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CLINICAL INDICATORS OF BONE DETERIORATION IN ALCOHOLIC LIVER CIRRHOSIS AND CHRONIC ALCOHOLISM WITHOUT CIRRHOSIS

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KLINIČKI POKAZATELJI PROPADANJA KOŠTANOG TKIVA U ALKOHOLNOJ CIROZI JETRE I HRONIČNOM ALKOHOLIZMU BEZ CIROZE

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Summary

Background. Although previous studies presented that chronic liver diseases (CLD) as well as excessive alcohol consumption lead to an increased prevalence of osteoporosis and pathological fractures due to disturbances in the bone formation and resorption balance, understanding bone fragility determinants is still modest in these individuals.

The aim of our study was to use a comprehensive method for clinical fracture risk assessment in patients with alcoholic liver cirrhosis (ALC), as well as patients with chronic alcohol abuse (CAA), without cirrhosis who have not previosly had femoral or vertebral fractures.

Material and methods. We analyzed the inter-group differences in laboratory analyses, osteodensitometry parameters of lumbar vertebrae, osteodensitometry and bone geometry parameters of different regions of proximal femora, as well as distinct in the concentration of specific bone markers between adult male patients with ALC (n=48), patients with CAA without liver cirrhosis (n=73) and healthy age- and sex-matched controls (n=51).

Results. Osteodensitometry confirms a statistically significant lower bone mineral density (BMD) in the intertrochanteric region of patients with ALC and CAA. The geometric parameters of the proximal femora after adjusting for body mass indeks (BMI) in patients with ALC verify the worse findings for endocortical diameter (ED) and cross-sectional area (CSA) of the femoral shaft. Also, comparison of CAA and control group showed that the most sensitive part of the femur was the shaft of patients who consume alcohol excessively. In comparison to ALC and control group, we verified significantly lower results in periosteal diameter (PD), CSA, moment of inertia on the cross-section (CSMI) and section modulus (SM) in CAA group, which all together makes the bone more fragile. The Fracture Risk Assessment Tool (FRAX) score risk of major osteoporosis-related fractures, as well as hip fractures, was clearly higher in both study groups. Lumbar spine T score was significantly lower in patients with ALC. It is particularly significant that the microarchitecture of the bone was the most damaged in patients with ALC. Significantly higher beta-C-terminal telopeptide (β-CTX) and osteoprotegerin (OPG) values were recorded in patients with ALC, while insulin-like growth factor 1 (IGF-1) and the receptor activator of nuclear factor kappa beta ligand (RANKL)/OPG ratio were decreased. At CAA, only a reduced RANKL/OPG ratio was verified.

Conclusion. The results of our study confirm that in patients with ALC and people who consume alcohol excessively, bone changes are presented even before pathological fractures occur. Decreased values of intertrochanteric BMD, and especially trabecular bone score (TBS) in patients with ALC indicate that these are potential places where pathological fractures could occur. In order to timely diagnose bone changes and prevent the occurrence of fractures, examination of bone metabolism is necessary in all patients with ALC, as well as those who consume alcohol excessively.

Keywords. Alcoholic liver cirrhosis, chronic alcohol abuse, Clinical fracture risk assessment, Osteoporosis, Osteodensitometry, Hip Structure Analisys, Bone turnover biomarkers.

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Sažetak

Uvod. Iako su prethodne studije pokazale da hronične bolesti jetre, kao i prekomerna konzumacija alkohola dovode do povećane prevalencije osteoporoze i patoloških fraktura zbog poremećaja ravnoteže u formiranju i resorpciji kostiju, razumevanje determinanti fragilnosti kod ovih osoba je još uvek oskudno.

Cilj našeg istraživanja bilo je sveobuhvatno ispitivanje promena na kostima koje nastaju kod bolesnika sa alkoholnom cirozom jetre (ALC), kao i bolesnika sa hroničnom zloupotrebom alkohola bez ciroze (CAA), koji prethodno nisu imali prelome butne kosti i/ili pršljenova.

Materijal i metode. Analizirali smo inter-grupne razlike u laboratorijskim analizama, parametrima osteodenzitometrije lumbalnih pršljenova, parametrima osteodenzitometrije i geometrije različitih regija proksimalnog okrajka femura, kao i razlike u koncentraciji specifičnih koštanih markera između odraslih muškaraca sa ALC (n=48), CAA bez ciroze jetre (n=73) i zdravih muškaraca odgovarajuće starosne dobi (n=51).

Rezultati. Osteodenzitometrija potvrđuje statistički značajno nižu vrednost BMD u intertrohanterečnoj regiji bolesnika sa ALC i CAA. Geometrijski parametri proksimalnog femura nakon prilagođavanja za indeks telesne mase (BMI), potvrđuju lošije nalaze kod bolesnika sa ALC za endokortikalni dijametar (ED) i indeks otpora na kompresivno opterećenje (*cross-sectional area* – CSA). Upoređivanjem CAA i kontrolne grupe dokazano je da je telo najosetljiviji deo butne kosti kod ljudi koji prekomerno konzumiraju alkohol. Dokazane su značajno niže vrednosti periostealnog dijametra (PD), CSA, momenta inercije na poprečnom preseku (*moment of inertia on the cross-section* – CSMI) i otpornog momenta na savijanje (*section modulus* – SM) u CAA grupi, što sve zajedno čini kost fragilnijom. *The Fracture Risk Assessment Tool* (FRAX) skor kojim se procenjuje rizik za prelome povezane sa osteoporozom bio je jasno veći u obe ispitivane grupe. T skor lumbalne kičme bio je značajno niži kod bolesnika sa ALC. Posebno je značajan rezultat da je kod bolesnika sa ALC veoma oštećena mikroarhitektura kosti. Kod bolesnika sa ALC zabeležene su značajno više vrednosti beta-C-terminalnog telopeptida (β-CTX) i osteoprotegerina (OPG), dok su insulin stimulišući faktor rasta (IGF-1) i odnos receptor activator nuklearnog faktora kapa beta liganda (RANKL)/OPG bili sniženi. U CAA je potvrđen samo smanjen odnos RANKL/OPG.

Zaključak. Rezultati našeg istraživanja potvrđuju da se kod bolesnika sa ALC i osoba koje prekomerno konzumiraju alkohol, promene na kostima javljaju pre nego što nastanu patološki prelomi. Snižene vrednosti intertrohanterične BMD, a posebno *trabecular bone score*-a (TBS) kod naših bolesnika sa ALC ukazuju da su to potencijalna mesta fraktura. Kako bi se pravovremeno dijagnostikovale koštane promene i sprečio nastanak preloma, potrebno je ispitati koštani metabolizam kod svih bolesnika sa ALC, kao i onih koji prekomerno konzumiraju alkohol.

Ključne riječi. Alkoholna ciroza jetre, hronična zloupotreba alkohola, klinička procena rizika preloma, osteoporoza, osteodenzitometrija, strukturna analiza kuka, koštani biomarkeri.

Naučna oblast: **Medicina**
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1. INTRODUCTION

1.1. Alcohol use disorder

Alcohol use disorder (AUD) is an impaired ability to stop or control alcohol use, despite negative social, occupational, or health consequences (1). The presence and severity of the disorder can be determined based on the symptoms defined in the Diagnostic and Statistical Manual of Mental Disorders (1). Depending on the presence of any of the 11 defined criteria, alcohol abuse disorder can be classified as mild (2-3 criteria), moderate (4-5 criteria) and severe (6 or more criteria). Binge drinking and frequent heavy alcohol use increase the risk of developing alcoholism. National Institute on Alcohol Abuse and Alcoholism (NIAAA) defines binge drinking as a pattern of drinking enough alcohol to reach a blood concentration of 0.08% (0.08 g/dL), which is an average of 4 – 5 alcoholic drinks consumed in 2 hours (1). On the other hand NIAAA defines heavy alcohol use for men as drinking 5 or more alcoholic drinks on any day or 15 or more during the week, and for women 4 or more alcoholic drinks on any day or 8 or more during the week (1). The amount of alcohol (ethanol) in the definition of a standard drink varies, so in Great Britain it is only 8 g, while it is the highest in Austria where it is 20 g. In Serbia, a standard drink has 13 g of ethanol, which represents about 350 ml of 5% beer or 150 ml of wine that contains 12% alcohol (2). According to recent data, 2.4 billion people (1.5 billion men and 900 million women) worldwide consume alcohol, of which almost 40% have episodes of excessive consumption, which globally represents the third leading cause of premature mortality, with over 3 million deaths, predominantly in the population between 15 and 49 years (3,4). Excessive alcohol consumption can be linked to more than 200 different diseases, and in addition to poor health, it also has a harmful effect on personal and social development (5). AUD affects 75 million people worldwide according to the World Health Organization (WHO) (6). In the population older than 15 years, the average consumption of alcohol in 2010 was 6.2 liters, and in the countries of Eastern Europe it was even up to 15 liters. On the other hand, the highest percentage of 15 – 19 year olds who drink heavily was highest in the countries of Western Europe (Germany, France and the Netherlands). Of particular concern is the fact that 5.9% of global mortality is linked to alcohol consumption (6). Alcoholism is one of the main addictive diseases and is one of the leading causes of death in the population under 20 years of age. In addition, the harmful impact on work ability and the economy is very pronounced and it is best illustrated by data from 2012, when it was estimated that there were 139 million disability-adjusted life years (DALYs) or 5.1% of the global burden of disease and injury associated with excessive alcohol consumption (7). AUD is associated with adverse effects on almost all organ systems, including the cardiovascular and central nervous system, gastrointestinal tract, pancreas, liver, and skeleton (8,9).

1.2. Liver cirrhosis

Liver cirrhosis represents a series of irreversible changes in the liver parenchyma that occurred as a result of remodeling and replacement of liver tissue with fibrotic interconnected septa. The described changes lead to the formation of nodules and altered vascularization, resulting in the development of portal hypertension (10). Numerous etiological factors lead to the liver cirrhosis, and the most common are: alcohol, non-alcoholic steatohepatitis (NASH), viral hepatitis, and autoimmune diseases (11).

Liver cirrhosis with its many complications causes more than 2 million deaths annually worldwide (12,13). In 2000, mortality from liver cirrhosis and hepatocellular carcinoma (HCC), as its most serious complications, was the 13th and 20th leading cause of death, respectively (12). Over the past 20 years, there has been an increase in the number of patients, and in 2020, HCC was in the 6th place of morbidity and even the third place of mortality among all malignant diseases. The European Association for the Study of the Liver (EASL) predicts that by 2040 the number of people suffering from and dying from liver cancer will increase by 55% is particularly worrying

(14,15). Also, the high economic impact and poor quality of life of these patients should not be ignored (16).

Apart from HCC, the most common complications of liver cirrhosis that could be life-threatening are: portal hypertension, ascites, variceal bleeding, hepatic encephalopathy and hepatorenal syndrome (17,18). Besides previously mentioned complications, liver disease is associated with skeletal changes previously defined as hepatic osteodystrophy. However, as hepatic osteodystrophy implies the presence of osteopenia, osteoporosis and osteomalacia, while osteomalacia is very rarely seen in patients with liver disease, except in isolated cases of advanced cholestasis and severe malabsorption in areas with limited exposure to sunlight, the term has been suppressed (19,20).

1.3. Osteoporosis – definition and epidemiology

During the last few decades we have witnessed a dramatic increase in the number of people with hip fractures all over the world. Due to changes in lifestyle, it is estimated that the number of hip fractures will increase from 1.3 – 1.7 million in 1990 to around 3 million by 2025 (21). It is known that the risk of hip fracture increases with age. In the age group of 50 – 59 years a 2.9% individuals have 10-years risk > 20%, while in those older than 80 years a 10-y risk > 20% rises to 27.4% (22). Bone fractures, especially in the elderly population, significantly affect the quality of life, reduce mobility, lead to more hospitalizations and increase the risk of death (23). Therefore, bone fragility has been increasingly investigated in recent years. There are many factors that contribute to the fragility of skeleton and therefore lead to the occurrence of fractures after on low impact force. Although there is a large number of contraversal data in the literature, today it is definitively established that the strength of bone tissue depends on both the quantity (the bone density and geometry) and quality (characteristics of microarchitecture, the degree of remodeling, the accumulation of microfractures, the degree of mineralization and the connections of collagen chains) of bones (24).

One of the main contributor of bone fragility is osteoporosis (25). It is estimated that more than 200 million people worldwide have osteoporosis, but even more worrying is the low quality of life of these patients, which results in more than 5 million DALYs globally per year (26). The main characteristics of osteoporosis are loss of bone mass and deterioration of bone microarchitecture (27). The gold standard for diagnosing and assessing the severity of osteoporosis is dual-energy X-ray absorptiometry (DEXA) (27). As life expectancy increases, osteoporosis and the consequent risk of pathological fractures become a major public health problem (28). Genetic predisposition combined with environmental factors are the main risk for osteoporosis (29). This implies low physical activity, alcohol consumption, weight loss and numerous other factors (30). In order to reduce the number of pathological fractions as much as possible, it is necessary to promptly identify and act preventively on all potential risk factors (31).

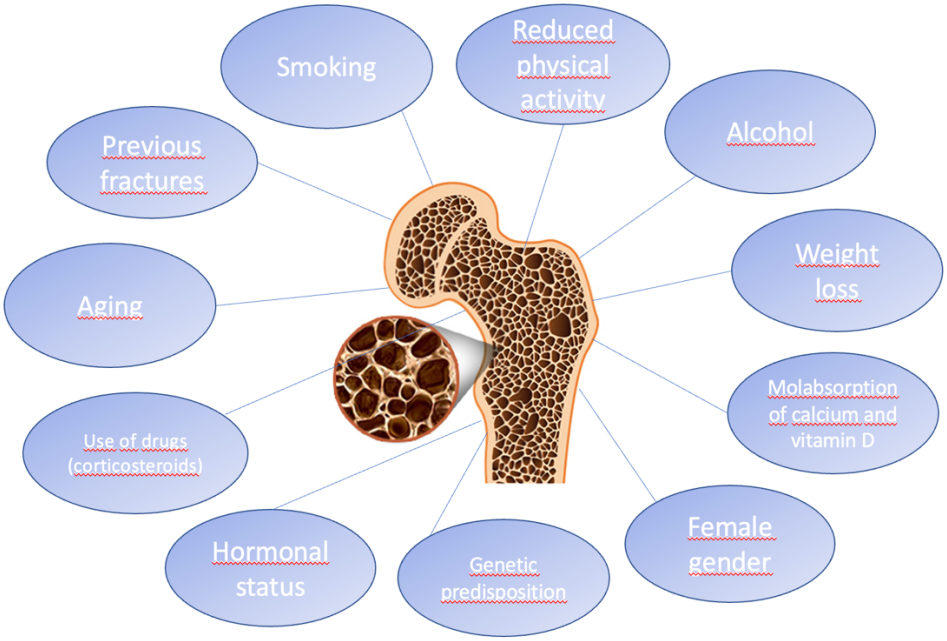

Figure 1. Schematic presentation of risk factors for osteoporosis (30).

Decreased bone mineral density (BMD) is golden standard for diagnostic of osteoporosis (32). After osteodensitometry, the diagnosis is made based on the WHO definition, when BMD is less than −2.5 standard deviations (SD) below the peak value obtained from normal adults and adjusted for gender (T-score ≤ −2.5 in postmenopausal women, or Z-score ≤ −2 in men less than 50 years old and premenopausal women) (33,34). Previously, it is necessary to exclude other reasons that would lead to changes in the bones, such as osteomalacia, extra-skeletal calcifications, osteophytes or calluses or other changes that occurred as a result of previous injuries (34,35). When the T score is reduced, but does not meet the criteria for osteoporosis and it is between −1 and −2.5, then we can conclude that the patient has osteopenia (33,34).

If osteoporosis is a consequence of aging or occurs in postmenopausal women, it is classified as primary, while secondary osteoporosis is characterized by the presence of another known risk factor, such as the liver cirrhosis, use of drugs (corticosteroid therapy) or alcohol, or it is idiopathic if it leads to a decrease in BMD and consequent bone fractures occurs in young adults without known cause (36).

Of particular concern is the fact that 40 – 60% of people diagnosed with osteoporosis actually have secondary osteoporosis. Premenopausal women, as well as younger men, are shown more often osteoporosis (37).

There are numerous comorbidities that can increase the risk of developing secondary osteoporosis and bone fragility: endocrinological disorders (hyperparathyroidism, hypercalciuria, Cushing syndrome, hypogonadism, hyperthyroidism, diabetes mellitus), malignant diseases, medications, liver diseases, especially cholestatic type, as well as lifestyle factors including alcohol, smoking and a sedentary lifestyle (36,37).

Although BMD is officially recommended by the WHO as the main parameter for the diagnosis of osteoporosis, pathological fractures can not be explained only by determining BMD. It is clear that bone fragility is influenced by several parameters and that their mutual interaction determines bone strength (38,39). If we know that only one third of all non-traumatic fractures are associated with reduced BMD, we can conclude that BMD has a limited ability to assess the risk of bone strength and fragility and that the application of other fracture risk factors is necessary (40,41).

The basis of a healthy skeleton is adequate bone remodeling, and when there is an imbalance in the function of osteoblasts and osteoclasts, there is a loss of quantity and a decrease in the quality of bone tissue (27,42). Microscopically, there are about one million sites where bone remodeling takes place called bone remodeling units (BRU) (42,43). This mechanism is tightly controlled by

different molecules and signaling pathways (44). When there is a disturbance in the regulation of this complex mechanism, in terms of increased resorption and inadequate bone formation, the osteoporosis process progresses (27,45). When bone is formed, a number of osteoblasts undergo apoptosis, while the rest differentiate into osteocytes or cells that line bones and survive for a long period (27,46). The main regulatory role in further remodeling is played by osteocytes. Depending on the bone's exposure to mechanical stress, osteocytes determine the number of osteoclasts and resorption, while on the other hand they control bone formation by modifying osteoblast-induced bone mineralization (27,33,34).

Bone microarchitecture is a very important parameter that determines bone strength and fragility (47). Initially, the microarchitecture is examined by bone biopsy and pathohistological analysis (48). However, with the discovery of the trabecular bone score (TBS), which is determined on the basis of previously performed osteodensitometry of the lumbar spine, a very reliable non-invasive score was obtained for the assessment of bone microarchitecture. Furthermore, TBS correlates well with three-dimensional parameters of bone microarchitecture such as the trabecular number, trabecular separation, connective density, and structure model index (48,49). What is even more important is that TBS, independently of BMD, can be used as a predictive parameter for the occurrence of pathological fractures (50).

1.4. Pathophysiological mechanisms of alcohol induced osteoporosis

Alcohol has been recognized as an independent risk factor for the onset and development of osteoporosis (51). It was found that 30% of people who consume alcohol excessively have osteoporosis, and in 36% of them pathological fractures of the vertebrae are verified radiologically (52). However, after the establishment of two years stable abstinence, there is an increase in the level of osteocalcin in the serum, as a marker of bone formation, along with the increase and recovery of BMD (53). Numerous studies have confirmed that excessive alcohol consumption has a harmful effect on bones, however, data on the potential benefit of light to moderate alcohol consumption is still controversial (54). According to the official American Dietary Guidelines, the permissible amount of alcohol is up to 2 drinks for men and one for women, on days when alcohol is consumed (55). In addition to the amount of alcohol, the sort of alcoholic beverage is also important, so it has been proven that with light to moderate consumption of beer there is an improvement in BMD in pre- and postmenopausal women (56), while another study showed that light consumption of alcohol, especially wine improved lumbar spine bone mass (57). The real challenge remains precisely defining what amount of alcohol has a benefit on bones, and when it begins to manifest a harmful effect. Most studies set that limit at 2 – 3 standard alcoholic drinks, which represents an average of 20 – 30 g of ethanol (58). However, a study conducted in Japan showed that men who consumed up to 55 g of ethanol per day had better BMD compared to those who consumed more (59).

Chronic excessive alcohol consumption leads to numerous changes in the body. Alcohol has a toxic effect on almost all organ systems, including a direct or indirect harmful effect on the skeleton. In people who consume alcohol excessively, a reduced concentration of calcium in the serum has been proven, as a result of reduced absorption from the digestive tract, and also a reduced level of testosterone, a hormone that has a stimulating effect on osteoblasts (60,61). Special attention is paid to the influence of increased levels of proinflammatory cytokines that could regulate osteoclastogenesis and adipocyte differentiation (62).

