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Full Length Article

Subregional areal bone mineral density (aBMD) is a better predictor of heterogeneity in trabecular microstructure of vertebrae in young and aged women than subregional trabecular bone score (TBS)

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ABSTRACT

Background: Currently, bone densitometry fails to identify nearly half of those elderly patients at immediate fracture risk. To improve clinical assessment of vertebral fracture risk, we aimed to determine how the DXA-based 2D parameter Trabecular Bone Score (TBS) relates to subregional variability in 3D trabecular microstructure in young and elderly women compared to aBMD.

Methods: T12 vertebrae from 29 women (11 young: 32 ± 6 years, 18 aged: 71 ± 5 years) were DXA-scanned ex vivo in anterior-posterior (AP) and lateral projection providing vertebral aBMD and TBS. Additionally, aBMD and TBS were measured for three horizontal (superior, mid-horizontal, inferior) and three vertical subregions (anterior, mid-vertical, posterior) and related to 3D microstructure indices, i.e. bone volume per tissue volume (BV/TV), trabecular number and thickness (Tb.N, Tb.Th), based on HRpQCT.

Results: Subregional high-resolution tomography showed significant differences in trabecular parameters for both age groups: In horizontal subregions, BV/TV was lowest superiorly, Tb.Th was highest mid-horizontally, and Tb.N was lowest mid-horizontally and highest inferiorly. Correspondingly, aBMD varied between horizontal subregions, with differences depending on projection direction. TBS varied only in lateral projections of the aged group, with lower values for the mid-horizontal subregion. In vertical subregions, BV/TV, Tb.N, and aBMD were highest posteriorly for both groups. TBS did not differ between vertical subregions. Regression analysis showed aBMD as a predictor explained more of the variance in subregional 3D microstructure compared to TBS. Stepwise multi-regression analysis revealed only three combinations of subregion, projection, and group where aBMD and TBS were both significant predictors.

Conclusions: Subregional aBMD reflects variations in trabecular bone microstructure better than subregional TBS for trisected regions. Specifically, lateral aBMD identifies microstructural heterogeneities independent of age and may improve prediction of vertebral strength and susceptibility to specific fracture types.

1. Introduction

Although the European Union spends > 37 billion Euros each year on osteoporosis management, only 5% of these costs are directed at preventing fractures and 3.5 million fractures occur annually within the EU [1]. For the last decades, the clinical assessment of osteoporosis and vertebral fracture risk, as the first step towards prevention, predominantly relied on areal bone mineral density (aBMD) measured by dual-energy x-ray absorptiometry (DXA) and the corresponding T-score, following WHO recommendations [2]. Using the DXA-based T-score as a diagnostic method for osteoporosis management is common worldwide and enables straightforward application with low radiation dose, high precision, and low costs. Therefore, it is unfortunate that 45% of patients that will sustain an osteoporotic fracture cannot be identified by a low aBMD and T-score, indicating a low predictive value of the current method [3]. The low predictive value of the T-score along with a decline of osteoporosis therapy initiation results in a significant treatment gap and failure to avoid a relevant number of fractures. This

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creates a need for improved diagnostic tools to allow better identification of imminent fracture risk and periods of high fracture risk [4].

Additional diagnostic measures such as the Fracture risk assessment tool (FRAX®) [5] or the trabecular bone score (TBS) [6] were created in an attempt to improve the prediction and prevention of fractures. Specifically, TBS, a parameter evaluating the gray-level texture of DXA scans, has received a lot of attention in recent years. A change in TBS is associated with altered fracture risk for patients, while TBS is partly independent from aBMD and other clinical risk factors [7].

Region-specific micro-computed tomography of human vertebrae confirmed significant variability in the subregional trabecular microstructure [8,9] and highlighted its importance for vertebral strength and fracture risk [10–13]. Both the WHO-recommended T-score and the parameter TBS are calculated from anterior-posterior 2D scans of the whole vertebra [2,7]. Thus, aBMD reflects the bone mass of both the vertebral body and the vertebral arch with processes, while TBS quantifies the 2D texture of the vertebra as a whole. In the context of vertebral fracture risk assessment, it is problematic that neither of these measures can distinguish between the changes in the vertebral body vs. changes of the posterior vertebral elements. Further, neither parameter can be used to detect subregional variations in trabecular microstructure. Therefore, a different approach is necessary to account for subregional variations in vertebral microstructure. Briggs et al. have shown significant subregional variations in vertebral aBMD in a small cohort of elderly donors using lateral DXA scans and related these to failure load [14,15]. Despite these efforts, most current studies investigating the relation between aBMD or TBS and bone microstructure and strength focus on the whole vertebral body or smaller extracted bone segments.

An early suggestion to improve DXA diagnostics has been to scan patients using a lateral projection instead of the common anterior-posterior projection. Several studies showed that lateral scans bear specific advantages for identifying patients with very low BMD [16], for determining bone mineral content (BMC) in osteopenic individuals [17], and also for assessing vertebral strength [18]. Whereas, other studies reported a negative influence on aBMD accuracy due to the lower ratio of bone to soft tissue in lateral projections [19].

As regional variation of trabecular microstructure in vertebrae changes with age [8], it is also imperative to investigate younger individuals regarding subregional variation in bone microstructure, aBMD, and TBS.

Consequently, our study sought to assess subregional 2D parameters aBMD and TBS based on dual energy x-ray absorptiometry in vertebral subregions of women and relate them to 3D trabecular microstructure based on high-resolution peripheral quantitative CT (HRpQCT). The specific aims of the study were:

1. to evaluate whether possible subregional heterogeneity in microstructural parameters measured by HR-pQCT is accompanied by subregional heterogeneity in aBMD and TBS,
2. to determine if aBMD alone, TBS alone, or a combination of aBMD and TBS would be the best predictor for subregional trabecular microstructure,
3. to investigate if the predictive ability of aBMD or TBS is influenced by
   a. the projection direction during DXA measurements (anterior-posterior compared to lateral) or
   b. the age of the individual (young compared to aged women).

2. Material and methods

2.1. Thoracic vertebrae from young and aged women

Thoracic vertebrae (T12) were obtained in cooperation with the Department of Forensic Medicine from 29 women postmortem according to local ethic guidelines (Ethics committee reference number WF-016/14) [20]. The twelfth thoracic vertebra was chosen since it is among the most frequent fracture sites in the spine for non-traumatic events [21]. To determine the influence of age on subregional variation and corresponding DXA-based parameters we collected vertebrae from 11 young (32 ± 6 years) and 18 aged (71 ± 5 years) women. Prior to analysis, soft tissue surrounding the vertebrae was removed and vertebrae were stored frozen at −21 °C in between measurements.

