent expressing their agreement to participate, after the details of its objectives and the implementation methodology were presented. The consent of the local ethics committee was obtained for conducting the study, on 14/02/2019.

4.2. Data collection and variables monitored

Patient data were obtained by interview, from the observation sheet or from the patient’s database. Information on patients' DXA and TBS assessment was obtained from the database of the DXA machine (General Electric Prodigy Lunar, Bedford, UK). They were entered into an Excel database for statistical processing.

Followed parameters were anthropometric, details of cause, duration, disease status, total serum calcium, phosphorus, (25-OH)VitD, PTH, bone turnover marker (alkaline phosphatase, osteocalcin, P1NP, serum beta-crosslaps), specific hormones of each endocrine pathology, DXA lumbar spine, hip ± radius 33%, TBS and profile dorso-lumbar spine radiographs.

Statistical analysis of the variables was performed using the IBM SPSS Statistics Data Editor version 20 program (IBM Corporation, Chicago, IL, USA). Descriptive, comparative analysis, univariate and multivariate Cox logistic regression and ROC curves were performed.

5. Results

5.1 Analysis of the entire patient cohort

The patient cohort included 418 subjects, of which 71 (17%) patients with acromegaly, 45 (10.8%) with Cushing's syndrome, 75 (17.9%) with hypogonadism, 73 (17.5%) with primary hyperparathyroidism and 154 (36.8%) with hyperthyroidism. Vertebral fractures had a prevalence of 15.55% and non-vertebral fractures of 3.3%. They were characterized by a DXA score of osteopenia, with the lowest bone density being at the lumbar spine, and by partially degraded bone according to the mean TBS value of 1.263±0.136.

Figure 5.1. Graphic representation of the distribution of endocrine pathologies in the cohort.

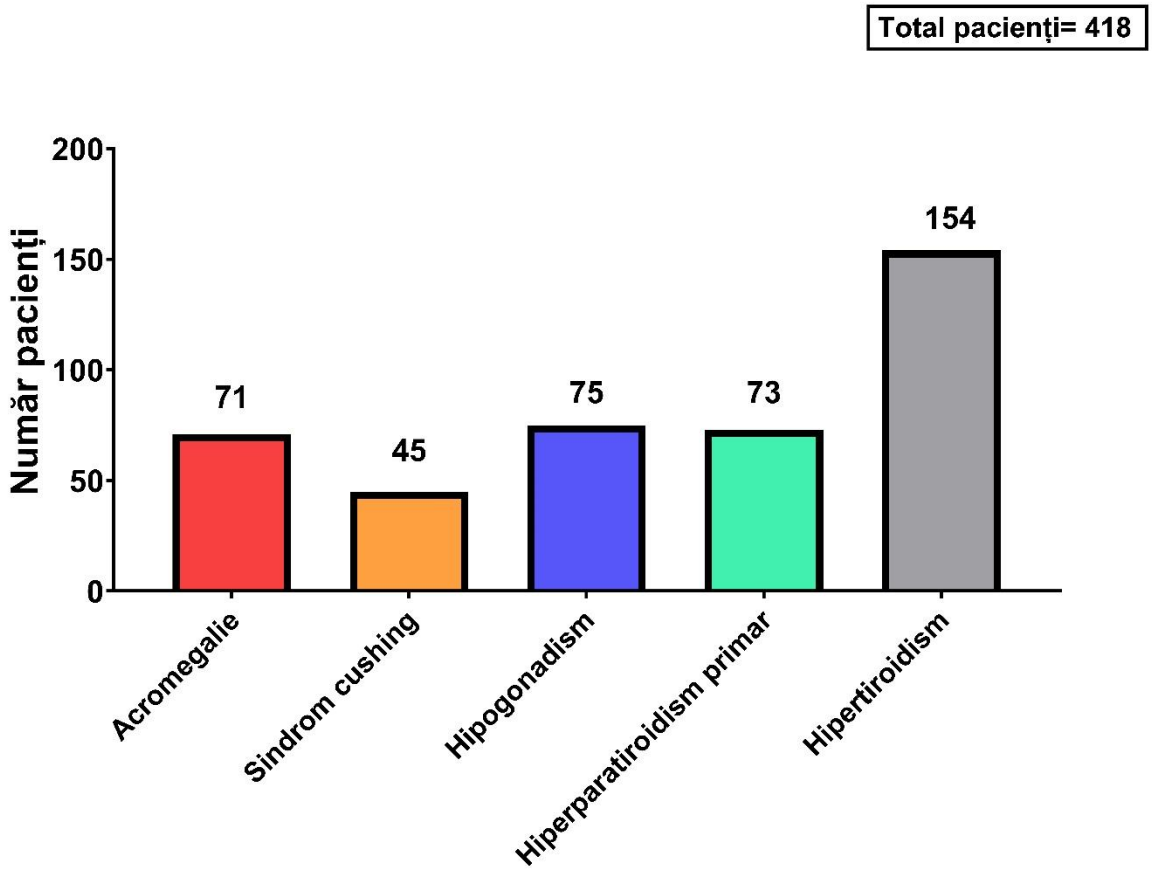


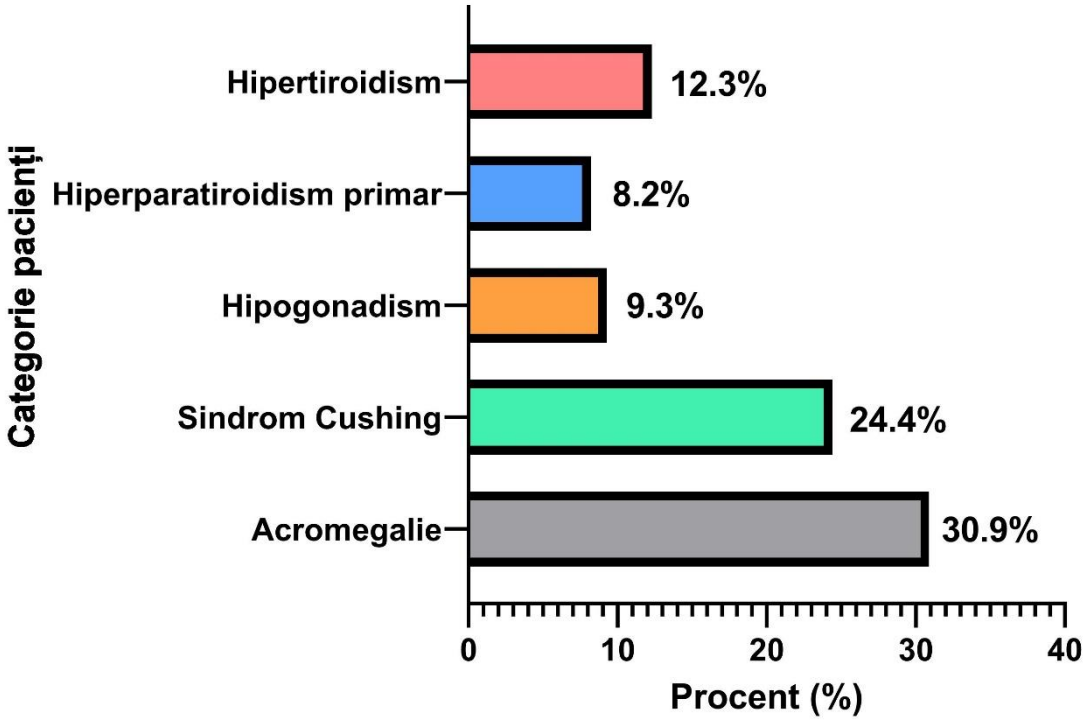
Table V.1. General characteristics of the entire batch of patients

