ORIGINAL ARTICLE

Methodological diversity in micro-CT evaluation of bone micro-architecture: importance for inter-study comparability

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Summary

Introduction: Micro-computed tomography (micro-CT) is a standard 3D technique for non-destructive analysis of bone micro-architecture. Although there have been many micro-CT studies in contemporary literature, inter-study comparability is often challenging due to a lack of methodological standardization, particularly regarding human bone analyses.

Aim: This study aimed to assess the consistency of micro-CT generated micro-architectural parameters obtained by two researchers (inter-observer reliability), by one researcher in two attempts (intra-observer reliability), as well as between manual and semi-automatic determination of the region of interest (ROI).

Material and methods: Superolateral femoral neck samples (n=8) were scanned with Bruker 1172 micro-CT system with a voxel size of 10 μm. We manually determined cortical and trabecular ROI (two authors, two attempts with a 45-day span). Also, trabecular ROI was determined using a semi-automatic method (round-shaped ROI with 6.5 mm diameter).

Results: The intraclass correlation coefficient (ICC) showed a high degree of consistency in the measurement of micro-architectural parameters of the superolateral femoral neck using the micro-CT (ICC range: 0.721-0.998; $p<0.05$). However, a detailed analysis revealed significant inter-observer and intra-observer differences, predominantly reflected in cortical porosity parameters (Student's *t*-test for dependent samples, $p<0.05$). On the other hand, the choice of ROI did not significantly affect trabecular micro-architectural parameters among researchers and between manual and semi-automatic demarcation methods (Student's *t*-test for dependent samples, $p>0.05$).

Conclusion: Our study emphasizes the importance of standardizing the methodology used in micro-CT evaluations of human bone samples, which could facilitate reliable inter-study comparison and ensure an adequate interpretation of results.

Keywords: Micro-CT, femoral neck, region of interest, bone micro-architecture, human

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INTRODUCTION

Micro-computed tomography (micro-CT) is based on sequential X-ray scanning of limited-size samples, usually resulting in images of transversal sections that can detect many small details [1]. As such, micro-CT opened up new possibilities for advanced analysis of mineralized and non-mineralized tissues in a non-destructive manner [1,2]. To analyze bone tissue, researchers predominantly used histomorphometric analysis of trabecular bone samples obtained by transiliac biopsy, which still represents the "gold standard" for clinical evaluation of bone status [3]. However, the preparation and procession of bone samples for histomorphometry has some disadvantages (for example, two-dimensionality, dependence on researchers' experience, and tissue destruction that makes it impossible to evaluate bone tissue using different methods) [4]. For all thise reasons, micro-CT has been increasingly described in contemporary literature as the method of choice for animal and human bone analyses [5].

Initially, micro-CT was used to calculate the mineralized bone ratio in an examined sample [6]. After that, the consistency of trabecular micro-architectural results obtained by histomorphometry and micro-CT was shown [1,7]. Soon enough, the importance of using micro-CT for the analysis of cortical bone was noted (which is of great significance in overall bone fragility), confirming comparability of the results obtained with micro-CT and histomorphometry [5,8]. Being a promising method, micro-CT has been applied in numerous human [9,10] or animal bone studies [11–14]. Considering the abundance of previous animal micro-CT studies, there was a need to compare the obtained results. This raised concerns about the validity of conclusions derived from these comparisons, considering different sample processing methodologies used in these studies. Therefore, protocols for semi-automatic differentiation of animal cortical and trabecular compartments were developed (with some persistent shortcomings), enabling a more reliable inter-study comparison using uniform methodological approaches [15–17]. On the other hand, studies conducted on human bone samples show significantly less uniformity in the micro-CT methodology of trabecular and cortical micro-architecture analyses. This indicates the necessity for standardization of micro-CT methodology to achieve well-grounded conclusions derived from comparing numerous human studies, especially in femoral studies, since these fractures are most severe in aged individuals [18].

This study aimed to determine the reliability of micro-CT derived micro-architectural femoral parameters obtained by two researchers (inter-observer reliability), by one researcher in two attempts (intra-observer reliability) as well as between manual and semi-automatic region of interest (ROI) determination.

MATERIALS AND METHODS

Material sampling for microstructural analysis

It has been shown that the trabeculae of the superolateral femoral neck represent the fracture-initiating site [19], while its cortex suffers the most significant forces during a side fall [20], so the reproducibility of the analysis of this part of the skeleton is of great importance. For this reason, we analyzed eight proximal femora collected from institutional osteological collection. The criteria for including samples in the study were as follows: fully preserved dry proximal left femora of an adult individual, without visible changes (e.g., cortical surface erosion, tumor-like masses, osteolytic changes, etc.) that would suggest the existence of structural bone damage.

Given that the micro-CT method cannot scan the entire human femur, samples of the superolateral part of the femoral neck were extracted **(Figure 1)** using Oscillating Autopsy Saw (HB-740-Accu-250, Kugel, Germany). The samples were then cleaned in an ultrasonic bath (SONOCOOL 255, Bandelin, Germany) and air-dried for at least two weeks. Finally, the samples were scanned using micro-CT system (1172 SkyScan, Bruker, Belgium).

Scanning of samples using the micro-CT method

To scan the transcervical region of the superolateral femoral neck, the samples were placed in the scanning chamber using identical sample orientation (basicervical parts facing the holder, cortical surface set perpendicular to the imaging camera). Orthodontic wax was used to attach the samples to the holder and prevent sample movement. The samples were scanned in dry conditions, using the following parameters: 80 kV, 124 μA, 10W, exposure time 1220 μs, voxel size of 10 μm, and Aluminium-Copper filter [10]. After scanning, the reconstruction of the projection images was made using NRecon software (version 1.6.9.8, Bruker, Belgium) accelerated with InstaRecon CBR software (2.0.2.1 version, InstaRecon, Illinois, USA). The reconstruction parameters were as follows: beam hardening correction of 25%, ring artefact correction of 5, smoothing correction of 2, and autogenerated compensation for thermal drift and misalignment.