2.2. Subregion-specific 3D evaluation of trabecular microstructure by HRpQCT

We performed a 3D analysis of the trabecular microstructure employing high-resolution peripheral quantitative computed tomography (HRpQCT) of vertebrae, which were scanned with an isotropic voxel size of 41 µm with an HRpQCT device (XtremeCT, Scanco Medical, Brüttisellen, Switzerland) using a custom-written protocol. All cross-section images were reconstructed as transverse slices and evaluated using a software suite (Scanco Medical, Brüttisellen, Switzerland), provided by the manufacturer. Reconstructed slices containing bone from the endplates were excluded to limit the region of interest to trabecular bone. On all remaining slices, trabecular bone was selected manually by creating a region of interest with its boundaries tracing along the inner surface of the cortical shell, keeping a distance to the cortical shell of 1–2 mm. Bone tissue was segmented from marrow tissue using a fixed threshold.

We determined parameters for the entire vertebral body, for three horizontal (superior, mid-horizontal, inferior) and for three vertical regions of the vertebral body (anterior, mid-vertical, posterior) as illustrated in Fig. 1, always excluding endplates. Vertical dimensions of subregions were equal to 1/3 of the height of the whole vertebral body ROI excluding endplates. Afterwards, BV/TV was calculated directly using triangulation [22], while trabecular number (Tb.N) and trabecular thickness (Tb.Th) were calculated based on distance transformation methods [23] (MicroCT Evaluation Software, Scanco Medical, Brüttisellen, Switzerland).

2.3. Subregion-specific 2D evaluation of aBMD and TBS based on DXA scans

Dual-energy x-ray absorptiometry (DXA) measurements were performed to relate observations from HRpQCT imaging to a clinically relevant method [2]. Ex vivo vertebrae were secured in a polyethylene container filled with saline solution. A container with a water column of 20 cm was used as a soft-tissue phantom, in accordance with recommendations of the device manufacturer. DXA measurements (Lunar iDXA, GE Healthcare, Chicago, IL, USA) were performed for samples in anterior-posterior (AP) and lateral projection. The spatial resolution was 0.30 × 0.25 mm [24]. We calculated values for the whole vertebral body excluding endplates and for specific subregions for aBMD and TBS in both projections. Boundaries of subregions were matched to the 3D regions of interest from HRpQCT. aBMD values were calculated with proprietary GE software and TBS values were calculated with TBS iNsight (Medimaps group, Geneva, Switzerland) after an initial calibration with a manufacturer-supplied standard. Subregional aBMD and TBS values were calculated for three horizontal (superior, mid-horizontal, inferior – on AP and lateral scans) and three vertical subregions of interest (anterior, mid-vertical, posterior – on lateral scans) of the vertebra (cf. Fig. 1) using the equivalent software as for standard clinical ROIs. Dimensions of subregions were equal to 1/3 of the height and width of the whole vertebral body ROI excluding endplates. Values for the whole vertebral body were calculated as average of the values of the three horizontal regions, which together cover the same region excluding endplates. Regions of interest (ROIs) were always selected by the same operator after an initial training period. The region of interest (ROIs) included only vertebral bone and excluded the endplates. In anterior-posterior scans, the ROI was horizontally confined by the

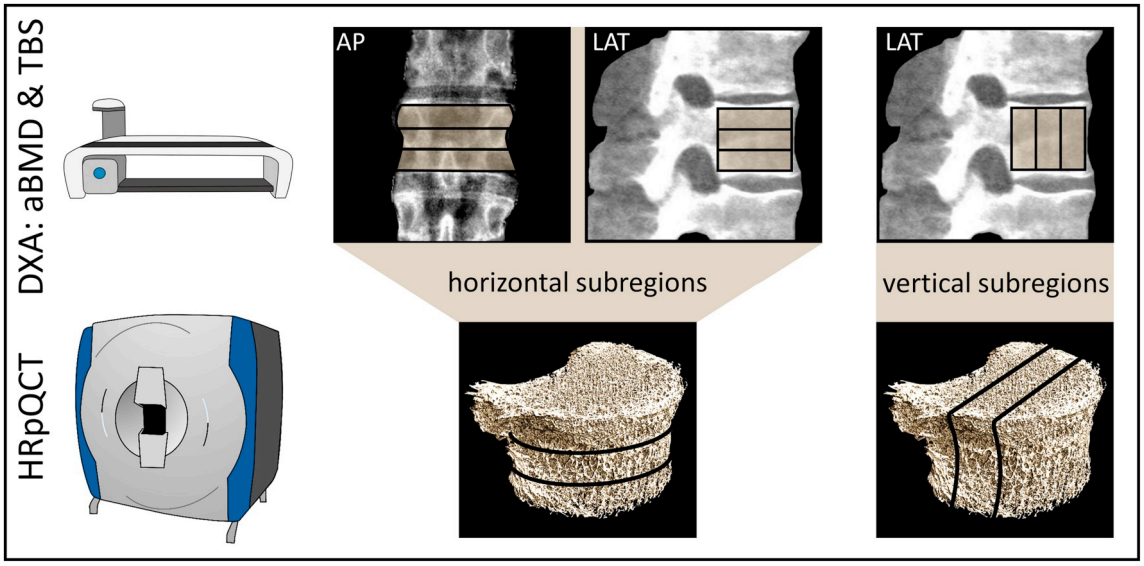

Fig. 1. Methods for analysis of vertebral subregions: Subregional evaluation of 3D trabecular microstructure was performed based on HRpQCT scans and subregional evaluation of 2D parameters, aBMD and TBS, based on dual energy x-ray absorptiometry scans.

Table 1
Age-related differences of vertebral characteristics on whole bone level and significance for age-group comparison.