Variables	*All subjects (N=418)*
Age (mean, years)	57.1±12

Gender (%)	
Female	344 (82.3%)
Male	74 (17.7%)
Smoking (%)	167 (40%)
Endocrine pathology (%)	
Acromegaly	71 (17%)
Cushing syndrome	45 (10.8%)
Hypogonadism	75 (17.9%)
Primary hyperparathyroidism	73 (17.5%)
Hyperthyroidism	154 (36.8%)
BMI (mean, kg/m^2)	27.82±6.5
Follow-up (average, months)	16.85±5.49
VFs (%)	65 (15.6%)
Non-VFs (%)	14 (3.3%)
Antiresorptive treatment (%)	83 (19.9%)
DZ type 2/secondary (%)	146 (34.9%)
Osteoporosis diagnosis (%)	116 (27.8%)
Laboratory tests	
Total serum calcium (mean, mg/dL)	9.81±0.92
Phosphorus (mean, mg/dL)	3.44±0.64
(25-OH)D3 (mean, ng/mL)	24.4±10.15
Parathyroid hormone (median, pg/mL)	47.4 (37.48-76.3)
Bone turnover markers	
Alkaline phosphatase (mean, U/L)	75 (60-95)
Osteocalcin (median, ng/mL)	23.46 (15.92-37.83)
Serum β-crosslaps (median, ng/mL)	0.5 (0.311-0.8)
P1NP (median, ng/mL)	61.45 (35.36-99.95)
DXA	
Lumbar spine	
BMD (mean, g/cm^2)	1.029±0.184
BMD evolution (median, g/cm^2)	+0.013 (-0.021-0.053)
T-score (median, SD)	-1.5 (-2.5- -0.5)
Z-score (median, SD)	-0.9 (-1.6-0.1)
Femoral neck	
BMD (mean, g/cm^2)	0.893±0.149
BMD evolution (median, g/cm^2)	-0.001 (-0.026-0.022)

T-score (median, SD)	-1.2 (-1.9- -0.4)
Z-score (median, SD)	-0.3 (-0.8-0.4)
Total hip	
BMD (mean, g/cm^2)	0.941±0.163
BMD evolution (median, g/cm^2)	+0.005 (-0.019-0.023)
T-score (median, SD)	-0.8 (-1.6-0.1)
Z-score (median, SD)	-0.1 (-0.8-0.7)
TBS (mean)	1.263±0.136
Evolution of TBS (median)	-0.004 (-0.06-0.05)

Figure 5.2. Prevalence of vertebral fractures according to endocrine pathology



To determine the factors associated with prevalent vertebral fractures, the comparative analysis of the group according to the presence or absence of radiographically detected vertebral fractures was performed. Thus, it was observed that patients with VFs were significantly older than those without VFs (62.57±9.05 years vs. 56.09±12.21 years, p<0.001), had significantly more frequently a diagnosis of acromegaly (33.84% vs. 13.88%, p<0.001), the diagnosis of Cushing's syndrome (16.92% vs. 9.63%, p=0.081) and that of

primary hyperparathyroidism (9.23% vs. 18.98%, p=0.057) being at the limit of statistical significance. Also, subjects with VFs had more frequently non-vertebral fractures (7.69% vs. 2.55%, p=0.034) as well as osteodensitometric diagnosis of osteoporosis (47.69% vs. 24.07%, p<0.001) and treatment with antiresorptive agents (43.07% vs. 15.86%, p<0.001). The group with vertebral fractures was characterized by significantly lower values of bone remodeling markers (osteocalcin, serum β-crosslaps and P1NP). Regarding BMD values, it was significantly lower in fractured patients compared to those without VFs analyzed all skeletal segments (lumbar spine: 0.959±0.199 g/cm2 vs. 1.04±1.178 g/cm2, p=0.001; femoral neck: 0.820±0.163 g/cm2 vs. 0.907±0.143 g/cm2, p<0.001; total hip: 0.850±0.174 g/cm2 vs. 0.958±0.155 g/cm2, p<0.001), as well as significantly lower TBS (1.206±0.13 vs. 1.274±0.134, p<0.001).

Table V.2. Group characteristics according to the presence of vertebral fractures

Variables	*With VFs (N=71)*	*No VFs (N=347)*	*p*
Age (mean, years)	62.57±9.05	56.09±12.21	***<0.001***
Gender (%)			0.594
Female	55 (84.61%)	289 (81.86%)	
Male	10 (15.38%)	64 (18.13%)	
BMI (mean, kg/m^2)	27.47±5.55	27.88±6.67	0.632
Smoking (%)	24 (36.92%)	143 (40.5%)	0.587
DZ type 2/secondary (%)	21 (32.3)	125 (35.41%)	0.630
Endocrine pathology (%)			
acromegaly	22 (33.84%)	49 (13.88%)	***<0.001***
Cushing syndrome	11 (16.92%)	34 (9.63%)	*0.081*
Hypogonadism	7 (6.15%)	68 (19.26%)	0.101
Primary hyperparathyroidism	6 (9.23%)	67 (18.98%)	*0.057*
Hyperthyroidism	19 (29.23%)	135 (38.24%)	0.166
Non-VFs (%)	5 (7.69%)	9 (2.55%)	***0.034***
Osteoporosis diagnosis (%)	31 (47.69 %)	85 (24.07%)	***<0.001***
Antiresorptive treatment (%)	28 (43.07%)	56 (15.86%)	***<0.001***
Laboratory tests			
Total serum calcium (mean, mg/dL)	9.62±1.17	9.84±0.87	0.085
Phosphorus (mean, mg/dL)	3.45±0.59	3.44±0.66	0.932
(25-OH)D3 (mean, ng/mL)	24.23±8.78	24.43±10.38	0.897
PTH (median, pg/mL)	47.65 (37.47-62.29)	47.4 (37.48-77.74)	0.579

Markers of bone remodeling			
ALP (median, U/L)	71 (57-97)	76 (60.25-95)	0.413
OC (median, ng/mL)	16.45 (12.87-31.21)	25.09 (17.85-38.41)	***0.003***
β-CTX (median, ng/mL)	0.376 (0.199-0.541)	0.53 (0.316-0.841)	***<0.001***
P1NP (median, ng/mL)	41.6 (22.31-77.17)	62.79 (39.17-122.15)	***0.006***
DXA			
Lumbar spine			
BMD (mean, g/cm^2)	0.959±0.199	1.04±1.178	***0.001***
BMD evolution (median, g/cm^2)	+0.021 (-0.005-0.058)	+0.01 (-0.02-0.05)	0.222
T-score (median, SD)	-2.0 (-3.3- -0.6)	-1.3 (-2.1- -0.3)	***0.001***
Z-score (median, SD)	-0.9 (-1.6-0)	-0.7 (-1.5-0.2)	0.599
Femoral neck			
BMD (mean, g/cm^2)	0.820±0.163	0.907±0.143	***<0.001***
BMD evolution (median, g/cm^2)	-0.001 (-0.02-0.02)	-0.001 (-0.02-0.02)	0.980
T-score (median, SD)	-1.6 (-2.4- -1.0)	-1.0 (-1.7- -0.3)	***<0.001***
Z-score (median, SD)	-0.5 (-1.0-0)	-0.1 (-0.8-0.4)	***0.013***
Total hip			
BMD (mean, g/cm^2)	0.850±0.174	0.958±0.155	***<0.001***
BMD evolution (median, g/cm^2)	+0.01 (-0.006-0.02)	+0.005 (-0.02-0.02)	0.295
T-score (median, SD)	-1.2 (-2.1- -0.5)	-0.5 (-1.3-0.3)	***<0.001***
Z-score (median, SD)	-0.5 (-1.0-0.4)	0 (-0.7-0.8)	***0.007***
TBS (mean)	1.206±0.13	1.274±0.134	**<0.001**
TBS evolution (median)	-0.006 (-0.07-0.04)	-0.002 (-0.06-0.05)	0.423