Using the updated version of the microstructural analysis software (CT.An 2020, Bruker, Belgium), all samples were standardized and marked so that the central 60% of the superolateral femoral neck's length (transcervical region of the neck) was analyzed **(Figure 1)**. The total length of the analyzed volume of interest (VOI) of trabecular and cortical bone was 1101 sections (central section ± 550). Using manually adjusted two-dimensional regions of interest (ROIs), two investigators independently marked trabecular and cortical VOIs. After 45 days, the two researchers repeated the manual ROI determination. Researchers consistently followed the rule that margin-

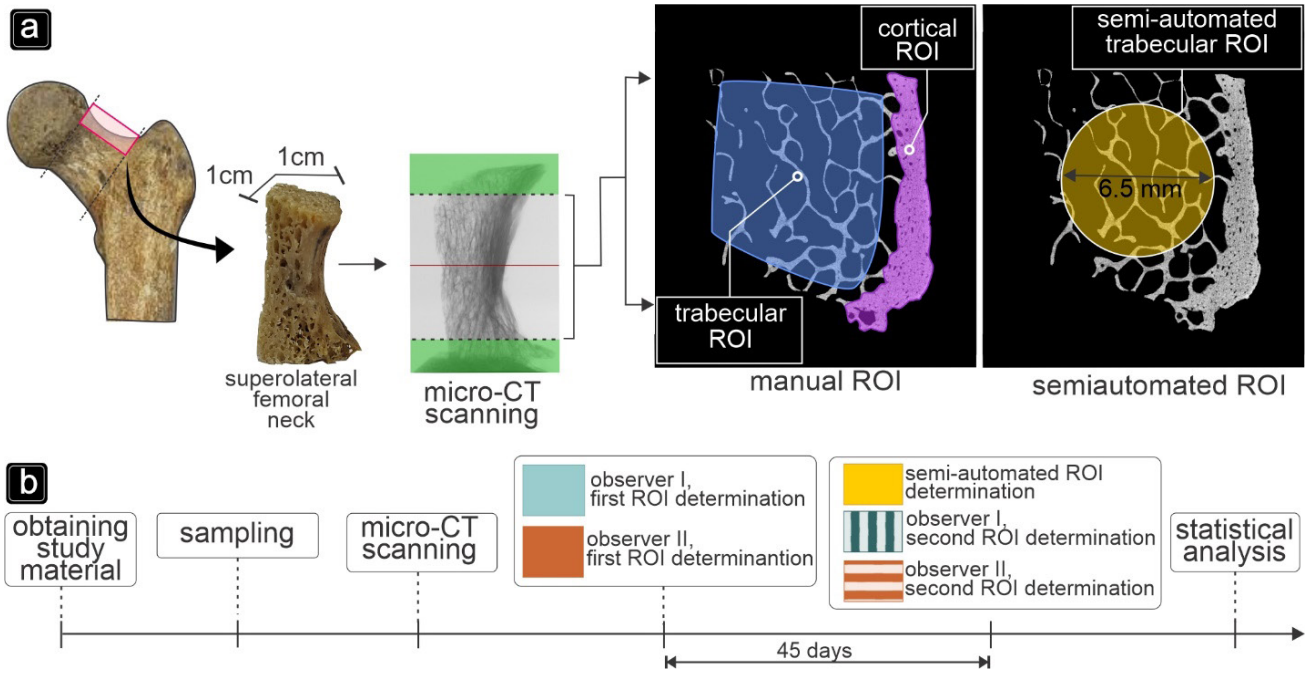

Figure 1. The schematic representation of the used methodology (a) and flow chart of the present study (b)
Abbreviations: Micro-CT – micro-computed tomography; observer I – early-career researcher; observer II – experienced researcher; ROI – region of interest.

alized damaged bone tissue was excluded from manually adjusted ROIs. For semi-automatic trabecular ROI determination, a centrally positioned round-shaped ROI was used (diameter: 6.5mm; **Figure 1**). Using CT.An 2020 software, the analysis of trabecular and cortical micro-architecture parameters was performed after segmenting the mineralized (greyscale value levels ranging from 95 to 255) and non-mineralized parts of the sample (greyscale value levels below 95), which was consistently used for all analyzed samples. The following parameters were generated and analyzed: cortical tissue volume (Ct.TV), cortical tissue surface (Ct.TS), cortical thickness (Ct.Th), total cortical porosity (Po.tot), closed cortical porosity (Po.cl), open cortical porosity (Po.op), total cortical porosity volume (Po.V.tot), closed cortical porosity volume (Po.V.cl), open cortical porosity volume (Po.V.op), cortical pore diameter (Po.Dm), cortical pore separation (Po.Sp), trabecular tissue surface (Tb.TS), trabecular tissue volume (Tb.TV), trabecular bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), connectivity density (Conn.Dn), degree of anisotropy (DA), fractal dimension (FD) and structural model index (SMI).

Statistical data analyses

The Kolmogorov-Smirnov test was used to assess the data distribution normality. The intraclass correlation coefficient (ICC) was used to evaluate the consistency of the results obtained by two researchers, as well as the results of semi-automatic and manual trabecular ROI determination [21]. Student *t*-test for dependent samples was used to evaluate the significance of the difference between researcher results (inter-observer differences), the difference of the results obtained during two ROI determinations by the same researcher (intra-observer differences), and the difference of results obtained through manual and semi-automatic ROI determination. Open-source statistical software (Easy R on R Commander, EZR) was used for statistical analyses with a significance level of 5% and a confidence interval of 95%.

RESULTS

Intra-observer differences in ROI determination

By comparing two manual ROIs marked in 45-day span, observer I (early-career researcher) marked a larger ROI during the second attempt, which was manifested by a larger cortical volume (Ct.TV, p=0.047; **Table 1**). As presented in Table 1, this ROI choice resulted in a statistically significantly higher Po.tot, Po.V.tot, Po.op, Po.V.op, Po.cl and Po.V.cl values (p=0.005, p=0.017, p =0.005, p=0.017, p=0.017, p=0.051, respectively). In contrast, the results of the second ROI determination by observer II (experienced researcher) only showed a trend towards the selection of a smaller volume of the cortex (Ct.TV, p=0.047), which resulted in a marginal decrease in the cortical pore separation (Po.Sp, p=0.041) **(Table 1)**. Unlike cortical bone, trabecular micro-architecture did not show a statistically significant difference between the two measurements by both observers (p>0.05, **Table 1**).