Parameter	Scan	Young	Aged	Group differences
Sample size	N/A	11	18	
Age [y]	N/A	31.6 ± 6.2	71.6 ± 4.8	**p < 0.001**[a]
BV/TV [%]	N/A	15.75 ± 6.31	6.83 ± 3.11	**p < 0.001**[b]
Tb.N [1/mm]	N/A	1.380 ± 0.238	0.940 ± 0.195	**p < 0.001**[b]
Tb.Th [mm]	N/A	0.182 ± 0.026	0.173 ± 0.018	p = 0.297[a]
aBMD by DXA [g/cm^2]	AP	1.109 ± 0.175	0.794 ± 0.232	**p = 0.001**[a]
	LAT	0.700 ± 0.101	0.488 ± 0.156	**p < 0.001**[b]
TBS	AP	1.144 ± 0.105	1.141 ± 0.141	p = 0.939[a]
	LAT	1.013 ± 0.114	0.998 ± 0.099	p = 0.716[a]

Note. Scan projection direction: AP = anterior-posterior, LAT = lateral.
Bold values highlight significant coefficients of determination.
[a] *t*-Test.
[b] Mann-Whitney *U* test.

automatically detected outline of the vertebra. In lateral scans, the ROI was defined as the largest rectangle that could be fitted between the anterior and posterior outline of the bone. Therefore our protocol slightly deviates from clinical practice where ROIs in anterior-posterior scans usually include the intervertebral discs, according to manufacturer recommendations. However, excluding the intervertebral discs and endplates enabled us to rule out influences of disc degeneration and draw more detailed conclusions about effects of variations in bone microstructure on DXA-based parameters.

2.4. Statistics

Statistical analysis was performed using SPSS Statistics 22 (IBM Corp., Armonk, NY, USA). The young and aged group were compared regarding their results from whole vertebral body measurements obtained by HRpQCT (BV/TV, Tb.N, Tb.Sp) and DXA (aBMD, TBS, in posterior-anterior direction and in lateral direction) using an independent samples *t*-test or non-parametric test (Mann Whitney U) depending on normality of data and homogeneity of variance.

To address aim 1, differences between subregions within a group were determined using Friedman's test followed by related-samples Wilcoxon signed rank test as posthoc test with Bonferroni correction. To address aim 2, single regression analyses were performed for all subregional trabecular parameters as dependent variables and either subregional aBMD or subregional TBS as predictor. Stepwise multiple regression analyses were performed for all subregional trabecular parameters as dependent variables and both subregional aBMD and subregional TBS as predictors. All regression analyses were performed for both age groups and both projection directions individually to address aim 3.

Single and multiple regression analyses were performed based on the absolute values. A p-value of 0.05 was used for determination of significance and corrected for multiple comparisons according to Bonferroni where necessary.

3. Results

Our combined analysis of microstructure, density, and "texture" in vertebral subregions revealed significant differences in trabecular parameters for both age groups in horizontal and vertical subregions, which could be predicted by aBMD. While BV/TV, Tb.Th, Tb.N, and aBMD showed a high variation between subregions, TBS rarely showed significant differences between subregions.

3.1. Group characteristics: age-related differences in whole vertebral body values for microstructure, aBMD, and TBS

Our whole vertebral body analysis (cf. Table 1) showed a significantly higher trabecular BV/TV and trabecular number, as well as aBMD (both in anterior-posterior and lateral DXA scans) in young compared to aged women. Trabecular thickness and TBS in either direction were not significantly different between the groups. Results of regression analyses with aBMD and TBS as predictor and trabecular parameters as dependent variables are displayed in Table 2: aBMD was a significant predictor for BV/TV in both projections and both groups. It further was a significant predictor for Tb.N in both projections in the aged and in lateral projection for the young group. For Tb.Th, aBMD was only a significant predictor in the young. TBS was a significant predictor in lateral projections of the aged group for BV/TV and Tb.N, but presented lower coefficients of determination than aBMD.

3.2. HRpQCT: subregion-specific differences in 3D trabecular microstructure (BV/TV, Tb.N, Tb.Th)

Young and aged individuals showed very similar significant

Table 2
Regression analyses of trabecular 3D microstructural parameters (BV/TV, Tb.N, Tb.Th) as dependent variables and aBMD or subregional TBS as predictors. The predictor aBMD shows significant coefficients of determination in anterior-posterior and lateral projection of both age groups. The predictor TBS shows significant coefficients of determination in lateral projection for the young group.

		Whole vertebral body values					
		Anterior-posterior projection			Lateral projection		
		BV/TV	Tb.N	Tb.Th	BV/TV	Tb.N	Tb.Th
Young	aBMD	**R^2 = 0.393**	R^2 = 0.296	**R^2 = 0.383**	**R^2 = 0.914**	**R^2 = 0.596**	**R^2 = 0.613**
	by DXA	**p = 0.039**	p = 0.083	**p = 0.042**	**p < 0.001**	**p = 0.005**	**p = 0.004**
	TBS	R^2 = 0.290	R^2 = 0.361	R^2 = 0.014	**R^2 = 0.368**	**R^2 = 0.441**	R^2 = 0.064
		p = 0.087	p = 0.050	p = 0.730	**p = 0.048**	**p = 0.026**	p = 0.454
Aged	aBMD	**R^2 = 0.563**	**R^2 = 0.702**	R^2 = 0.020	**R^2 = 0.567**	**R^2 = 0.714**	R^2 = 0.009
	by DXA	**p < 0.001**	**p < 0.001**	p = 0.577	**p < 0.001**	**p < 0.001**	p = 0.713
	TBS	**R^2 = 0.248**	R^2 = 0.133	R^2 = 0.001	R^2 = 0.022	R^2 = 0.007	R^2 = 0.186
		p = 0.035	p = 0.136	p = 0.921	p = 0.558	p = 0.745	p = 0.074

Bold values with gray background highlight significant coefficients of determination.