Studies conducted on animal models also confirm the harmful effects of alcohol on bones. It was found that alcohol administration for 4 months in interleukin (IL)-6 gene-knockout mice can induce the production of IL-6 in wild-type mice with reduced osteoblast function (63). In addition, receptor activator of nuclear factor kappa-B ligand (RANKL) messenger ribonucleic acid (mRNA) was verified only in bone marrow cultures of the wild-type mice, concluding that alcohol-induced IL-6 and RANKL affect osteoblast function and osteoclastogenesis, which may lead to reduced bone turnover (64).

Alcohol manifest a direct toxic effect on osteoblasts and osteoclasts (65). However, there are numerous other, indirect ways in which alcohol can have a harmful effect on bones. First of all, there are changes in the activation of Wingless-related integration site (Wnt) and the mammalian target of rapamycin (mTOR) signaling pathways, and then metabolic disorders in the form of hormonal status disturbances (66), the inability of the parathyroid gland to respond adequately to reduced vitamin D levels (67,68), disturbed growth hormone (GH) and insulin-like growth factor-1 (IGF-1) axis (69), as well as a strong influence of oxidative stress (63).

1.4.1. The Wnt/b-catenin pathway

Since its discovery in the 1980s, the Wnt signaling pathway has been linked to numerous conditions and pathogenic processes. The largest number of studies refer to the role in carcinogenesis, but numerous other processes are certainly significant: hair loss, pigmentation disorders, wound healing, neurodegenerative diseases, proper functioning of the liver, chronic obstructive pulmonary disease, development and renewal of the epithelium of the small intestine with the promotion of differentiation of Paneth cells (70). In recent years, the role of the Wnt signaling pathway in bone formation has been investigated (58).

The main role of b-Catenin is gene transcription in the cytoplasm and translocation to the nucleus. All key mechanisms in bone formation are regulated precisely by the Wnt/b-catenin pathway. Further, through its mediation, the proliferation, differentiation and apoptosis of osteoblasts and osteoclasts take place (71). This pathway is activated when appropriate Wnt proteins bind to the co-receptor complex, such as lipoprotein receptor-related protein 5 (LRP5) or lipoprotein receptor-related protein 6 (LRP6) (72). In addition to the direct harmful effect on osteoblasts and osteoclasts, chronic heavy alcohol consumption also exerts significant toxicity on the bone marrow, including bone marrow mesenchymal stem cells (BMMSC), which can differentiate into osteoblasts and adipocytes (73,74). As a consequence of the effects of alcohol, these multipotent progenitor stem cells differentiate to a greater extent into adipocytes, which leads to the accumulation of fat in the bone marrow. As a result, there is a loss of bone mass and the development of osteopenia and osteoporosis. The Wnt/b-catenin pathway, as well as the mTOR pathway, participate in this process (75,76).

Activation of adipogenetic genes and peroxisome proliferator-activated receptor c (PPAR-c) in bone marrow stem cells results in inhibition of the Wnt/b-catenin pathway. In addition, individuals who consume alcohol excessively have significantly higher levels of sclerostin, which is synthesized in osteocytes and has the ability to bind to LRP5 and LRP6, acting antagonistically with Wnt signaling. In this way, bone formation is disturbed, reducing the function of osteoblasts and stimulating osteoclastogenesis (77,78). Another way in which the Wnt/b-catenin signaling pathway due to excessive alcohol consumption affects bone loss is by triggering oxidative stress (71).

1.4.2. The phosphoinositide 3 kinase/AKT/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway

mTOR belongs to the PI3K family of protein kinases and in interaction with other proteins, regulates the differentiation and proliferation of numerous cells (79). In this way, with excessive alcohol consumption, inhibition of the mTOR pathway affects bone formation in several ways: the activity of the adipogenetic gene PPAR-c increases, the osteogenetic gene Runt-related transcription factor 2 (Runx2) is inhibited, the differentiation is reduced and the apoptosis of osteoclasts is promoted (80,81).

In vivo and in vitro BMMSC lines were investigated to determine whether excessive alcohol consumption has a detrimental effect on bone through mTOR pathways. It has been proven that high doses of ethanol through the PI3K/AKT/mTOR pathway, due to the increased level of

PI3K/AKT/P70S6K, reduce osteogenic differentiation and at the same time affect enhanced adipogenic differentiation (64,75).

1.4.3. Estrogen

Estrogen has a protective effect on bones, what is one of the reasons for the lower risk of osteoporosis in women before menopause. The predominant form of estrogen in men is estradiol, which is of great importance in the normal function of the reproductive tract (82). In young men who chronically and excessively consume alcohol, there is a decrease in the level of estradiol (E2), as well as osteocalcin (66,83). In contrast, light alcohol consumption results in a slight increase in estrogen in postmenopausal women and E2 and its receptor levels in men, leading to increased remodeling (84). Examination of mouse osteoblasts showed that under the influence of acetaldehyde, which is the main byproduct of alcohol metabolism, there is an increased production of oxygen radicals as well as RANKL mRNA through extracellular signal-regulated kinase (ERK). The protective role of estrogen is reflected in the fact that it has an antioxidant effect, while E2 activates ERK (85).

1.4.4. Status of the PTH – vitamin D axis in alcohol abuse

Alcohol can significantly disrupt bone mineralization, as well as calcium metabolism (67). In addition, due to disturbances in nutrition, there is a reduced absorption of vitamin D. Apart from its influence on calcium homeostasis, vitamin D plays a role in the differentiation of osteoblasts and in the microarchitectural bone remodeling (51). However, the expected response to low levels of vitamin D is absent, and people who consume alcohol excessively also have reduced PTH values. The described negative feedback disorder is explained by the fact that alcohol temporarily and reversibly blocks the secretion of PTH (86). Moreover, in order for PTH to affect the number of osteoblast cells, transcription factors such as Runx2 and cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) are needed, which could be inhibited by alcohol. Furthermore, it has been proven that administration of intermittent PTH improves bone mineralization via the Wnt signaling pathway, but that PTH can not prevent alcohol-induced bone marrow adiposity (68,87). In individuals who consume alcohol excessively and have reduced levels of vitamin D, there is excessive production of RANKL and consequently increased bone resorption. The mentioned regularity can be reversed by vitamin D supplementation, which affects the reduction of the RANKL/osteoprotegerin (OPG) ratio (88,89).

1.4.5. GH – IGF-1 signaling

It has been proven in vitro that there is increased differentiation and proliferation of osteoblasts and chondrocytes under the influence of GH, as well as that the stimulatory mechanism may be regulated by the synthesis and secretion of IGF-1 (90,91). Chronic excessive alcohol consumption, as well as binge drinking, reduce the synthesis and secretion of GH and IGF-1, thereby disrupting the balance between osteogenesis and adipogenesis (91,92).

Heavy alcohol consumption reduces IGF-1 activity, while simultaneously increasing the synthesis of IGF-1 binding protein (IGFBP-1), which directly affects the reduced differentiation of osteoblasts and chondrocytes (93). Furthermore, after acute alcohol intake, there is a decrease in the phosphorylation of ribosomal protein S6 kinase-1 (S6K-1/Thr389; S6K-1/Thr421), through the mTOR pathway, which is a consequence of reduced IGF-1 activity (94,95). The exact mechanism of reduced function and increased resistance of IGF has not been fully clarified, given that phosphorylation of another mTOR substrate, 4E-binding protein-1 (4E-BP1), is not inhibited (96).

1.4.6. Oxidative stress

Reactive oxygen species (ROS) have an important role in the inhibition of bone formation (97). Under the influence of alcohol, the intracellular synthesis of ROS occurs, which results in damage of the cell membrane (98). In an animal model of rats, it was determined that there is an increase in the expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) in osteoblasts, which accelerates the synthesis of ROS (99). The plasma membrane is affected by NOX 1, 2 and 4, with NOX 4 being found to have the greatest role in the generation of superoxide in these cells and it is considered the main regulator of cell proliferation and apoptosis, what is associated with increased bone resorption (100). Furthermore, an imbalance between increased ROS production and detoxification capacity leads to reduced bone formation (98).

Also, increased production of RANKL mRNA in rat osteoblasts under the influence of oxidative stress has been proven (101). However, with light alcohol consumption, E2 can inhibit the production of NOX, which suppresses the expression of RANKL mRNA (76). It has been experimentally proven that diphenyleneiodonium, through NOX blockade, reduces the synthesis of RANKL mRNA in osteoblasts (101).

On the other hand, there is not enough data on the role of NOX in the process of osteoclastogenesis (102). So far, it has been proven that alcohol through NOX 1 and 2 stimulates the activity of osteoclasts, while preventing their apoptosis (99). Moreover, NOX4 influences the increased differentiation of preadipocytes. In addition, ethanol via mitogen-activated protein kinase and signal transducer and activator of transcription 3 signaling pathway induce the synthesis of ROS, which consequently affects osteoclasts or other precursors regulated by RANKL signaling (103).

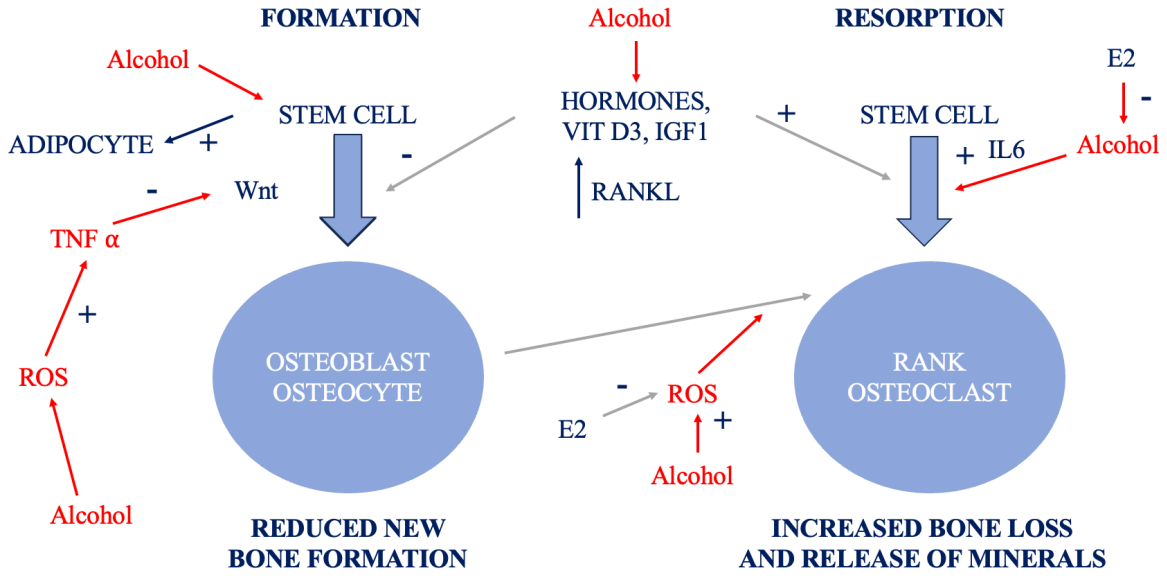

Figure 2. Schematic representation of different mechanisms of alcohol's effect on bones (104).
Abbreviations: TNF α – tumor necrosis factor α, Wnt – wingless-related integration site, ROS – reactive oxygen species, RANKL – receptor activator of nuclear factor kappa-B ligand, E2 – estradiol, IL – interleukin, Vit D – Vitamin D, IGF-1 – insulin-like growth factor-1.

However, many studies have shown that moderate amounts of alcohol as well as some of the ingredients in alcoholic beverages could have a protective effect on bone. It is known that light alcohol consumption slightly increases the level of estrogen in women and estradiol (E2) in men (105). In addition, it has been proven that the non-alcoholic fraction has a significant benefit on bone strength and fragility. Resveratrol (a stilbene with phytoestrogenic properties), which is dominantly found in red wine, has a similar protective effect on bones as estrogen (106). Also,

phenolic acids, which are dominantly found in beer, are associated with the neutralization of ROS along with the downregulation of inflammatory mediators, which all together affect the achievement of an adequate balance between osteoblasts and osteoclasts (107). Furthermore, silicon from malt found in wine and beer affects the regeneration of connective tissue and improves bone mineralization (108).

Light to moderate alcohol consumption is linked to cultural and religious heritage and is a common way of diet for certain populations. The Mediterranean diet, as an example of such concept, has proven health benefits (109). Furthermore, exept moderation in quantity, also the way of consuming alcohol with meals, and not in the form of excessive drinking is a very significant characteristic of the Mediterranean diet (110). The initial theory that the main benefit of the Mediterranean diet is actually the type of alcohol, since it is based on drinking red wine, was abandoned after similar results were obtained in people who drank beer. Moreover, regardless of whether wine or beer is drunk, in the way predicted by the Mediterranean diet, there is a health benefit (111). This population has a lower risk of hip fracture in middle-aged and older men and women, as well as lower mortality compared to those who drink alcohol excessively or do not drink alcohol at all (112). However, much remains unclear when it comes to the effects of alcohol on health (113).

Light alcohol consumption affects the metabolism of parathyroid hormone (PTH). A lower concentration of PTH decreased bone turnover markers (serum osteocalcin and C-terminal telopeptide (CTx)), what results a decrease in the level of bone remodeling (114). In addition, there is an increase in the concentration of estrogen, as well as calcitonin, and a consequent decrease in bone resorption and improvement in BMD, especially in older women (115).

1.5. Pathophysiological mechanisms of liver cirrhosis induced osteoporosis

Liver cirrhosis is a significant risk factor for osteoporosis. It has been proven that there is a decrease in BMD and a disruption of bone microarchitecture, which all together makes bones more prone to pathological fractures. It is known that 10 – 40% of patients with liver cirrhosis have osteoporosis (116). The exact mechanism of osteoporosis development in patients with liver disease has not been fully elucidated (117). Changes occur as a result of an imbalance in the activity of osteoblasts and osteoclasts (27). Chronic inflammation as a consequence of the underlying disease and activated cells of the immune system accelerate the complex process of developing osteoporosis (118). The etiology of liver disease, as well as other comorbid conditions and risk factors, significantly affect the incidence of osteoporosis. If it is diagnosed or if pathological fractures occur, the outcome of the treatment of the underlying liver disease itself worsens, with a significantly lower quality of life (116,119).

Depending on the etiology, in end-stage liver disease, osteoporosis is verified up to eight times more often compared to healthy controls, while one third of these patients during their lifetime make a pathological vertebral fracture (119). The incidence of other fractures compared to vertebral fractures is significantly lower, which indicates that in patients with liver cirrhosis, the trabecular bone is significantly more affected than the cortical bone (120,121). What is of particular importance, pathological fractures in patients with liver cirrhosis occur at a significantly younger age. It was determined that the cumulative fracture risk of patients with liver diseases younger than 45 years corresponds to the risk in the population of healthy controls over 75 years of age (122).

The mechanism of bone damage in patients with liver cirrhosis is very complex. Etiology and stage of liver disease significantly influence the degree of bone damage. Patients with cholestatic diseases and non-alcoholic fatty liver disease (NAFLD) are prone to developing osteoporosis due to increased resorption (119). The bone remodeling process is very complex and consists of several segments: activation, resorption, reversal, formation, and termination phase. Disruption of any segment affects remodeling and leads to decreased bone density and increases the risk of bone fragility (123). The molecular mechanisms that most affect bone resorption are

RANK/RANKL/OPG, activated proinflammatory cytokines (primarily IL-1, IL-6 and TNF-α) (124).

Except increased osteoclastogenesis, bone formation is reduced in patients with liver cirrhosis also, as a result of the toxic substances that are not metabolized (116,119,124). In addition to reduced bone mineralization, the proliferation and differentiation of osteoblasts was also inhibited (125). It has been proven that patients with alcoholic liver cirrhosis have a reduced concentration of osteocalcin. After synthesis in osteoblasts, it significantly affects bone mineralization and it is clear that its deficiency affects reduced bone formation (126). In patients with liver cirrhosis, the expected response of mesenchymal stem cells and osteoblast precursors, through the Wnt signaling pathway, bone morphogenetic protein (BMP), and fibroblast growth factor (FGF) signaling, is absent (119,127).

1.5.1. RANK/RANKL/OPG

RANKL is a type II TNF ligand with a C-terminal extracellular domain, RANK is a transmembrane TNF receptor, while OPG is a soluble TNF receptor family member. RANK is located on the osteoclast precursor and acts competitively with OPG for binding to RANKL. Since the binding of RANK and RANKL is necessary for the process of osteoclastogenesis, in order to initiate the cascade, it is clear that if OPG binds to RANKL, this process is blocked and bone resorption is prevented (128). Matrix metallo-proteinases cleave the C-terminal extracellular domain of RANKL and in this way soluble RANKL (sRANKL) in the extracellular space is obtained which can also bind to RANK. Considering that the binding of membrane RANKL as well as sRANKL can initiate the cascade of osteoclastogenesis, it is clear that the OPG/sRANKL ratio affects the preservation of bone mass. It was found that the concentration of sRANKL is increased in patients with liver diseases. The increase in the level of sRANKL in these patients affects the increased bone turnover. As a protective response, there is also an increase in OPG levels (126,129).

1.5.2. Proinflammatory cytokines

Liver cirrhosis is a state of chronic inflammation in which proinflammatory cytokines directly and indirectly stimulate osteoclast activation (130). IL-1 and IL-6 can directly improve osteoclast function, while at the same time they stimulate the synthesis of RANKL in osteoblasts and thus indirectly affect osteoclast differentiation (131,132). It is known that in the liver diseases there is an increased production of IL-6 which stimulates liver regeneration (133). Also, in alcoholic liver cirrhosis, the underlying disease and alcohol itself influence the increased production of IL-6 and TNF-α which stimulates the expression of RANKL in osteoblasts. In addition, TNF-α has a stimulating effect on osteoclast precursors through increased expression of the colony stimulating factor-1 (CSF-1) receptor gene. All of this affects the increased differentiation and activity of osteoclasts (134,135).

1.5.3. IGF-1

In the text above it is described that IGF-1 has an anabolic effect on bone growth. IGF-1 is produced in hepatocytes and is stimulated by GH. Its synthesis is reduced with the weakening of liver function and the loss of GH receptors on hepatocytes (136). The importance of the effect of IGF-1 on bones is well explained by the fact, that osteoporosis can be verified, in women with reduced values of IGF-1 (137). A study in rats with liver cirrhosis found that administration of low doses of IGF-1 could increase bone mass (138).

1.5.4. Hypogonadism

Patients with alcoholic liver cirrhosis and advanced portal hypertension have hypogonadism (116,139). Testosterone, through the stimulation of IGF-1 expression, affects the differentiation and proliferation of osteoblasts and chondrocytes, while at the same time, it reduces the activity of IL-6, which activates osteoclasts (140). In addition, the reduced concentration of testosterone stimulates the synthesis of RANKL, which promotes osteoclastogenesis and affects the reduction of BMD (141,142). Therefore, testosterone directly improves the synthesis of trabecular bone, while in patients with alcoholic liver cirrhosis there is increased osteoclastogenesis, due to reduced testosterone levels (143). Furthermore, although in liver cirrhosis androgens are converted to estrogen peripherally, the protective effect of the estrogen produced in this way is weak to protect bones from osteopenia and osteoporosis (44,144).

1.5.5. Malnutrition and gut microbiota in liver cirrhosis

Malnutrition in patients with liver cirrhosis is a significant risk factor for osteoporosis. Even 50 % to 90% of patients with liver cirrhosis are malnourished (145,146). Numerous factors influence the development of sarcopenia: reduced intake of nutrients, malabsorption and hypercatabolism (146,147). The condition is further aggravated by external risk factors in the form of alcohol consumption, numerous comorbidities, tendencies towards the development of infections, but also metabolic changes that are a consequence of the underlying disease such as hyperammonemia, low testosterone levels, reduced GH and high levels of endotoxins (147,148). Moreover, due to the reduction of glycogen stores and the need for gluconeogenesis, there is additional increase in muscle proteolysis (148,149). Sarcopenia significantly affects BMD and increases the risk of developing osteoporosis many times over (150). In recent years, more and more studies have been conducted on the disruption of the microbiota and the consequences on the entire organism (151). Given that liver cirrhosis leads to dysbiosis and the appearance of "leaky gut syndrome", as well as the fact that there is a proven link between microbiota disorders and the occurrence of osteoporosis, it is clear that the intestinal flora disorder is another additional risk factor for increased bone fragility (152,153).