Statistically significant variables in the comparative analysis of the group according to the presence of vertebral fractures were entered into the Cox binary regression model: age, diagnosis of acromegaly, Cushing's syndrome, primary hyperparathyroidism, diagnosis of osteoporosis, non-vertebral fractures, osteocalcin, β -CTX, P1NP, BMD lumbar spine, femoral neck, hip and TBS. Results are represented as OR with confidence interval and statistical significance. All variables maintained their statistical significance in the association with vertebral fractures, the diagnosis of primary hyperparathyroidism and Cushing's syndrome remaining at the limit of statistical significance. Subsequently, these variables were simultaneously entered into a multivariate regression model to determine independent risk factors for vertebral fractures. Following this analysis, the diagnosis of acromegaly, Cushing's syndrome and BMD at the hip remained significant. Thus, the

diagnosis of acromegaly increases the risk of vertebral fractures by 13.51 times (OR=13.51 95%CI: 4.07-44.79, p<0.001), the diagnosis of Cushing's syndrome increasing the risk of vertebral fractures by 9.63 times (OR=9.63, 95%CI : 2.34-39.63, p=0.002) and BMD at the hip level being a protective factor for VFs, increasing its value by 0.1 mg/cm2 decreases the risk of vertebral fractures by 99%.

Table V.3. Cox logistic regression for risk factors associated with vertebral fractures.

Variables	*Univariate Cox regression*			*Multivariate Cox regression*		
	OR	*95%CI*	*p*	*OR*	*95%CI*	*p*
Acromegaly	**3.17**	**1.74-5.7**	**<0.001**	**13.51**	4.07-44.79	***<0.001***
Cushing syndrome	**1.91**	**0.91-4**	0.086	**9.63**	2.34-39.63	***0.002***
Primary hyperparathyroidism	0.434	0.18-1.04	0.063	-	-	-
Age	1.054	1.027-1.082	***<0.001***	-	-	-
Non-VFs	3.18	1.032-9.83	***0.044***	-	-	-
Osteocalcin	0.978	0.959-0.999	***0.039***	-	-	-
Serum β-crosslaps	0.111	0.028-0.435	***0.002***	-	-	-
P1NP	0.990	0.982-0.999	***0.028***	-	-	-
Lumbar spine BMD	0.072	0.015-0.348	***0.001***	-	-	-
Femoral neck BMD	0.079	0.014-0.455	***0.004***	-	-	-
Hip BMD	**0.013**	**0.002-0.08**	**<0.001**	**0.001**	0.001-0.006	***<0.001***
TBS	0.024	0.003-0.183	***<0.001***	-	-	-
Osteoporosis diagnosis	2.87	1.66-4.95	***<0.001***	-	-	-

ROC analysis performed for age, BMD lumbar spine, femoral neck, hip and TBS highlighted the predictive ability of these continuous variables for VFs. Thus, it turned out that all variables have an acceptable predictive capacity, the area under the curve (AUC) for age being 0.664, for lumbar spine BMD being 0.638, for femoral neck BMD 0.664, for hip BMD 0.681 and for TBS 0.691. The threshold value with the best sensitivity and specificity for the prediction of VFs was identified for the variables entered in the ROC analysis, cut-off value for age 55.5 years having an accuracy of 51.32%, a sensitivity of 80%, a specificity of 46.02%, value positive predictive value (PVP) of 21.49% and negative predictive value (PNV) of 92.57%. The cut-off value of 0.970 for lumbar spine BMD showed an accuracy of 65.07%, a sensitivity of 60%, specificity of 66.01%, VPP of 24.53% and NPV of 89.96%.

Femoral neck BMD of 0.91 had an accuracy of 55.02%, sensitivity of 75.38%, specificity of 51.27%, VPP of 22.17% and NPV of 91.88%. The hip BMD threshold value of 0.855 was associated with an accuracy of 70.57%, sensitivity of 49.23%, specificity of 74.5%, VPP of 26.23% and NPV of 88.85%. The TBS value of 1.196 was associated with an accuracy of 70.33%, sensitivity of 50.77%, specificity of 73.94%, VPP of 26.4% and NPV of 89.08%. All this information is also reproduced in Table V.4.

Table V.4. Predictive analysis of age, BMD and TBS for vertebral fractures

Variables	*Cut-off*	*Accuracy*	*Specificity*	*Sensitivity*	*PPV*	*NPV*
Age (years)	**55.5**	51.32%	46.02%	80%	21.49%	92.57%
Lumbar spine BMD (g/cm^2)	**0.970**	65.07%	66.01%	60%	24.53%	89.96%
Femoral neck BMD (g/cm^2)	**0.91**	55.02%	51.27%	75.38%	22.17%	91.88%
Hip BMD (g/cm^2)	**0.855**	70.57%	74.5%	49.23%	26.23%	88.85%
TBS	**1.196**	70.33%	73.94%	50.77%	26.4%	89.08%

5.2. Analysis of the acromegaly group

The group of patients with acromegaly included 71 subjects. The batch was characterized by normal bone mineral density, BMD, mean/median T-score and Z-score at the level of all analyzed skeletal segments but partially degraded bone according to the mean value of TBS, being 1.259±0.122. The prevalence of vertebral fractures was 30.09%.

When comparing patients with acromegaly according to the presence of vertebral fractures, it was observed that fractured patients were significantly older (62.19±9.03 years vs. 52.94±11.87 years, p=0.002) and a significantly older age at diagnosis (53.19± 11.18 years vs. 45.18±11.73 years, p=0.01). They also had significantly lower values of bone formation markers (alkaline phosphatase, osteocalcin, P1NP) but no significant differences in bone resorption markers. Fractured patients had a more frequent osteodensitometric diagnosis of osteoporosis (33.3% vs. 8%, p=0.007), being more frequently under antiresorptive treatment (23.8% vs. 6%). Thus, BMD was significantly lower in all skeletal segments compared to patients without VFs (lumbar spine: 1.03±0.2 g/cm2 vs. 1.131±0.187 g/cm2, p=0.046, femoral neck: 0.904±0.117 g/cm2 vs. 1.012±0.142 g/cm2, p=0.003, hip: 0.933±0.13 g/cm2 vs. 1.049±0.144 g/cm2, p=0.002) but without significant differences in TBS values (1.242±0.131 vs. 1.266±0.119 , p=0.466).

Table V.5. Characteristics of the acromegaly group according to the presence of vertebral fractures

Variables	*With VFs (N=22)*	*No VFs (N=49)*	*p*
Age (mean, years)	62.19±9.03	52.94±11.87	***0.002***
Gender (%)			0.904
Female	15 (71.42%)	35 (70%)	
Male	6 (28.57%)	15 (30%)	
BMI (mean, kg/m^2)	29.44±3.23	28.67±5.11	0.528
Smoking (%)	5 (23.8%)	16 (32%)	0.490
DZ type 2/secondary (%)	6 (28.6%)	22 (44%)	0.225
Tumor size – macroadenoma (%)	18 (85.71%)	43 (86%)	0.975
Age at diagnosis (mean, years)	53.19±11.18	45.18±11.73	***0.01***
Active disease (%)	18 (85.71%)	45 (90%)	0.602
Duration of illness (median, months)	69 (17.5-189.5)	56.5 (4.75-123)	0.426
Prolactin co-secretion (%)	3 (14.28%)	11 (22%)	0.456
Hypogonadism (%)	14 (66.66%)	30 (60%)	0.597
Osteoporosis diagnosis (%)	7 (33.3%)	4 (8%)	***0.007***
Antiresorptive treatment (%)	5 (23.8%)	3 (6%)	***0.03***
Laboratory tests			
IGF-1 (median, ng/mL)	243.4 (179.3-306.6)	320.7 (188.8-491.6)	0.199
IGF-1 x ULN (median)	1.06 (0.76-1.27)	1.31 (0.82-2.1)	0.197
GH random (median, ng/ml)	0.73 (0.4-2)	1.34 (0.4-3.2)	0.217
Prolactin (median, ng/mL)	3.7 (0.8-8.5)	5.17-0.44-9.5)	0.955
Total serum calcium (mean, mg/dL)	9.31±1.43	9.51±1.09	0.516
Phosphorus (median, mg/dL)	3.6 (3.25-3.8)	3.69 (3.37-4.1)	0.171
(25-OH)D3 (mean, ng/mL)	21.83±8.9	24.12±8.15	0.363
Parathyroid hormone (mean, pg/mL)	44.01±14.96	40.88±16.72	0.564
Bone turnover markers			
Alkaline phosphatase (median, U/L)	70 (57-91.75)	74.5 (54.7-90.25)	**0.766**
Osteocalcin (median, ng/mL)	15.3 (12.5-25.18)	25.67 (17-40.04)	***0.041***
Serum β-crosslaps (median, ng/mL)	0.396 (0.25-0.54)	0.543 (0.36-0.87)	0.066
P1NP (median, ng/mL)	27.44 (21.62-66.13)	67.5 (34.9-99.7)	***0.023***
DXA			
Lumbar spine			
BMD (mean, g/cm^2)	1.03±0.2	1.131±0.187	***0.046***
BMD evolution (median, g/cm^2)	+0.009 (-0.01-0.4)	+0.004 (-0.04-0.04)	0.694