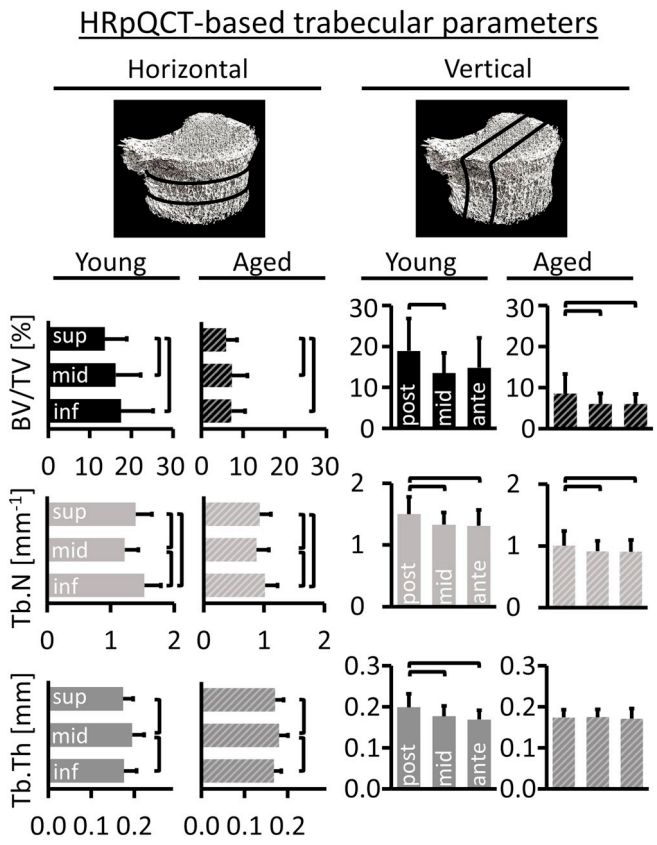

Fig. 2. HRpQCT analysis of subregional 3D microstructure: Horizontal and vertical subregions show significant differences in trabecular parameters in the young and aged group, Friedman test followed by related-samples Wilcoxon signed rank tests (adjusted $p < 0.0167$).

differences regarding trabecular parameters between horizontal subregions (cf. Fig. 2, left side). In both groups, BV/TV was lower in the superior subregion compared to the mid-horizontal and inferior subregion, Tb.N was lowest in the mid-horizontal and highest in the inferior subregion, Tb.Th was higher in the mid-horizontal subregion compared to the inferior and superior subregion.

When comparing vertical subregions, the posterior subregion showed consistently higher values for all parameters in both groups except for Tb.Th in the aged group (cf. Fig. 2, right side). Varying from our horizontal analysis, our vertical analysis showed small differences between the young and the aged group. While in the young group, BV/TV was only significantly higher in the posterior subregion compared to the mid-vertical subregion, in the aged group, the posterior subregion presented with the highest BV/TV compared to both other regions. The parameter Tb.N was distributed in the same manner for both groups with highest values in the posterior region. While in the young group Tb.Th was correspondingly the highest in the posterior subregion, the aged group did not show any significant differences among vertical subregions.

3.3. DXA: subregion-specific differences in 2D parameters (aBMD and TBS)

Between horizontal subregions, aBMD displayed significant variations for both groups and both projection directions (cf. Fig. 3). In anterior-posterior projections of both groups, aBMD was significantly lower in the superior subregion than in the inferior subregion, in the aged group aBMD was additionally lower superiorly than mid-horizontally. Lateral projections showed a slightly different pattern: The aBMD values of both superior and mid-horizontal subregions were lower than those of the inferior one for both age groups. In contrast, TBS showed only differences in lateral projections for the aged group, where inferior values were significantly higher than values from the other subregions.

In vertical subregions and similar to the pattern observed for BV/TV, aBMD displayed higher values in the posterior subregion (cf. Fig. 4). TBS showed no significant differences in either group.

3.4. Regression analysis of prediction of 3D trabecular parameters by 2D DXA-based parameters

The individual coefficients of determination and corresponding p-values from single linear regressions are displayed in Table 3 (horizontal regions of young group), Table 4 (horizontal regions of aged group), and Table 5 (vertical regions of both groups). Scatterplots with regression lines can be found as Supplementary Fig. S1 for horizontal subregions in lateral projection, Fig. S2 for horizontal subregions in AP projection, and Fig. S3 for vertical subregions in lateral projection.

Subregional aBMD values were predominantly good predictors of subregional BV/TV or subregional Tb.N (R^2 = 0.393–0.935). In the aged group, subregional aBMD was a significant predictor in all horizontal and vertical subregions in both projection directions for both BV/TV and Tb.N, except for BV/TV in the anterior subregion in lateral projection (Tables 4, 5). In the young group, subregional aBMD performed equally well for all subregions in lateral projections for BV/TV

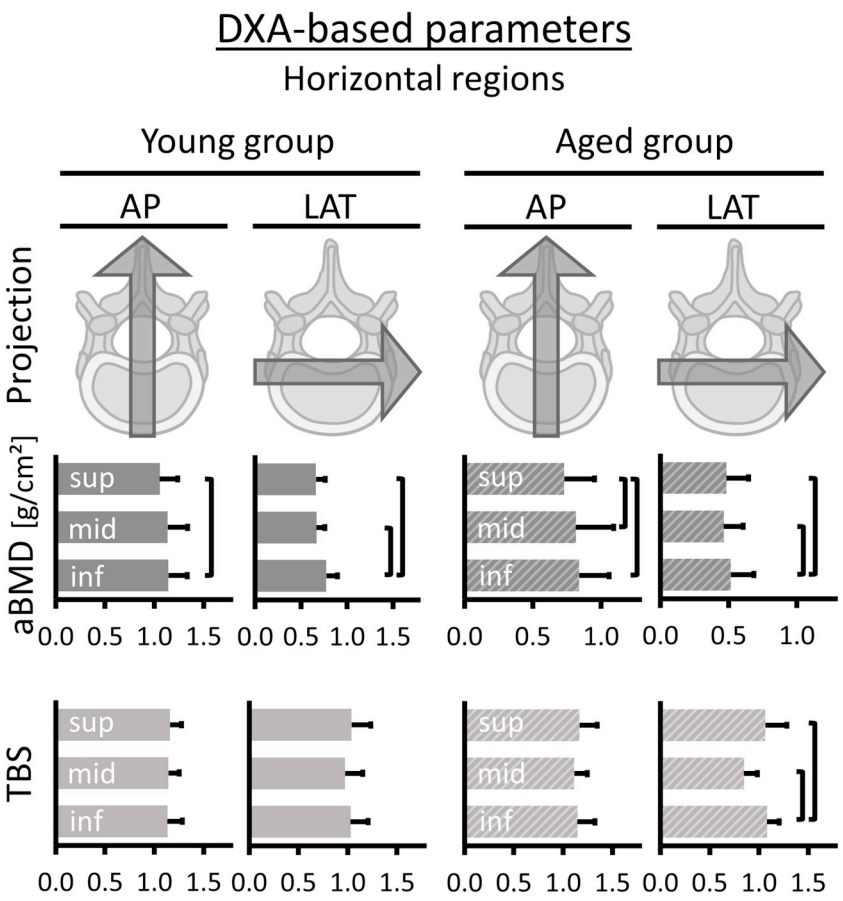

Fig. 3. 2D analysis of horizontal subregions: Analysis of horizontal subregions showed significant variations of DXA-based parameter aBMD for the young and aged group in both projection direction (AP: anterior-posterior, LAT: lateral), TBS varied only for the aged group in lateral projection, Friedman test followed by related-samples Wilcoxon signed rank tests (adjusted $p < 0.0167$).

and Tb.N as dependent variables, whereas in AP projections aBMD was only significant in the mid-horizontal subregion (Tables 3, 5). Interestingly, in aged individuals, R^2 values for aBMD as predictor of subregional Tb.N were in all but one case higher than for aBMD as predictor of subregional BV/TV (Table 4). Regression analysis with Tb.Th as dependent variable showed aBMD as significant predictor only in a few subregions for the young group (Tables 3, 5) but in no subregion in the aged group (Tables 4, 5).