Table 1. Intraobserver differences in trabecular and cortical micro-architectural parameters obtained by micro-CT

Parameters	Observer I 1st attempt (mean± SD)	Observer I 2nd attempt (mean± SD)	(*p*)	Observer II 1st attempt (mean± SD)	Observer II 2nd attempt (mean ± SD)	(*p*)
Ct.TS (mm^2)	339.73 ± 61.24	329.34 ± 52.99	0.401	355.94 ± 47.17	365.23 ± 51.42	**0.050**
Ct.TV (mm^3)	95.86 ± 32.46	109.13 ± 24.33	**0.047**	107.42 ± 27.68	106.93 ± 27.28	**0.047**
Ct.Th (mm)	0.29 ± 0.12	0.29 ± 0.13	0.158	0.30 ± 0.14	0.30 ± 0.14	0.302
Po.Dm (mm)	0.35 ± 0.13	0.39 ± 0.15	0.067	0.19 ± 0.06	0.20 ± 0.06	0.466
Po.Sp (mm)	0.27 ±0.08	0.27 ± 0.07	0.238	0.27 ± 0.06	0.28 ± 0.08	**0.041**
Po.tot (%)	21.95 ± 11.60	26.23 ± 11.62	**0.005**	18.99 ± 10.43	18.78 ± 10.36	0.450
Po.cl (%)	0.34 ± 0.23	0.36 ± 0.24	**0.017**	0.37 ± 0.24	0.37 ± 0.24	0.525
Po.op (%)	21.67 ± 11.67	25.96 ± 11.68	**0.005**	18.69 ± 10.48	18.49 ± 10.41	0.451
Po.V.tot (mm^3)	19.11 ± 7.99	27.03 ± 9.26	**0.017**	18.44 ± 6.99	18.11 ± 6.85	0.209
Po.V.cl (mm^3)	0.26 ± 0.18	0.30 ± 0.22	0.051	0.33 ± 0.23	0.33 ± 0.23	0.251
Po.V.op (mm^3)	18.85 ± 8.08	26.72 ± 9.32	**0.017**	18.11 ± 7.08	17.78 ± 6.94	0.212
Tb.TS (mm^2)	465.17 ± 97.08	461.15 ± 76.45	0.712	533.81 ± 93.49	540.15 ± 96.44	0.411
Tb.TV (mm^3)	500.18 ± 170.23	505.27 ± 162.67	0.758	630.14 ± 168.19	633.00 ± 182.12	0.878
BV/TV (%)	22.88 ± 7.29	22.72 ± 7.40	0.431	22.38 ± 7.25	22.37 ± 7.14	0.964
Tb.N (1/mm)	1.28 ± 0.41	1.26 ± 0.37	0.475	1.26 ± 0.39	1.27 ± 0.40	0.418
Tb.Th (mm)	0.19 ± 0.07	0.18 ± 0.07	0.371	0.18 ± 0.06	0.18 ± 0.06	0.205
Tb.Sp (mm)	0.88 ± 0.18	0.88 ± 0.16	0.993	0.88 ± 0.17	0.87 ± 0.16	0.150
Conn.Dn (1/mm)	22.051 ± 31.50	21.56 ± 28.98	0.698	21.64 ± 29.81	22.64 ± 32.38	0.347
DA	2.13 ± 0.36	2.13 ± 0.39	0.872	2.11 ± 0.37	2.11 ± 0.38	0.934
FD	2.42 ± 0.07	2.42 ± 0.06	0.912	2.44 ± 0.07	2.44 ± 0.06	0.865
SMI	-1.00 ± 2.58	-0.84 ± 2.24	0.292	-0.88 ± 2.38	-0.79 ± 2.23	0.323

Student's t-test for two dependent samples was used to assess microstructural differences between the two attempts in the region of interest determination in two independent investigators (the significant difference is presented in bold).
Abbreviations: SD – Standard deviation; observer I – early-career researcher; observer II – experienced researcher; Ct.TS – Cortical tissue surface; Ct.TV – Cortical tissue volume; Ct.Th – Cortical thickness; Po.Dm – Cortical pore diameter; Po.Sp – Cortical pore separation; Po.tot – Total cortical porosity; Po.cl – Closed cortical porosity; Po.op – Open cortical porosity; Po.V.tot – Total volume of cortical porosity; Po.V.cl – Volume of closed cortical porosity; Po.V.op – Volume of open cortical porosity; Tb.TS – Trabecular tissue surface; Tb.TV – Trabecular tissue volume; BV/TV – Trabecular bone volume fraction; Tb.N – Trabecular number; Tb.Th – Trabecular thickness; Tb.Sp – Trabecular separation; Conn.Dn – Connectivity density; DA – Degree of anisotropy; FD – Fractal dimension; SMI – Structural model index.

Inter-observer differences in ROI determination

Although ICC showed a high degree of consistency of data on cortical and trabecular micro-architecture of the femoral neck between two researchers (ICC range: 0.721-0.998; p<0.05), suggesting a high degree of reliability in measuring micro-architectural parameters by micro-CT, a significant inter-observer inconsistency was shown in the values of the diameter of the cortical pores (ICC value: 0.026; p=0.565). To determine the cause of these inter-observer inconsistencies in the microstructural parameter values, the Student's *t*-test was used to compare the second ROI determination of the younger researcher and the experienced researcher. It was shown