1.5.6. Vitamin K, Vitamin D and PTH

Patients with liver cirrhosis can often have reduced levels of vitamin K. Considering that vitamin K is necessary for the synthesis of osteocalcin, it has been proven that its supplementation can have a positive effect on BMD (154).

The largest part of vitamin D is the result of cutaneous synthesis under the influence of ultraviolet (UV) rays. The active form of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)2D), is formed after hydroxylation first in the liver and then in the kidneys (155). The main transporter of vitamin D in the circulation is the vitamin-D-binding protein (GC or DBP) which is expressed in the liver. Therefore, the reduced concentration of vitamin D in the circulation is not only responsible for reduced hydroxylation, but with the progression of liver disease, there is also a reduced expression of DBP (156,157). In patients with liver cirrhosis, there is a reduced absorption of calcium and phosphorus from the digestive tract, due to the deficit of 1,25(OH)2D, which consequently affects the increased release from the bones and the reduction of bone mineralization (158). Furthermore, vitamin D affects the inhibition of osteoclastogenesis (159). However, in patients with cirrhosis of the liver, the activation of the negative feedback loop is absent, and in contrast to the expected elevated values of PTH, decreased values are recorded. The exact mechanism has not been clarified (86).

However, despite the fact that there is a clear interaction between liver disease and increased bone fragility, there are still no guidelines that would precisely indicate the importance of timely diagnosis and appropriate therapy (124).

2. AIMS

The aims of the dissertation are to examine the changes in bone tissue in patients with alcoholic liver cirrhosis (ALC), as well as patients with chronic alcohol abuse (CAA), without cirrhosis. The specific objectives are:

- to examine the significance of the difference in mineral density of the proximal femur and lumbar spine, as well as structural parameters of the proximal femur in CAA group in relation to the control group matched by sex and age;

- to examine the significance of the difference in mineral density of the proximal femur and lumbar spine, as well as structural parameters of the proximal femur in persons with ALC in relation to the control group matched by sex and age;

- to examine the significance of the difference between the two examinated groups (CAA and ALC);

- to examine the significance of the use of Fracture Risk Assessment Tool (FRAX) and trabecular bone score (TBS) in predicting the risk of pathological bone fractures in patients with CAA and ALC;

- to determine whether there is a relationship between the results obtained by osteodensitometry and biochemical parameters, which include markers of bone metabolism (vitamin D, PTH, osteocalcin, β-CTX, osteoprotogerin, RANKL, IGF-1) and hormonal status (testosterone, estradiol, LH, FSH, SHBG) in patients with alcoholic liver cirrhosis and persons who excessively consume alcohol;

- to examine whether there was a difference in all examined parameters in the group of patients with ALC classified according to the degree of liver insufficiency according to the Child Pugh classification.

3. MATERIAL AND METHODS

3.1. Study sample

We conducted a cross-sectional study of 172 male patients treated at the Clinic for gastroenterohepatology (Clinic for GEH), University Clinical Center of Serbia (UCCS) and the Special Hospital for Addiction Diseases. A detailed anamnesis was taken from all study participants, including a detailed socio-epidemiological survey, with special reference to habits related to alcohol consumption (frequency, amount, length of consumption, type of alcoholic beverage). Anthropometric measurements (body weight, body height, body mass index (BMI)), biochemical analyses, as well as abdominal ultrasound were performed. Based on the anamnestic data and the examination, the patients were divided into 3 groups: patients with ALC (48), patients with CAA without liver cirrhosis (73) and a healthy control group (51).

The ALC group included 48 patients who were hospitalized at the Clinic for GEH, UCCS. In order to establish a distinction from the direct effect of alcohol on bones, all patients in this group were at least one year of stable abstinence. On the basis of previous medical documentation (50 g of pure alcohol/day for more than five years) and examination (physical signs of alcoholic chronic liver disease, indirect signs of portal hypertension verified by abdominal ultrasound and/or upper endoscopy, specific serum biochemical profile), was made a diagnosis of ethylic liver cirrhosis. Subsequently, all patients with cirrhosis was classified according to Child Pugh stages of the disease, into three subgroups: A, B and C (score A $\leq$ 6, B 7 – 9, C $\geq$ 10) (Table 2) (160).

The second group included 73 patients who were hospitalized at the Special Hospital for Addiction Diseases. They met the NIAAA criteria for AUD and by the day of admission to the hospital had consumed excessive amounts of alcohol in the form of heavy alcohol use or binge drinking.

The control group included 51 age-matched male patients in whom hemorrhoidal disease was confirmed endoscopically without any other pathological findings and who denied alcohol use. Age and anthropometric parameters of all three groups are shown in Table 1.

The exclusion criteria included: female gender, positive history of endocrine and metabolic diseases affecting the skeletal system (hyperparathyroidism, hypogonadism, thyroid dysfunction, diabetes, obesity, autoimmune, hereditary or viral liver disease), hereditary musculoskeletal disorders, the presence of fractures of the femur and / or vertebrae, confirmed by X ray, the presence of solitary and / or metastatic malignant lesions, as well as the usage of drugs that affect bone metabolism (antiepileptics, cytostatics, corticosteroids, vitamin D, bisphosphonates and others). After biochemical analyses, subjects with elevated transaminases were not included in the control group, while alcoholics who were not diagnosed with liver cirrhosis were excluded in case of verified impairment of liver synthetic function and/or secondary hypersplenism. Also, if the ultrasound examination of the abdomen in subjects of the control group indicated a fatty liver or any pathological findings in the abdomen, they were excluded from the study. It is a significant fact that we radiologically confirmed that 6 patients with ALC, who initially met all criteria, had pathological vertebral fractures, and that they were then excluded from the study. No vertebral fractures were verified in the CAA and control group, nor were hip fractures noted in all included subjects.

Table 1. Age and anthropometric parameters

	N	Age (average ± SD)	Height (cm ± SD)	Weight (kg. ± SD)	BMI (kg/m^2 ± SD)
ALC group	48	51.96 ± 7.5	176.36 ± 8.09	85.95 ± 14.03	27.64 ± 4.18
CAA group	73	49.37 ± 8.82	176.97 ± 7.31	80.65 ± 12.34	25.68 ± 3.20
Control group	51	49.93 ± 11.92	180.75 ± 5.95	92.25 ± 16.16	28.24 ± 4.84
Total	172	50.20 ± 9.44	177.83 ± 7.36	85.27 ± 14.61	26.92 ± 4.10

Abbreviations: ALC – alcoholic liver cirrhosis, CAA – chronic alcohol abuse, SD – standard deviation.

Table 2. Child Pugh score (160)

Factor	1 point	2 point	3 point
Total bilirubin (μmol/L)	< 34	34 – 50	> 50
Albumin (g/L)	> 35	28 – 35	< 28
INR	< 1.7	1.71 – 2.30	> 2.30
Ascites	None	Mild	Moderate to severe
Hepatic encephalopathy	None	Grade I – II (or suppressed with medication)	Grade III – IV (or refractory)

Class A (score 5 – 6), Class B (7 – 9), Class C (10 – 15).

3.2. Biochemical blood tests and serum markers of bone metabolism

Standard blood tests were performed in all study participants (blood cell count, coagulation profile with special reference to the synthetic function of the liver, hepatocyte integrity tests with a check of cholestasis enzymes also, excretory function of the liver, nutritional and lipid status, renal and pancreatic function, electrolyte status with special reference on parameters important for bone metabolism, along with other standard biochemical analyses), vitamin D level and parathyroid gland function, osteocalcin, analysis of sex hormones (total and free testosterone, estradiol, luteinizing hormone – LH, follicle-stimulating hormone – FSH, dehydroepiandrosterone sulfate – DHEAS and sex hormone-binding globulin – SHBG). Blood was sampled in the early morning hours, after an overnight fasting. Vacutainers without additives were used. After centrifugation at 3500 rpm, and separation, the sample was stored in a deep freezer (- 80 C). Special attention was paid to analyzing specific bone biomarkers (beta-C-terminal telopeptide – β-CTX, receptor activator of nuclear factor kappa beta ligand – RANKL, insulin-like growth factor 1 – IGF-1 and osteoprotegerin – OPG). According to the manufacturer's strictly prescribed instructions, the analysis was performed at the Institute of Medical and Clinical Biochemistry, Faculty of Medicine in Belgrade. Adequate commercially available ELISA kits (Abcam plc, Cambridge, UK and Abbexa, Cambridge, UK) were used.

3.3. Ostedensitometry (DXA)

All study participants were initially X-rayed on both hips and the lumbar spine in order to exclude patients with possible pathological fractures.

Measurement of bone mineral density of the proximal femur and lumbar spine was performed using dual-energy X-ray absorptiometry (DXA) on a HOLOGIC 1000 W device (Hologic QDR 1000 / W; Hologic, Waltham, MA) in cooperation with the Institute for Health Protection of Workers of "Serbian Railways", in Belgrade.

Furthermore, using standard software, bone mineral content (BMC; g) and bone mineral density (BMD; g/cm^2) was determined in the standard regions of the proximal femur (narrow neck, intertrochanteric region and shaft) and lumbar spine. Then, for all patients, we calculated the FRAX and TBS, as the main indicators of the risk of potential pathological fractures.

The FRAX score was created by experts from The University of Sheffield in the United Kingdom in order to more accurately assess the risk of bone fracture depending on the presence of certain risk factors. It presents an algorithm that estimates the ten-year risk of major osteoporotic fracture (including hip, spine, forearm, and proximal humerus fractures) and hip fracture. It consists of 11 parameters, most of which carry an individual risk for bone fracture: age, sex, weight, height, previous fracture, parental hip fracture, smoking, glucocorticoid use, rheumatoid arthritis, secondary osteoporosis, alcohol consumption. Bone mineral density (BMD) at the femoral neck is the 12th parameter that is optional and can be used if osteodensitometry was performed. Excessive alcohol consumption, according to the FRAX algorithm, represents taking 3 or more standard doses per day (161). FRAX values ≥ 20% for major osteoporotic fracture or ≥ 3% for hip fracture are considered significant and such patients should be considered for drug therapy.

3.4. Hip Structure Analysis (HSA) of the proximal femur

Geometrical and mechanical characteristics of the proximal end of the femur represent the structural analysis of images obtained by osteodensitometry. Realizing that BMD is not the only parameter that affects the assessment of the risk of pathological fractures, there was a need to determine numerous other parameters that can more precisely explain the resistance of the proximal end of the femur to forces coming from various directions and leading to bending and ultimately to fracture. The idea initially arose in 1984, and then Beck and his collaborators developed a special software program – HSA with which these measurements could be performed (162).

However, in order for this idea to come in practical work, certain mathematical models had to be created that relate to the shape and symmetry of the proximal femur, as well as to the assessment of the thickness of the cortex. For this reason, the proximal end of the femur is conceived as a continuous curved beam, with repeated measurements being performed on the intertrochanteric region, the neck and shaft of the femur, in regions 5 mm wide. Special attention is paid to the shafts that pass through the mentioned segments of the femur, so that the measurements can also be carried out in transverse planes (163).

The regions of interest that we examined are:

1. narrow neck of the femur (NN), transverse section in relation to the longitudinal axis, made at the narrowest point on the neck;
2. intertrochanteric region (IT), a transverse section along the intertrochanteric line connecting the greater and lesser trochanters of the femur;
3. femoral body shaft (FS), transverse section made 2 cm below the midpoint of the lesser trochanter of the femur.

Using the previously mentioned software program, the following parameters were directly obtained for each of the listed regions of interest:

1. Periosteal diameter (PD, cm) is measured between the outer edges of the respective region of interest on a line perpendicular to the longitudinal axis of the region;
2. The index of resistance to compressive load (cross-sectional area, CSA, cm^2) is defined as the cross-sectional area of the bone after subtracting all cavities filled with bone marrow. CSA is an index of resistance to load directed along the longitudinal axis. It is calculated using the following formula:

$$CSA = \underline{BMD \times PD}$$

ρm

(BMD – bone mineral density, PD – periosteal diameter; ρm – degree of mineralization, which is 1.05 g/cm^2 in a bone that is completely mineralized);

3. The moment of inertia on the cross-section (CSMI, cm^4) represents the distribution of mass in relation to the center of bending, i.e. the axis of bending. The point farthest from the center suffers maximum bending stress, while the point in the center suffers virtually no stress:

$$CSMI = \frac{\pi}{4} \left[\frac{(PD)^4}{2} - \frac{\rho(ED)^4}{2} \right]$$

(PD – periosteal diameter, ED – estimated endocortical diameter, ρ – trabecular porosity);

4. The section modulus (SM, cm^3) is an indicator of bending strength for maximum bending stress in the image plane. It is calculated by dividing the cross-sectional moment of inertia of the corresponding region of interest by half the periosteal diameter:

$$SM = \frac{CSMI}{PD/2}$$

(CSMI – moment of inertia on the cross-section, PD – periosteal diameter)

5. Endocortical diameter (ED, cm) is calculated indirectly using the model, while periosteal diameter is measured directly from DXA scans. Beck (163) based his model for estimating cortical thickness on previous results obtained by Kuper et al. (164) and Bell et al. (165) examining total cortical and trabecular bone mass in *in vitro* samples of proximal end of the femur using quantitative computed tomography. They determined that the total part of the cortical bone mass is 60% in the neck, 70% in the intertrochanteric region, and all 100% in the femoral body shaft. If we consider the femur as a model in which the cortical bone mass is distributed in this way, we can calculate the endocortical diameter:

$$ED = 2 \times \sqrt{(PD/2)^2 - fc \times CSA/\pi}$$

(PD – periosteal diameter, CSA – cross-sectional area, fc – constant for cortical mass and ranges from 0.6 for the neck to 1 for the body of the femur).

In order to apply this model for estimating the endocortical diameter, it is assumed that the cortex is symmetrically placed in relation to the periosteal diameter and that it can be approximately imagined as an ideal circle around the neck and body of the femur, or as an ellipse around the intertrochanteric region;

6. The thickness of the cortex (CTh, cm) is calculated from the difference between the periosteal and endocortical diameters:

$$CTh = PD - ED$$

(PD – periosteal diameter, ED – endocortical diameter)

7. The buckling ratio (BR, dimensionless) is a mechanical index of the buckling stability of the tube wall and is calculated when half of the periosteal diameter is divided by the thickness of the cortex. High values indicate that the bone has become unstable due to thinning of the cortex.

$$BR = \frac{PD/2}{CTh}$$

(PD – periosteal diameter, CTh – thickness of the cortex)

3.5. Statistical analysis

The sample size was determined using the statistical software G * Power (version 3.1., for Windows operating system) in order to achieve a study power of 80% at a significance level of 5% ($\beta = 0.2$ and $\alpha = 0.05$).

Statistical analysis of the data: The collected results were first analyzed using the Kolmogorov-Smirnov test to check the data distribution of all variables according to the theoretical normal distribution. A probability value of the null hypothesis that is greater than 0.05 confirms the agreement of the empirical with the normal distribution. After the analysis, it was determined that all data behave according to a normal distribution ($p>0.05$; Table 3 – 8) and that they can be analyzed by parametric statistical tests.

Table 3. Kolmogorov-Smirnov test for anthropometric parameters and FRAX

	N	Z	*p*
Age	172	0.76	0.604
Height	172	0.92	0.361
Weight	172	0.65	0.788
BMI	172	0.85	0.469
FRAX major osteoporotic fracture	172	1.38	0.044
FRAX hip fracture	172	3.96	< 0.001

Abbreviations: BMI – body mass index, FRAX – Fracture Risk Assessment Tool.

Tabela 4. Kolmogorov-Smirnov test for hip parameters obtained by DXA

	N	Z	*p*
BMCneck	140	0.79	0.560
BMCintertroch	139	0.68	0.751
BMCtot	140	0.68	0.752
BMDneck	140	0.86	0.455
BMDintertroch	139	0.77	0.596
BMDtot	140	1.10	0.180
T score hip	139	1.07	0.202
Z score hip	140	1.10	0.178

Abbreviations: BMC – bone mineral contetnt, BMD – bone mineral density, intertroch – intertrochanteric, tot – total.

Tabela 5. Kolmogorov-Smirnov test for parameters obtained by analysis of structural parameters of the proximal femur

	N	Z	*p*
PDneck	139	0.94	0.337
EDneck	139	0.90	0.391
PDintertroch	139	0.77	0.590
EDintertroch	139	0.62	0.835
PDfs	136	3.03	< 0.001
EDfs	136	1.44	0.032
CSAneck	139	0.85	0.469
CSAintertroch	139	0.76	0.609
CSAfs	136	1.90	0.002
CSMIneck	139	1.16	0.133
CSMIintertroch	139	0.83	0.500
CSMIfs	136	3.40	< 0.001
SMneck	139	0.99	0.286
SMintertroch	139	1.20	0.113
SMfs	136	3.09	< 0.001
CThneck	139	2.68	< 0.001
CThintertroch	139	0.71	0.699
CThfs	136	0.88	0.420
BRneck	139	0.96	0.313
BRintertroch	139	1.16	0.136
BRfs	136	1.76	0.004

Abbreviations: PD – periosteal diameter, ED – endocortical diameter, CSA – cross-sectional area, CSMI – moment of inertia on the cross-section, SM – section modulus, CTh – cortical thickness, BR – buckling ratio, intertroch – intertrochanteric, fs – femoral body shaft.

Tabela 6. Kolmogorov-Smirnov test for spine parameters obtained by DXA

	N	Z	*p*
BMCspine	139	0.89	0.414
BMDspine	139	0.93	0.357
T score spine	139	0.86	0.454
Z score spine	139	0.95	0.333
TBS	129	0.96	0.314

Abbreviations: BMC – bone mineral contetnt, BMD – bone mineral density, TBS – trabecular bone score.

Tabela 7. Kolmogorov-Smirnov test for laboratory parameters

	N	Z	*p*
PT	156	3.37	< 0.001
Fibrinogen	156	0.75	0.627
Albumin	158	1.87	0.002
Total bilirubin	158	3.68	< 0.001
Direct bilirubin	146	4.05	< 0.001
AST	158	2.77	< 0.001
ALT	158	2.45	< 0.001
ALP	158	2.52	< 0.001
GGT	158	2.90	< 0.001
Ca	147	1.02	0.246
Ca^{2+}	149	1.04	0.233
P	152	0.90	0.391
Vitamin D	115	1.27	0.080
PTH	142	1.82	0.003
Osteocalcin	145	0.73	0.663
Testosteron total	132	1.15	0.145
Testosteron free	74	1.04	0.233
Estradiol	97	1.99	0.001
LH	97	2.03	0.001
FSH	97	2.15	< 0.001
DHEAS	61	1.29	0.071
SHBG	95	0.91	0.381

Abbreviations: PT – prothrombin time, AST – aspartate aminotransferase, ALT – alanine aminotransferase, ALP – alkaline phosphatase, GGT – gamma-glutamyltransferase, Ca – calcium, Ca^{2+} – ionized calcium, P – phosphorus, PTH – parathyroid hormone, LH – luteinizing hormone, FSH – follicle-stimulating hormone, DHEAS – dehydroepiandrosterone sulfate, SHBG – sex hormone binding globulin.

Tabela 8. Kolmogorov-Smirnov test for specific bone biomarkers parameters

	N	Z	*p*
β-CTX	94	0.86	0.446
IGF-1	81	2.57	< 0.001
Osteoprotegerin	81	1.00	0.272
RANKL	81	1.74	0.005
RANKL/osteoprotegerin	81	1.579	0.014

Abbreviations: β-CTX – beta-C-terminal telopeptide, IGF-1 – insulin-like growth factor 1, RANKL – receptor activator of nuclear factor kappa beta ligand.

Levine's test was used to determine the data homogeneity before conducting an analysis of covariance (ANCOVA) with Bonferroni posthoc correction to estimate intergroup differences in mean values of the examined osteodensitometry parameters (covariates appearing in the corrected model were evaluated at a BMI value of 27.73 kg/m2). Analysis of variance (ANOVA) with Bonferroni posthoc correction was conducted to assess the significance of the difference in biochemical blood parameters and bone turnover biomarkers between the ALC, CAA, and control groups. All parameters that did not follow normal distribution were analyzed by adequate nonparametric tests (KruskalWallis and MannWhitney tests). Statistical analyses were conducted using SPSS statistical software (version 21, IBM Corp, Armonk, NY, USA) at a significance level of 5% (0.05).