TBS did not show a consistent pattern: Subregional TBS was a significant predictor of trabecular microstructure in only a minority of the regressions examined (seven regressions out of the 54 subregional regressions for all combinations of projection direction, age group and horizontal or vertical regions as presented in Tables 3–5). Moreover, in all except two cases (Tb.N, young group, for posterior subregion (lateral) and inferior subregion (AP), cf. Tables 3 and 5) regression models with TBS as predictor showed lower R^2 values than those with aBMD as predictor.

Stepwise multiple regression analyses showed that for three cases in the horizontal subregion analysis adding TBS as a predictor to the regression "BV/TV vs. aBMD" improved the prediction of BV/TV. In the young group, adjusted R^2 improved from 0.837 to 0.893 in the superior subregion in lateral projection ($BV/TV_{sup} = -0.209 + 0.402\,cm^2/g * aBMD_{sup} + 0.077 * TBS_{sup}$). In the aged group, adjusted R^2 improved from 0.377 to 0.538 in the superior subregion in anterior-posterior projection ($BV/TV_{sup} = -0.063 + 0.07\,cm^2/g * aBMD_{sup} + 0.061 * TBS_{sup}$) and from 0.454 to 0.571 in the inferior subregion in lateral projection ($BV/TV_{inf} = -0.098 + 0.113\,cm^2/g * aBMD_{inf} + 0.104 * TBS_{inf}$). Although TBS showed no significant differences in between vertical regions, in respect to the prediction of Tb.N, stepwise multi-regression showed TBS as additional significant predictor for the anterior subregion in lateral projection for the aged group with an increase in adjusted R^2 from 0.355 to 0.473 ($Tb.N_{ante} = 0.977 + 0.756\,cm^2/g * aBMD_{ante} - 0.415 * TBS_{ante}$). In no further combination of age group and trabecular parameter did the addition of TBS improve the prediction model.

3.5. Influence of projection direction and age of the individual on the ability of aBMD or TBS to predict microstructural parameters

Coefficients of determination were compared between results from anterior-posterior projections and lateral projections for horizontal regions and revealed an influence of projection direction on the predictive ability of aBMD (cf. Tables 3 and 4): In both age groups, coefficients of determination were higher when aBMD values calculated from lateral were used as predictors of BV/TV compared to those from AP projection. This was also true for Tb.N as dependent variable in the young but not in the aged group.

Comparing the two age groups in AP projection, the young group showed lower coefficients of determination with aBMD as predictor for BV/TV or Tb.N as dependent variable compared to the aged. Comparing the two age groups in lateral projections, the young group displayed higher R^2 for prediction of BV/TV using aBMD as predictor while the aged group showed higher coefficients for Tb.N as dependent variable.

TBS as predictor did not display a clear influence of projection direction or age for the few significant cases.

4. Discussion

Our study showed how subregional evaluation of aBMD can reveal microstructural heterogeneity in both young and aged women and confirmed the advantages of lateral over anterior-posterior DXA scanning. Further, TBS displayed minor potential for this specific prediction of subregional heterogeneities in vertebrae.

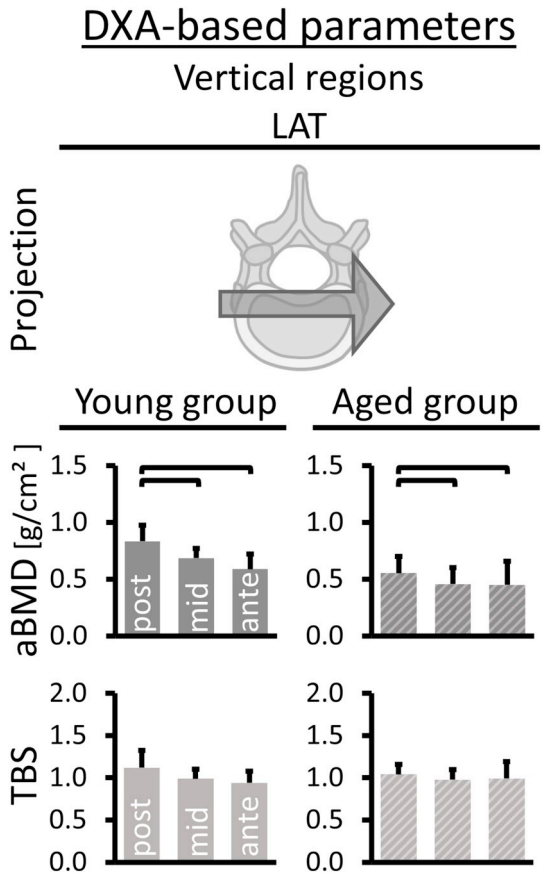

Fig. 4. 2D analysis of vertical subregions: Analysis of vertical subregions showed significant variations of DXA-based parameter aBMD for the young and aged group in lateral projection, TBS varied only for the young group, Friedman test followed by related-samples Wilcoxon signed rank tests (adjusted $p < 0.0167$).

Regression analysis for the prediction of whole bone values of BV/TV with aBMD as predictor showed R^2 in a similar range as previous studies (R^2 = 0.41–0.65, [25]), but deviate in so far as that R^2 was only much higher in lateral scans compared to anterior-posterior scans for the young group in our study. The aged group showed similar R^2 values for regression with BV/TV as dependent variable and aBMD as predictor for lateral (0.567) and AP (0.563) whole bone scans. This deviation from previously published results might be caused by our exclusion of vertebral endplates or the prevalence of osteophytes in the aged group.