T-score (median, SD)	-1.6 (-2.7- -0.5)	-0.8 (-1.6-0.8)	*0.051*
Z-score (median, SD)	-0.2 (-1.5-0.1)	-0.3 (-1.3-0.7)	*0.329*
Femoral neck			
BMD (mean, g/cm^2)	0.904±0.117	1.012±0.142	***0.003***
BMD evolution (median, g/cm^2)	-0.008 (-0.04-0.01)	-0.01 (-0.04-0.01)	*0.955*
T-score (median, SD)	-1.4 (-1.6- -0.3)	-0.3 (-1.0-0.6)	***0.003***
Z-score (median, SD)	-0.1 (-0.7-0.5)	0.4 (-0.2-1.1)	***0.055***
Total hip			
Hip BMD (mean, g/cm^2)	0.933±0.13	1.049±0.144	***0.002***
BMD evolution (median, g/cm^2)	+0.002 (-0.01-0.02)	-0.007 (-0.04-0.01)	0.257
T-score (median, SD)	-1.0 (-1.4- -0.2)	0.2 (-0.8-0.8)	***0.002***
Z-score (median, SD)	-0.2 (-0.7-0.6)	0.6 (-0.2-1.1)	***0.042***
TBS (mean)	1.242±0.131	1.266±0.119	0.466
TBS evolution (median)	-0.044 (-0.07-0.02)	-0.01 (-0.08-0.051)	0.363

Statistically significant variables in the comparative analysis of the group with acromegaly according to the presence of vertebral fractures were entered into a Cox binary regression model: age, age at diagnosis, diagnosis of osteoporosis, alkaline phosphatase, osteocalcin, P1NP, BMD lumbar spine, femoral neck and hip. Results are represented as OR with confidence interval and statistical significance. The variables that retained their statistical significance in association with vertebral fractures after univariate logistic regression were age, age at diagnosis, diagnosis of osteoporosis, lumbar spine, femoral neck and hip BMD. These variables were entered into a multivariate regression model to determine independent risk factors for vertebral fractures in acromegaly. This analysis found that only age at diagnosis and low hip BMD were independent risk factors for vertebral fractures in acromegaly. The details of the analysis are shown in Table V.7.

Table V.6. Cox logistic regression for risk factors associated with vertebral fractures in acromegaly

Variables	*Univariate Cox regression*			*Multivariate Cox regression*		
	OR	*95%CI*	*p*	*OR*	*95%CI*	*p*
Age	1.097	1.032-1.166	***0.003***	-	-	-
Age at diagnosis	1.071	1.019-1.125	***0.007***	**1.095**	1.011-1.186	***0.025***
Alkaline phosphatase	0.996	0.981-1.011	0.557	-	-	-
Osteocalcin	0.959	0.905-1.016	0.160	-	-	-
P1NP	0.982	0.961-1.004	0.103	-	-	-
Lumbar spine BMD	0.037	0.002-0.762	***0.033***	-	-	-
Femoral neck BMD	0.005	0.001-0.337	***0.013***	-	-	-
Hip BMD	0.003	0.001-0.187	**0.006**	**0.003**	0.001-0.242	***0.01***
Osteoporosis diagnosis	5.25	1.347-20.46	**0.017**	-	-	-

5.3. Analysis of the Cushing syndrome cohort

The group of patients with Cushing's syndrome consisted of 45 subjects. Vertebral fractures had a prevalence of 24.4% and non-vertebral fractures of 6.7% (3 events). During the DXA evaluation, an average T score of osteopenia was observed at the level of the lumbar spine and the femoral neck, and the average TBS value of 1.216±0.12 suggests partially degraded bone.

The patients were compared according to the presence of vertebral fractures and an association with the exogenous cause of the disease was observed, all 4 patients having vertebral fractures ($p<0.001$), the diagnosis of osteoporosis was at the limit of statistical significance ($p=0.062$) but the patients fractured had antiresorptive treatment more frequently (45.5% vs. 8.8%, $p=0.006$). There were no significant differences in bone remodeling markers and in terms of BMD, in the presence of vertebral fractures, BMD at the femoral neck and hip were significantly lower (femoral neck: 0.78±0.09 vs. 0.938±0.12, $p < 0.001$, hip: 0.826±0.105 vs. 1.023±0.125, $p<0.001$). There were no significant differences in lumbar spine BMD or TBS.

Table V.7. Characteristics of the group with Cushing's syndrome according to the presence of vertebral fractures

Variables	*With VFs (N=11)*	*No VFs (N=34)*	*p*
Age (mean, years)	61.45±10.3	58.85±9.65	0.449
Gender (%)			0.978
Female	10 (90.9%)	31 (91.2%)	
Male	1 (9.1%)	3 (8.8%)	
Smoking (%)	7 (63.6%)	21 (61.8%)	0.911
BMI (mean, kg/m^2)	27.52±5.53	30.77±5.29	0.087
Active disease (%)	7 (63.6%)	28 (82.4%)	0.194
Non-VFs (%)	2 (18.2%)	1 (2.9%)	0.078
Antiresorptive treatment (%)	5 (45.5%)	3 (8.8%)	***0.006***
DZ type 2/secondary (%)	6 (54.5%)	23 (67.6%)	0.430
Osteoporosis diagnosis (%)	5 (45.5%)	5 (17.6%)	*0.062*
Cause of disease (%)			
exogenous	4 (36.4%)	0 (%)	***<0.001***
ACTH-dependent	3 (27.3%)	16 (47.1%)	0.248
ACTH-independent	4 (36.4%)	18 (52.9%)	0.339
Laboratory tests			
ACTH (median, ng/mL)	13.53 (6.3-111.97)	34.9 (8.05-71.25)	0.648
Basal cortisol (median, microg/dL)	9.35 (5.59-13.17)	13.13 (10.22-18.48	0.07
Cortisol after DXM inhibition (median, microg/dL)	3.99 (2.55-10.75)	4.99 (2.02-8.39)	0.965
Total serum calcium (mean, mg/dL)	9.51±0.57	9.7±0.41	0.237
Phosphorus (mean, mg/dL)	3.66±0.51	3.6±0.58	0.902
(25-OH)D3 (mean, ng/mL)	28.11±9.91	27.38±9.47	0.848
Parathyroid hormone (mean, pg/mL)	38.9±8.88	43.85±20.93	0.526
Bone turnover markers			
Alkaline phosphatase (mean, U/L)	64.44±17.4	61.55±14.9	0.644
Osteocalcin (median, ng/mL)	14.36 (3.51-21.37)	12.7 (10.21-15.58)	0.861
Serum β-crosslaps (median, ng/mL)	0.16 (0.102-0.48)	0.252 (0.205-0.312)	0.124
P1NP (median, ng/mL)	29.01 (11.91-55.42)	33.29 (26.29-47.35)	0.535
DXA			
Lumbar spine			
BMD (mean, g/cm^2)	1.006±0.164	1.074±0.153	0.221
BMD evolution (median, g/cm^2)	+0.04 (0.005-0.08)	0.01 (-0.02-0.03)	0.164
T-score (median, SD)	-1.3 (-2.7- -0.3)	-0.9 (-1.8-0)	0.223