3.6. Ethical considerations

The Ethics Committee of the Faculty of Medicine, University of Belgrade, confirmed that the study was conducted by the Guidelines for Good Clinical Practice, the Declaration of Helsinki, and local laws and regulations (approval no. 1322/IX-11). The study protocol was approved by the Joint Research and Ethics Committee (University Clinical Center of Serbia, approval no 890/9; Special Hospital on Addiction, approval no 2964). Written informed consent was obtained from all participants included in the study.

4. RESULTS

4.1. Biochemical analysis

Routine laboratory analyzes were performed to all subjects included in the study. A statistically significant difference was obtained for almost all compared parameters except ALT, osteocalcin, total testosterone and estradiol (Table 9 and 10). Then, an individual post-hoc analysis was performed, which more precisely determined intergroup differences.

The analysis of the two examined groups showed that patients with ALC had a statistically significantly weakened synthetic and excretory function of the liver, the concentration of total and ionized calcium was also lower, and the values of AST and ALP were higher, while there was no statistically significant difference in other compared parameters (Table 11 and 12).

The comparison of laboratory parameters of patients with ALC and the control group showed, as expected, a statistically significantly reduced synthetic and excretory function of the liver in the ALC group, as well as elevated values of AST and cholestasis enzymes, and significantly lower values of bone metabolism parameters. By analyzing sex hormones, statistically significantly lower values of free testosterone were obtained, along with higher values of SHBG and LH in the ALC group (Table 13 and 14).

Comparing patients who consumed alcohol excessively with healthy subjects, statistically significantly higher values of fibrinogen, GGT, phosphorus, LH and SHBG were obtained, while the levels of albumin, vitamin D and PTH were significantly lower in the CAA group (Tables 15 and 16).

Table 9. Comparison of laboratory parameters with normal distribution

	ALC gorup		CAA group		Control group		Overall p value
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	
Fibrinogen (g/L)	51	2.79 ± 0.14	73	3.98 ± 0.11	32	3.38 ± 0.12	< 0.001
Ca (mmol/L)	45	2.34 ± 0.02	69	2.40 ± 0.01	33	2.43 ± 0.02	0.001
Ca^{2+} (mmol/L)	46	1.27 ± 0.01	70	1.30 ± 0.01	33	1.29 ± 0.01	0.025
P (mmol/L)	48	1.07 ± 0.04	71	1.02 ± 0.03	33	0.89 ± 0.03	0.004
Osteocalcin (µg/L)	48	17.54 ± 1.71	65	17.27 ± 1.24	32	19.91 ± 1.25	> 0.05
Testosteron total (nmol/L)	48	16.19 ± 1.27	51	19.13 ± 1.30	33	20.04 ± 1.48	> 0.05
Testosteron free (nmol/L)	23	4.68 ± 0.84	20	7.83 ± 1.37	31	9.98 ± 0.96	0.002
SHBG (nmol/L)	37	69.82 ± 3.40	27	59.34 ± 3.77	31	44.54 ± 3.24	< 0.001

Abbreviations: ALC – alcoholic liver cirrhosis, CAA – chronic alcohol abuse, SE – standard error, Ca – calcium, Ca^{2+} – ionized calcium, P – phosphorus, SHBG – sex hormone binding globulin.

Table 10. Comparison of laboratory parameters that did not follow normal distribution

	ALC gorup N	ALC gorup Mean (Min-Max)	CAA group N	CAA group Mean (Min-Max)	Control group N	Control group Mean (Min-Max)	Overall p value
PT (s)	51	16.99 (10.6-29.5)	73	11.89 (9.8-16.9)	32	11.50 (10.7-12.5)	< 0.001
Albumin (g/L)	51	36.47 (26.0-49.0)	73	43.96 (36.0-52.0)	34	46.56 (38.0-52.0)	< 0.001
Total bilirubin (μmol/L)	51	47.84 (2.2-256.4)	73	8.72 (3.4-52.9)	34	14.11 (6.2-36.1)	< 0.001
Direct bilirubin (μmol/L)	49	25.44 (0.7-167.5)	63	2.68 (0.6-23.1)	34	4.06 (1.6-11.8)	< 0.001
AST (U/L)	51	47.74 (12.0-189.0)	73	31.18 (9.0-178.0)	34	24.23 (12.0-63.0)	< 0.001
ALT (U/L)	51	32.61 (11.0-98.0)	73	35.33 (11.0-246.0)	34	32.74 (14.0-96.0)	> 0.05
ALP (U/L)	51	112.63 (36.0-283.0)	73	70.89 (33.0-137.0)	34	69.23 (33.0-100.0)	< 0.001
GGT (U/L)	51	95.04 (20.0-669.0)	73	71.20 (14.0-322.0)	34	30.44 (11.0-100.0)	0.001
Vitamin D (nmol/L)	42	31.48 (8.8-84.9)	42	29.99 (8.8-88.9)	31	45.93 (14.4-98.0)	0.002
PTH (pg/mL)	49	37.41 (8.0-133.0)	60	36.82 (7.0-122.0)	33	71.12 (20.0-149.0)	< 0.001
Estradiol (pmol/L)	38	164.05 (88.0-650.0)	28	140.03 (88.0-243.0)	31	131.22 (88.0-206.0)	> 0.05
LH (mIU/mL)	38	6.87 (1.0-27.8)	29	6.67 (2.4-27.7)	30	3.09 (1.3-6.5)	0.001
FSH (mIU/mL)	37	9.07 (1.1-37.3)	29	8.92 (2.0-58.8)	31	5.49 (1.3-18.7)	> 0.05
DHEAS (μmol/L)	29	4.46 (0.4-27.8)	22	7.09 (0.3-20.2)	10	4.28 (1.7-9.6)	> 0.05

Abbreviations: ALC – alcoholic liver cirrhosis, CAA – chronic alcohol abuse, PT – prothrombin time, AST – aspartate aminotransferase, ALT – alanine aminotransferase, ALP – alkaline phosphatase, GGT – gamma-glutamyltransferase, PTH – parathyroid hormone, LH – luteinizing hormone, FSH – follicle-stimulating hormone, DHEAS – dehydroepiandrosterone sulfate.

Table 11. Comparison of laboratory parameters of two investigated groups with normal distribution

	ALC gorup N	ALC gorup Mean ± SE	CAA group N	CAA group Mean ± SE	p value
Fibrinogen (g/L)	51	2.79 ± 0.14	73	3.98 ± 0.11	< 0.001
Ca (mmol/L)	45	2.34 ± 0.02	69	2.40 ± 0.01	0.010
Ca^{2+} (mmol/L)	46	1.27 ± 0.01	70	1.30 ± 0.01	0.024
P (mmol/L)	48	1.07 ± 0.04	71	1.02 ± 0.03	0.720
Osteocalcin (μg/L)	48	17.54 ± 1.71	65	17.27 ± 1.24	> 0.05
Testosteron total (nmol/L)	48	16.19 ± 1.27	51	19.13 ± 1.30	> 0.05
Testosteron free (nmol/L)	23	4.68 ± 0.84	20	7.83 ± 1.37	0.156
SHBG (nmol/L)	37	69.82 ± 3.40	27	59.34 ± 3.77	0.111

Abbreviations: ALC – alcoholic liver cirrhosis, CAA – chronic alcohol abuse, SE – standard error, Ca – calcium, Ca^{2+} – ionized calcium, P – phosphorus, SHBG – sex hormone binding globulin.

Table 12. Comparison of laboratory parameters between two investigated groups that did not follow normal distribution

	ALC gorup		CAA group		p value
	N	Mean (Min-Max)	N	Mean (Min-Max)	
PT (s)	51	16.99 (10.6-29.5)	73	11.89 (9.8-16.9)	< 0.001
Albumin (g/L)	51	36.47 (26.0-49.0)	73	43.96 (36.0-52.0)	< 0.001
Total bilirubin (μmol/L)	51	47.84 (2.2-256.4)	73	8.72 (3.4-52.9)	< 0.001
Direct bilirubin (μmol/L)	49	25.44 (0.7-167.5)	63	2.68 (0.6-23.1)	< 0.001
AST (U/L)	51	47.74 (12.0-189.0)	73	31.18 (9.0-178.0)	0.002
ALT (U/L)	51	32.61 (11.0-98.0)	73	35.33 (11.0-246.0)	> 0.05
ALP (U/L)	51	112.63 (36.0-283.0)	73	70.89 (33.0-137.0)	< 0.001
GGT (U/L)	51	95.04 (20.0-669.0)	73	71.20 (14.0-322.0)	0.279
Vitamin D (nmol/L)	42	31.48 (8.8-84.9)	42	29.99 (8.8-88.9)	1.000
PTH (pg/mL)	49	37.41 (8.0-133.0)	60	36.82 (7.0-122.0)	1.000
Estradiol (pmol/L)	38	164.05 (88.0-650.0)	28	140.03 (88.0-243.0)	> 0.05
LH (mIU/mL)	38	6.87 (1.0-27.8)	29	6.67 (2.4-27.7)	1.000
FSH (mIU/mL)	37	9.07 (1.1-37.3)	29	8.92 (2.0-58.8)	> 0.05
DHEAS (μmol/L)	29	4.46 (0.4-27.8)	22	7.09 (0.3-20.2)	> 0.05

Abbreviations: ALC – alcoholic liver cirrhosis, CAA – chronic alcohol abuse, PT – prothrombin time, AST – aspartate aminotransferase, ALT – alanine aminotransferase, ALP – alkaline phosphatase, GGT – gamma-glutamyltransferase, PTH – parathyroid hormone, LH – luteinizing hormone, FSH – follicle-stimulating hormone, DHEAS – dehydroepiandrosterone sulfate.

Table 13. Comparison of laboratory parameters between ALC and control group with normal distribution

	ALC gorup		Control group		p value
	N	Mean ± SE	N	Mean ± SE	
Fibrinogen (g/L)	51	2.79 ± 0.14	32	3.38 ± 0.12	0.015
Ca (mmol/L)	45	2.34 ± 0.02	33	2.43 ± 0.02	0.002
Ca^{2+} (mmol/L)	46	1.27 ± 0.01	33	1.29 ± 0.01	0.195
P (mmol/L)	48	1.07 ± 0.04	33	0.89 ± 0.03	0.003
Osteocalcin (µg/L)	48	17.54 ± 1.71	32	19.91 ± 1.25	> 0.05
Testosteron total (nmol/L)	48	16.19 ± 1.27	33	20.04 ± 1.48	> 0.05
Testosteron free (nmol/L)	23	4.68 ± 0.84	31	9.98 ± 0.96	0.001
SHBG (nmol/L)	37	69.82 ± 3.40	31	44.54 ± 3.24	< 0.001

Abbreviations: ALC – alcoholic liver cirrhosis, SE – standard error, Ca – calcium, Ca^{2+} – ionized calcium, P – phosphorus, SHBG – sex hormone binding globulin.

Table 14. Comparison of laboratory parameters between ALC and control group that did not follow normal distribution

	ALC gorup		Control group		p value
	N	Mean (Min-Max)	N	Mean (Min-Max)	
PT (s)	51	16.99 (10.6-29.5)	32	11.50 (10.7-12.5)	< 0.001
Albumin (g/L)	51	36.47 (26.0-49.0)	34	46.56 (38.0-52.0)	< 0.001
Total bilirubin (µmol/L)	51	47.84 (2.2-256.4)	34	14.11 (6.2-36.1)	< 0.001
Direct bilirubin (µmol/L)	49	25.44 (0.7-167.5)	34	4.06 (1.6-11.8)	< 0.001
AST (U/L)	51	47.74 (12.0-189.0)	34	24.23 (12.0-63.0)	< 0.001
ALT (U/L)	51	32.61 (11.0-98.0)	34	32.74 (14.0-96.0)	> 0.05
ALP (U/L)	51	112.63 (36.0-283.0)	34	69.23 (33.0-100.0)	< 0.001
GGT (U/L)	51	95.04 (20.0-669.0)	34	30.44 (11.0-100.0)	0.001
Vitamin D (nmol/L)	42	31.48 (8.8-84.9)	31	45.93 (14.4-98.0)	0.009
PTH (pg/mL)	49	37.41 (8.0-133.0)	33	71.12 (20.0-149.0)	< 0.001
Estradiol (pmol/L)	38	164.05 (88.0-650.0)	31	131.22 (88.0-206.0)	> 0.05
LH (mIU/mL)	38	6.87 (1.0-27.8)	30	3.09 (1.3-6.5)	0.002
FSH (mIU/mL)	37	9.07 (1.1-37.3)	31	5.49 (1.3-18.7)	> 0.05
DHEAS (µmol/L)	29	4.46 (0.4-27.8)	10	4.28 (1.7-9.6)	> 0.05

Abbreviations: ALC – alcoholic liver cirrhosis, PT – prothrombin time, AST – aspartate aminotransferase, ALT – alanine aminotransferase, ALP – alkaline phosphatase, GGT – gamma-glutamyltransferase, PTH – parathyroid hormone, LH – luteinizing hormone, FSH – follicle-stimulating hormone, DHEAS – dehydroepiandrosterone sulfate.

Table 15. Comparison of laboratory parameters between CAA and control group with normal distribution

	CAA group		Control group		p value
	N	Mean ± SE	N	Mean ± SE	
Fibrinogen (g/L)	73	3.98 ± 0.11	32	3.38 ± 0.12	0.007
Ca (mmol/L)	69	2.40 ± 0.01	33	2.43 ± 0.02	0.882
Ca^{2+} (mmol/L)	70	1.30 ± 0.01	33	1.29 ± 0.01	1.000
P (mmol/L)	71	1.02 ± 0.03	33	0.89 ± 0.03	0.034
Osteocalcin (µg/L)	65	17.27 ± 1.24	32	19.91 ± 1.25	> 0.05
Testosteron total (nmol/L)	51	19.13 ± 1.30	33	20.04 ± 1.48	> 0.05
Testosteron free (nmol/L)	20	7.83 ± 1.37	31	9.98 ± 0.96	0.469
SHBG (nmol/L)	27	59.34 ± 3.77	31	44.54 ± 3.24	0.015

Abbreviations: CAA – chronic alcohol abuse, SE – standard error, Ca – calcium, Ca^{2+} – ionized calcium, P – phosphorus, SHBG – sex hormone binding globulin.

Table 16. Comparison of laboratory parameters between CAA and control group that did not follow normal distribution

	CAA group		Control group		p value
	N	Mean (Min-Max)	N	Mean (Min-Max)	
PT (s)	73	11.89 (9.8-16.9)	32	11.50 (10.7-12.5)	1.000
Albumin (g/L)	73	43.96 (36.0-52.0)	34	46.56 (38.0-52.0)	0.015
Total bilirubin (µmol/L)	73	8.72 (3.4-52.9)	34	14.11 (6.2-36.1)	1.000
Direct bilirubin (µmol/L)	63	2.68 (0.6-23.1)	34	4.06 (1.6-11.8)	1.000
AST (U/L)	73	31.18 (9.0-178.0)	34	24.23 (12.0-63.0)	0.629
ALT (U/L)	73	35.33 (11.0-246.0)	34	32.74 (14.0-96.0)	> 0.05
ALP (U/L)	73	70.89 (33.0-137.0)	34	69.23 (33.0-100.0)	1.000
GGT (U/L)	73	71.20 (14.0-322.0)	34	30.44 (11.0-100.0)	0.036
Vitamin D (nmol/L)	42	29.99 (8.8-88.9)	31	45.93 (14.4-98.0)	0.003
PTH (pg/mL)	60	36.82 (7.0-122.0)	33	71.12 (20.0-149.0)	< 0.001
Estradiol (pmol/L)	28	140.03 (88.0-243.0)	31	131.22 (88.0-206.0)	> 0.05
LH (mIU/mL)	29	6.67 (2.4-27.7)	30	3.09 (1.3-6.5)	0.007
FSH (mIU/mL)	29	8.92 (2.0-58.8)	31	5.49 (1.3-18.7)	> 0.05
DHEAS (µmol/L)	22	7.09 (0.3-20.2)	10	4.28 (1.7-9.6)	> 0.05

Abbreviations: CAA – chronic alcohol abuse, PT – prothrombin time, AST – aspartate aminotransferase, ALT – alanine aminotransferase, ALP – alkaline phosphatase, GGT – gamma-glutamyltransferase, PTH – parathyroid hormone, LH – luteinizing hormone, FSH – follicle-stimulating hormone, DHEAS – dehydroepiandrosterone sulfate.

4.2. Hip osteodensitometry findings

Analysis of the covariance of the osteodensitometric parameters obtained on the hip showed that there is a statistically significant difference in BMC and BMD in the intertrochanteric region, while the T score was at borderline statistical significance (Table 17).

Although DXA findings of all investigated parameters, except intertrochanteric BMD, were worse in patients with ALC compared to CAA, statistically significant differences were noted only in intertrochanteric BMC (Table 18).

Comparing the DXA findings of patients with ALC and the control group, lower values of all parameters were obtained in the examined group, but a statistically significant difference was recorded only in intertrochanteric BMD and T score (Table 19).

Comparing patients who consumed alcohol excessively and healthy subjects, a statistically significant difference was obtained only by analyzing BMC and BMD in the intertrochanteric region, while lower values of T and Z scores were recorded in CAA, but not statistically significant (Table 20).

Table 17. Comparison of the hip osteodensitometry findings

	ALC gorup		CAA group		Control group		Overall p value
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	
BMCneck (g)	43	4.85 ± 0.14	52	4.92 ±0.13	45	5.21 ± 0.14	0.150
BMCintertroch (g)	43	32.80 ± 1.10	52	38.20 ± 1.02	44	34.39 ± 1.10	0.001
BMCtotal (g)	43	46.07 ± 1.26	52	49.06 ± 1.16	45	49.05 ± 1.25	0.146
BMDneck (g/cm^2)	43	0.83 ± 0.19	52	0.87 ± 0.18	45	0.88 ± 0.19	0.163
BMDintertroch (g/cm^2)	43	1.08 ± 0.02	52	1.07 ± 0.02	44	1.16 ± 0.02	0.002
BMDtotal (g/cm^2)	43	0.96 ± 0.02	52	0.98 ± 0.02	45	1.02 ± 0.02	0.130
T score	43	-0.49 ± 0.12	52	-0.30 ± 0.11	44	-0.07 ± 0.12	0.052
Z score	43	-0.13 ± 0.20	52	0.08 ± 0.11	45	0.23 ± 0.12	0.107

Abbreviations: ALC – alcoholic liver cirrhosis, CAA – chronic alcohol abuse, SE – standard error, BMC – bone mineral contetnt, BMD – bone mineral density, intertroch – intertrochanteric.
Data were BMI-adjusted to avoid the covariant effect on the study results (BMI value appearing in corrected model was 26.87 kg/m2).

Table 18. Comparison of the hip osteodensitometry findings between two investigated groups

	ALC gorup		CAA group		p value
	N	Mean ± SE	N	Mean ± SE	
BMCneck (g)	43	4.85 ± 0.14	52	4.92 ±0.13	1.000
BMCintertroch (g)	43	32.80 ± 1.10	52	38.20 ± 1.02	0.001
BMCtotal (g)	43	46.07 ± 1.26	52	49.06 ± 1.16	0.249
BMDneck (g/cm^2)	43	0.83 ± 0.19	52	0.87 ± 0.18	0.432
BMDintertroch (g/cm^2)	43	1.08 ± 0.02	52	1.07 ± 0.02	1.000
BMDtotal (g/cm^2)	43	0.96 ± 0.02	52	0.98 ± 0.02	1.000
T score	43	-0.49 ± 0.12	52	-0.30 ± 0.11	0.777
Z score	43	-0.13 ± 0.20	52	0.08 ± 0.11	0.642

Abbreviations: ALC – alcoholic liver cirrhosis, CAA – chronic alcohol abuse, SE – standard error, BMC – bone mineral contetnt, BMD – bone mineral density, intertroch – intertrochanteric.
Data were BMI-adjusted to avoid the covariant effect on the study results (BMI value appearing in corrected model was 26.87 kg/m2).