Previously shown correlation between TBS and trabecular BV/TV for whole vertebra values [26–28] could be partially reproduced. In this study, the coefficients of determination (cf. Table 2) were significant for the aged group in AP projection (R^2 = 0.248, similar to Hans et al. [28]) and the young group in lateral projection (R^2 = 0.368, similar to Pothuaud et al. [27]). The lack of a significant regression for the predictor TBS vs. BV/TV in lateral projections of the aged group might be attributed to osteophyte occurrence, which contributes to x-ray attenuation relatively more in lateral than in AP projections. The calculated R^2 for the young group in AP projection for TBS vs. BV/TV was in a similar range as for the aged group but not significant ($p = 0.087$). The observed significant regression for the young group in lateral projection showed similar values as Pothuaud et al. found. This similarity is reasonable since Pothuaud et al. evaluated TBS based on artificial projections of only the trabecular compartment and our lateral scans reduce the influence of non-trabecular bone, due to no overlay of vertebral processes and exclusion of the endplates [27]. In addition, our R^2 for regression with TBS as predictor and Tb.N as dependent variable in the young group in lateral projection also yields comparable results to the ones published by Pothuaud et al., while the regression for AP projection was at the threshold of significance ($p = 0.05$).

4.1. Aim 1: subregion-specific differences in 3D trabecular microstructure and 2D parameters (aBMD and TBS)

Our microstructural analysis of the whole vertebral body showed results in agreement with previous literature [8,9,29], specifically a significantly higher trabecular BV/TV and trabecular number, as well as aBMD (for both projection directions) in young compared to aged women. Analyzing both horizontal and vertical subregions showed significant differences in microarchitecture and aBMD values. The determined absolute values for BV/TV and aBMD for the aged group agree very well with the data provided by Briggs et al. [13], for both vertical and horizontal subregions. Comparable data for subregions in young

Table 3
Results for horizontal regions of the young group: Regression analyses of subregional trabecular 3D microstructural parameters (BV/TV, Tb.N, Tb.Th) as dependent variables and subregional aBMD or subregional TBS as predictors show more significant coefficients of determination for the predictor aBMD, specifically in lateral projection.

		Horizontal: young group											
		Anterior-posterior projection						Lateral projection					
		BV/TV		Tb.N		Tb.Th		BV/TV		Tb.N		Tb.Th	
aBMD by DXA	sup	R^2 =	0.235	R^2 =	0.067	**R^2 =**	**0.421**	**R^2 =**	**0.854**	**R^2 =**	**0.472**	**R^2 =**	**0.537**
		p =	0.131	p =	0.442	**p =**	**0.031**	**p =**	**0.000**	**p =**	**0.020**	**p =**	**0.010**
	mid	**R^2 =**	**0.523**	**R^2 =**	**0.500**	R^2 =	0.256	**R^2 =**	**0.837**	**R^2 =**	**0.506**	**R^2 =**	**0.677**
		p =	**0.012**	**p =**	**0.015**	p =	0.112	**p =**	**0.000**	**p =**	**0.014**	**p =**	**0.002**
	inf	R^2 =	0.293	R^2 =	0.201	R^2 =	0.299	**R^2 =**	**0.880**	**R^2 =**	**0.654**	**R^2 =**	**0.460**
		p =	0.085	p =	0.167	p =	0.081	**p =**	**0.000**	**p =**	**0.003**	**p =**	**0.022**
TBS	sup	R^2 =	0.223	R^2 =	0.228	R^2 =	0.035	**R^2 =**	**0.449**	**R^2 =**	**0.424**	R^2 =	0.035
		p =	0.143	p =	0.138	p =	0.582	**p =**	**0.024**	**p =**	**0.030**	p =	0.583
	mid	R^2 =	0.066	R^2 =	0.130	R^2 =	0.081	R^2 =	0.009	R^2 =	0.010	R^2 =	0.209
		p =	0.446	p =	0.276	p =	0.395	p =	0.778	p =	0.773	p =	0.158
	inf	R^2 =	0.262	**R^2 =**	**0.370**	R^2 =	0.022	R^2 =	0.317	R^2 =	0.252	R^2 =	0.258
		p =	0.107	**p =**	**0.047**	p =	0.665	p =	0.070	p =	0.116	p =	0.111

Note. Bold values with gray background highlight significant coefficients of determination, sup = superior, mid = mid-horizontal, inf = inferior.

Table 4
Results for horizontal regions of the aged group: Regression analyses of subregional trabecular 3D microstructural parameters (BV/TV, Tb.N, Tb.Th) as dependent variables and subregional aBMD or subregional TBS as predictors show more significant coefficients of determination for the predictor aBMD, specifically in lateral projection.

		Horizontal: aged group					
		Anterior-posterior projection			Lateral projection		
		BV/TV	Tb.N	Tb.Th	BV/TV	Tb.N	Tb.Th
aBMD by DXA	sup	**R^2 = 0.413**	**R^2 = 0.654**	R^2 = 0.048	**R^2 = 0.479**	**R^2 = 0.635**	R^2 = 0.029
		p = 0.004	**p = 0.000**	p = 0.383	**p = 0.001**	**p = 0.000**	p = 0.501
	mid	**R^2 = 0.635**	**R^2 = 0.780**	R^2 = 0.025	**R^2 = 0.674**	**R^2 = 0.734**	R^2 = 0.001
		p = 0.000	**p = 0.000**	p = 0.529	**p = 0.000**	**p = 0.000**	p = 0.898
	inf	**R^2 = 0.448**	**R^2 = 0.513**	R^2 = 0.005	**R^2 = 0.486**	**R^2 = 0.654**	R^2 = 0.002
		p = 0.002	**p = 0.001**	p = 0.770	**p = 0.001**	**p = 0.000**	p = 0.872
TBS	sup	**R^2 = 0.232**	R^2 = 0.071	R^2 = 0.022	R^2 = 0.026	R^2 = 0.105	R^2 = 0.048
		p = 0.043	p = 0.286	p = 0.556	p = 0.521	p = 0.190	p = 0.384
	mid	R^2 = 0.116	R^2 = 0.073	R^2 = 0.026	R^2 = 0.000	R^2 = 0.002	R^2 = 0.001
		p = 0.166	p = 0.279	p = 0.522	p = 0.978	p = 0.874	p = 0.914
	inf	R^2 = 0.187	R^2 = 0.202	R^2 = 0.021	**R^2 = 0.321**	R^2 = 0.131	R^2 = 0.139
		p = 0.074	p = 0.062	p = 0.567	**p = 0.014**	p = 0.140	p = 0.127

Note. Bold values with gray background highlight significant coefficients of determination, sup = superior, mid = mid-horizontal, inf = inferior.

individuals have not been reported previously, but our values for whole vertebrae were in the same range as literature data [30].