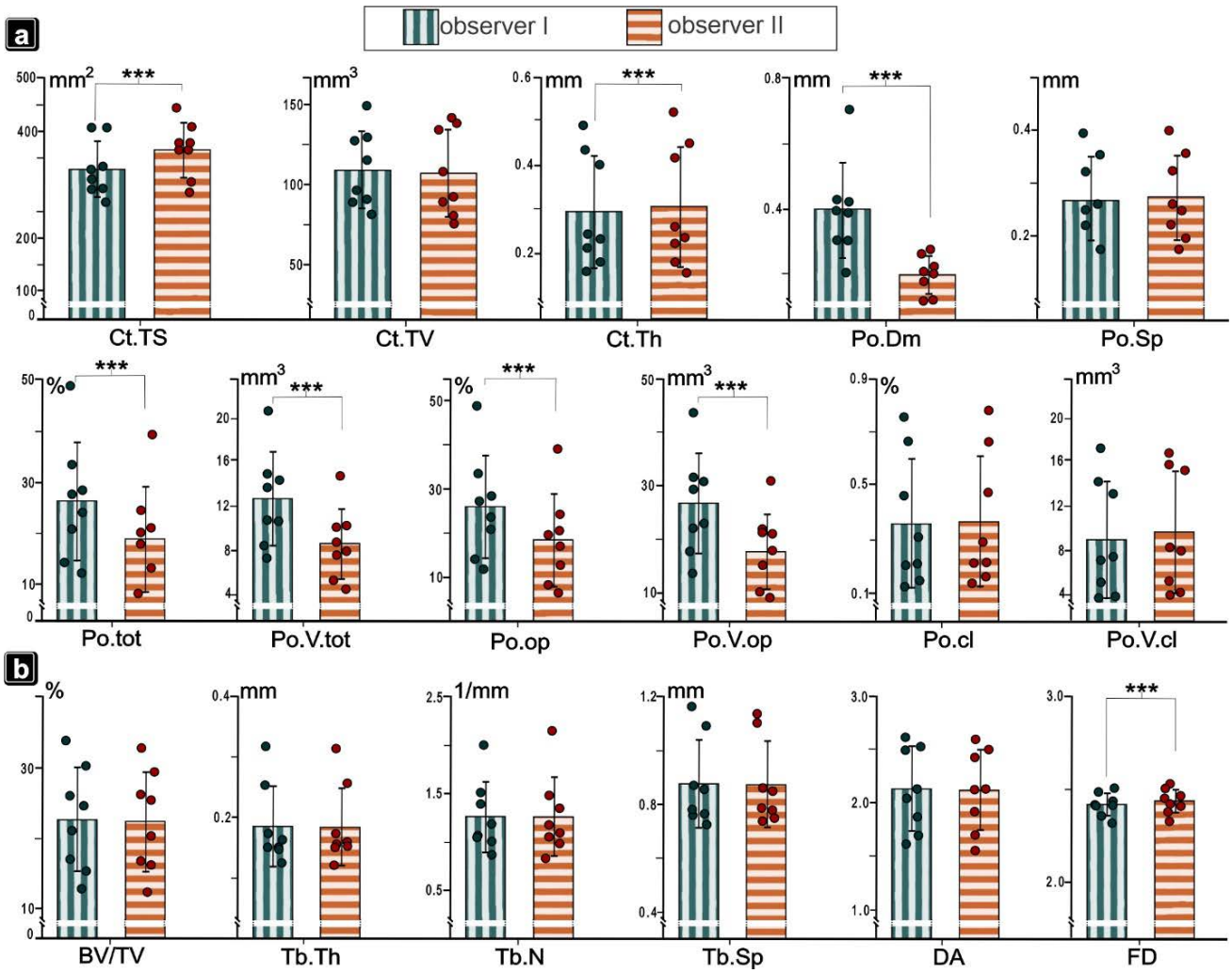

Figure 2. The inter-observer reliability for micro-CT assessment of femoral cortical and trabecular bone

Student's t-test for two dependent samples was used to assess the difference in microstructural parameters obtained by two independent investigators (*** p<0.05). Bar graphs represent the data as mean ± standard deviation, including the individual data points.

Abbreviations: Observer I – early-career researcher; Observer II – experienced researcher; Ct.TS – Cortical tissue surface; Ct.TV – Cortical tissue volume; Ct.Th – Cortical thickness; Po.Dm – Cortical pore diameter; Po.Sp – Cortical pore separation; Po.tot – Total cortical porosity; Po.V.tot – Total volume of cortical porosity; Po.op – Open cortical porosity; Po.V.op – Volume of open cortical porosity; Po.cl – Closed cortical porosity; Po.V.cl – Volume of closed cortical porosity; BV/TV – Trabecular bone volume fraction; Tb.Th – Trabecular thickness; Tb.N – Trabecular number; Tb.Sp – Trabecular separation; DA – Degree of anisotropy; FD – Fractal dimension;

that the early-career researcher chose a smaller Ct.TS (p=0.005), which resulted in a significantly thinner cortex (p=0.021) that was substantially more porous (Po.tot, p<0.001; Po.V.tot p=0.001; **Figure 2**). Increased Po.tot when marking was done by the early-career researcher was conditioned by increased Po.op and Po.V.op (p<0.001, p=0.001, respectively). Pore diameter was significantly greater (p=0.011), while cortical Po.Sp was lower in the cortex marked by the observer I (p=0.005, **Figure 2**). On the other hand, the experienced researcher analyzed a statistically significantly larger Tb.TS and Tb.TV (p<0.001, p<0.001, respectively) and obtained higher FD values (p=0.013, **Figure 2**).

Differences between manual and semi-automatic ROI determination

The ICC analysis showed a high degree of consistency of femoral neck trabecular micro-architecture data between manual and semi-automatic ROI determination (ICC range: 0.658-0.994; p<0.05), suggesting that the micro-CT method is highly reliable in evaluation of trabecular micro-architecture in superolateral femoral neck. However, it was shown that there was also a significant inconsistency in the DA values (ICC value: 0.165; p=0.739).

The possibility of selecting an irregularly shaped ROI that includes a larger part of the trabecular zone is reflected by a significantly larger Tb.TS and Tb.TV obtained by the manual method (p=0.001, p=0.003 respectively), and the selected trabeculae demonstrated higher degree of anisotropy (p=0.002, **Figure 3**).

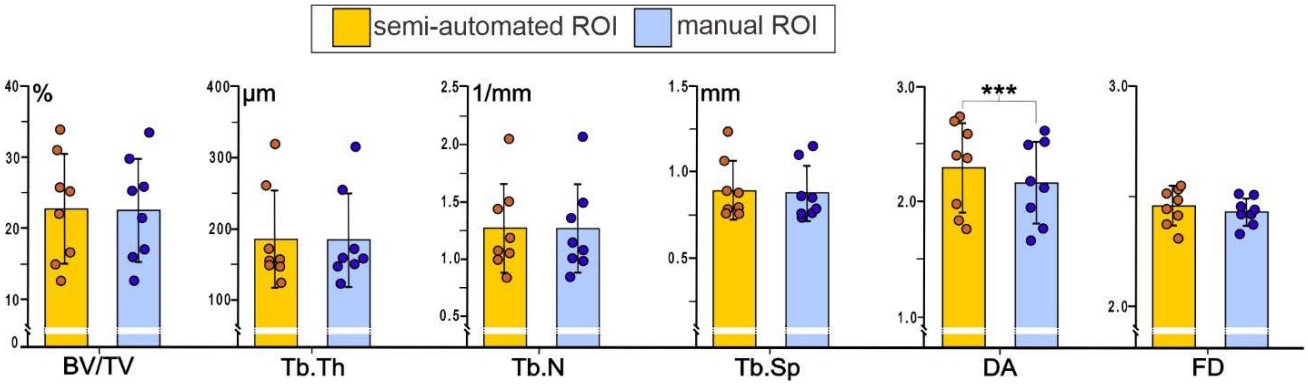

Figure 3. Differences in trabecular micro-architecture parameters due to manual and semi-automatic ROI demarcation methods.
Student's t-test for two dependent samples was used to assess the difference in microstructural parameters between manual and semi-automatic demarcation methods (*** $p<0.05$). Bar graphs represent the data as mean ± standard deviation, including the individual data points.
Abbreviations: BV/TV – Trabecular bone volume/total volume; Tb.Th – Trabecular thickness; Tb.N – Trabecular number; Tb.Sp – Trabecular separation; DA – Degree of anisotropy; FD – Fractal dimension