Table 19. Comparison of the hip osteodensitometry findings between ALC and control group

	ALC gorup		Control group		p value
	N	Mean ± SE	N	Mean ± SE	
BMCneck (g)	43	4.85 ± 0.14	45	5.21 ± 0.14	0.199
BMCintertroch (g)	43	32.80 ± 1.10	44	34.39 ± 1.10	0.925
BMCtotal (g)	43	46.07 ± 1.26	45	49.05 ± 1.25	0.284
BMDneck (g/cm^2)	43	0.83 ± 0.19	45	0.88 ± 0.19	0.218
BMDintertroch (g/cm^2)	43	1.08 ± 0.02	44	1.16 ± 0.02	0.011
BMDtotal (g/cm^2)	43	0.96 ± 0.02	45	1.02 ± 0.02	0.142
T score	43	-0.49 ± 0.12	44	-0.07 ± 0.12	0.046
Z score	43	-0.13 ± 0.20	45	0.23 ± 0.12	0.107

Abbreviations: ALC – alcoholic liver cirrhosis, SE – standard error, BMC – bone mineral contetnt, BMD – bone mineral density, intertroch – intertrochanteric.
Data were BMI-adjusted to avoid the covariant effect on the study results (BMI value appearing in corrected model was 26.87 kg/m2).

Table 20. Comparison of the hip osteodensitometry findings between CAA and control group

	CAA group		Control group		p value
	N	Mean ± SE	N	Mean ± SE	
BMCneck (g)	52	4.92 ±0.13	45	5.21 ± 0.14	0.392
BMCintertroch (g)	52	38.20 ± 1.02	44	34.39 ± 1.10	0.040
BMCtotal (g)	52	49.06 ± 1.16	45	49.05 ± 1.25	1.000
BMDneck (g/cm^2)	52	0.87 ± 0.18	45	0.88 ± 0.19	1.000
BMDintertroch (g/cm^2)	52	1.07 ± 0.02	44	1.16 ± 0.02	0.004
BMDtotal (g/cm^2)	52	0.98 ± 0.02	45	1.02 ± 0.02	0.538
T score	52	-0.30 ± 0.11	44	-0.07 ± 0.12	0.489
Z score	52	0.08 ± 0.11	45	0.23 ± 0.12	1.000

Abbreviations: CAA – chronic alcohol abuse, SE – standard error, BMC – bone mineral contetnt, BMD – bone mineral density, intertroch – intertrochanteric.
Data were BMI-adjusted to avoid the covariant effect on the study results (BMI value appearing in corrected model was 26.87 kg/m2).

4.3. Hip structural analysis

In order to determine more precisely the regions of the proximal end of the femur, which have a higher risk of fracture, after the DXA, an HSA analysis was performed. Covariance analysis showed that there are statistically significant intergroup differences in the comparison of ED, PD and BR in the neck region, PD, ED, CSMI and SM of the intertrochanteric region and PD, ED, CSA, CSMI, SM, BR on the femoral body shaft (Table 21).

Comparing the two examined groups showed that patients who excessively and actively consumed alcohol had somewhat worse structual parameters of proximal femur, but a statistically significant difference was found only in CSMI of the intertrochanteric region, BR neck region and femoral shaft PD (Table 23 and 24).

Comparison of structual parameters of proximal femur of patients with ALC and healthy controls did not show a statistically significant difference in any parameter in the neck and intertrochanteric regions. ED and CSA of femoral shaft were statistically significantly worse in patients with ALC, while femoral shaft BR was surprisingly worse in the control group (Table 25 and 26).

The largest number of differences in the structural parameters of the proximal femur was found in the comparison of the CAA and control group, predominantly in the femoral shaft region (PD, CSA, CSMI, SM), but differences were also found in the intertrochanteric (CSMI, SM) and neck region (PD) (Table 27 and 28).

Table 21. Comparison of structural parameters of proximal femur with normal distribution

	ALC gorup		CAA group		Control group		Overall p value
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	
PDneck (cm)	43	3.41 ± 0.04	52	3.32 ± 0.04	44	3.46 ± 0.04	0.043
PDintertroch (cm)	43	7.08 ± 0.12	52	6.68 ± 0.11	44	7.03 ± 0.12	0.033
EDneck (cm)	43	3.02 ± 0.05	52	2.89 ± 0.04	44	3.05 ± 0.05	0.037
EDintertroch (cm)	43	5.36 ± 0.12	52	4.99 ± 0.11	44	5.37 ± 0.12	0.030
CSAneck (cm^2)	43	3.29 ± 0.08	52	3.36 ± 0.08	44	3.51 ± 0.08	0.157
CSAintertroch (cm^2)	43	6.90 ± 0.20	52	6.66 ± 0.19	44	7.30 ± 0.20	0.072
CSMIneck (cm^4)	43	3.16 ± 0.13	52	3.09 ± 0.12	44	3.47 ± 0.13	0.089
CSMIintertroch (cm^4)	43	28.85 ± 1.46	52	23.65 ± 1.34	44	30.73 ± 1.46	0.002
SMneck (cm^3)	43	1.66 ± 0.06	52	1.71 ± 0.05	44	1.85 ± 0.06	0.063
SMintertroch (cm^3)	43	7.23 ± 0.39	52	6.41 ± 0.36	44	8.03 ± 0.39	0.013
CThintertroch (cm)	43	0.84 ± 0.03	52	0.85 ± 0.03	44	0.81 ± 0.03	0.693
CThfs (cm)	43	0.71 ± 0.02	52	0.64 ± 0.02	41	0.68 ± 0.02	0.098
BRneck	43	10.10 ± 0.33	52	8.99 ± 0.31	44	9.47± 0.33	0.054
BRintertroch	43	4.76 ± 0.18	52	4.49 ± 0.16	44	5.02 ± 0.18	0.099

Abbreviations: ALC – alcoholic liver cirrhosis, CAA – chronic alcohol abuse, SE – standard error, PD – periosteal diameter, ED – endocortical diameter, CSA – cross-sectional area, CSMI – moment of inertia on the cross-section, SM – section modulus, CTh – cortical thickness, BR – buckling ratio, intertroch – intertrochanteric, fs – femoral body shaft. Data were BMI-adjusted to avoid the covariant effect on the study results (BMI value appearing in corrected model was 26.84 kg/m2).

Table 22. Comparison of structural parameters of proximal femur that did not follow normal distribution.

	ALC gorup N	ALC gorup Mean (Min-Max)	CAA group N	CAA group Mean (Min-Max)	Control group N	Control group Mean (Min-Max)	Overall p value
PDfs (cm)	43	2.07 (1.82-2.39)	52	2.03 (1.68-3.08)	41	2.34 (1.76-3.66)	0.015
EDfs (cm)	43	0.67 (0.25-1.31)	52	0.75 (0.22-1.89)	41	0.97 (0.23-2.51)	0.022
CSAfs (cm^2)	43	3.27 (0.71-4.17)	52	3.35 (2.30-4.73)	41	4.07 (2.66-7.29)	0.002
CSMIfs (cm^4)	43	1.45 (0.76-2.27)	52	1.38 (0.80-3.72)	41	2.38 (0.87-7.69)	0.026
SMfs (cm^3)	43	1.33 (0.79-1.88)	52	1.30 (0.90-2.36)	41	1.84 (0.93-5.34)	0.025
CThneck (cm)	43	0.20 (0.13-1.30)	52	0.21 (0.16-1.15)	44	0.21 (0.14-0.31)	0.282
BRfs	43	1.58 (1.20-2.60)	52	1.72 (1.20-2.90)	41	1.81 (1.20-3.20)	0.043

Abbreviations: ALC – alcoholic liver cirrhosis, CAA – chronic alcohol abuse, SE – standard error, PD – periosteal diameter, ED – endocortical diameter, CSA – cross-sectional area, CSMI – moment of inertia on the cross-section, SM – section modulus, CTh – cortical thickness, BR – buckling ratio, fs – femoral body shaft.

Table 23. Comparison of structural parameters of proximal femur with normal distribution between two investigated groups.

	ALC gorup N	ALC gorup Mean ± SE	CAA group N	CAA group Mean ± SE	p value
PDneck (cm)	43	3.41 ± 0.04	52	3.32 ± 0.04	0.303
PDintertroch (cm)	43	7.08 ± 0.12	52	6.68 ± 0.11	0.051
EDneck (cm)	43	3.02 ± 0.05	52	2.89 ± 0.04	0.150
EDintertroch (cm)	43	5.36 ± 0.12	52	4.99 ± 0.11	0.072
CSAneck (cm^2)	43	3.29 ± 0.08	52	3.36 ± 0.08	1.000
CSAintertroch (cm^2)	43	6.90 ± 0.20	52	6.66 ± 0.19	1.000
CSMIneck (cm^4)	43	3.16 ± 0.13	52	3.09 ± 0.12	1.000
CSMIintertroch (cm^4)	43	28.85 ± 1.46	52	23.65 ± 1.34	0.029
SMneck (cm^3)	43	1.66 ± 0.06	52	1.71 ± 0.05	1.000
SMintertroch (cm^3)	43	7.23 ± 0.39	52	6.41 ± 0.36	0.382
CThintertroch (cm)	43	0.84 ± 0.03	52	0.85 ± 0.03	1.000
CThfs (cm)	43	0.71 ± 0.02	52	0.64 ± 0.02	0.094
BRneck	43	10.10 ± 0.33	52	8.99 ± 0.31	0.048
BRintertroch	43	4.76 ± 0.18	52	4.49 ± 0.16	0.821

Abbreviations: ALC – alcoholic liver cirrhosis, CAA – chronic alcohol abuse, SE – standard error, PD – periosteal diameter, ED – endocortical diameter, CSA – cross-sectional area, CSMI – moment of inertia on the cross-section, SM – section modulus, CTh – cortical thickness, BR – buckling ratio, intertroch – intertrochanteric, fs – femoral body shaft. Data were BMI-adjusted to avoid the covariant effect on the study results (BMI value appearing in corrected model was 26.84 kg/m2).

Table 24. Comparison of structural parameters of proximal femur between two investigated groups that did not follow normal distribution

	ALC gorup		CAA group		p value
	N	Mean (Min-Max)	N	Mean (Min-Max)	
PDfs (cm)	43	2.07 (1.82-2.39)	52	2.03 (1.68-3.08)	0.022
EDfs (cm)	43	0.67 (0.25-1.31)	52	0.75 (0.22-1.89)	0.198
CSAfs (cm^2)	43	3.27 (0.71-4.17)	52	3.35 (2.30-4.73)	0.967
CSMIfs (cm^4)	43	1.45 (0.76-2.27)	52	1.38 (0.80-3.72)	0.092
SMfs (cm^3)	43	1.33 (0.79-1.88)	52	1.30 (0.90-2.36)	0.229
CThneck (cm)	43	0.20 (0.13-1.30)	52	0.21 (0.16-1.15)	0.485
BRfs	43	1.58 (1.20-2.60)	52	1.72 (1.20-2.90)	0.075

Abbreviations: ALC – alcoholic liver cirrhosis, CAA – chronic alcohol abuse, PD – periosteal diameter, ED – endocortical diameter, CSA – cross-sectional area, CSMI – moment of inertia on the cross-section, SM – section modulus, CTh – cortical thickness, BR – buckling ratio, fs – femoral body shaft.

Table 25. Comparison of structural parameters of proximal femur with normal distribution between ALC and control group.

	ALC gorup		Control group		p value
	N	Mean ± SE	N	Mean ± SE	
PDneck (cm)	43	3.41 ± 0.04	44	3.46 ± 0.04	1.000
PDintertroch (cm)	43	7.08 ± 0.12	44	7.03 ± 0.12	1.000
EDneck (cm)	43	3.02 ± 0.05	44	3.05 ± 0.05	1.000
EDintertroch (cm)	43	5.36 ± 0.12	44	5.37 ± 0.12	1.000
CSAneck (cm^2)	43	3.29 ± 0.08	44	3.51 ± 0.08	0.180
CSAintertroch (cm^2)	43	6.90 ± 0.20	44	7.30 ± 0.20	0.495
CSMIneck (cm^4)	43	3.16 ± 0.13	44	3.47 ± 0.13	0.290
CSMIintertroch (cm^4)	43	28.85 ± 1.46	44	30.73 ± 1.46	1.000
SMneck (cm^3)	43	1.66 ± 0.06	44	1.85 ± 0.06	0.070
SMintertroch (cm^3)	43	7.23 ± 0.39	44	8.03 ± 0.39	0.449
CThintertroch (cm)	43	0.84 ± 0.03	44	0.81 ± 0.03	1.000
CThfs (cm)	43	0.71 ± 0.02	41	0.68 ± 0.02	0.835
BRneck	43	10.10 ± 0.33	44	9.47± 0.33	0.544
BRintertroch	43	4.76 ± 0.18	44	5.02 ± 0.18	0.883

Abbreviations: ALC – alcoholic liver cirrhosis, SE – standard error, PD – periosteal diameter, ED – endocortical diameter, CSA – cross-sectional area, CSMI – moment of inertia on the cross-section, SM – section modulus, CTh – cortical thickness, BR – buckling ratio, intertroch – intertrochanteric, fs – femoral body shaft.
Data were BMI-adjusted to avoid the covariant effect on the study results (BMI value appearing in corrected model was 26.84 kg/m2).

Table 26. Comparison of structural parameters of proximal femur between ALC and control group that did not follow normal distribution

	ALC gorup N	Mean (Min-Max)	Control group N	Mean (Min-Max)	p value
PDfs (cm)	43	2.07 (1.82-2.39)	41	2.34 (1.76-3.66)	0.410
EDfs (cm)	43	0.67 (0.25-1.31)	41	0.97 (0.23-2.51)	0.009
CSAfs (cm^2)	43	3.27 (0.71-4.17)	41	4.07 (2.66-7.29)	0.003
CSMIfs (cm^4)	43	1.45 (0.76-2.27)	41	2.38 (0.87-7.69)	0.239
SMfs (cm^3)	43	1.33 (0.79-1.88)	41	1.84 (0.93-5.34)	0.095
CThneck (cm)	43	0.20 (0.13-1.30)	44	0.21 (0.14-0.31)	0.521
BRfs	43	1.58 (1.20-2.60)	41	1.81 (1.20-3.20)	0.017

Abbreviations: ALC – alcoholic liver cirrhosis, PD – periosteal diameter, ED – endocortical diameter, CSA – cross-sectional area, CSMI – moment of inertia on the cross-section, SM – section modulus, CTh – cortical thickness, BR – buckling ratio, fs – femoral body shaft.

Table 27. Comparison of structural parameters of proximal femur with normal distribution between CAA and control group

	CAA group N	Mean ± SE	Control group N	Mean ± SE	p value
PDneck (cm)	52	3.32 ± 0.04	44	3.46 ± 0.04	0.044
PDintertroch (cm)	52	6.68 ± 0.11	44	7.03 ± 0.12	0.116
EDneck (cm)	52	2.89 ± 0.04	44	3.05 ± 0.05	0.051
EDintertroch (cm)	52	4.99 ± 0.11	44	5.37 ± 0.12	0.065
CSAneck (cm^2)	52	3.36 ± 0.08	44	3.51 ± 0.08	0.546
CSAintertroch (cm^2)	52	6.66 ± 0.19	44	7.30 ± 0.20	0.068
CSMIneck (cm^4)	52	3.09 ± 0.12	44	3.47 ± 0.13	0.107
CSMIintertroch (cm^4)	52	23.65 ± 1.34	44	30.73 ± 1.46	0.002
SMneck (cm^3)	52	1.71 ± 0.05	44	1.85 ± 0.06	0.256
SMintertroch (cm^3)	52	6.41 ± 0.36	44	8.03 ± 0.39	0.010
CThintertroch (cm)	52	0.85 ± 0.03	44	0.81 ± 0.03	1.000
CThfs (cm)	52	0.64 ± 0.02	41	0.68 ± 0.02	0.973
BRneck	52	8.99 ± 0.31	44	9.47± 0.33	0.913
BRintertroch	52	4.49 ± 0.16	44	5.02 ± 0.18	0.097

Abbreviations: CAA – chronic alcohol abuse, SE – standard error, PD – periosteal diameter, ED – endocortical diameter, CSA – cross-sectional area, CSMI – moment of inertia on the cross-section, SM – section modulus, CTh – cortical thickness, BR – buckling ratio, intertroch – intertrochanteric, fs – femoral body shaft.
Data were BMI-adjusted to avoid the covariant effect on the study results (BMI value appearing in corrected model was 26.84 kg/m2).

Table 28. Comparison of structural parameters of proximal femur between CAA and control group that did not follow normal distribution.

	CAA group		Control group		p value
	N	Mean (Min-Max)	N	Mean (Min-Max)	
PDfs (cm)	52	2.03 (1.68-3.08)	41	2.34 (1.76-3.66)	0.012
EDfs (cm)	52	0.75 (0.22-1.89)	41	0.97 (0.23-2.51)	0.070
CSAfs (cm^2)	52	3.35 (2.30-4.73)	41	4.07 (2.66-7.29)	0.002
CSMIfs (cm^4)	52	1.38 (0.80-3.72)	41	2.38 (0.87-7.69)	0.011
SMfs (cm^3)	52	1.30 (0.90-2.36)	41	1.84 (0.93-5.34)	0.009
CThneck (cm)	52	0.21 (0.16-1.15)	44	0.21 (0.14-0.31)	1.000
BRfs	52	1.72 (1.20-2.90)	41	1.81 (1.20-3.20)	0.363

Abbreviations: CAA – chronic alcohol abuse, PD – periosteal diameter, ED – endocortical diameter, CSA – cross-sectional area, CSMI – moment of inertia on the cross-section, SM – section modulus, CTh – cortical thickness, BR – buckling ratio, fs – femoral body shaft.

4.4. Assessment of the risk of bone fractures

By analyzing patients with ALC and those who consume alcohol excessively, similar FRAX values were obtained, but there is an evidently higher risk of major osteoporosis-related fractures as well as of hip fractures compared to the control group (Table 29, 30 and 31).

Table 29. Comparison of FRAX score

	ALC gorup		CAA group		Control group		Overall p value
	N	Mean (Min-Max)	N	Mean (Min-Max)	N	Mean (Min-Max)	
FRAX major osteoporotic fracture	51	3.45 (1.80-5.90)	73	3.44 (1.70-6.50)	48	2.63 (1.30-5.20)	0.001
FRAX hip fracture	51	0.13 (0.00-0.90)	73	0.20 (0.00-1.00)	48	0.08 (0.00-1.10)	< 0.001

Abbreviations: ALC – alcoholic liver cirrhosis, CAA – chronic alcohol abuse, FRAX – Fracture Risk Assessment Tool.

Table 30. Comparison of FRAX score between two investigated groups

	ALC gorup N	Mean (Min-Max)	CAA group N	Mean (Min-Max)	p value
FRAX major osteoporotic fracture	51	3.45 (1.80-5.90)	73	3.44 (1.70-6.50)	1.000
FRAX hip fracture	51	0.13 (0.00-0.90)	73	0.20 (0.00-1.00)	0.22

Abbreviations: ALC – alcoholic liver cirrhosis, CAA – chronic alcohol abuse, FRAX – Fracture Risk Assessment Tool.

Table 31. Comparison of FRAX score between ALC and control group

	ALC gorup N	Mean (Min-Max)	Control group N	Mean (Min-Max)	p value
FRAX major osteoporotic fracture	51	3.45 (1.80-5.90)	48	2.63 (1.30-5.20)	0.003
FRAX hip fracture	51	0.13 (0.00-0.90)	48	0.08 (0.00-1.10)	0.002

Abbreviations: ALC – alcoholic liver cirrhosis, FRAX – Fracture Risk Assessment Tool.

Table 32. Comparison of FRAX score between CAA and control group

	CAA group N	Mean (Min-Max)	Control group N	Mean (Min-Max)	p value
FRAX major osteoporotic fracture	73	3.44 (1.70-6.50)	48	2.63 (1.30-5.20)	0.001
FRAX hip fracture	73	0.20 (0.00-1.00)	48	0.08 (0.00-1.10)	< 0.001

Abbreviations: CAA – chronic alcohol abuse, FRAX – Fracture Risk Assessment Tool.

4.5. Osteodensitometry of lumbar spine

Analysis of covariance of the parameters obtained by DXA of the lumbar spine did not indicate statistically significant differences in BMC and BMD, although lower values were recorded in patients with ALC. However, the T score was statistically significantly the lowest in patients with ALC in comparison with CAA and the control group. Furthermore, TBS stood out as the best indicator of damaged bone microarchitecture. Patients with ALC had significantly lower TBS in comparison with CAA and the control group (Table 33, 34, 35 and 36).

