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SHEI "State Medical University by I. Ya. Horbachevsky"

**AGE AS A DETERMINANT OF VERTEBRAL TRABECULAR BONE STATUS IN HODGKIN LYMPHOMA PATIENTS AFTER COMPLETION OF CHEMOTHERAPY: A PROSPECTIVE STUDY**

AGE AS A DETERMINANT OF VERTEBRAL TRABECULAR BONE STATUS IN HODGKIN LYMPHOMA PATIENTS AFTER COMPLETION OF CHEMOTHERAPY: A PROSPECTIVE STUDY – Bone density alterations are often reported in patients with solid tumors and hematologic malignancies except cases of Hodgkin lymphoma, when this problem remains understudied. The aim of this study was to investigate the significance of patients' age and ABVD standard chemotherapy regimen as the determinants of vertebral trabecular bone tissue status changes. The study included 40 HL patients of different age groups (median age 35.38±2.22). Densitometric and structural parameters were obtained from pre-treatment and after chemo CT scans. Analysis of these parameters after chemotherapy compared to the initial values revealed significant decrease of bone density (maximum up to 24.9 %) in all age groups, as well as in patients who were treated with ABVD regimen. Structural parameters values (BV/TV, SMI, BS, FD, textural parameters) had also suggested trabecular transformation dependent on patients' age and chemotherapy treatment. Age and ABVD standard chemotherapy may be considered as predisposing risk factors for trabecular bone structural alteration and there is a need for timely diagnosis and prevention.

ВІК ЯК ДЕТЕРМІНАНТА СТАНУ ТРАБЕКУЛЯРНОЇ ТКАНИНИ ХРЕБЦІВ У ПАЦІЄНТІВ ІЗ ЛІМФОМОЮ ХОДЖКІНА ПІСЛЯ ЗАВЕРШЕННЯ ХІМІОТЕРАПІЇ – Ураження кісткової тканини часто беруть до уваги у пацієнтів із солідними пухлинами та гематологічною патологією, оминаючи пацієнтів з лімфомою Ходжкіна, в яких ця проблема залишається недостатньо вивченою. Метою цього дослідження було вивчити значення віку пацієнта та стандартного протоколу хіміотерапії (ABVD) як детермінант змін стану трабекулярної тканини хребців. У дослідження було залучено 40 пацієнтів різних вікових груп (середній вік ((35,38±2,22) року). Денситометричні та структурні параметри досліджували, використовуючи комп'ютерні томограми до та після хіміотерапії. У результаті порівняльного аналізу після хіміотерапії з вихідними значеннями отримали значне зменшення кісткової щільності (максимально до 24,9 %) у всіх вікових групах, а також і в пацієнтів, які отримали хіміотерапію згідно з протоколом ABVD. Зміна структурних параметрів після отримання хіміотерапії (BV/TV, SMI, BS, FD, текстури) також вказувала на трабекулярну трансформацію як в різних вікових групах, так і у пацієнтів після хіміотерапії згідно з протоколом ABVD. Вік та проведення стандартної хіміотерапії за протоколом ABVD можуть вважатися факторами ризику щодо виникнення ураження трабекулярної тканини хребців, що є підставою для потреби їх вчасної діагностики та профілактики виникнення.

ВОЗРАСТ КАК ДЕТЕРМИНАНТА СОСТОЯНИЯ ТРАБЕКУЛЯРНОЙ ТКАНИ ПОЗВОНКОВ У ПАЦИЕНТОВ С ЛИМФОМОЙ ХОДЖКИНА ПОСЛЕ ОКОНЧАНИЯ ХИМИТЕРАПИИ – Поражение костной ткани часто принимают во внимание у пациентов с солідными опухолями и гематологической патологией, минуя пациентов с лимфомой Ходжкіна, в которых эта проблема остается недостаточно изученной. Целью этого исследования было изучить значение возраста пациента и стандартного протокола химиотерапии (ABVD) как детерминант изменений состояния трабекулярной ткани позвонков. В исследование было вовлечено 40 пациентов различных возрастных групп (средний возраст (35,38±2,22) лет)). Денситометрические и структурные параметры изучали, используя компьютерные томограммы до и после химиотерапии. В результате сравнительного анализа после химиотерапии с исходными значениями, получили значительное уменьше-