DISCUSSION

To analyze the bone sample with micro-CT, it is necessary to select an adequate VOI, which illustrates the importance of adequately standardized segmentation of different tissue compartments [22]. Analyzing various human and animal skeletal sites, numerous techniques for selecting bone tissue of interest have been applied (semi-automatic segmentation by determining the threshold of mineralized tissue [5,23], circular or rectangular ROIs of constant area and manual demarcation of the cortex and trabeculae [22]). Although the micro-CT methodology has been increasingly present in the human bone research practice, clear guidelines for manual ROI determination have not been agreed upon yet [22–24]. There are numerous dilemmas about manual ROI determination in the bone research field, which compromises the validity of the conclusion derived from inter-study comparison. It may arise due to inter- and intra-group, as well as inter- and intra-observer differences in research practice. It is important to emphasize that previous studies on human bone tissue used either a unique ROI selection methodology (methodology mentioned once in the work of one research group) or a methodology that was not fully described to enable exact reproducibility [23,24]. In addition, the methodology of including the transitional cortico-trabecular zone is rarely described (given that it is susceptible to subjectivity), which can have a significant impact on the data interpretation and interstudy comparison [1,10]. On the other hand, due to often conflicting results and a complex understanding of the effects of various conditions on animal models [25–31], guidelines for standardized micro-CT analyses of rodent bone tissue were recommended [32].

To our knowledge, this is the first study that investigated the inter-observer and intra-observer consistency of micro-CT derived trabecular and cortical micro-architectural parameters in the human superolateral femoral neck. In addition, the variability of the results obtained by manual and semi-automatic demarcation methods was investigated. Our study demonstrated a high degree of consistency in the results of trabecular micro-architectural parameters, except for a statistically significant difference in ROI size. A potential explanation may lie in the distance of the demarcation edge from the tissue contaminated with fragments created during sampling **(Figure 4, Figure 5)**. Given that manual marking is time-consuming and susceptible to subjective evaluation, there have been attempts to develop a protocol for a semi-automatic method of segmenting cortical from trabecular bone [11,16,17]. The protocols were based on different greyscale thresholds, and they had shortcomings that were primarily based on the conditions of sample size and sample surroundings, which is the rationale for the absence of consensus regarding methodology standards. Nevertheless, an unambiguous description of semi-automatic demarcation methodology in future studies may contribute to more reliable inter-study comparisons [11,16,17].

Although the effect of choosing the cortical ROI has not been investigated, the deterioration of cortical micro-architecture conditioned by various factors can be of great importance in the occurrence of increased bone fragility [33,34]. It is important to emphasize that cortical micro-architecture was more susceptible to inter-observer and intra-observer variability in our study **(Figure 4)**. One of the primary causes of this finding may arise from different approaches in ROI inclusion of transitional cortico-trabecular zone **(Figure 5)**. If the transitional cortico-trabecular zone (in some studies reported as "cortical trabecularization") is considered part of the cortical compartment, there is a possibility of overestimating cortical porosity [35]. An additional cause of cortical variability may be transcortical vascular pores **(Figure 5)** [36,37]. Although bone vascularization is increasingly analyzed in animal and human bone [36–38], it is not clear whether and to what extent vascular pores can influence bone fragility. This suggests that a clear explanation of the manual demarcation protocol in cortical tissue (with particular reference to the transitional corti-

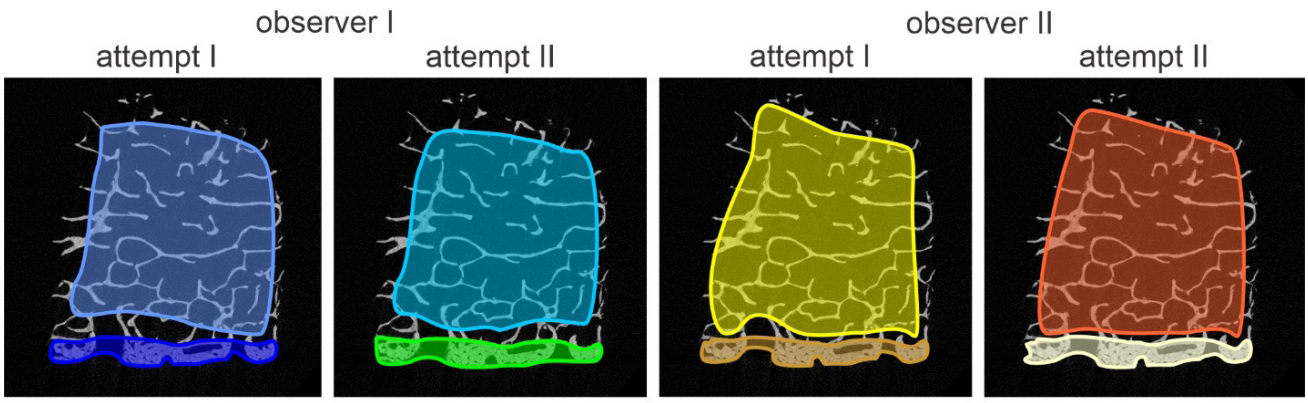

Figure 4. Graphic representation of diversity of manual ROI demarcation methods used in our study.