Z-score (median, SD)	-0.1 (-0.9-0.2)	-0.6 (-1.3-0.7)	0.516
Femoral neck			
BMD (mean, g/cm^2)	0.78±0.09	0.938±0.12	***<0.001***
BMD evolution (median, g/cm^2)	+0.005 (-0.01-0.02)	-0.01 (-0.03-0.02)	0.120
T-score (median, SD)	-1.9 (-2.4- -1.3)	-0.7 (-1.2- -0.2)	***<0.001***
Z-score (median, SD)	-0.5 (-1.0- -0.3)	0.3 (-0.3-0.6)	***0.001***
Total hip			
BMD (mean, g/cm^2)	0.826±0.105	1.023±0.125	***<0.001***
BMD evolution (median, g/cm^2)	+0.016 (0.002-0.018)	-0.01 (-0.02-0.015)	0.07
T-score (median, SD)	-1.7 (-2.1- -1.0)	0.1 (-0.4-0.6)	***<0.001***
Z-score (median, SD)	-0.5 (-0.8-0.2)	0.6 (0-1.3)	***<0.001***
TBS (mean)	1.225±0.12	1.213±0.122	0.779
TBS evolution (median)	-0.09 (-0.15-0.06)	+0.01 (-0.05-0.11)	0.058

Osteoporosis diagnosis, femoral neck and hip BMD were analyzed by bivariate Cox regression. The exogenous cause of Cushing's syndrome could not be analyzed because all 4 patients with exogenous Cushing's syndrome had vertebral fractures. Femoral neck and hip BMD were significantly associated with vertebral fractures on analysis and entered together in a multivariate regression model. Thus, only hip BMD turned out to be a risk factor for vertebral fractures, behaving as a protective factor (OR=0.001, 95%CI: 0.001-0.048, p=0.006).

Table V.8. Cox logistic regression for risk factors associated with vertebral fractures in Cushing's syndrome

Variables	*Univariate Cox regression*			*Multivariate Cox regression*		
	OR	*95%CI*	*p*	*OR*	*95%CI*	*p*
Osteoporosis diagnosis	3.88	0.88-17.05	0.072	-	-	-
Femoral neck BMD	0.001	0.001-0.012	***0.002***	-	-	-
Hip BMD	0.001	0.001-0.007	***0.001***	**0.001**	0.001-0.048	***0.006***

5.4. Analysis of the group with hypogonadism

The hypogonadism group included 75 patients. Vertebral fractures were present in 9.3% of patients and non-vertebral fractures in 4%. Through the DXA evaluation, the group was characterized by an osteopenia score and 34.7% had an osteodensitometric score of osteoporosis and 9.3% were under treatment with antiresorptive agents. According to the mean TBS value of 1.287±0.145, the patients had partially degraded bone.

Patients were compared according to the presence of vertebral fractures and statistically significant differences were observed in terms of femoral neck, hip and TBS BMD, which were significantly lower in fractured patients (femoral neck: 0.745±0.2 g/cm2 vs. 0.889 ±0.155 g/cm2, p=0.026, hip: 0.789±0.198 g/cm2 vs. 0.923±0.142 g/cm2, p=0.026, TBS: 1.168±0.09 vs. 1.299±0.144, p=0.023).

Table V.9. Characteristics of the group with hypogonadism according to the presence of vertebral fractures

Variables	*With VFs (N=7)*	*No VFs (N=68)*	*p*
Age (mean, years)	58.43±13.66	50.63±13.16	0.141
Gender (%)			0.633
Female	4 (57.1%)	45 (66.2%)	
Male	3 (42.9%)	23 (33.8%)	
Smoking (%)	3 (42.9%)	26 (38.2%)	0.811
BMI (mean, kg/m^2)	25.87±2.48	28.49±7.06	0.335
Non-VFs (%)	1 (14.3%)	2 (2.9%)	0.145
Antiresorptive treatment (%)	1 (14.3%)	6 (8.8%)	0.636
DZ type 2/secondary (%)	0 (0%)	12 (17.6%)	0.225
Osteoporosis diagnosis (%)	4 (57.1%)	22 (32.4%)	0.189
Cause of disease (%)			0.838
Hypogonadotropic	5 (71.4%)	46 (67.6%)	
Hypergonadotropic	2 (28.6%)	22 (32.4%)	
Hormone replacement therapy (%)	3 (42.9%)	24 (35.5%)	0.691
Age at diagnosis (mean, years)	42.86±27.35	37.88±15.26	0.462
Duration of illness (median, months)	180 (6-396)	108 (48-240)	0.846
Laboratory tests			
FSH (median, mIU/mL)	10 (2.9-19.8)	2.91 (1.62-47.5)	0.461
LH (median, mIU/mL)	2.7 (1.58-8)	1.2 (0.24-17.5)	0.415
Testosterone (median, ng/mL)	0.15 (0.13-1)	1.36 (0.12-2.35)	0.944

Estradiol (median, pg/mL)	10 (5.81-47.5)	10 (10-20.5)	0.866
Prolactin (median, ng/mL)	14.12 (6.18-27.45)	8.15 (5.11-14.16)	0.412
Total serum calcium (mean, mg/dl)	9.22±0.51	9.4±0.4	0.133
Phosphorus (mean, mg/dl)	3.71±0.43	3.63±0.68	0.785
(25-OH)D3 (mean, ng/mL)	22.33±6.6	30.26±14.79	0.202
Parathyroid hormone (mean, pg/mL)	47.69±8.46	49.81±20.39	0.839
Bone turnover markers			
Alkaline phosphatase (mean, U/L)	71.16±25.67	74.1±25.19	0.787
Osteocalcin (mean, ng/mL)	14.27±3.43	21.26±11.6	0.242
Serum β-crosslaps (mean, ng/mL)	0.358±0.04	0.449±0.22	0.428
P1NP (median, ng/mL)	41.6 (20.84-71)	50.93 (29-68.7)	0.746
DXA			
Lumbar spine			
BMD (mean, g/cm^2)	0.948±0.27	0.988±0.14	0.529
BMD evolution (median, g/cm^2)	0.017 (-0.01-0.1)	+0.003 (-0.03-0.04)	0.363
T-score (median, SD)	-2.2 (-3.6- -0.8)	-1.5 (-2.6- -0.9)	0.208
Z-score (median, SD)	-1.4 (-2.6-0.8)	-1.4 (-2.1- -0.7)	0.750
Femoral neck			
BMD (mean, g/cm^2)	0.745±0.2	0.889±0.155	***0.026***
BMD evolution (median, g/cm^2)	+0.004 (-0.01-0.03)	-0.004 (-0.03-0.01)	0.406
T-score (median, SD)	-2.4 (-3.0- -2.0)	-1.2 (-2.0- -0.5)	***0.01***
Z-score (median, SD)	-1.2 (-2.1- -0.6)	-0.6 (-1.2-0)	***0.07***
Total hip			
BMD (mean, g/cm^2)	0.789±0.198	0.923±0.142	***0.026***
BMD evolution (median, g/cm^2)	-0.003 (-0.04-0.037)	+0.002 (-0.02-0.01)	0.954
T-score (median, SD)	-2.1 (-3.0- -1.6)	-0.8 (-1.7- -0.1)	***0.01***
Z-score (median, SD)	-1.2 (-2.2- -0.4)	-0.6 (-1.1-0)	0.068
TBS (mean)	1.168±0.09	1.299±0.144	**0.023**
TBS evolution (median)	+0.01 (-0.03-0.05)	-0.002 (-0.06-0.04)	0.551

The variables that were significantly associated with vertebral fractures in the comparative analysis were analyzed using univariate Cox logistic regression: femoral neck BMD, hip BMD and TBS. All retained their statistical significance but when entered into a multivariate logistic regression model, none of these proved to be an independent risk factor for vertebral fractures, with femoral neck BMD and TBS being at the limit of statistical significance.