Table 33. Comparison of the lumbar spine osteodensitometry findings

	ALC gorup		CAA group		Control group		Overall p value
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	
BMC spine (g)	43	70.17 ± 2.57	51	75.57 ± 2.39	45	74.87 ± 2.55	0.257
BMD spine (g/cm^2)	43	0.98 ± 0.02	51	1.04 ± 0.02	45	1.03 ± 0.02	0.112
T score spine	43	-1.03 ± 0.21	52	-0.33 ± 0.19	44	-0.47 ± 0.21	0.039
Z score spine	43	-0.64 ± 0.21	51	-0.10 ± 0.20	45	-0.20 ± 0.21	0.151
TBS	39	1.18 ± 0.02	52	1.28 ± 0.02	38	1.31 ± 0.02	< 0.001

Abbreviations: ALC – alcoholic liver cirrhosis, CAA – chronic alcohol abuse, BMC – bone mineral contetnt, BMD – bone mineral density, TBS – trabecular bone score.
Data were BMI-adjusted to avoid the covariant effect on the study results (BMI value appearing in corrected model was 26.84 kg/m2).

Table 34. Comparison of the lumbar spine osteodensitometry findings between two investigated groups

	ALC group		CAA group		p value
	N	Mean ± SE	N	Mean ± SE	
BMC spine (g)	43	70.17 ± 2.57	51	75.57 ± 2.39	0.377
BMD spine (g/cm^2)	43	0.98 ± 0.02	51	1.04 ± 0.02	0.159
T score spine	43	-1.03 ± 0.21	52	-0.33 ± 0.19	0.044
Z score spine	43	-0.64 ± 0.21	51	-0.10 ± 0.20	0.198
TBS	39	1.18 ± 0.02	52	1.28 ± 0.02	0.004

Abbreviations: ALC – alcoholic liver cirrhosis, CAA – chronic alcohol abuse, BMC – bone mineral contetnt, BMD – bone mineral density, TBS – trabecular bone score.
Data were BMI-adjusted to avoid the covariant effect on the study results (BMI value appearing in corrected model was 26.84 kg/m2).

Table 35. Comparison of the lumbar spine osteodensitometry findings between ALC and control group

	ALC gorup		Control group		p value
	N	Mean ± SE	N	Mean ± SE	
BMC spine (g)	43	70.17 ± 2.57	45	74.87 ± 2.55	0.584
BMD spine (g/cm^2)	43	0.98 ± 0.02	45	1.03 ± 0.02	0.290
T score spine	43	-1.03 ± 0.21	44	-0.47 ± 0.21	0.185
Z score spine	43	-0.64 ± 0.21	45	-0.20 ± 0.21	0.425
TBS	39	1.18 ± 0.02	38	1.31 ± 0.02	< 0.001

Abbreviations: ALC – alcoholic liver cirrhosis, BMC – bone mineral contetnt, BMD – bone mineral density, TBS – trabecular bone score.
Data were BMI-adjusted to avoid the covariant effect on the study results (BMI value appearing in corrected model was 26.84 kg/m2).

Table 36. Comparison of the lumbar spine osteodensitometry findings between CAA and control group

	CAA group		Control group		p value
	N	Mean ± SE	N	Mean ± SE	
BMC spine (g)	51	75.57 ± 2.39	45	74.87 ± 2.55	1.000
BMD spine (g/cm^2)	51	1.04 ± 0.02	45	1.03 ± 0.02	1.000
T score spine	52	-0.33 ± 0.19	44	-0.47 ± 0.21	1.000
Z score spine	51	-0.10 ± 0.20	45	-0.20 ± 0.21	1.000
TBS	52	1.28 ± 0.02	38	1.31 ± 0.02	0.597

Abbreviations: CAA – chronic alcohol abuse, BMC – bone mineral contetnt, BMD – bone mineral density, TBS – trabecular bone score.
Data were BMI-adjusted to avoid the covariant effect on the study results (BMI value appearing in corrected model was 26.84 kg/m2).

4.6. Bone turnover biomarkers

Intergroup analysis of bone turnover biomarkers indicated statistically significant differences in the comparison of β-CTX, OPG, RNKL/OPG ratio and IGF-1 (Table 37).

By comparing the two examined groups, statistically significantly higher values of β-CTX and OPG were obtained in patients with ALC, while there were no significant differences in other parameters (Table 38).

In the comparison of ALC with the control group, statistically significantly different values were obtained for all parameters except RANKL (Table 39).

Post-hoc analysis of CAA and the control group showed that the synthesis of IGF-1 was statistically significantly lower in patients with CAA, and the RANKL/OPG ratio was also reduced (Table 40).

Table 37. Comparasion of bone turnover biomarkers

	ALC gorup		CAA group		Control group		Overall p value
β-CTX (pg/mL)	N 31	6202.10 ± 437.96	N 39	2819.23 ± 390.46	N 24	4224.12 ± 497.75	< 0.001
OPG (pg/mL)	N 30	391.88 ± 19.48	N 27	309.58 ± 20.54	N 24	252.59 ± 21.73	< 0.001
RANKL (pg/mL)	N 30	2584 (1171-5657)	N 27	2737 (1408-4980)	N 24	2701.79 (1408-7900)	> 0.05
RANKL/OPG ratio	N 30	7.65 (1.43-21.02)	N 27	7.80 (3.27-23.50)	N 24	11.28 (5.11-27.49)	0.003
IGF-1 (ng/mL)	N 30	38.60 (23.97-122.90)	N 27	35.31 (24.21-136.30)	N 24	52.72 (31.30-233.50)	< 0.001

Abbreviations: ALC – alcoholic liver cirrhosis, CAA – chronic alcohol abuse, β-CTX – beta-C-terminal telopeptide, OPG – osteoprotegerin, RANKL – receptor activator of nuclear factor kappa beta ligand, IGF-1 – insulin-like growth factor 1.
Data with a normal distribution are reported as Mean ± standard error, while data that did not follow normal distribution are presented as Mean (Min-Max).

Table 38. Comparasion of bone turnover biomarkers between two investigated groups

	ALC gorup		CAA group		Overall p value
β-CTX (pg/mL)	N 31	6202.10 ± 437.96	N 39	2819.23 ± 390.46	< 0.001
OPG (pg/mL)	N 30	391.88 ± 19.48	N 27	309.58 ± 20.54	0.014
RANKL (pg/mL)	N 30	2584 (1171-5657)	N 27	2737 (1408-4980)	> 0.05
RANKL/OPG ratio	N 30	7.65 (1.43-21.02)	N 27	7.80 (3.27-23.50)	0.502
IGF-1 (ng/mL)	N 30	38.60 (23.97-122.90)	N 27	35.31 (24.21-136.30)	0.660

Abbreviations: ALC – alcoholic liver cirrhosis, CAA – chronic alcohol abuse, β-CTX – beta-C-terminal telopeptide, OPG – osteoprotegerin, RANKL – receptor activator of nuclear factor kappa beta ligand, IGF-1 – insulin-like growth factor 1.
Data with a normal distribution are reported as Mean ± standard error, while data that did not follow normal distribution are presented as Mean (Min-Max).

Table 39. Comparasion of bone turnover biomarkers between ALC and control group

	ALC gorup		Control group		Overall p value
β-CTX (pg/mL)	N 31	6202.10 ± 437.96	N 24	4224.12 ± 497.75	0.011
OPG (pg/mL)	N 30	391.88 ± 19.48	N 24	252.59 ± 21.73	< 0.001
RANKL (pg/mL)	N 30	2584 (1171-5657)	N 24	2701.79 (1408-7900)	> 0.05
RANKL/OPG ratio	N 30	7.65 (1.43-21.02)	N 24	11.28 (5.11-27.49)	0.003
IGF-1 (ng/mL)	N 30	38.60 (23.97-122.90)	N 24	52.72 (31.30-233.50)	0.001

Abbreviations: ALC – alcoholic liver cirrhosis, β-CTX – beta-C-terminal telopeptide, OPG – osteoprotegerin, RANKL – receptor activator of nuclear factor kappa beta ligand, IGF-1 – insulin-like growth factor 1.
Data with a normal distribution are reported as Mean ± standard error, while data that did not follow normal distribution are presented as Mean (Min-Max).

Table 40. Comparasion of bone turnover biomarkers between CAA and control group

	CAA group		Control group		Overall p value
β-CTX (pg/mL)	N 39	2819.23 ± 390.46	N 24	4224.12 ± 497.75	0.087
OPG (pg/mL)	N 27	309.58 ± 20.54	N 24	252.59 ± 21.73	0.182
RANKL (pg/mL)	N 27	2737 (1408-4980)	N 24	2701.79 (1408-7900)	> 0.05
RANKL/OPG ratio	N 27	7.80 (3.27-23.50)	N 24	11.28 (5.11-27.49)	0.004
IGF-1 (ng/mL)	N 27	35.31 (24.21-136.30)	N 24	52.72 (31.30-233.50)	< 0.001

Abbreviations: CAA – chronic alcohol abuse, β-CTX – beta-C-terminal telopeptide, OPG – osteoprotegerin, RANKL – receptor activator of nuclear factor kappa beta ligand, IGF-1 – insulin-like growth factor 1.
Data with a normal distribution are reported as Mean ± standard error, while data that did not follow normal distribution are presented as Mean (Min-Max).

4.7. Analysis of patients with ALC classified according to the Child Pugh classification

The study included 17 patients who were in Child Pugh A stage, 16 were Child Pugh B and 12 Child Pugh C stage. A statistically significant difference was verified in terms of BMI, while there was no difference in the analysis of age, height and weight. We made an adjustment for BMI, and we present the results of DXA and HSA obtained in this way.

As expected, the synthetic and excretory function of the liver weakened with the progression of liver cirrhosis, and the worst values were recorded in patients in the Child Pugh C stage. Hepatogram analysis showed that the value of AST was statistically significantly lowest in patients in the terminal phase of liver cirrhosis, while the other parameters (ALT, ALP and GGT) did not differ. Vitamin D values were reduced in all three analyzed groups, while at the same time there was no negative feedback response, and PTH values were within the reference value, but with a trend of decreasing from Child Pugh stage A to C. Osteocalcin values decreased with the progression of liver failure, but a statistically significant difference was recorded only between stage A and C. The analysis of sex hormones showed a statistically significant difference only in the values of total testosterone, whose value was lower with the progression of liver insufficiency, and DHEAS, whose value was the highest in Child Pugh stage C (Table 41).

Contrary to expectations, DXA of the hip showed the worst results in patients in Child Pugh stage A, while a statistically significant difference was reported only in total BMC and neck BMD (Table 42).

The analysis of structural parameters of proximal femur is based on the findings obtained by osteodensitometry, and in the Child Pugh stage A compared to the stage B group, statistically significantly lower values of CSA, SM and CTh in the area of the femoral neck were verified, while there was no significant difference in all other parameters (Table 43).

Osteodensitometry of the spine did not show statistically significant differences according to Child-Pugh stages (Table 44).

Bone turnover biomarkers were not statistically significantly different in ALC patients in different stages of liver insufficiency according to the Child Pugh classification (Table 45).

Table 41. Comparison of laboratory parameters of patients with ALC according to Child Pugh classiffication

	Stage A Mean ± SE	Stage B Mean ± SE	Stage C Mean ± SE	Overall p value	A vs. B	A vs. C	B vs. C
PT (s)	14.27 ± 1.65	17.39 ± 2.00	22.73 ± 3.88	< 0.001	0.003	< 0.001	< 0.001
Fibrinogen (g/L)	3.25 ± 0.99	2.51 ± 0.92	2.05 ± 0.83	0.004	0.076	0.004	0.610
Albumin (g/L)	39.47 ± 1.16	35.06 ± 1.20	30.83 ± 1.38	< 0.001	0.034	< 0.001	0.077
Total bilirubin (μmol/L)	19.27 ± 11.53	54.04 ± 11.88	98.63 ± 13.72	< 0.001	0.135	< 0.001	0.055
Direct bilirubin (μmol/L)	7.71 ± 8.20	32.73 ± 8.46	52.03 ± 10.20	0.005	0.119	0.005	0.459
AST (U/L)	34.24 ± 7.40	42.50 ± 7.63	81.33 ± 8.81	0.001	1.000	0.001	0.005
ALT (U/L)	27.24 ± 4.65	28.06 ± 4.80	42.50 ± 5.54	0.082	1.000	0.122	0.166
ALP (U/L)	115.47 ± 12.75	103.13 ± 13.15	141.92 ± 15.18	0.162	1.000	0.568	0.180
GGT (U/L)	126.59 ± 26.98	80.50 ± 27.81	78.67 ± 32.12	0.399	0.723	0.779	1.000
Ca (mmol/L)	2.35 ± 0.04	2.35 ± 0.04	2.21 ± 0.05	0.055	1.000	0.099	0.089
Ca^{2+} (mmol/L)	1.27 ± 0.02	1.28 ± 0.02	1.23 ± 0.02	0.341	1.000	0.540	0.584
P (mmol/L)	1.15 ± 0.07	1.04 ± 0.07	1.05 ± 0.08	0.492	0.825	1.000	1.000
Vitamin D (nmol/L)	41.07 ± 5.06	22.59 ± 5.41	27.28 ± 6.75	0.047	0.052	0.333	1.000
PTH (pg/mL)	44.94 ± 6.31	38.07 ± 6.72	32.18 ± 7.85	0.445	1.000	0.638	1.000
Osteocalcin (μg/L)	21.07 ± 3.08	19.94 ± 2.88	9.92 ± 3.32	0.036	1.000	0.055	0.084
Testosteron total (nmol/L)	17.81 ± 2.20	18.03 ± 2.20	9.72 ± 2.46	0.027	1.000	0.056	0.047
Testosteron free (nmol/L)	4.57 ± 1.39	5.91 ± 1.23	1.88 ± 1.50	0.141	1.000	0.610	0.154
Estradiol (pmol/L)	161.00 ± 30.05	166.71 ± 27.82	181.22 ± 34.70	0.905	1.000	1.000	1.000
LH (mIU/mL)	9.16 ± 1.55	5.89 ± 1.44	5.52 ± 1.79	0.217	0.396	0.403	1.000
FSH (mIU/mL)	11.18 ± 2.50	9.28 ± 2.40	6.40 ± 2.89	0.465	1.000	0.660	1.000
DHEAS (μmol/L)	2.50 ± 1.69	3.41 ± 1.53	9.71 ± 2.07	0.029	1.000	0.038	0.067
SHBG (nmol/L)	74.28 ± 5.88	72.36 ± 5.44	59.38 ± 7.20	0.250	1.000	0.357	0.480

Abbreviations: SD – standard deviation, PT – prothrombin time, AST – aspartate aminotransferase, ALT – alanine aminotransferase, ALP – alkaline phosphatase, GGT – gamma-glutamyltransferase, Ca – calcium, Ca^{2+} – ionized calcium, P – phosphorus, PTH – parathyroid hormone, LH – luteinizing hormone, FSH – follicle-stimulating hormone, DHEAS – dehydroepiandrosterone sulfate, SHBG – sex hormone binding globulin.

Table 42. Comparison of the hip osteodensitometry findings of patients with ALC according to Child Pugh classiffication

	Stage A Mean ± SE	Stage B Mean ± SE	Stage C Mean ± SE	Overall p value	A vs. B	A vs. C	B vs. C
BMCneck (g)	4.50 ± 0.26	5.35 ± 0.28	5.05 ± 0.31	0.097	0.118	0.495	1.000
BMCintertroch (g)	30.91 ± 1.75	37.08 ± 1.92	32.81 ± 2.08	0.086	0.085	1.000	0.475
BMCtotal (g)	43.19 ± 2.01	51.88 ± 2.22	46.85 ± 2.40	0.030	0.026	0.718	0.450
BMDneck (g/cm^2)	0.76 ± 0.04	0.93 ± 0.04	0.86 ± 0.04	0.012	0.012	0.199	0.815
BMDintertroch (g/cm^2)	1.03 ± 0.04	1.15 ± 0.05	1.12 ± 0.05	0.154	0.223	0.503	1.000
BMDtotal (g/cm^2)	0.91 ± 0.03	1.03 ± 0.04	0.99 ± 0.04	0.059	0.068	0.399	1.000
T score	- 0.80 ± 0.21	- 0.03 ± 0.24	- 0.27 ± 0.26	0.061	0.076	0.337	1.000
Z score	- 0.41 ± 0.20	0.37 ± 0.23	0.02 ± 0.24	0.055	0.056	0.534	0.944

Abbreviations: SE – standard error, BMC – bone mineral contetnt, BMD – bone mineral density, intertroch – intertrochanteric.
Data were BMI-adjusted to avoid the covariant effect on the study results (BMI value appearing in corrected model was 27.37 kg/m2).

Table 43. Comparison of structural parameters of proximal femur of patients with ALC according to Child Pugh classiffication.

	Stage A Mean ± SE	Stage B Mean ± SE	Stage C Mean ± SE	Overall p value	A vs. B	A vs. C	B vs. C
PDneck (cm)	3.39 ± 0.09	3.50 ± 0.10	3.46 ± 0.11	0.712	1.000	1.000	1.000
PDintertroch (cm)	6.84 ± 0.18	7.48 ± 0.20	7.15 ± 0.22	0.089	0.089	0.830	0.867
PDfs (cm)	2.07 ± 0.03	2.07 ± 0.04	2.09 ± 0.04	0.843	1.000	1.000	1.000
EDneck (cm)	3.02 ± 0.10	3.05 ± 0.11	3.06 ± 0.12	0.974	1.000	1.000	1.000
EDintertroch (cm)	5.24 ± 0.20	5.52 ± 0.23	5.34 ± 0.24	0.674	1.000	1.000	1.000
EDfs (cm)	0.73 ± 0.07	0.61 ± 0.08	0.60 ± 0.09	0.422	0.887	0.772	1.000
CSAneck (cm^2)	3.00 ± 0.14	3.73 ± 0.15	3.48 ± 0.17	0.005	0.005	0.093	0.852
CSAintertroch (cm^2)	6.51 ± 0.37	7.77 ± 0.41	7.10 ± 0.45	0.108	0.111	0.940	0.891
CSAfs (cm^2)	3.24 ± 0.17	3.47 ± 0.19	3.25 ± 0.21	0.654	1.000	1.000	1.000
CSMIneck (cm^4)	2.90 ± 0.20	3.51 ± 0.22	3.52 ± 0.24	0.063	0.155	0.141	1.000
CSMIintertroch (cm^4)	26.66 ± 2.57	33.40 ± 2.83	31.09 ± 3.06	0.222	0.297	0.796	1.000
CSMIfs (cm^4)	1.40 ± 0.08	1.51 ± 0.08	1.52 ± 0.09	0.503	1.000	0.900	1.000
SMneck (cm^3)	1.53 ± 0.08	1.86 ± 0.09	1.75 ± 0.09	0.023	0.027	0.220	1.000
SMintertroch (cm^3)	6.91 ± 0.56	8.05 ± 0.62	7.66 ± 0.67	0.392	0.579	1.000	1.000
SMfs (cm^3)	1.32 ± 0.05	1.40 ± 0.05	1.37 ± 0.06	0.524	0.833	1.000	1.000
CThneck (cm)	0.18 ± 0.01	0.23 ± 0.01	0.20 ± 0.01	0.022	0.018	0.591	0.442
CThintertroch (cm)	0.80 ± 0.05	0.95 ± 0.05	0.83 ± 0.06	0.146	0.165	1.000	0.504
CThfs (cm)	0.67 ± 0.04	0.74 ± 0.04	0.77 ± 0.05	0.207	0.700	0.284	1.000
BRneck	10.89 ± 0.78	9.27 ± 0.86	9.74 ± 0.93	0.368	0.558	1.000	1.000
BRintertroch	5.08 ± 0.29	4.11 ± 0.32	4.73 ± 0.35	0.116	0.119	1.000	0.659
BRfs	1.66 ± 0.09	1.49 ± 0.10	1.46 ± 0.10	0.259	0.634	0.430	1.000

Abbreviations: SE – standard error, PD – periosteal diameter, ED – endocortical diameter, CSA – cross-sectional area, CSMI – moment of inertia on the cross-section, SM – section modulus, CTh – cortical thickness, BR – buckling ratio, intertroch – intertrochanteric, fs – femoral body shaft.
Data were BMI-adjusted to avoid the covariant effect on the study results (BMI value appearing in corrected model was 27.37 kg/m2).