ние костной плотности (максимально до 24,9 %) во всех возрастных группах, а также и в пациентов, получивших химиотерапию по протоколу ABVD. Изменение структурных параметров после получения химиотерапии (BV/TV, SMI, BS, FD, текстуры) также указывали на трабекулярную трансформацию как в разных возрастных группах, так и у пациентов после химиотерапии по протоколу ABVD. Возраст и проведения стандартной химиотерапии по протоколу ABVD могут считаться факторами риска возникновения поражения трабекулярной ткани позвонков, что является основанием для необходимости их своевременной диагностики и профилактики возникновения.

**Key words:** Hodgkin lymphoma, vertebral trabecular bone status, chemotherapy, ABVD regimen, age

**Ключові слова:** лімфома Ходжкіна, трабекулярна кісткова тканина хребців, хіміотерапія, протокол ABVD, вік.

**Ключевые слова:** лимфома Ходжкіна, трабекулярная костная ткань позвонков, химиотерапия, протокол ABVD, возраст.

**INTRODUCTION** Patients with solid tumors, acute and chronic leukemias are more commonly diagnosed with bone density alterations, however, only a few studies have been published exploring the dynamics of bone mineral density in Hodgkin lymphoma (HL) patients [9,10]. Although several studies have reported bone mineral density changes in patients with HL [2], there are no prospective studies indicating the impact of age and chemotherapy regimen on vertebral trabecular bone status (VTBS) and it remains unclear.

The aim of the work was to investigate the impact of age as a determinant of VTBS changes; to explore the VTBS changes in Hodgkin lymphoma patients at the diagnostics and after treatment with ABVD chemotherapy regimen.

**MATERIALS AND METHODS** 40 patients with newly diagnosed HL were hospitalized in Ternopil Regional Oncology Hospital and involved in this study. A definitive diagnosis was been established according to histopathologic subtype of HL (Figure 1), immunohistochemical pattern and national diagnostic criteria.

HL patients were divided according to median age in order to investigate the impact of this determinant on VTBS. We performed an estimation of VTBS parameters at the mid-vertebral levels using CT slices (performed on Philips Brilliance 64 CT Scanner and Siemens SOMATOM Definition AS) and obtained densitometric values of thoracic trabecular bone at diagnosis and after completion of chemotherapy. Densitometry was performed in trabecular bone of thoracic vertebrae using CT image analysis software DICOM format – ClearCanvas Workstation. Direct measurements were conducted at mid-vertebral slices of vertebrae in 5 areas avoiding the cortical bone. The results were obtained in Hounsfield units (HU) [7].

For assessing the structure of VTBS at the mesoscopic level we chose a method of virtual computer-based biopsy of thoracic and lumbar vertebrae (image resolution 1 pixel

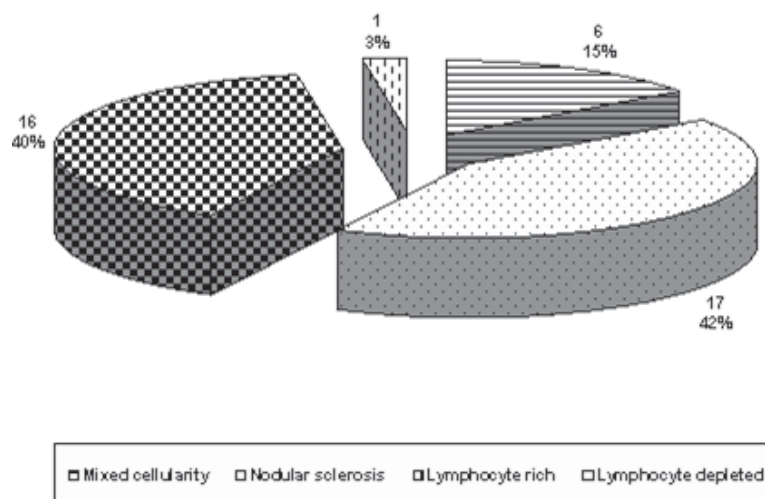


Figure 1. Histological subtypes of Hodgkin lymphoma.