Tabel V.10. Cox logistic regression for factors associated with vertebral fractures in hypogonadism

Variables	*Univariate Cox regression*			*Multivariate Cox regression*		
	OR	*95%CI*	*p*	*OR*	*95%CI*	*p*
Femoral neck BMD	0.001	0.001-0.415	***0.027***	0.001	0.001-2.09	*0.078*
Hip BMD	0.001	0.001-0.6	***0.034***	-	-	-
TBS	0.001	0.001-0.495	***0.031***	0.001	0.001-1.87	*0.071*

5.5. Analysis of the group with primary hyperparathyroidism

There were 73 patients diagnosed with primary hyperparathyroidism. The prevalence of vertebral fractures was 8.2% and of non-vertebral fractures 2.7%. Osteodensitometrically, the group was characterized by osteopenia, the lowest T score being at the level of the radius 33% and the average TBS value of 1.248±0.138 showed that the subjects had partially degraded bone.

After comparing the group of patients with primary hyperparathyroidism according to the presence of vertebral fractures, statistically significant differences were observed regarding the prevalence of osteoporosis diagnosis and BMD values at the femoral neck and hip. Fractured patients had a higher osteodensitometric diagnosis of osteoporosis (100% vs. 43.3%, p=0.008) and had lower neck and hip BMD (femoral neck: 0.735±0.163 g/cm2 vs. 0.849 ±0.123 g/cm2, p=0.039, hip: 0.766±0.193 g/cm2 vs. 0.911±0.135 g/cm2, p=0.017).

Table V.11. Characteristics of patients with primary hyperparathyroidism according to the presence of vertebral fractures

Variables	*With VFs (N=6)*	*No VFs (N=67)*	*p*
Age (mean, years)	65.67±9.6	60.81±11.65	0.325
Gender (%)			0.308
Female	6 (100%)	57 (85.1%)	
Male	0 (0%)	10 (14.9%)	
Smoking (%)	2 (33.3%)	23 (34.3%)	0.961
BMI (mean, kg/m^2)	24.31±2.2	27.8±4.27	0.053
Non-VFs (%)	0 (0%)	2 (3%)	0.668
Antiresorptive treatment (%)	3 (50%)	16 (23.9%)	0.162

DZ type 2/secondary (%)	2 (33.3%)	11 (16.4%)	0.299
Osteoporosis diagnosis (%)	6 (100%)	29 (43.3%)	***0.008***
Active disease (%)	5 (83.5%)	56 (83.6%)	0.987
Cause of disease (%)			0.370
Parathyroid adenoma	6 (100%)	59 (88.1%)	
Parathyroid hyperplasia	0 (0%)	8 (11.9%)	
Age at diagnosis (mean, years)	67.4±8.2	59.23±11.73	0.132
Laboratory tests			
Total serum calcium (mean, mg/dL)	11.65±0.99	10.94±0.86	0.06
Phosphorus (mean, mg/dL)	2.51±0.52	2.77±0.52	0.253
(25-OH)D3 (mean, ng/mL)	24.86±10.67	22.69±7.9	0.567
Parathormone (median, pg/mL)	223.5 (113.3-435.7)	124.95 (87.95-177.65)	0.064
Bone turnover markers			
Alkaline phosphatase (median, U/L)	103 (63.5-151.5)	76 (66-88)	0.344
Osteocalcin (median, ng/mL)	29.18 (13.66-113)	33.44 (21.96-41.76)	0.727
Serum β-crosslaps (median, ng/mL)	0.384 (0.152-1.187)	0.572 (0.34-0.93)	0.427
P1NP (median, ng/mL)	74.16 (30.31-97)	63.26 (44.65-99.16)	1
DXA			
Lumbar spine			
BMD (mean, g/cm^2)	0.956±0.205	1.012±0.181	0.479
BMD evolution (median, g/cm^2)	+0.05 (0.04-0.07)	+0.02 (-0.01-0.06)	0.148
T-score (median, SD)	-2.2 (-3.1-0.1)	-1.6 (-2.3- -0.5)	0.494
Z-score (median, SD)	-0.7 (-1.5-0.5)	-05 (-1.7-0.5)	0.811
Femoral neck			
BMD (mean, g/cm^2)	0.735±0.163	0.849±0.123	***0.039***
BMD evolution (median, g/cm^2)	-0.02 (-0.03-0.01)	+0.005 (-0.01-0.02)	0.507
T-score (median, SD)	-1.7 (-3.1- -1.3)	-1.4 (-2.0- -0.8)	0.142
Z-score (median, SD)	-0.9 (-1.5- -0.4)	-0.4 (-1.0-0.1)	0.12
Total hip			
BMD (mean, g/cm^2)	0.766±0.193	0.911±0.135	***0.017***
BMD evolution (median, g/cm^2)	+0.004 (0-0.006)	+0.011 (-0.005-0.02)	0.579
T-score (median, SD)	-1.4 (-3.2- -0.6)	-0.8 (-1.6-0)	0.095
Z-score (median, SD)	-0.4 (-0.9-0.3)	-0.2 (-0.7-0.7)	0.532
Radius 33%			

BMD (mean, g/cm^2)	0.503±0.1	0.587±0.188	0.285
BMD evolution (median, g/cm^2)	-0.001 (-0.01-0.002)	0.008 (-0.01-0.02)	0.773
T-score (median, SD)	-2.8 (-4.5- -1.7)	-1.6 (-2.5 -0.6)	0.055
Z-score (median, SD)	-1.3 (-2- -0.1)	-0.5 (-1.6-0.3)	0.320
TBS (mean)	1.205±0.11	1.252±0.14	0.429
TBS evolution (median)	+0.033 (-0.008-0.05)	+0.001 (-0.06-0.048)	0.245

Femoral neck and hip BMD were analyzed using binary Cox logistic regression. The diagnosis of osteoporosis could not be included in the model because patients with vertebral fractures had a 100% prevalence of osteodensitometric diagnosis of osteoporosis. These remained significant in association with vertebral fractures on univariate Cox analysis. When both were entered into a multivariate regression model, only hip BMD emerged as a risk factor for vertebral fractures, being a protective factor (OR=0.001, 95%CI:0.001-0.543, p =0.026).

Table V.12. Cox logistic regression for risk factors associated with vertebral fractures in primary hyperparathyroidism

Variables	*Univariate Cox regression*			*Multivariate Cox regression*		
	OR	*95%CI*	*p*	*OR*	*95%CI*	*p*
Femoral neck BMD	0.001	0.001-0.979	***0.049***	-	-	-
Hip BMD	0.001	0.001-0.453	***0.026***	**0.001**	0.001-0.453	***0.026***

5.6. Analysis of the group with hyperthyroidism

One hundred and fifty-four patients with hyperthyroidism were recruited. The prevalence of vertebral and non-vertebral fractures was 12.3% and 3.9%, respectively. The group was characterized by osteopenia and partially degraded bone having an average TBS of 1.275±0.139.