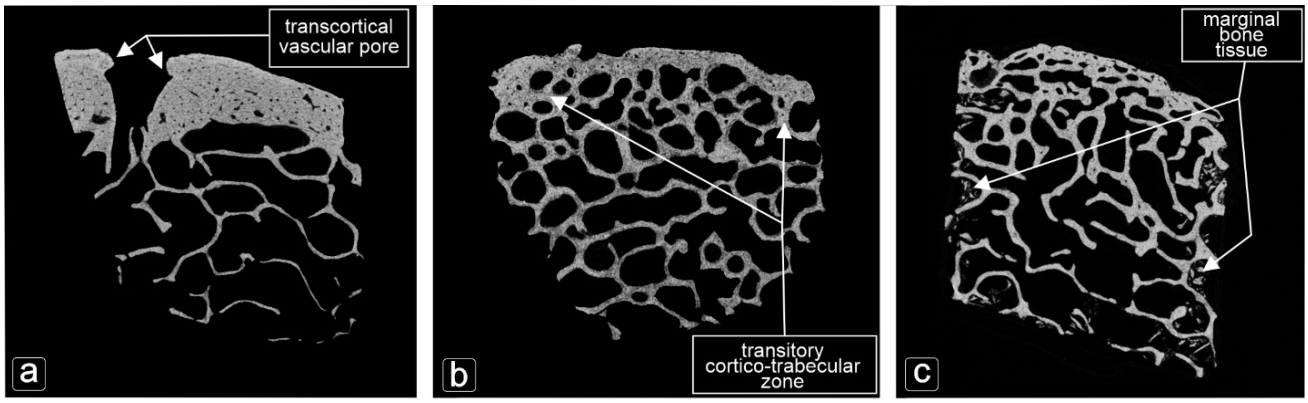

Figure 5. Representative findings that can significantly influence the values of bone micro-architectural parameters obtained by micro-CT analysis.

Transcortical vascular pores (a), transitional cortico-trabecular zone (b), and marginally damaged trabecular tissue (c) should be taken into account and clearly reported in the manuscript methodology section to ensure proper data interpretation and reproducibility.

co-trabecular zone and transcortical vascular pores) could contribute to greater reliability of the interpretation of the findings of different studies.

It is necessary to mention our study limitations. Although a statistically significant difference in specific parameters of bone micro-architecture was shown, and the number of samples is comparable to previous research on human material [39], the interpretation of our results could be complemented by including a larger number of bone samples derived from various skeletal sites. The correctness of mineralized and non-mineralized tissue differentiation was done using fixed greyscale intensity values by comparing to the original scanning image, which may result in the subjective estimation of bone fraction, especially when analyzing limited-volume samples with a small amount of bone tissue (such as in postmenopausal osteoporosis [40]). Lastly, our data could be subject to bias because included investigators (both from medical backgrounds) belong to the same research group and had similar training for micro-CT application in bone research.

CONCLUSION

Our study demonstrated that trabecular micro-architectural parameters were not significantly sensitive to various approaches in ROI demarcation methodology, given that a high degree of consistency was noted between two observers, between two attempts of the same observer and between manual and semi-automatic ROI-determination methods. In contrast, ROI determination had a significant effect on cortical microstructural parameters. As a result of the non-standardized ROI determination methodology, inter-observer and intra-observer differences were mainly reflected in cortical porosity parameters. Thus, our data highlight a need to standardize the methodology used in micro-CT evaluations of human bone samples in order to facilitate reliable inter-study comparison and ensure adequate result interpretation.

Ethical approval and patient consent: All procedures performed in this study were under the institutional ethics committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Author contribution statement: Conceptualization: JJ; Data acquisition: UA and JJ; Data interpretation: all authors; Data visualization: JJ and UA; Writing – original draft: UA; Writing – Review and Editing: JJ and MD; Project administration and funding: MD; Approval of the submitted manuscript version: all authors.