– 1 millimeter). We used specialized medical image analysis software ImageJ with BoneJ plugin which is freely distributed by The National Institute of Health (USA). In order to standardize and stabilize an image obtained, we used Mexican hat wavelet filtering [4]. Trabecular structural parameters calculated in this study were the following [12]: bone surface (BS) [5] which is a basic 2D parameter of vertebral trabecular structure, bone volume fraction (BV/TV) [1,11] reflecting bone mineralization, structure model index (SMI) [13] for determining trabecular geometry, fractal dimension (FD map-counting and box-counting algorithm) [3] that reflects the complexity of branching trabeculae and texture parameters (Angular Second Moment that reflects image uniformity, Inverse Difference Moment, Entropy, Contrast

describing image heterogeneity and may reflect trabecular structural transformation) [8]. All statistical analyses were performed with statistical software (OpenStat® (v. 3.5.5) and Statgraphics (v. 3.0). The robust Brown-Forsythe Levene test was used as a main criterion for statistical analysis [6].

**RESULTS AND DISCUSSIONS** We conducted a comparative analysis of bone density changes (HU units) at the diagnostics and after completion of chemotherapy in male, female and common group. The differences between median age in those groups were not statistically significant (median age in male group –  $36.86 \pm 2.50$ , in female group –  $33.74 \pm 3.82$ , in common group –  $35.38 \pm 2.22$ ).

Table 1 summarizes bone density values in all age groups of HL patients at diagnosis and after completion

**Table 1. Densitometric values (HU) of thoracic and lumbar vertebral trabecular tissue in older, younger and common group of HL patients at diagnosis and after completion of chemotherapy**

HU	AT DIAGNOSIS			AFTER CHEMOTHERAPY		
	Common group (n=40)*	Younger age group (n=18) <sup>Δ</sup>	Older age group (n=22) <sup>Ω Θ</sup>	Common group (n=29)*	Younger age group (n=16) <sup>Δ</sup>	Older age group (n=13) <sup>Ω Θ</sup>
Th <sub>1</sub>	241,59±6,16	260,23±9,42	226,34±6,69 Θ	227,07±7,68	239,18±9,01	212,17±12,20
Th <sub>2</sub>	240,13±6,38	257,53±9,51	225,89±7,48 Θ	216,21±8,07*	220,18±11,22 Δ	166,42±12,13 <sup>Ψ</sup>
Th <sub>3</sub>	236,19±5,88	249,13±10,07	225,59±6,14	213,49±8,49*	216,79±12,09	185,43±9,89 <sup>Ω Ψ</sup>
Th <sub>4</sub>	229,68±6,55	243,69±11,05	218,22±7,09	205,23±6,32*	213,10±8,36 Δ	180,54±8,76 <sup>Ω Ψ</sup>
Th <sub>5</sub>	225,48±5,97	244,50±9,06	209,93±6,35 Θ	190,32±8,31*	204,20±9,04 Δ	173,15±8,59 <sup>Ω Ψ</sup>
Th <sub>6</sub>	218,28±6,63	232,69±9,78	206,48±8,40	195,79±6,79*	199,08±9,40	175,08±12,50 <sup>Ω</sup>
Th <sub>7</sub>	216,67±6,54	226,97±9,85	208,24±8,53	190,77±6,62*	199,34±10,61 Δ	170,74±12,88
Th <sub>8</sub>	220,48±7,06	235,64±9,39	208,07±9,68	187,60±7,31*	207,53±9,50 Δ	168,51±11,47 <sup>Ω Ψ</sup>
Th <sub>9</sub>	223,92±7,14	244,22±9,12	207,31±9,37 Θ	192,98±8,13*	209,74±9,04 Δ	165,08±13,09 <sup>Ω Ψ</sup>
Th <sub>10</sub>	222,74±5,85	241,47±7,79	207,41±7,09 Θ	194,23±8,58*	213,31±9,34 Δ	211,32±11,89
Th <sub>11</sub>	217,08±6,35	234,38±8,54	202,92±8,17	186,14±7,56*	200,48±8,78 Δ	209,43±12,16
Th <sub>12</sub>	209,52±5,94	227,50±8,27	194,81±7,15	182,54±7,89*	196,73±8,31 Δ	195,54±9,28
	AT DIAGNOSIS			AFTER CHEMOTHERAPY		
	Common group (n=30)*	Younger age group (n=14) <sup>Δ</sup>	Older age group (n=16) <sup>Ω Θ</sup>	Common group (n=21)*	Younger age group (n=10)	Older age group (n=11) <sup>Ω</sup>
L <sub>1</sub>	208,36±8,97	233,49±8,68	184,80±13,00 Θ	176,80±9,44*	184,96±13,07 Δ	169,38±13,74
L <sub>2</sub>	202,67±7,96	225,39±8,68	181,36±10,83 Θ	167,32±9,41*	181,42±13,75 Δ	154,51±12,20
L <sub>3</sub>	211,91±10,48	241,96±11,28	185,63±14,25 Θ	167,25±8,58*	183,48±13,55 Δ	152,49±9,22
L <sub>4</sub>	224,71±13,88	272,20±18,57	183,15±13,78 Θ	191,08±16,63*	214,42±29,85	167,74±12,50
L <sub>5</sub>	224,16±10,31	262,63±10,78	188,44±10,32 Θ	201,97±13,90	219,80±21,37	184,14±17,00