The group of patients with hyperthyroidism was analyzed according to the presence of vertebral fractures. The fractured patients were older (64.16±6.3 years vs. 57.03±11.46 years, p=0.009) and had a more frequent osteodensitometric diagnosis of osteoporosis (57.9% vs. 21.5%, p=0.001) as well as antiresorptive treatment (47.4% vs. 8.9%, p<0.001). They more frequently had toxic multinodular goiter (TMNG) (68.4% vs. 33.3%, p-0.003) and toxic adenoma (15.8% vs. 4.4%, p=0.048) and less frequently Basedow's disease (15.8%

vs. 62.2 %, p<0.001) and etiology. Ophthalmopathy was also less frequent in the group without VFs. The value of parathormone was significantly higher in patients with VFs (55.43±27.5 pg/mL vs. 43.76±16.4 pg/mL, p=0.036). BMD at lumbar spine (0.859±0.193 g/cm2 vs. 1.041±0.179 g/cm2, p<0.001) and hip (0.810±0.2 g/cm2 vs. 0.949±0.165 g/cm2, p=0.001) was significantly lower in subjects with VFs, BMD at the level of the femoral neck being at the limit of statistical significance (0.789±0.18 g/cm2 vs. 0.885±0.2 g/cm2, p=0.058). TBS was significantly lower in fractured patients (1.167±0.164 vs. 1.29±0.128, <0.001).

Table V.13. Characteristics of the group with hyperthyroidism according to the presence of vertebral fractures

Variables	*With VFs (N=19)*	*No VFs (N=135)*	*p*
Age (mean, years)	64.16±6.3	57.03±11.46	**0.009**
Gender (%)			0.157
Female	19 (100%)	122 (90.4%)	
Male	0 (0%)	13 (9.6%)	
Smoking (%)	7 (36.8%)	57 (42.2%)	0.656
BMI (mean, kg/m^2)	28.08±5.21	27.77±6	0.834
Non-VFs (%)	2 (10.5%)	4 (3%)	0.111
Antiresorptive treatment (%)	9 (47.4%)	12 (8.9%)	**<0.001**
DZ type 2/secondary (%)	4 (21.1%)	37 (27.4%)	0.557
Osteoporosis diagnosis (%)	11 (57.9%)	29 (21.5%)	***0.001***
Cause of disease (%)			***<0.001***
Basedow-Graves disease	3 (15.8%)	84 (62.2%)	***<0.001***
TMNG	13 (68.4%)	45 (33.3%)	***0.003***
Toxic adenoma	3 (15.8%)	6 (4.4%)	***0.048***
Ophthalmopathy (%)	1 (5.3%)	39 (28.9%)	***0.028***
Glucocorticoid treatment (%)	0 (0%)	17 (12.6%)	0.101
Active/uncontrolled disease (%)	12 (63.3%)	78 (57.8%)	0.656
Time since diagnosis (median, years)	24 (1-100)	11 (1-42)	0.140
Laboratory tests			
FT4 (median, pmol/L)	15 (12.07-16.29)	13.4 (11.77-21.21)	0.967
TSH (median, microIU/mL)	0.18 (0.001-1.01)	0.3 (0.02-1.74)	0.135
Total serum calcium (mean, mg/dL)	9.58±0.37	9.65±0.51	0.559
Phosphorus (mean, mg/dL)	3.52±0.53	3.52±0.53	0.996
(25-OH)D3 (mean, ng/mL)	24.5±8.25	21.34±7.8	0.195

Parathyroid hormone (mean, pg/mL)	55.43±27.5	43.76±16.4	***0.036***
Bone turnover markers			
Alkaline phosphatase (mean, U/L)	86.24±29.06	99.05±47.33	0.344
Osteocalcin (median, ng/mL)	22.97 (14.8-39.36)	30.66 (20.45-51.28)	0.098
Serum β-crosslaps (median, ng/mL)	0.399 (0.25-0.53)	0.7 (0.39-1.21)	***0.006***
P1NP (median, ng/mL)	74.87 (37.8-125.22)	99.95 (54.33-199)	0.172
DXA			
Lumbar spine			
BMD (mean, g/cm^2)	0.859±0.193	1.041±0.179	***<0.001***
BMD evolution (median, g/cm^2)	+0.02 (-0.01-0.05)	+0.01 (-0.01-0.07)	1
T-score (median, SD)	-3.3 (-3.8- -1.2)	-1.2 (-2.1- -0.2)	***<0.001***
Z-score (median, SD)	-1.2 (-2.4-0.2)	-0.5 (-1.2-0.2)	0.067
Femoral neck			
BMD (mean, g/cm^2)	0.789±0.18	0.885±0.2	0.058
BMD evolution (median, g/cm^2)	+0.009 (-0.02-0.3)	0.012 (-0.007-0.027)	0.692
T-score (median, SD)	-1.9 (-2.4- -0.9)	-1.0 (-1.7- -0.4)	**0.004**
Z-score (median, SD)	-0.5 (-0.9-0.5)	0 (-0.6-0.4)	0.236
Total hip			
BMD (mean, g/cm^2)	0.810±0.2	0.949±0.165	***0.001***
BMD evolution (median, g/cm^2)	+0.01 (-0.004-0.04)	+0.01 (-0.007-0.04)	0.828
T-score (median, SD)	-1.6 (-2.5- -0.5)	-0.5 (-1.3-0.2)	***0.005***
Z-score (median, SD)	0 (-1.0-0.4)	0 (0-0.8)	0.117
TBS (mean)	1.167±0.164	1.29±0.128	**<0.001**
TBS evolution (median)	+0.02 (-0.007-0.05)	-0.01 (-0.04-0.04)	0.376

On univariate logistic regression analysis, age, diagnosis of Grave's disease, toxic multinodular goiter, parathyroid hormone, lumbar spine BMD, hip, and TBS retained statistical significance. After entered into a multivariate logistic regression model, the variables that proved to be protective factors for vertebral fractures were lumbar spine BMD (OR=0.011, 95%CI: 0.001-0.329, p=0.009) and TBS (OR=0.015, 95%CI: 0.001-0.965, p=0.048).

Table V.14. Cox logistic regression for risk factors associated with vertebral fractures in hyperthyroidism

Variables	*Cox univariate regression*			*Cox multivariate regression*		
	OR	*95%CI*	*p*	*OR*	*95%CI*	*p*
Age	1.074	1.017-1.134	***0.011***	-	-	-
Grave's disease	0.114	0.032-0.410	***0.001***	-	-	-
TMNG	4.33	1.545-12.15	***0.005***	-	-	-
Toxic adenoma	4.031	0.918-17.7	0.065	-	-	-
Parathormone	1.030	1.001-1.061	***0.046***	-	-	-
Lumbar spine BMD	0.003	0.001-0.065	***<0.001***	**0.011**	0.001-0.329	***0.009***
Femoral neck BMD	0.211	0.03-1.467	0.116	-	-	-
Hip BMD	0.008	0.001-0.171	***0.002***	-	-	-
TBS	0.002	0.001-0.071	***0.001***	**0.015**	0.001-0.965	***0.048***

6. Conclusions and original contributions

6.1. Conclusions

The results of the evaluation of a group of 418 patients with endocrine pathologies considered to be secondary causes of osteoporosis and associated with bone fragility showed an increased prevalence of vertebral fractures, of 15.55%. Risk factors for vertebral fractures in the entire patient cohort were diagnosis of acromegaly, Cushing's syndrome, and low hip BMD. TBS was found to be a risk factor for vertebral fractures only in the group of patients with hyperthyroidism. Low hip BMD is a risk factor for vertebral fractures in acromegaly, Cushing's syndrome, and primary hyperparathyroidism, and lumbar spine BMD is a risk factor for vertebral fractures in hyperthyroidism. Femoral neck BMD and TBS were at the borderline of statistical significance as risk factors for vertebral fractures in patients with hypogonadism. Another independent risk factor for vertebral fractures in acromegaly is older age at diagnosis.