Using high-resolution peripheral quantitative CT we identified a significantly weaker trabecular microstructure in the superior subregion and stronger microstructure in the posterior subregion of the vertebral body. Overall, this heterogeneity pattern was similar for both age groups in horizontal subregions. It is important to note that in vertical subregions in the young group BV/TV was not significantly different between anterior and posterior subregions, while this was the case for the aged group. This could be explained by predominant bone loss in the anterior region, which was previously reported by Sandor et al. [31]. Further, Tb.Th did not show significant differences in vertical subregions of the aged group. The apparent homogeneity in Tb.Th between vertical subregions of the aged group might be caused by a more complex distribution pattern: While Chen et al. found no significant differences in Tb.Th when comparing different positons in the vertebra (anterosuperior, anteroinferior, central, posterosuperior, and posteroinferior) [8], Gong et al. showed that trabecular thickness varied significantly when comparing mean values for superior, mid-horizontal and inferior subregions grouped from different positions in the vertebra [9]. Wang et al. presented significantly higher Tb.Th values in the peripheral compared to the central region, for both superior and inferior subregions [32]. Consequently, the complex distribution of Tb.Th along all anatomical planes is likely not entirely captured by analyzing three vertebral subregions, as chosen in our study design. According to literature another contributing factor could be compensatory thickening of trabeculae occurring with trabecular bone loss as described by Parfitt et al. [33], which Gong et al. mentioned as potential reason for regional differences in Tb.Th in vertebrae [9]. As evaluation of trabecular thickening was not the central aim of our

Table 5
Results for vertical regions of the young and the aged group: Regression analyses of subregional trabecular 3D microstructural parameters (BV/TV, Tb.N, Tb.Th) as dependent variables and subregional aBMD or subregional TBS as predictors show more significant coefficients of determination for the predictor aBMD.

		Young group			Aged group		
		BV/TV	Tb.N	Tb.Th	BV/TV	Tb.N	Tb.Th
aBMD by DXA	ante	**R^2 = 0.841**	**R^2 = 0.642**	R^2 = 0.136	R^2 = 0.194	**R^2 = 0.393**	R^2 = 0.042
		p = 0.000	**p = 0.003**	p = 0.264	p = 0.067	**p = 0.005**	p = 0.411
	mid	**R^2 = 0.935**	**R^2 = 0.740**	R^2 = 0.286	**R^2 = 0.483**	**R^2 = 0.704**	R^2 = 0.011
		p = 0.000	**p = 0.001**	p = 0.090	**p = 0.001**	**p = 0.000**	p = 0.672
	post	**R^2 = 0.839**	**R^2 = 0.489**	**R^2 = 0.856**	**R^2 = 0.797**	**R^2 = 0.792**	R^2 = 0.094
		p = 0.000	**p = 0.017**	**p = 0.000**	**p = 0.000**	**p = 0.000**	p = 0.217
TBS	ante	**R^2 = 0.366**	R^2 = 0.218	R^2 = 0.181	R^2 = 0.030	R^2 = 0.001	R^2 = 0.002
		p = 0.049	p = 0.147	p = 0.191	p = 0.494	p = 0.906	p = 0.863
	mid	R^2 = 0.013	R^2 = 0.059	R^2 = 0.207	R^2 = 0.000	R^2 = 0.003	R^2 = 0.046
		p = 0.735	p = 0.472	p = 0.159	p = 0.968	p = 0.815	p = 0.394
	post	R^2 = 0.281	**R^2 = 0.642**	R^2 = 0.173	R^2 = 0.018	R^2 = 0.048	R^2 = 0.001
		p = 0.094	**p = 0.003**	p = 0.203	p = 0.598	p = 0.380	p = 0.901

Note. Bold values with gray background highlight significant coefficients of determination, ante = anterior, mid = mid-vertical, post = posterior.

study, the chosen resolution of our scans was not ideal to observe very small variations in trabecular thickness. Smaller regions of interest and a resolution of 5–10 µm might yield further insight into these mechanisms.

4.2. Aim 2: regression analysis of prediction of 3D trabecular parameters by 2D DXA-based parameters

In respect to the role of subregional aBMD and subregional TBS as predictors of trabecular microstructure (Aim 2), the complex variations of trabecular microstructure parameters in subregions could be predicted by aBMD, while TBS showed few significant coefficients of determination. Here, aBMD always showed a higher R^2 than TBS for prediction of trabecular parameters except for Tb.N in the inferior subregion of AP projections and posterior subregions of the lateral projections of the young group.

In subregions, coefficients of determination (TBS vs. BV/TV) were mostly non-significant, but if significant they displayed values similar to literature (R^2 = 0.232–0.642, Tables 3, 4, 5). In the aged group, the two cases with significant R^2 for TBS as predictor of BV/TV (AP: superior, lateral: inferior) show R^2 values similar to literature values [26–28]. In the two cases, R^2 was 0.232 (AP, superior, Table 4) and 0.321 (lateral, inferior, Table 4), whereas prior studies showed R^2 = 0.397 (R = −0.63) when calculating TBS for artificial projections of vertebral trabecular bone without cortical shell [27], R^2 = 0.270 (R = 0.52) when calculating TBS based on DXA with a rectangular ROI [28], or R^2 = 0.336 (R = 0.58) when calculating TBS based on DXA with ROI following vertebral contours and including endplates [26]. While the mentioned studies also show significant R^2 values for TBS as predictor of Tb.N, these were here only found for two subregions in the young group (lateral, superior and posterior).

Possible reasons for non-significant or lower coefficients of determination between subregional TBS as predictor and trabecular parameters in this study might be that TBS as a texture parameter represents the variation in the 2D projection of a 3D vertebra. Using smaller subregions likely reduces the amount of variation within the analyzed subregion and consequently results in more homogeneous TBS values.

Using TBS as an additional predictor in a stepwise multi-regression model only improved the R^2 moderately for the prediction of BV/TV in three cases (young in lateral scan: superior; aged in AP scan: superior and lateral scan: inferior). The only cases where TBS alone was a better predictor than aBMD alone were for Tb.N as dependent variable in subregions the young group, which only includes individuals far below the age of the target group for osteoporosis diagnostics. Thus, for the specific aim of investigating subregional differences using TBS is not providing additional information.