References

1. L.A. Feldkamp, S.A. Goldstein, M.A. Parfitt, G. Jesion, M. Kleerekoper, The direct examination of three-dimensional bone architecture in vitro by computed tomography, J. Bone Miner. Res. 4 (1989) 3–11. https://doi.org/10.1002/jbmr.5650040103.
2. P. Heimel, N.V. Swiadek, P. Slezak, M. Kerbl, C. Schneider, S. Nürnberger, H. Redl, A.H. Teuschl, D. Hercher, Iodine-Enhanced Micro-CT Imaging of Soft Tissue on the Example of Peripheral Nerve Regeneration, Contrast Media Mol. Imaging. 2019 (2019) 1–15. https://doi.org/10.1155/2019/7483745.
3. P. Chavassieux, R. Chapurlat, Interest of Bone Histomorphometry in Bone Pathophysiology Investigation: Foundation, Present, and Future, Front. Endocrinol. (Lausanne). 13 (2022) 1–20. https://doi.org/10.3389/fendo.2022.907914.
4. T. Mawatari, H. Miura, H. Higaki, K. Kurata, T. Moro-Oka, T. Murakami, Y. Iwamoto, Quantitative analysis of three-dimensional complexity and connectivity changes in trabecular microarchitecture in relation to aging, menopause, and inflammation, J. Orthop. Sci. 4 (1999) 431–438. https://doi.org/10.1007/s007760050126.
5. N.J. Wachter, P. Augat, G.D. Krischak, M. Mentzel, L. Kinzl, L. Claes, Prediction of Cortical Bone Porosity in Vitro by Microcomputed Tomography, Calcif. Tissue Int. 68 (2001) 38–42. https://doi.org/10.1007/BF02685001.
6. F.S.L. Wong, J.C. Elliott, P. Anderson, G.R. Davis, Mineral concentration gradients in rat femoral diaphyses measured by X-ray microtomography, Calcif. Tissue Int. 56 (1995) 62–70. https://doi.org/10.1007/BF00298746.
7. T. Uchiyama, T. Tanizawa, H. Muramatsu, N. Endo, H.E. Takahashi, T. Hara, A morphometric comparison of trabecular structure of human ilium between microcomputed tomography and conventional histomorphometry, Calcif. Tissue Int. 61 (1997) 493–498. https://doi.org/10.1007/s002239900373.
8. H. Fonseca, D. Moreira-Gonçalves, M. Vaz, M.H. Fernandes, R. Ferreira, F. Amado, M.P. Mota, J.A. Duarte, Changes in proximal femur bone properties following ovariectomy and their association with resistance to fracture, J. Bone Miner. Metab. 30 (2012) 281–292. https://doi.org/10.1007/s00774-011-0308-2.
9. T. Oláh, X. Cai, L. Gao, F. Walter, D. Pape, M. Cucchiarini, H. Madry, Quantifying the Human Subchondral Trabecular Bone Microstructure in Osteoarthritis with Clinical CT, Adv. Sci. 9 (2022) 1–14. https://doi.org/10.1002/advs.202201692.
10. J. Jadzic, P. Milovanovic, D. Cvetkovic, M. Ivovic, N. Tomanovic, M. Bracanovic, V. Zivkovic, S. Nikolic, M. Djuric, D. Djonic, Mechano-structural alteration in proximal femora of individuals with alcoholic liver disease: Implications for increased bone fragility, Bone. 150 (2021) 116020. https://doi.org/10.1016/j.bone.2021.116020.
11. J.S. Sankaran, M. Varshney, S. Judex, Differences in bone structure and unloading-induced bone loss between C57BL/6N and C57BL/6J mice, Mamm. Genome. 28 (2017) 476–486. https://doi.org/10.1007/s00335-017-9717-4.
12. K. Kerschan-Schindl, M. Papageorgiou, U. Föger-Samwald, M. Butylina, M. Weber, P. Pietschmann, Assessment of Bone Microstructure by Micro CT in C57BL/6J Mice for Sex-Specific Differentiation, Int. J. Mol. Sci. 23 (2022). https://doi.org/10.3390/ijms232314585.
13. K. Wada, M. Kawano, Y. Hemmi, R. Suzuki, K. Kunoki, H. Sakagami, H. Kawazu, S. Yokose, Effect of Low-intensity Pulsed Ultrasound on Healing of Bone Defects in Rat Tibia as Measured by Reconstructed Three-dimensional Analysis of Micro CT Images, In Vivo (Brooklyn). 36 (2022) 643–648. https://doi.org/10.21873/INVIVO.12748.
14. J. Shim, C. Iwaya, C.G. Ambrose, A. Suzuki, J. Iwata, Micro-computed tomography assessment of bone structure in aging mice, Sci. Rep. 12 (2022) 1–16. https://doi.org/10.1038/s41598-022-11965-4.
15. E.C. Herbst, A.A. Felder, L.A.E. Evans, S. Ajami, B. Javaheri, A.A. Pitsillides, A new straightforward method for semi-automated segmentation of trabecular bone from cortical bone in diverse and challenging morphologies, R. Soc. Open Sci. 8 (2021) 210408. https://doi.org/10.1098/rsos.210408.
16. P. Saparin, J.S. Thomsen, J. Kurths, G. Beller, W. Gowin, Segmentation of bone CT images and assessment of bone structure using measures of complexity, Med. Phys. 33 (2006) 3857–3873. https://doi.org/10.1118/1.2336501.
17. S. Lublinsky, E. Ozcivici, S. Judex, An automated algorithm to detect the trabecular-cortical bone interface in micro-computed tomographic images, Calcif. Tissue Int. 81 (2007) 285–293. https://doi.org/10.1007/s00223-007-9063-8.
18. T.M. Lawrence, C.T. White, R. Wenn, C.G. Moran, The current hospital costs of treating hip fractures, Injury. 36 (2005) 88–91. https://doi.org/10.1016/j.injury.2004.06.015.
19. P.M. de Bakker, S.L. Manske, V. Ebacher, T.R. Oxland, P.A. Cripton, P. Guy, During sideways falls proximal femur fractures initiate in the superolateral cortex: Evidence from high-speed video of simulated fractures, J. Biomech. 42 (2009) 1917–1925. https://doi.org/10.1016/j.jbiomech.2009.05.001.
20. E. Verhulp, B. van Rietbergen, R. Huiskes, Load distribution in the healthy and osteoporotic human proximal femur during a fall to the side, Bone. 42 (2008) 30–35. https://doi.org/10.1016/j.bone.2007.08.039.
21. D. Liljequist, B. Elfving, K.S. Roaldsen, Intraclass correlation – A discussion and demonstration of basic features, PLoS One. 14 (2019) e0219854. https://doi.org/10.1371/journal.pone.0219854.
22. J. Jadzic, D. Cvetkovic, P. Milovanovic, N. Tomanovic, V. Zivkovic, S. Nikolic, M. Djuric, D. Djonic, The micro-structural analysis of lumbar vertebrae in alcoholic liver cirrhosis, Osteoporos. Int. 31 (2020) 2209–2217. https://doi.org/10.1007/s00198-020-05509-7.
23. V. Bousson, F. Peyrin, C. Bergot, M. Hausard, A. Sautet, J.D. Laredo, Cortical bone in the human femoral neck: Three-dimensional appearance and porosity using synchrotron radiation, J. Bone Miner. Res. 19 (2004) 794–801. https://doi.org/10.1359/JBMR.040124.
24. S.D. Stout, B.S. Brunsden, C.F. Hildebolt, P.K. Commean, K.E. Smith, N.C. Tappen, Computer-assisted 3D reconstruction of serial sections of cortical bone to determine the 3D structure of osteons, Calcif. Tissue Int. 65 (1999) 280–284. https://doi.org/10.1007/s002239900699.
25. J.H. Waarsing, J.S. Day, J.A.N. Verhaar, A.G.H. Ederveen, H. Weinans, Bone loss dynamics result in trabecular alignment in aging and ovariectomized rats, J. Orthop. Res. 24 (2006) 926–935. https://doi.org/10.1002/jor.20063.
26. S.K. Boyd, P. Davison, R. Müller, J.A. Gasser, Monitoring individual morphological changes over time in ovariectomized rats by in vivo micro-computed tomography, Bone. 39 (2006) 854–862. https://doi.org/10.1016/j.bone.2006.04.017.
27. J.H. Waarsing, J.S. Day, J.C. Van Der Linden, A.G. Ederveen, C. Spanjers, N. De Clerck, A. Sasov, J.A.N. Verhaar, H. Weinans, Detecting and tracking local changes in the tibiae of individual rats: A novel method to analyse longitudinal in vivo micro-CT data, Bone. 34 (2004) 163–169. https://doi.org/10.1016/j.bone.2003.08.012.
28. A. Laib, J.L. Kumer, S. Majumdar, N.E. Lane, The temporal changes of trabecular architecture in ovariectomized rats assessed by microCT, Osteoporos. Int. 12 (2001) 936–941. https://doi.org/10.1007/s001980170022.
29. G.M. Campbell, H.R. Buie, S.K. Boyd, Signs of irreversible architectural changes occur early in the development of experimental osteoporosis as assessed by in vivo micro-CT, Osteoporos. Int. 19 (2008) 1409–1419. https://doi.org/10.1007/s00198-008-0581-7.
30. J.I. Francisco, Y. Yu, R.A. Oliver, W.R. Walsh, Relationship between age, skeletal site, and time post-ovariectomy on bone mineral and trabecular microarchitecture in rats, J. Orthop. Res. 29 (2011) 189–196. https://doi.org/10.1002/jor.21217.
31. P.Y. Hsu, M.T. Tsai, S.P. Wang, Y.J. Chen, J. Wu, J.T. Hsu, Cortical bone morphological and trabecular bone microarchitectural changes in the mandible and femoral neck of ovariectomized rats, PLoS One. 11 (2016) e0154367. https://doi.org/10.1371/journal.pone.0154367.
32. M.L. Bouxsein, S.K. Boyd, B.A. Christiansen, R.E. Guldberg, K.J. Jepsen, R. Müller, Guidelines for assessment of bone microstructure in rodents using micro-computed tomography, J. Bone Miner. Res. 25 (2010) 1468–1486. https://doi.org/10.1002/jbmr.141.
33. E. Seeman, T.J. Martin, Antiresorptive and anabolic agents in the prevention and reversal of bone fragility, Nat. Rev. Rheumatol. 15 (2019) 225–236. https://doi.org/10.1038/s41584-019-0172-3.