Note. Here and in the following tables : Δ; Ω; Ψ – statistically significant difference ( $0,001 < p < 0,05$ ) between groups according to the Brown-Forsythe Levene test (\* – between common group at diagnosis and after completion of chemotherapy; Δ – in younger age group at diagnosis and after completion of chemotherapy; Ω – in older age group at diagnosis and after completion of chemotherapy; Θ – younger and older age groups at at diagnosis; Ψ – between younger and older age group after completion of chemotherapy).

of chemotherapy. Comparative analysis of bone density changes revealed a significant decrease of BD in thoracic (up to 12.4 %) and lumbar (up to 17.2 %) vertebrae in common age group after completion of chemo compared to the same group at diagnosis, in older age group (up to 17.9 %) compared to the same age group after chemotherapy, in younger age group in thoracic (up to 13.5 %) and lumbar (up to 21.5 %) vertebrae compared to the same age group after chemo, in common group (up to 9.96 %) compared to the same group at the presentation of the disease, in older age group after chemo (up to 17.4 %) compared to younger age group, in older age group in thoracic (up to 13.9 %) and lumbar (up to 24.9 %) vertebrae after chemo compared to younger age group at diagnosis. The calculation of pair correlation by Pearson criterion indicated reliable inverse (negative) correlation between age and densitometric density in all investigated vertebral areas.

Among parameters reflecting trabecular structure and mineralization BS values were significantly higher in older group than in younger group after chemotherapy in almost all thoracic vertebrae as well as BV/TV index was higher in older group (Th2, Th5, L3) compared with younger age group values at diagnosis, in younger age group after chemo compared to the same age group at diagnosis (Th8, Th9), in common group after chemo compared to the same age group at diagnosis. BV values were significantly lower in the following groups: in older age group than in younger age group after completion of chemotherapy, in all lumbar vertebrae in common group after chemo compared to the same group at diagnostics, in younger age group after chemo compared to the same age group at diagnosis. FD values were significantly lower in the following groups: in older age group (Th1, Th8) compared to younger age group at diagnosis, in common group (Th7, L4, L5) after chemo compared to the same group at diagnosis, in younger age group after chemo (Th8, Th11, L2, L4).