Table 44. Comparison of the lumbar spine osteodensitometry findings of patients with ALC according to Child Pugh classiffication

	Stage A Mean ± SE	Stage B Mean ± SE	Stage C Mean ± SE	Overall p value	A vs. B	A vs. C	B vs. C
BMC spine (g)	64.75 ± 5.30	81.07 ± 5.84	71.85 ± 6.32	0.152	0.164	1.000	0.937
BMD spine (g/cm^2)	0.96 ± 0.05	1.04 ± 0.06	1.02 ± 0.06	0.534	0.915	1.000	1.000
T score spine	- 1.22 ± 0.48	- 0.46 ± 0.53	- 0.67 ± 0.57	0.553	0.934	1.000	1.000
Z score spine	- 0.76 ± 0.48	- 0.02 ± 0.53	- 0.35 ± 0.57	0.593	0.956	1.000	1.000
TBS	1.18 ± 0.04	1.20 ± 0.05	1.17 ± 0.05	0.888	1.000	1.000	1.000

Abbreviations: SE – standard error, BMC – bone mineral contetnt, BMD – bone mineral density, intertroch – intertrochanteric.
Data were BMI-adjusted to avoid the covariant effect on the study results (BMI value appearing in corrected model was 27.37 kg/m2).

Table 45. Comparison of bone turnover biomarkers of patients with ALC according to Child Pugh classiffication

	Stage A Mean ± SE	Stage B Mean ± SE	Stage C Mean ± SE	Overall p value	A vs. B	A vs. C	B vs. C
β-CTX (pg/mL)	5631.75 ± 718.87	6942.11 ± 830.08	6252.38 ± 830.08	0.499	0.731	1.000	1.000
OPG (pg/mL)	391.76 ± 40.45	445.54 ± 44.71	341.03 ±47.43	0.293	1.000	1.000	0.364
RANKL (pg/mL)	2336.55 ± 307.74	2570.44 ± 340.22	3022.63 ± 360.85	0.363	1.000	0.481	1.000
IGF-1 (ng/mL)	41.78 ± 7.71	38.62 ± 8.52	37.04 ± 0.04	0.918	1.000	1.000	1.000

Abbreviations: SE – standard error, β-CTX – beta-C-terminal telopeptide, OPG – osteoprotegerin, RANKL – receptor activator of nuclear factor kappa beta ligand, IGF-1 – insulin-like growth factor 1.

5. DISCUSSION

The systemic changes that develop in liver cirrhosis also affect the skeleton. Osteopenia and osteoporosis that develop over time increase the risk of fractures, which leads to a greater number of hospitalizations and surgical interventions, what in patients with liver cirrhosis have a significantly higher risk compared to the healthy population (166). Special attention should be paid to eventual fractures of the vertebrae, considering that one third are asymptomatic, while fractures of the femoral neck occur much less often compare to vertebrae, and average 10 years after the fracture of the vertebrae (167). In addition, the health of the skeletal system has probably the greatest impact on the quality of life in general. For this reason, all people who have some risk factors, which certainly include liver cirrhosis and excessive alcohol consumption, need to be examined in a timely manner in order to detect changes in time and prescribe adequate therapy for prevention bone fractures (166).

Epidemiological studies show that the prevalence of osteoporosis in patients suffering from liver cirrhosis is significantly higher than in the general population and, according to different authors, ranges from 12% to even 75% (168,169). Of particular concern is the fact that, depending on the etiology and stage of the liver disease, 7% to 40% of patients experience some vertebral and/or non-vertebral fracture. The changes in the bones of these patients prove that there is damage to both the trabecular and cortical component of the bones, as well as a disruption of the microarchitecture (27,170). Moreover, if hypogonadism is pronounced, which is a common finding in patients with liver cirrhosis, especially alcoholic etiology, bone fractures regardless of trauma intensity are even more frequent and reach as much as 71% (171).

It is confirmed that alcohol is a risk factor per se by studies conducted on patients who consume alcohol excessively and do not have cirrhosis (52). Consuming large amounts of alcohol has a damaging effect on bone at many different levels. First of all, the degree of differentiation of stem cells into osteogenic lineage cells decreases (172). In addition, alcohol has a direct toxic effect on osteocytes and osteoblasts with a consequent increase in osteoclast activity, which leads to a decrease in BMD (173). Furthermore, alcohol has a harmful effect on both trabecular and cortical bone, significantly disrupting bone microarchitecture (173). Of particular concern is the fact that alcohol in larger quantities blocks bone regeneration after a fracture (174). It was found that in comparison with people who do not consume alcohol, drinking three or four standard alcoholic drinks per day significantly increases the risk of hip fracture (relative risk 1.33 and 1.59 respectively) (175). However, previous studies have not fully examined the influence of different types of alcoholic beverages, since it is assumed that the non-alcoholic component has an important role in the eventual protective effect on the bones. So far, it is known that flavanoids have a positive effect on numerous organ systems, including bones, since they promote the proliferation of osteoblasts and suppress the differentiation of osteoclasts (106). With their antioxidant effect, they reduce the synthesis of pro-inflammatory cytokines, and consequently the expression of RANKL (176). In this regard, it was concluded that people who drink red wine, which contains the highest concentration of flavanoids, has a lower risk of hip fractures (177).

In addition to the usual risk factors, poor prognostic signs in patients with liver cirrhosis are: older age, malnutrition, sarcopenia and low BMI, history of previous fracture, cholestasis, hyperbilirubinemia, iron and copper accumulation, use of drugs that affect bone loss (169). Once a fracture occurs, a special challenge is the choice of technique that will be used to repair the fracture. Although surgical techniques and the equipment used are constantly advancing, in patients with liver cirrhosis, in addition to poor bone quality, the challenge is also the inability to heal adequately, which results in an increased rate of infections. Prolonged recovery significantly disturbs the quality of life and affects the increased mortality rate of these patients (134,169). In this regard, although vertebral fractures are much more common, hip fractures, due to the fact that they are mostly solved surgically, have a higher risk of death (178). Studies conducted in Taiwan (179) and Denmark (180) on a large series of patients with hip fractures confirm that patients with liver cirrhosis had a significantly higher number of complications in the form of infection, sepsis, osteomyelitis, urinary

tract infections, as well as peptic ulcers. In addition, a significantly higher mortality rate was demonstrated in patients with liver cirrhosis within one month, 3 months, as well as in the first year after the fracture. These studies clearly points to the great importance of timely diagnosis of bone changes, in order to prescribe adequate therapy, because if a fracture occurs, the degree of complications and the risk of death are significantly higher in patients with liver cirrhosis.

Alcohol per se is a major risk factor, while numerous authors agree that nutritional deficiency is one of the main causes of secondary osteoporosis in patients with liver cirrhosis (33,34). In order to focus on these two investigated risk factors for the occurrence of secondary osteoporosis, we excluded women from our study, in order to homogenize the sample as much as possible.

The etiology of liver disease significantly affects the degree of manifestation of bone changes. Most of the studies dealt with cholestatic forms of liver disease, at the first place primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), but also liver cirrhosis caused by viral hepatitis (181,182). In recent years, because of the application of new antiviral therapy, the number of these patients has been significantly reduced (183). Due to the sedentary lifestyle, but also the increasing use of alcohol, the most common factors of cirrhosis worldwide are fatty liver disease and alcohol. In some parts of the world, the prevalence of alcoholic liver cirrhosis is as high as 80% of all cirrhosis. In almost all regions of the world, an increase in the number of patients with alcoholic liver cirrhosis is recorded, and the highest increase was noticed in the United Arab Emirates and amounted to 863.93% (184). There is a need to pay special attention to the skeletal health of these patients and it is the best demonstrated by the fact that 39.4% of them have osteoporosis (86).

Some studies promote BMI as a factor affecting BMD, so patients with liver cirrhosis and BMI less than 18 had significantly lower BMD values compared to healthy ones, while in patients with BMI greater than 24 there was no statistically significant difference in BMD (185). The mentioned data could explain the fact that in our both study groups was obtained no significant difference for the hip and lumbar spine BMD (BMI > 25 in all groups), considering that we excluded patients with verified pathological fractures. In our sample, the hip T score indicated that osteopenia ($-2.5 < T \text{ score} \leq 1.0$) was verified in 12 patients with ALC, 21 patients in CAA group, while there were 5 patients in the control group who had osteopania. There were no patients with hip osteoporosis ($T \text{ score} \leq 2.5$) in all three groups. However, although examination of the lumbar spine showed 6 patients with osteoporosis and 16 with osteopenia, according to T score, in ALC group, and 5 patients with osteoporosis and even 27 with osteopenia in CAA group, while in the control group there were no patients with osteoporosis, and 16 had osteopenia, the inter group comparison did not establish a statistically significant difference. Nevertheless, the results of the TBS analysis show that the vertebal micro-architecture was disturbed in ALC group.

The analysis of laboratory parameters in our study showed the expected results in terms of weakened synthetic and excretory liver function in patients with alcoholic liver cirrhosis. The mean values of vitamin D in all groups were below the referent value, with statistically significantly lower values recorded in both examined groups compared to the control group. The lack of vitamin D combined with expected reduced concentration of albumin to which calcium binds, explains the significantly lower calcium values in ALC compared to CAA and the control group. In response to decreased calcium values, an increased concentration of phosphorus was observed in both investigated groups. It is known that calcium plays a very important role in the body and that its concentration in the serum is strictly controlled by numerous mechanisms. As the priority is to preserve the concentration of calcium in the serum, if hypocalcemia occurs for any reason, calcium will be withdrawn from the bones, which weakens mineralization (186). Also, due to the reduced values of vitamin D and calcium, it was expected that a calcium – PTH negative feedback loop would be activated. However, contrary to expectations, significantly lower PTH values were recorded in both investigated groups compared to the control. The mechanism that leads to this in patients with alcoholic cirrhosis is not known, while some studies present evidence that alcohol transiently and reversibly affects the reduction of PTH secretion (86,187). Special attention should

be paid to vitamin D level and bone mineralization in patients who are candidates for liver transplantation. It was proven that almost 90% of them have reduced vitamin D values. In the same study, 36% of patients had vertebral fractures, some of whom had multiple fractures. In ALC, 32% of patients have vitamin D values below 20 ng/mL, which are recorded as the disease progresses (188,189). In addition, the elevated value of ALP in patients with liver cirrhosis can be related to vitamin D deficiency (190), which is consistent with our results. Numerous studies confirm that low levels of vitamin D and PTH, poor nutrition along with hypogonadism, are the main reasons for disturbed bone metabolism (187,191).

Normal concentration and activity of sex hormones is necessary for adequate functioning of the entire organism. Their influence on the skeletal system is extremely important also. The best indicator are the changes that occur in postmenopause, when physiologically the concentration of estrogen decreases, and consequently primary osteoporosis develops. The same happens in men with hypogonadism for any reason (192,193). Testosterone is synthesized in Leydig cells. The whole process is regulated by the hypothalamic–pituitary–testicular (HPT) axis. GnRH synthesized in the hypothalamus stimulates the secretion of LH from the pituitary gland, which controls the secretion of testosterone. DHEAS synthesized in the adrenal gland after conversion to testosterone may also contribute to androgenic effects to a small extent. Testosterone has a great influence on the whole organism. It acts on: brain (raises libido and mood), bones (increases BMD), male sexual organs (positively affects penile growth, spermatogenesis and prostate growth and function), muscles (increase in strength and volume), skin (body hair growth, male pattern balding, sebum production), metabolism (increased lipolysis, reduction of insulin resistance), bone marrow (stimulation of red blood cell production, possible suppression of immune system) (194).

The basic mechanism of osteoporosis is an increase in the activity of osteoclasts and at the same time a decrease in the activity of osteoblasts. In addition to the direct harmful effect of alcohol on bones in the form of suppressed formation, which is proven by reduced osteocalcin values and bone biopsies in alcoholics, it has been proven that alcohol also leads to hypogonadism. However, the described changes are reversible and after establishing stable abstinence, bone recovery occurs (53,143,195). Preserved liver function is of crucial importance for the normal metabolism of sex hormones. Gynecomastia occurs when there is an imbalance and a predominance of estrogen in relation to androgens (196). As liver failure progresses, there is a further decline in testosterone levels and the development of sarcopenia, bone changes, loss of male sexual characteristics and anemia. In this regard, testosterone can be used as a prognostic parameter in liver cirrhosis, independent of the MELD score (197). Furthermore, sarcopenia, which develops in patients with liver cirrhosis, among other things, due to testosterone deficiency, is also a prognostic parameter and its progression indicates an increased risk of mortality (198). Because of its importance, some studies have been conducted examining testosterone replacement. Testosterone replacement in hypogonadal patients has been shown to increase BMD with enhanced bone formation (199,200). However, due to its toxicity even when administered transdermally, instead of oral administration, and a proven slightly higher risk for the development of hepatocellular carcinoma, its substitution did not enter the official guidelines (201). Testosterone can exert its effect directly or after aromatization into estradiol which also has a major impact on BMD (194,199). Androgen receptors to which testosterone binds have been verified in rat models on osteoblasts with the function of stimulating bone formation, but also on osteoclasts with the function of inhibiting resorption (199). SHBG is produced in the liver and binds testosterone with extremely high affinity. Also, albumin binds testosterone with a slightly lower affinity so that only 2% of total testosterone can be found in the circulation in free form (194).

In some studies has been proven that women who consume light to moderate amounts of alcohol have a higher concentration of estrogen. Considering that estrogen has a protective effect on bones and is a strong regulator of adequate bone turnover, it is clear that its higher concentration affects the increase of BMD in both women and men (58,202).

The results of our study confirm that there is an adequate negative feedback response to low testosterone levels, given that the values of LH and SHBG in both examined groups were

significantly higher compared to the control group. However, despite the decreased concentration of albumin in patients with ALC, there is a significant decrease in the concentration of the free fraction of testosterone due to the increased secretion of SHBG. The level of estradiol was higher in both examined groups, but no statistically significant difference was found in comparison with the control group.

Numerous studies have examined BMD in patients with liver cirrhosis and in people who consume alcohol. In our study it has been shown that the intertrochanteric part of the femur was the most affected by the changes, because significantly lower BMD values were recorded there compared to the control group, while BMD neck and BMD total femur were not significantly different in the examined and control groups. Also, similar BMD spine values were recorded in all three groups. However, the literal data are very heterogeneous. While some studies show that hip BMD was worse in patients with ALC (86,203), others support the fact that there was no significant difference in the compared groups (190). The situation is similar with spine BMD, where some authors conclude that lower BMD was observed in ALC (86,190,203), while in some examined groups there was no difference compared to the control (204). When it comes to the effect of alcohol on BMD, studies generally agree on the harmfulness of using excessive amounts (205), while a small dose of alcohol can have a protective effect (105). People who consume up to two standard alcoholic drinks per day have better BMD at both the lumbar spine and hip compared to non-drinkers, while those who consume only one alcoholic drink have better BMD values only at the femoral neck (175). Another study did not find a statistically significant difference between people who consume more than 30 g of alcohol per day and those who consume up to 19 g of alcohol per day in terms of lumbar, femur neck, and total femur BMD, although lower values were obtained in people who consumed larger amounts of alcohol. In addition, BMD has been shown to increase at most sites in light to moderate drinkers compared to non-drinkers, and then decrease when the amount exceeds 30 g of alcohol per day (206). The inconsistency of the obtained results could possibly be explained by the different methodology of the conducted studies. The reasons can be diverse, from the heterogeneity of the examined and control groups, insufficient number of participants, not classifying according to etiology and stage of liver disease, gender, age, different definitions of light, moderate and heavy alcohol consumption (51,86,121,203,207). Also, adequate adjustment of the results is of crucial importance for their interpretation. The best example of this is the study conducted by Zheng et al (185). The obtained results for the entire sample indicated significantly lower values of hip and spine BMD in ALC compared to the control group. After adjusting for sex, identical results were obtained in women, while in men only a significant difference in hip BMD was maintained. If the groups are stratified by age, significantly worse values of both hip and spine BMD of patients over 60 years of age are observed, while there was no difference in people under 40 years of age. Alcohol, as an etiological factor, carried a higher risk of developing osteoporosis (185). A study conducted on rats confirms that after constant exposure to alcohol, although there was no significant decrease in BMD, a reduced presence of collagen in the femoral neck trabeculae was verified, which can significantly increase bone fragility (208). Apart from the usual predilection sites of fractures, some authors examined the fragility of other bones as well. It was found that the changes can be verified in patients with ALC and on the radius, but they were somewhat more pronounced on the tibia (209).

It is known that patients who are on the waiting list for a liver transplantation must be in stable abstinence from alcohol. However, prior excessive alcohol consumption did not correlate with BMD at the time of DXA, and they also had, contrary to expectations, fewer vertebral fractures compared to patients who did not consume alcohol (120). The explanation could be the fact that among the patients who are on the waiting list for a liver transplantation, and who did not consume alcohol, there are a large number of those who basically have an autoimmune disease that was previously treated with corticosteroids, which are a major risk factor for the occurrence of osteoporosis and consequently bone fractures. Therefore, a more precise selection of the patients included in the study is needed in order to examine the influence of individual risk factors in the best possible way.

Considering that BMD analysis shows inconsistent results on the basis of which we cannot fully assess the risk of potential fractures, there is a need to analyze other parameters that could better assess bone fragility. One of them is TBS, which best describes bone microarchitecture. Studies show that TBS is decreased in 35% of patients with liver cirrhosis and that TBS correlates best with pathological vertebral fractures. In comparing TBS with other parameters, a positive correlation with BMD and a negative correlation with age and BMI were found (41). Our results are consistent with literal data (41), considering that TBS was decreased in both examined groups, but a statistically significant difference was found only in the comparison of ALC and the control group.

Special attention is drawn to patients with pathological femoral fractures who did not have decreased BMD. For this reason, there was a need for further testing to verify additional parameters that affect bone fragility (61). In order to more accurately determine the potential fracture site, osteodensitometry parameters, as well as the geometry of the proximal femur, can be determined in several regions, most often the neck, intertrochanteric region and shaft of the femur. A study conducted on patients who consumed alcohol excessively (patients with or without cirrhosis) proves that the results in individual regions can differ significantly. While compared to the control group, DXA and HSA parameters did not differ in the intertrochanteric part, a difference was verified in the region of the femoral neck, considering that patients with alcoholic liver disease had significantly lower BMD and CSA values (210). Also, in the group of patients with ALC, decreased values of BMD, CSA and CTh of the intertrochanteric region were obtained. The same study proves that the microarchitecture of the trabecular and cortical components of the bone is most damaged in the intertrochanteric region (210). However, observed results are not consistent with some other studies, while one study indicate an altered microarchitecture predominantly in the region of the narrow neck (121), the another one verified significantly thinner cortex and lower ability to resist compression forces in intertrochanteric segment (210). The reliability of the obtained HSA results based on DXA was checked in several studies by comparison with the parameters obtained by quantitative computed tomography. It was found that the HSA results obtained on DXA were comparable and consistent with the findings obtained on CT and high-resolution quantitative CT (211,212).

Our results showed that the HSA changes in the examined patients were most pronounced in the femoral shaft region, while some parameters were changed in the intertrochanteric and femoral neck region. Osteoporotic fractures are caused by both cortical thinning and trabecular bone loss. The highest BR value in the region of the femoral neck was recorded in patients with ALC. As one of the main indicators of cortical stability, it indicates that the neck of the femur in these patients could be the predilection site of the fracture. CSA was decreased in patients in both studied groups, but statistical significance was recorded only in femoral shaft CSA. The lowest value of CSMI in all three regions, as a parameter of distribution of mass in relation to the center of bending, was recorded in CAA without cirrhosis. SM, as an indicator of the bending resistance of the bone, was decreased in patients with ALC and CAA in all three regions, but statistical significance was recorded only in the comparison of SM intertrochanteric and femoral shaft between CAA and the control group, which represent a greater risk of fracturing in these patients.

The FRAX score was created so that people with an increased risk of hip fracture or other major osteoporotic fracture could be recognized in time to start their treatment. Our results prove that patients with ALC as well as CAA without cirrhosis have a significantly higher risk of pathological fracture in the next 10 years and are in line with the data from the literature (161,213). In order to obtain the most reliable data, the score contains 11 parameters plus DXA results, which on the other hand makes it quite complex. Inadequate cooperation of respondents, as well as insufficient knowledge of certain parameters required for analysis, can make it difficult to obtain conclusive data. Consuming 3 or more standard alcoholic drinks is considered an isolated risk factor. A study conducted in Australia confirms that each patient consuming more than 30 g of alcohol per day, had elevated both fractions of the FRAX score (161). Due to its high negative predictive value for values over 6.6% (for major fracture without BMD) it could also be used in the selection of patients who need DXA (214). In order to achieve the best possible prediction of

potential fractures, numerous studies were conducted on various parameters that could further improve the FRAX score. It was found that adjusting the FRAX score according to TBS could improve its predictive power (49).