34. E.J. Samelson, K.E. Broe, H. Xu, L. Yang, S. Boyd, E. Biver, P. Szulc, J. Adachi, S. Amin, E. Atkinson, C. Berger, L. Burt, R. Chapurlat, T. Chevalley, S. Ferrari, D. Goltzman, D.A. Hanley, M.T. Hannan, S. Khosla, C.-T. Liu, M. Lorentzon, D. Mellstrom, B. Merle, M. Nethander, R. Rizzoli, E. Sornay-Rendu, B. Van Rietbergen, D. Sundh, A.K.O. Wong, C. Ohlsson, S. Demissie, D.P. Kiel, M.L. Bouxsein, Cortical and trabecular bone microarchitecture as an independent predictor of incident fracture risk in older women and men in the Bone Microarchitecture International Consortium (BoMIC): a prospective study, Lancet Diabetes Endocrinol. 7 (2019) 34–43. https://doi.org/10.1016/S2213-8587(18)30308-5.

35. P. Salmon, Editorial: Putting the "Why" back into bone "Archytecture," 2016. https://doi.org/10.3389/fendo.2016.00014.

36. V. Gatti, M.J. Gelbs, R.B. Guerra, M.B. Gerber, S.P. Fritton, Interstitial fluid velocity is decreased around cortical bone vascular pores and depends on osteocyte position in a rat model of disuse osteoporosis, Biomech. Model. Mechanobiol. 20 (2021) 1135–1146. https://doi.org/10.1007/s10237-021-01438-4.

37. L.L. Loundagin, A.J. Pohl, W.B. Edwards, Stressed volume estimated by finite element analysis predicts the fatigue life of human cortical bone: The role of vascular canals as stress concentrators, Bone. 143 (2021) 115647. https://doi.org/10.1016/j.bone.2020.115647.

38. P. Uniyal, P. Sihota, K. Tikoo, N. Kumar, Anatomical variation in intracortical canal network microarchitecture and its influence on bone fracture risk, J. Mech. Behav. Biomed. Mater. 123 (2021) 104770. https://doi.org/10.1016/j.jmbbm.2021.104770.

39. J. Van Dessel, Y. Huang, M. Depypere, I. Rubira-Bullen, F. Maes, R. Jacobs, A comparative evaluation of cone beam CT and micro-CT on trabecular bone structures in the human mandible, Dentomaxillofacial Radiol. 42 (2013) 20130145. https://doi.org/10.1259/dmfr.20130145.

40. S. Tassani, V. Korfiatis, G.K. Matsopoulos, Influence of segmentation on micro-CT images of trabecular bone, J. Microsc. 256 (2014) 75–81. https://doi.org/10.1111/jmi.12159.

RAZLIČITI METODOLOŠKI PRISTUPI PROCENI MIKROARHITEKTURE KOSTI POMOĆU MIKRO-KOMPJUTERIZOVANE TOMOGRAFIJE: ZNAČAJ ZA POREĐENJE REZULTATA RAZLIČITIH STUDIJA

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Sažetak

Uvod: Mikro-kompjuterizovana tomografija (mikro-CT) je često korišćen 3D metod za nedestruktivnu analizu mikro-arhitekture koštanog tkiva. Iako su u savremenoj literaturi dostupne brojne mikro-CT studije, poređenje nalaza ovih studija je otežano izostankom standardizacije metodologije, posebno tokom analize humanog materijala.

Cilj: Ova studija je imala za cilj da utvrdi konzistentnost mikro-arhitekturnih parametara dobijenih mikro-CT analizom, između dva istraživača (međuposmatračka pouzdanost), jednog istraživača u dva pokušaja (unutarposmatračka pouzdanost), kao i između manuelnog i poluautomatskog metoda određivanja regiona od interesa (ROI).

Materijal i metode: Uzorci superolateralnog dela vrata humanih butnih kostiju (n=8) skenirani su Bruker 1172 mikro-CT sistemom koristeći veličinu voksela od 10 µm. Određivanje ROI-a kortikalne i trabekularne kosti je vršeno manuelno (dva istraživača, u dva vremena sa razmakom od 45 dana). Takođe, određivanje ROI-a trabekularne kosti vršeno je i poluautomatski pomoću ROI-a okruglog oblika (prečnik 6,5 mm).

Rezultati: Intraklasni koeficijent korelacije (*intraclass correlation coefficient* - ICC) pokazao je visok stepen konzistentnosti mikro-CT metodologije u merenju mikro-arhitekturnih parametara superolateralnog dela vrata femura (opseg ICC vrednosti: 0,721-0,998; p<0,05). Međutim, detaljnija analiza je pokazala značajne razlike među istraživačima, kao i između načina obeležavanja jednog istraživača, koje se pretežno ogledaju u parametrima kortikalne poroznosti (Studentov t-test za zavisne uzorke, p<0,05). S druge strane, izbor ROI-a nije značajno uticao na parametre trabekularne mikro-arhitekture, kako među istraživačima, tako i između manuelnog i poluautomatskog metoda obeležavanja (Studentov t-test za zavisne uzorke, p>0,05).

Zaključak: Rezultati ove studije ukazuju na to da postoji potreba za standardizacijom metodologije mikro-CT analize koštanog tkiva humanog porekla kako bi se olakšalo poređenje i omogućila adekvatna interpretacija rezultata različitih studija.

Ključne reči: mikro-CT, vrat butne kosti, region od interesa, mikro-arhitektura kosti, humani materijal

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