SMI values were significantly higher in the following groups: in older age group compared to younger age

group at diagnosis, in older age group compared to the same age group at diagnosis, in common group compared to the same group at diagnosis and SMI was lower in younger age group than in the same age group after chemo. Texture parameters were also studied by using comparative analysis that showed significant decrease of angular second moment, Correlation, Inverse Difference moment values in older age group compared to young age group after completion of chemotherapy, Angular Second moment values were significantly lower after chemo in older age group compared to the same group at diagnosis, Contrast and Angular Second moment values were lower in younger age group after chemo than in the same group at diagnosis, Inverse difference moment values were significantly lower in older age group compared to younger group after chemo and in common group after it (L1, L5).

We also conducted a comparative analysis of densitometric values (listed in table 2) of thoracic and lumbar vertebral trabecular tissue in groups of HL patients who received ABVD chemotherapy regimen, which is the most common protocol for HL treatment, compared to initial values (HU) at diagnosis.

There were no statistically significant differences between BD values (listed in table 1) in common group of HL patients and the group of patients at diagnosis who would receive ABVD chemotherapy regimen. Analysis showed significant decrease (up to 13.8 % in thoracic and up to 19.9 % in lumbar vertebrae) of BD values in a group of HL patients who received 4-6 cycles of chemotherapy and the approximate total treatment duration and therefore the time of bone density loss is 4-6 months respectively.

Further analysis of structural parameters showed significantly higher values of BV (Th5-Th12) on a group of patients who received chemotherapy compared to patients at diagnosis, BV/TV index was significantly higher after chemo compared to a group of patients at diagnosis. SMI that indicates trabecular transformation was significantly higher in patients after chemotherapy

**Table 2. Densitometric values (HU) of thoracic and lumbar vertebral trabecular tissue in groups of HL patients who received ABVD chemotherapy regimen compared to initial values at diagnosis**

HU	AT Diagnosis	AFTER CHEMOTHERAPY
	ABVD (n=27)	ABVD (n=21)
Th <sub>1</sub>	236,56±6,54	228,54±9,64
Th <sub>2</sub>	238,10±7,94	215,09±10,58
Th <sub>3</sub>	240,20±7,44	216,96±10,55 *
Th <sub>4</sub>	232,50±8,25	206,19±8,22 *
Th <sub>5</sub>	220,91±9,59	184,24±10,52 *
Th <sub>6</sub>	226,53±7,93	199,89±8,61 *
Th <sub>7</sub>	213,90±8,62	188,64±8,08 *
Th <sub>8</sub>	217,51±8,72	188,22±9,42
Th <sub>9</sub>	220,69±9,94	188,59±9,79 *
Th <sub>10</sub>	221,42±7,62	188,02±10,22 *
Th <sub>11</sub>	220,07±7,55	184,14±9,28 *
Th <sub>12</sub>	209,78±7,51	179,37±10,46 *
	ABVD (n=16)	ABVD (n=15)
L <sub>1</sub>	204,54±11,99	172,72±12,26 *
L <sub>2</sub>	201,83±11,78	159,49±11,32 *
L <sub>3</sub>	208,73±15,08	160,43±10,20 *
L <sub>4</sub>	223,35±21,16	192,11±23,44
L <sub>5</sub>	215,99±12,79	194,51±18,28

Note. Here and in the following tables: \* – statistically significant difference between groups at diagnosis and after completion of chemotherapy.

compared to those patients at diagnosis. FD values were controversial (significantly lower in Th7 and higher in Th11, Th120 after chemotherapy. Analysis of texture parameters revealed that Angular Second moment values were significantly lower (Th7, Th8) after chemotherapy than in patients at diagnosis, however Entropy values tended to increase after chemotherapy in thoracic vertebrae. No statistically significant differences were found in lumbar vertebrae.

**CONCLUSIONS** The above mentioned findings suggest that age and standard chemotherapy regimen for HL treatment are important predisposing factors for VTB qualitative and quantitative changes of structural trabecular parameters and therefore may cause structural alteration. Significant percentage of bone density loss in both thoracic and lumbar segments of the vertebral column during 4–6 months of chemotherapy should be taken into account as a major risk factor in all age groups of HL patients for prior evaluation of VTBTS and timely prevention of these side effects.

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