4.3. Aim 3: (a) influence of projection direction and (b) age of the individual, on the ability of aBMD or TBS to predict microstructural parameters

When investigating the influence of the projection direction (Aim 3a), comparing regression results for horizontal regions showed more significances and higher R^2 values in lateral projections for aBMD as predictor of BV/TV than in AP projections for both age groups. In the young group, analysis with aBMD as predictor and Tb.N and Tb.Th as dependent variables showed the same.

Over the last two decades anterior-posterior DXA became the norm for assessing osteoporosis and fracture risk. AP scanning is clearly a useful and convenient tool during standard patient care, benefitting from established reference cohorts, healthcare funding schemes, and an abundance of studies applying it to a vast amount of diseases. Nonetheless, the increasing treatment gap [1] and aging of the population create an immense need for more accurate diagnostic tools [4]. Certainly, there are obstacles associated with lateral scanning, such as lower precision when scanning in decubitus position [19], less accessible vertebrae or the lack of a reference collective. But these could be overcome with the use of rotating c-arm scanners, balanced by excluding the posterior elements, and integration of lateral scans in larger studies. Other clinically relevant issues of lateral scans are not as easily overcome such as longer scanning time and radiation dose [34] as well as increased fat inhomogeneity in lateral scans of obese patients [35]. Nonetheless, the creation of a reference cohort would facilitate diagnoses based on scans with a better sensitivity [36], strong predictive ability regarding fracture risk [37] and potential for identifying the risk for specific fracture types [13]. Our results support this notion.

When investigating the influence of the age group (Aim 3b), the high coefficients of determination for aBMD and BV/TV with R^2 ranging from 0.479 to 0.674 for the aged and from 0.837 to 0.880 for the young group expanded the established relationship between these two parameters in lateral projectionr [25] to subregional analyses to both age groups. Establishing this relationship on a subregional level is important since both BV/TV and aBMD are highly predictive for vertebral failure strength [25].

While Briggs et al. [13–15] reported the predictive power of subregional aBMD and BMC values regarding trabecular bone parameters and failure strength in lumbar vertebrae from aged individuals in lateral scans, relating to Aim 3b of the study, we were able to show the transferability of the relationship to young individuals and thoracic vertebrae. Further, our study highlights detectable subregional differences in anterior-posterior scans albeit confirming their limitations in comparison to lateral scans.

One main advantage of subregional vertebral analysis is its potential for predicting specific fracture types. The most prominent atraumatic vertebral fracture types are wedge fractures, where the anterior subregion of the vertebra collapses, and biconcave fractures, where endplates deflect and damage the superior subregion [38]. These fracture types are likely associated with microstructural deterioration of a subregion compared to the neighboring subregions within the vertebral body. Detecting these subregionally deteriorated microstructures using subregional aBMD could allow anticipating an impending wedge or biconcave fracture. Predicting specific fracture types based on structurally pathological subregions would be particularly important for the osteopenic population where fracture risk is currently underestimated using DXA.

5. Limitations

Our study has a few limitations. However, the validity of our used methods is emphasized by the close agreement of the measured values for the whole vertebral body analysis with the literature. Nonetheless, TBS values measured in our study of 29 ex vivo vertebrae lack a significant difference between age groups, whereas large cohort studies showed a decline of TBS with aging [39,40]. The lack of a significantly different TBS value between the age groups might be attributed to the exclusion of endplates in our setup or due to a comparatively small sample size. The exclusion of endplates might further have contributed to our absolute TBS values being slightly lower than values obtained in a clinical setting. An additional influence on absolute values may be introduced by the use of a water phantom, which, although necessary for ex vivo measurements, might influence absorption patterns. TBS calculations based on patient data showed values in the normal range, confirming the correct calibration of the system. Since the main aim of our study was to investigate intravertebral differences, the slightly lower values for absolute TBS should not influence our conclusions about regional heterogeneity.

The segmentation of the vertebrae into three subregions clearly allows the detection of overall heterogeneity. Nonetheless is it likely that three subregions do not reflect a full picture of specific local heterogeneities. When investigating differences between horizontal or vertical subregions a possible gradient from the inferior posterior subregion

towards the anterior superior subregion would be impossible to detect. Thus, extending the evaluation of subregional differences to a 3 × 3 matrix might improve the predictive ability.

Although not significant, the anterior subregion shows slightly lower values for aBMD than the mid-vertical subregion in the young group, while the values of BV/TV are slightly higher. This seems counterintuitive but has been reported previously by Briggs et al. [13] and can be explained the differently sized volumes that are being projected onto same sized areas (ROIs): all subregional ROIs share the same height and width, but while the corresponding 3D volume of the posterior and the mid-vertical subregion have also a similar depth and thereby similar volume, the anterior subregion has a smaller volume that is projected on to the analyzed area due to its half-cylindrical shape. Projecting and consequently evaluating less bone volume may result in a smaller aBMD. This might explain the lack of a significant R^2 for the anterior region of the aged group with BV/TV as dependent variable and aBMD as predictor. This effect could be accounted for by scaling the anterior values to a theoretical similar volume [14]. In this study, we refrained from correcting the values since we aimed to investigate if easily applied changes to the ROI could already yield meaningful results. For future applications with increased sample size, using a correction factor would be advisable to gain the highest accuracy possible.

6. Conclusions

Our study determining the relationship between aBMD, TBS and 3D microstructure in vertebral subregions showed that already a straightforward horizontal or vertical trisection of the vertebral region of interest allows the detection of heterogeneities in trabecular microstructure. Subregional evaluation of aBMD reflects microstructural heterogeneities well, even more so when calculated based on lateral projections. Thus, our findings confirm the capacity for improved fracture risk assessment using lateral scans and additionally highlight the potential for assessing the susceptibility to specific fracture types by subregional evaluation of aBMD, less so for TBS. Further studies extending subregional evaluation of aBMD from lateral DXA-scans to larger cohorts and investigating more detailed subregions related to typical vertebral failure regions could aid in improving vertebral fracture risk assessment, specifically for the osteopenic at-risk population.

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Authors' roles

Study design: AvS, BB, and PM. Study conduct: AvS, EFGS, KP and CP. Data collection: AvS, EFGS, and CP. Data analysis: AvS. Data interpretation: AvS, BB, and PM. Drafting manuscript: AvS. Revising manuscript content: AvS, BB, and PM. Approving final version of manuscript: AvS, EFGS, CP, MA, BB, and PM. AvS takes responsibility for the integrity of the data analysis.

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