Tissue sampling and its pathohistological analysis are very often the best way to establish any final diagnosis. It is similar with the changes that occur in the bones where histomorphometry could be applied. Computer-assisted microscopic analysis of bone tissue, obtained by iliac crest, femoral neck and vertebras, enables direct insight into the degree of bone formation and remodeling, as well as the microarchitectural structure of the bone (215,216). However, the procedure itself, as well as the obtained results, have numerous drawbacks. An invasive procedure that involves taking biopsies of a potentially osteoporotic fragile bone can be complicated by a fracture or possibly an infection. Then, the obtained sample of the iliac bone does not necessarily reflect the state of other bones, especially microarchitecture. Also, there is a lack of a three-dimensional view that gives a complete picture of the state of the skeleton. Because of all of the above, bearing in mind the high cost of the procedure and the risk of complications, without obtaining absolutely conclusive results, non-invasive methods of examination of the skeletal system are used much more often (217).

One of the non-invasive ways to assess the bone condition is the analysis of serum parameters of bone turnover. Due to the fact that bones are constantly undergoing a remodeling process, bone homeostasis is very complex and possible only if there is an adequate balance between formation and degradation. Osteocalcin is one of the well-known parameters of bone formation. It is produced in osteoblasts, and in the serum it is mostly in the carboxylated form (218). Osteocalcin has a circadian rhythm with the highest serum concentration at midnight and around noon (219). However, laboratory tests for the analysis of osteocalcin are adapted for blood sampling in the early morning hours, after an overnight fasting (220). It is excreted by the kidneys. As an indicator of bone formation, osteocalcin is elevated several months after a fracture, but also in hyperthyroidism (221). Apart from its role in bone formation, it has been proven that osteocalcin has a significant effect on the functionality of 3 other hormones: it increases the secretion of insulin by influencing the stimulation of pancreas β-cell proliferation (222), increases the secretion of adiponectin from adipocytes what reduces insulin resistance and finally (222), it increases the secretion of testosterone from the Leydig cells (223). In addition, it has been proven that it passes the bloodbrain barrier and that it can inhibit the production of γ-aminobutyric acid (GABA) at the level of the striatum and hippocampus while simultaneously stimulating the production of serotonin, dopamine and norepinephrine (224). A study conducted on mice proved that osteocalcin reduces anxiety and depression levels while increasing memory and learning abilities (225). In patients with liver cirrhosis, osteocalcin is decreased in 85% of cases (121). In our study, osteocalcin was decreased in both investigated groups, but no statistical significance was determined in comparison ALC and CAA patients with the controls. The reason could be the fact that patients with fractures were initially excluded.

Telopeptides of type 1 collagen are the most studied biomarkers of bone resorption. Depending on the cross-link forming site they differ: carboxy-terminal crosslinked (CTX-1 or β-CTX) and amino-terminal crosslinked (NTX-1). Both are produced by collagen degradation (226). Apart from the diagnostic importance, β-CTX can also be important for monitoring the effect of therapy in patients with osteoporosis receiving bisphosphonates (227). It is interesting to note that eating can affect the reduction of serum β-CTX levels, so blood sampling must be done in the morning after an overnight fast. Also, as it is excreted by the kidneys, its concentration in the urine can be determined. However, more reliable values are obtained by determining the blood concentration because the need for correction according to creatinine excretion is avoided (228,229). CTX coluld be transformed into 4 isoforms through the process of racemization and isomerization: the native form (á-L) and three age-related forms which are an isomerized form (â-L), a racemized form (á-D) and an isomerized/racemized (â-D) form. It is known that an increased áCTX/âCTX ratio can be an indicator of increased bone turnover. The mentioned situation is characteristic of Paget's disease or bone metastases and in postmenopausal women with rapid bone

loss (230). ELISA, radioimmunoassay (RIA) and electrochemiluminescence assay are different models for determining the concentration of β-CTX. The use of specific antibodies for the determination of â-CTX fragments proved to be a reliable method considering that the obtained results correlate with bone loss and fracture risk (231). Our previous study showed that 37% of patients with liver cirrhosis had elevated β-CTX levels (121). Similar results were obtained in other studies in which it was concluded that β-CTX values were higher in patients with liver cirrhosis compared to the control group (232,233). On the other hand, analyzing β-CTX values as one of the main parameters of bone resorption, it is concluded that mild to moderate alcohol consumption has a suppressive effect on bone resorption (234). If we analyze the values of osteocalcin and β-CTX in the subjects in our study, we conclude that in patients with ALC the dominant process of bone damage was increased resorption, with relatively preserved formation, bearing in mind that statistically significantly higher values of β-CTX were obtained compared to CAA and the control group, while osteocalcin values did not differ significantly. On the other hand, in people who consume alcohol excessively, there was no significant difference in both investigated parameters compared to healthy controls.

A study conducted on women who consume alcohol in moderation confirms its protective effect on bones, since lower concentrations of NTX, PTH, and osteocalcin were verified, on the basis of which it is concluded that in this case there was a decrease in bone turnover. Furthermore, after the cessation of alcohol intake, the concentration of all mentioned markers increases (114,235).

The concentration of IGF-1 is conditioned by numerous parameters. It depicts the specific connection between bone metabolism and liver function, given that it is produced to the greatest extent by hepatocytes, but also by numerous other cells, including osteoblasts (236). By binding to one of the 6 potential regulatory proteins, its function can be suppressed or enhanced. Finally, the acid labile subunit is responsible for the long half-life of IGF-1 in serum (237). In one animal study it has been proven that mice in which the concentration of IGF-1 is decreased have a dominantly reduced BMD of the cortical bone (238,239). Furthermore, mice in which the secretion of IGF-1 from the liver was suppressed, which reduces the total concentration by 75%, had significantly reduced periosteal circumference and CSA, only 6% shorter femur, while cortical bone BMD was lower by 26%, with spared trabecular bone (236). In addition to the direct effect on bones, it was found that the expression of RANKL was also reduced in these mice (240). Some studies (241) have examined the effect of treatment with recombinant human IGF-1 (rhIGF-1) in children with GH resistance and consequent IGF-1 deficiency. Although the benefit in the form of increased bone mass has been proven, the application of rhIGF-1 was not included in the official recommendations due to the accompanying side effects of the therapy, in the form of hypoglycemia and hypophosphatemia. It is known that with aging, as well as in liver failure, there is a decrease in the production of IGF-1 (190,237,242). In addition, reduced concentrations of IGF-1 are recorded in people who drink large amounts of alcohol (more than 150 g per day) for a long period of time (243). In our study we found significantly lower concentracion of IGF-1 in patients with ALC as well as in CAA compared to healthy controls.

The RANKL-RANK-OPG connection is crucial for understanding the development of the osteoporosis process (128). Apart from bones, RANK and RANKL can be expressed in numerous other cells, with a particularly important role in the activation of the immune system through T cells. In vitro studies have proven that RANKL affects the stimulation of antigen presentation in T cells by prolonging the life of dendritic cells (244). Studies conducted on mice have proven that T cells are key mediators in the process of osteoporosis. Athymic mice lacking T cells were found to be protected from bone loss. In this regard, it is concluded that chronic inflammation is of great importance for the development of osteoporosis. Considering that liver cirrhosis is a state of chronic inflammation in which proinflammatory cytokines are produced, it is clear that these patients are more susceptible to the development of osteoporosis (131,245). Increased concentration of IL-6 in liver cirrhosis directly and indirectly promotes osteoclastogenesis. In addition to directly influencing osteoclast activation, it stimulates osteoblasts to synthesize RANKL, which in turn

leads to osteoclast activation (130,132). Also, one of the most potent cytokines from the IL-1 family, which contains 11 different pro-inflammatory cytokines, is IL-1β, which is proven to be a significant stimulant of osteoclastogenesis and enhanced bone resorption (246). The described relationship is confirmed by studies conducted on mice, which proved that knockout mice have increased BMD of the femur, as well as better trabecular and cortical bone findings (247).

Apart from osteoblasts, OPG can be synthesized in numerous other cells (heart, kidney, liver and spleen) (128). Studies on mice have shown that B cells in the bone marrow are the main ones for the synthesis of osteoprotegerin, since B cell-deficient mice have pronounced osteoporosis (128). The importance of OPG is reflected in the fact that binding to RANKL stops the process of osteoclast activation. It has been proven that in patients with juvenile Paget's disease who had homozygous partial deletions of the OPG gene, there is a significantly higher rate of bone loss with an increased risk for pathological fractures (128).

Numerous studies have investigated RANKL and OPG as well as their relationship. Our results show that the highest OPG values were recorded in ALC and those were statistically significant compared to CAA and the control group. Patients who consumed alcohol excessively also had higher OPG values compared to the control group, but without a statistically significant difference. Many studies confirm the results of our study, considering that despite elevated OPG values in patients with liver cirrhosis, significantly elevated RANKL values were not verified (248,249). In addition, it was proven that OPG values were significantly higher in patients with liver cirrhosis compared to patients with chronic liver disease without cirrhosis (249). Elevated OPG values are also verified in patients who consume alcohol excessively (248). The main reason for the aforementioned increase in OPG values in patients with ALC and CAA is increased bone resorption that occurs as a result of chronic inflammation (250). However, data from the literature are not consistent. In patients with liver cirrhosis, elevated RANKL values can be verified along with decreased OPG values, which results in an increased RANKL/OPG ratio. As a consequence of the growth of the mentioned ratio, there is increased osteoclastogenesis (251,252). An increased value of RANKL does not have to be a consequence of increased osteoclastogenesis, but can also be interpreted as a consequence of increased activity of osteoblasts. On the other hand, its reduced concentration indicates low bone turnover, which results in increased bone fragility (253).

Cirrhosis of the liver, a condition in which the liver is irreversibly damaged, is a risk factor for osteoporosis and pathological fractures. The degree of liver failure and the cause of cirrhosis are the main risk factors for bone changes. The largest number of studies were conducted on patients who are on the waiting list for liver transplantation, given that the state of bone metabolism is of exceptional importance both before and after transplantation (254). However, numerous studies have not confirmed that the degree of liver failure, which is defined according to the Child Pugh stages or MELD score, correlates with osteoporotic changes in the bones and/or the presence of fractures (121,209). This is supported by a study that highlights a significant difference between the BMD of the lumbar spine and femoral neck of patients with liver cirrhosis compared with healthy ones, while there was no difference in the comparison of patients classified according to the Child Pugh criteria or MELD score (255). Our results are in accordance with the mentioned literal data, considering that statistically significantly lower values of neck BMD and total BMC were recorded in patients in Child Pugh stage A. In addition, the analysis of the structural parameters of the proximal edge of the femur showed statistically significantly lower values of CSA, SM and CTh in the area of the neck in the Child Pugh stage A compared to the stage B group, while there was no significant difference in all other parameters.

A study examining BMD using quantitative computed tomography did not indicate a significant difference according to the stage of liver disease through the Child Pugh classification (256). However, some published studies confirm that advanced liver cirrhosis increases the risk of pathological fractures (41), and that MELD score negatively correlate with total hip BMD (120). Also, it was noted that the level of osteocalcin was lower in patients with Child Pugh B and C stages of cirrhosis (121). In our study, a drastic decrease in the value of osteocalcin was recorded in the terminal phase of liver failure. Considering that IGF-1 is mostly synthesized in the liver, the

weakening of the liver synthetic function leads to a decrease in its synthesis, so studies have confirmed that the concentration of IGF-1 is negatively correlated with the MELD score, INR and spleen size, while it is positively correlated with albumins (257,258). In addition, some studies showed that IGF-1 values less than 30 ng/mL represent a negative prognostic parameter and have a high risk for death in the next 6 months (259), while the 3-month survival in those with values less than 13 ng/mL is only 63.1% (260). The results of studies examining OPG and RANKL values and liver insufficiency are not consistent. Some authors verify a positive correlation of OPG with total bilirubin, AST, ALP, GGT and CRP, with a negative correlation with serum calcium, albumin, hemoglobin and platelets (249). On the other hand, the degree of liver insufficiency of ethylic liver cirrhosis did not affect OPG values (250). All 4 examined bone markers in our study (IGF-1, β-CTX, OPG and RANKL) did not differ according to the degree of liver insufficiency. Based on the obtained results, as well as data from the literature, it is concluded that liver cirrhosis per se significantly affects bone metabolism, regardless of the degree of liver insufficiency.

6. CONCLUSION

The results of our study confirm that in patients with alcoholic liver cirrhosis and people who consume alcohol excessively, bone changes occur even before pathological fractures occur.

Osteodensitometry confirms a significantly lower BMD value in the intertrochanteric region of patients with ALC and CAA. Significantly the lowest value of T score on the hip was recorded in patients with ALC. A comparison of the two examined groups showed that ALC patients had significantly lower intertrochanteric BMC values. Osteodensitometric parametars on lumbar spine showed significant differences in the T score and TBS.

A comparison of the two examined groups showed that CAA patients had the worse structural parameters of proximal femur, but a statistically significant difference was found only in intertrochanteric CSMI and femoral shaft PD. HSA of proximal femur of patients with ALC and healthy controls showed that ED and CSA of femoral shaft were statistically significantly worse in patients with ALC. Comparison of CAA and control group showed that the most sensitive part of the femur was the shaft of patients who consume alcohol excessively. We verified significantly worse results in PD, CSA, CSMI and SM in CAA group, which all together makes the bone more fragile.

The FRAX score risk of major osteoporosis-related fractures, as well as hip fractures, was clearly higher in both study groups compared to the control group.

Significantly higher β-CTX and OPG values were recorded in patients with ALC, while IGF-1 and the RANKL/OPG ratio were decreased. At CAA, reduced IGF-1 and RANKL/OPG ratio were verified.

The analysis of patients with ALC classified through the degree of liver insufficiency according to Child Pugh criteria did not indicate significant differences, and it is concluded that the dominant bone damage occurs during the process of development of liver cirrhosis.

In order to prescribe adequate therapy on time, it is necessary to create a specific algorithm that predicts patients with an increased risk of pathological fractures as comprehensively as possible.

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Published thesis articles:

1. Stulic M, Jadzic J, Dostanic N, Zivkovic M, Stojkovic T, Aleksic J, Stojkovic S, Stojkovic Lalosevic M, Vojnovic M, Vlaisavljevic Z, Martinov Nestorov J, Nikolic T , Culafic Vojinovic V, Culafic D, Djonic D. Clinical Indicators of Bone Deterioration in Alcoholic Liver Cirrhosis and Chronic Alcohol Abuse: Looking beyond Bone Fracture Occurrence. Diagnostics 2024; 14(5): 510. (doi.org/10.3390/diagnostics14050510).
2. Štulić M, Ćulafić Đ, Đonić D. Uticaj alkohola na fragilnost kosti. Medicinski podmladak. DOI 10.5937/mp76-49230.

Author′s short biography

Dr. Miloš Štulić was born on 20th September 1984. in Valjevo, where he finished primary and music school and Gymnasium with excellent results. He graduated from the Faculty of Medicine of the University of Belgrade on 31st January 2011. with an average grade of 8.32 and obtained the title of Doctor of Medicine. He passed the professional exam for doctors of medicine in 2011. He enrolled in specialist academic studies in the field of the digestive system in the academic year 2011/2012. and passed all exams with an average grade of 9.09. He defended his final thesis "Immunosuppressive therapy in liver transplantation" in March 2014 and earned the title of academic specialist. He has been employed at the Clinic for Gastroenterohepatology, University Clinical Center of Serbia since October 2013. He enrolled in doctoral studies in the field of Skeletal Biology at the Faculty of Medicine of the University of Belgrade in 2015. In the same year, he enrolled in the specialization in internal medicine, and by passing the specialist exam with excellent results in October 2020, he obtained the title of specialist in internal medicine. He was elected to the position of clinical assistant at the Department of Internal Medicine at the Faculty of Medicine in Belgrade in May 2021. He trained in the country and abroad in the field of transplantation hepatology and digestive endoscopy. He is a member of numerous domestic and international professional associations. He is currently the president of the board of young gastroenterologists at the Association of Gastroenterologists of Serbia and the general secretary of the board of directors of the Gastroenterological Endoscopy Association of Serbia. He is the author/co-author of 19 papers, 17 of which were published in journals indexed in SCI, as well as two chapters in a book - a textbook within specialist studies. His works have been cited 90 times according to SCOPUS and the h-index is 7.

Kratka biografija autora

Dr Miloš Štulić rođen je 20. septembra 1984. god. u Valjevu gde je završio osnovnu i muzičku školu, kao i Gimnaziju sa odličnim uspehom. Na Medicinskom fakultetu Univerziteta u Beogradu diplomirao je 31.01.2011. godine sa prosečnom ocenom 8,32 i stekao zvanje doktora medicine. Stručni ispit za doktore medicine položio je 2011. godine. Specijalističke akademske studije iz oblasti digestivnog sistema, upisao je školske 2011/2012. godine i položio sve zakonski predviđene ispite sa srednjom ocenom 9,09. Završni rad "Imunosupresivna terapija u transplantaciji jetre" odbranio je u martu 2014. godine i stekao zvanje akademskog specijaliste. Zaposlen je u Klinici za gastroenterohepatologiju, Univerzitetskog kliničkog centra Srbije od oktobra 2013. godine. Doktorske studije iz oblasti Biologije skeleta na Medicinskom fakultetu Univerziteta u Beogradu upisao je 2015. godine. Iste godine upisao je specijalizaciju iz interne medicine, a polaganjem specijalističkog ispita sa odličnim uspehom u oktobru 2020. godine, stekao je zvanje lekara specijaliste interne medicine. U zvanje kliničkog asistenta na katedri interne medicine na Medicinskom fakultetu u Beogradu izabran je u maju 2021. godine. Usavršavao se u zemlji i inostranstvu na polju transplantacione hepatologije i digestivne endoskopije. Član je brojnih domaćih i internacionalnih strukovnih udruženja. Aktuelni je predsednik borda mladih gastroenterologa, pri Udruženju gastroenterologa Srbije i generalni sekretar upravnog odbora Gastroenterološkog endoskopskog udruženja Srbije. Autor/koautor je u 19 radova, od kojih je 17 publikovano u časopisima indeksiranim u SCI, kao i dva poglavlja u knjizi - udžbeniku u okviru specijalističkih studija. Njegovi radovi citirani su 90 puta prema SCOPUS-u i h indeks iznosi 7.

образац изјаве о ауторству

Изјава о ауторству

Име и презиме аутора: Милош Штулић

Број индекса БС-01/15

Изјављујем

да је докторска дисертација под насловом:

Клинички показатељи пропадања коштаног ткива у алкохолној цирози јетре и хроничном алкохолизму без цирозе

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Изјава о истоветности штампане и електронске верзије докторског рада

Име и презиме аутора: Милош Штулић

Број индекса: БС-01/15

Студијски програм: биологија скелета

Наслов рада: Клинички показатељи пропадања коштаног ткива у алкохолној цирози јетре и хроничном алкохолизму без цирозе

Ментори: Проф. др Ђорђе Ђулафић и Проф. др Данијела Ђонић

Изјављујем да је штампана верзија мог докторског рада истоветна електронској верзији коју сам предао/ла ради похрањивања у **Дигиталном репозиторијуму Универзитета у Београду.**

Дозвољавам да се објаве моји лични подаци везани за добијање академског назива доктора наука, као што су име и презиме, година и место рођења и датум одбране рада.

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Потпис аутора

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образац изјаве о коришћењу

Изјава о коришћењу

Овлашћујем Универзитетску библиотеку „Светозар Марковић“ да у Дигитални репозиторијум Универзитета у Београду унесе моју докторску дисертацију под насловом:

Клинички показатељи пропадања коштаног ткива у алкохолној цирози јетре и хроничном алкохолизму без цирозе,

која је моје ауторско дело.

Дисертацију са свим прилозима предао/ла сам у електронском формату погодном за трајно архивирање.

Моју докторску дисертацију похрањену у Дигиталном репозиторијуму Универзитета у Београду и доступну у отвореном приступу могу да користе сви који поштују одредбе садржане у одабраном типу лиценце Креативне заједнице (Creative Commons) за коју сам се одлучио/ла.

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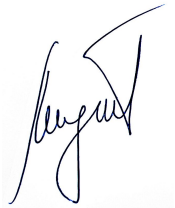

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