Vertebral fractures are the most common osteoporotic fractures and their prevalence increases with age. Their frequency increases significantly after the age of 70, when they can be identifiend in up to 20% of the population. Under the age of 60, the prevalence of vertebral fractures detected by imaging is around 3%[16,17]. Our cohort, with a mean age of 57 years, had a 5-fold increased prevalence of vertebral fractures for this age group. This reiterates the

fact that the endocrine diseases included in the study have a significant impact on bone fragility. The highest prevalence of vertebral fractures was detected in the acromegaly group, of 31% and in the Cushing syndrome group, of 24%, and the lowest in the group of patients with primary hyperparathyroidism, of 8.2%, all being higher compared to the expected value for the age group <60 years. Given the presence of a considerable number of vertebral fractures in the groups diagnosed with acromegaly and Cushing's syndrome, these two diseases were detected as independent risk factors for vertebral fractures. These results are consistent with information from the literature, with reported data on the prevalence of vertebral fractures in these pathologies ranging from 10% to 70-80%[18]. Thus, the presence of acromegaly increases the risk of vertebral fractures by 13.5 times and the presence of Cushing's syndrome increases the risk of VFs by 9.6 times.

Despite the fact that fractured patients were older than the non-fractured ones, age was not detected as a risk factor for vertebral fractures. This was true for the entire cohort as well as for each subgroup. The only group in which age at diagnosis was a risk factor for vertebral fractures was the acromegaly group. This result could be explained by the lack of early diagnosis of the disease, it being a known fact that acromegaly is a pathology that faces a delay of up to 10 years in the diagnosis from the time of onset given the rarity of the disease and the insidious installation clinical manifestations. Although disease activity was not an independent risk factor for VFs, the duration of active disease could significantly contribute to vertebral fragility.

The osteodensitometric diagnosis of osteoporosis was present in a quarter of the patients, but it did not represent an independent risk factor for VFs, the group being characterized by osteopenia. Hip BMD was revealed as an independent risk factor for VFs, both in the whole group and in the subgroups of patients with acromegaly, Cushing's syndrome and primary hyperparathyroidism. In patients with hyperthyroidism, low lumbar spine BMD has been identified as a factor in vertebral fractures. In patients with hypogonadism, BMD at the femoral neck was at the limit of statistical significance as a risk factor. The threshold value of 0.855 g/cm2 for BMD at the hip level had a sensitivity of 49.23% and a specificity of 74.5% in the prediction of vertebral fractures in the whole group of patients. This shows that an increase of 0.1 g/cm^2 in hip BMD decreases fracture risk by 99%.

The TBS value in the studied cohort was 1.263±0.136, suggesting that the whole group is characterized by partially degraded bone. Despite the fact that patients with vertebral

fractures had lower TBS values, 1.206±0.13 versus 1.274±0.134, it was not detected as an independent risk factor for prevalent vertebral fractures. TBS is considered to be an indirect index of evaluating the alteration of the bone quality, of the degradation of bone microarchitecture[19]. Thus, the results of our study show that all patients suffering from one of the endocrine diseases included in the study have bone quality impairment, but this does not significantly contribute to the occurrence of vertebral fractures. In the prediction analysis of TBS for vertebral fractures, the threshold value of 1.196 had a sensitivity of 50.77% and specificity of 73.94% in the prediction of vertebral fractures. The only groups in which TBS was below this threshold value were those of patients with hypogonadism and hyperthyroidism in the presence of vertebral fractures. TBS was detected as a risk factor for vertebral fractures only in the group with hyperthyroidism, in the group with hypogonadism being at the limit of statistical significance (p=0.071). This could be due to the small number of events, with only 7 cases of vertebral fractures being present in the group of patients with hypogonadism.

Diabetes mellitus was present in 35% of patients. This is known to be a cause of increased bone fragility, with diabetic patients usually presenting with vertebral fractures in conditions of a normal BMD[20]. However, vertebral fractures were not significantly associated with the presence of diabetes in any of the pathologies. We can deduce that the presence of carbohydrate metabolism changes frequently present in acromegaly, Cushing's syndrome or hyperthyroidism is not the cause of their bone fragility.

The association of hypogonadism in patients with acromegaly is one of the mechanisms of bone fragility proposed in the literature. In our group, the presence of hypogonadism did not significantly differ between the group with and without vertebral fractures, and considering the higher prevalence of vertebral fractures in the group with acromegaly compared to the one with hypogonadism, we can deduce that hypogonadism is not the cause of fractures in patients with acromegaly.

Considering the interest given in recent years to the usefulness of TBS in medical practice, we believe that this research contributes substantially to the development of studies evaluating the predictive role of TBS on incident vertebral fractures. The fact that bone microarchitecture is altered in all endocrine pathologies evaluated in this paper, by the presence of a low TBS, is essential information for further research. Establishing a causal relationship between the value of TBS or BMD and the occurrence of incident vertebral fractures in endocrine pathologies could help develop diagnostic and prevention guidelines

in this field. This is the research direction that is wanted to be pursued as a continuation of the doctoral research. Thus, follow-up of patients with re-evaluation at least 3 years after enrollment in the study will be considered, with the help of bone remodeling markers, DXA, TBS and vertebral fractures assessment (VFA). The reason for using VFA for patient follow-up is to limit their exposure to the significant radiation doses used in performing conventional radiological evaluations. The objectives are to determine the incidence of vertebral fractures in endocrine diseases and the risk factors for their occurrence.

6.2. Original contributions

The doctoral research addressed a topic of great interest in the field of endocrinology. Bone fragility is an element that contributes significantly to the morbidity of patients with endocrine diseases, additionally altering their quality of life. While some of the pathologies included in the study, such as primary hyperparathyroidism or hyperthyroidism, have good curative potential, changes in bone quantity and quality do not appear to be completely reversible, and thus the predisposition to fragility fractures remains high. This is all the more important in patients with chronic diseases, such as hypogonadism, or in whom curative interventions have not been successful, such as cases of acromegaly or Cushing's syndrome. In our study the prevalence of vertebral fractures did not differ significantly between patients with controlled or uncontrolled disease with hyperthyroidism, patients with acromegaly with cured or active disease, those with active or cured Cushing's syndrome, or patients with hypogonadism with or without a history of hormone replacement therapy.

The original contribution of this paper is related to highlighting the link between prevalent vertebral fractures and TBS. It is the only study that cumulatively analyzed the prevalence of vertebral fractures in endocrine diseases, associated risk factors, and the relationship between vertebral fractures and TBS. In acromegaly, this was studied only in 2 studies, both by Kužma et al., who found volumetric BMD at the hip but not TBS as a predictive factor for vertebral fractures, similar to our results[21,22]. Most studies showed significantly lower TBS in patients with Cushing's syndrome, but assessment of the association with vertebral fractures was studied in only two studies. Belaya and colleagues had similar results to ours, with fractured patients having low TBS but not being an independent risk factor for vertebral fractures[23]. Ferraù et al identified altered TBS with a value <1,300 as an independent risk factor for vertebral fractures[5]. Data on the association between TBS and vertebral fractures in patients with hypogonadism were not found in the literature. We only mention the study by Nguyen et al., which observed a significant

correlation between fragility fractures of any type and a TBS<1.35. Regarding the association between TBS values and prevalent vertebral fractures in primary hyperparathyroidism, the data from the 4 studies that analyzed this connection are contradictory[24–27]. In addition, our study is the first to consider the determination of the predictive value of TBS for vertebral fractures in patients with hyperthyroidism.

Thus, we consider that the contribution to the field of research regarding the role of TBS in the prediction of vertebral fractures in endocrine diseases is significant and the continuation of research in this field may bring a benefit in the preventive approach of patients with endocrine diseases associated with bone fragility.

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