

Structural dependence of trabecular bone on bone volume fraction: a comparison between osteoarthritis and control

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Aims

The structural characterization of trabecular bone is becoming a mandatory issue for the study of mechanical behavior of cancellous bone [1-4]. Along the years many structural parameters were studied for the mechanical characterization of the tissue [5-8]. The analysis of trabecular structure was faced even for the study of bone pathologies, such as osteoarthritis and osteoporosis [9-11], even if it is still not clear how osteoarthritic disease affects the structure and the mechanical behavior of cancellous bone. Differences in mechanical behavior, structural properties and tissue composition of cancellous bone have been found between osteoarthritic (OA) and non-pathologic bone. In particular, it has been shown that bone volume fraction (BV/TV) is the most important parameter in describing the mechanical properties of cancellous bone [12]. However, trabecular microstructure was also found to have an important role in the trabecular tissue mechanics [1,13]. Nonetheless, few works tried to investigate if some correlations between BV/TV and structure exist [14].

It is known that OA affect bone mass usually increasing BV/TV [15], but it is not clear if different stages of the pathology could be bounded to different trabecular structures.

The aim of the present work was to verify if structural parameters obtained from micro-CT examination of trabecular bone specimens are or not dependent on bone volume fraction. Is the structure independent on BV/TV or not? Does OA bone have different trabecular structure or all the changes are bounded to bone volume fraction?

Method

Sixty trabecular bone specimens, originated from the femoral heads of two groups of Caucasian donors, a non pathological group (hereafter called control group), and a OA group, were extracted. The 30 control specimens were obtained from deceased persons without musculoskeletal disorders (22 males, 8 females, age 67 ± 10) thanks to a donor program (International Institute for the Advance of Medicine, Jessup, PA, USA). The 30 osteoarthritic ones originated from patients underwent hip replacements (13 males, 17 females, age 66 ± 9). They were selected from an internal database of OA patients as presenting severe osteoarthritis. The severity of the pathology was evidenced by the presence of a discontinuity in the cartilage (severe OA, figure 1).

The following procedure for the specimen preparation and micro-CT examination was applied to both the groups.

The heads were stored in a 70% ethanol solution for at least four weeks before testing in order to prevent the transmission of infectious diseases during laboratory handling. A cylindrical trabecular bone specimen was retrieved from the primary compression region of each head, as described in previous studies [16]. Using a diamond saw under constant water irrigation, a 26-mm-thick bone slice was obtained, about 5 mm from the articular surface of the femoral head. A hollow diamond-coated milling cutter, with the slice immersed in water, was used to extract a cylindrical trabecular bone specimen (height 26 mm diameter 10 mm).



Figure 1: A femoral head with a discontinuity of the cartilage, affected by severe osteoarthritis

Specimens were examined by microCT (model Skyscan 1072, Skyscan, Kontich, Belgium) with a previously described protocol [17]. The microCT scan settings were: complete rotation over 185°, tube voltage of 50 kVp, tube current of 200 μ A, 1-mm-thick aluminium filter for beam hardening reduction, field of view 20 mm \times 20 mm and an isotropic pixel size of 19.5 μ m. The cross-section images were reconstructed using a filtered back-projection algorithm (software “Cone_rec”, Skyscan, Belgium) [18], and storing each cross-section as an 8-bit image (256 grey levels), 1024 \times 1024 pixels in size. To calculate the structural parameters, the cross-sections were binarized using a uniform threshold (software “3D-Calculator” Skyscan, Belgium), set according to a previously published protocol [17]. For each cross-section, a circular region of interest (ROI) with a diameter of 9 mm was defined, centered on the bone specimen, containing only trabecular bone. The volume of interest (VOI) over which the structural parameters were calculated was composed of a stack of 1000 consecutive ROIs, resulting in a cylindrical VOI of 9 mm in diameter and of 19.5 mm in height [16]. For each specimen, the following structural parameters were determined over the VOI (software “3D-Calculator”, Skyscan, Belgium): bone volume fraction (BV/TV) [5], direct trabecular thickness (Tb.Th*) [19]; structure model index (SMI)[20]; the eigenvalues (E1, E2, E3) of the anisotropy tensor and their normalizations (H1, H2, H3), computed using the normalization proposed by Turner et al. (1990), obtained from the three principal directions of the ellipsoid (MIL method) [21].

A correlation matrix was used to control the dependence of the parameters among themselves. The matrix was computed for both OA and control groups. All the parameters resulting correlated to BV/TV ($R > 0.5$) were also analyzed by means of a regression analysis splitting the data in control and OA groups. The coefficient of determination (R^2) was calculated for each regression. The regression lines of the two groups were compared by analysis of variance (ANOVA). For each parameter in fact, the difference between slope and intercept was analysed.

Finally, for parameters not correlated to BV/TV, an unpaired t-test was used to make a comparison and to find statistically significant differences between the two groups, the control and the OA ones.

For statistical analysis the software StatView (StatView version 5.0.1, SAS institute inc., Cary, NC, USA) was used. Differences were deemed to be statistically significant at a probability of $p < 0.05$.

Results

A correlation matrix was performed in order to verify the dependence among the structural parameters and the bone volume fraction. The matrix highlighted that the biggest part of the structural parameters is correlated to BV/TV, with the exception of CD and the three normalizations of the eigenvalues H_1 , H_2 , H_3 . A linear regression analysis was used, for parameters correlated to BV/TV, in order to study the way in which each parameter depends on BV/TV.

As a result of the regression analysis, a great part of the structural parameters was found linearly dependent on BV/TV: some were strong dependent both in the OA and control (SMI, Direct Tb.Th., Ash Density) whereas others showed smaller dependence in one of the two groups (E_1 , E_2 , E_3 , Tb.Sp., Tb.N.) (Table 1).

	Determination coefficient		ANOVA analysis	
	CONTROL	OA	Slope P value	Intercept P value
SMI	0.89	0.86	0.596	<0.0001
Direct Tb. Th.	0.72	0.77	0.008*	<0.0001
Tb. Sp.	0.84	0.65	0.246	<0.0001
Tb. N.	0.64	0.36	0.339	<0.0001
Ash Density	0.94	0.83	0.060	<0.0001
E_1	0.57	0.26	0.207	<0.0001
E_2	0.51	0.24	0.202	<0.0001
E_3	0.45	0.18	0.113	<0.0001

Table 1: Determination coefficients and p-values of slope and intercept calculated for each regressions for control and OA group

*p-value<0.05

The regression analysis showed that all the parameters had the same slope and different intercept, but Direct Trabecular Thickness that has different slopes for the two groups ($p=0.0089$). Only the ash density is near the limit ($p=0.0606$).

In order to further analyze the differences between the two groups, the control one and the osteoarthritic one, an unpaired t-test was performed for parameters not correlated to BV/TV. In this way parameters' differences depending on pathology, and not on BV/TV, can be emphasized. In particular, among the independent parameters, the test pointed out significant differences between the two groups in the normalizations of the eigenvalues H_1 and H_2 : the OA specimen had larger H_1 value and lower H_2 value than the control specimens. On the other hand, no statistically significant differences were found in the normalization of the eigenvalue H_3 and in the connectivity density (Table 2).

	CONTROL	OA	p-value
H₁	0.409 (\pm 0.015)	0.421 (\pm 0.004)	0.014
H₂	0.314 (\pm 0.009)	0.305 (\pm 0.003)	0.008
H₃	0.277 (\pm 0.002)	0.274 (\pm 0.002)	0.277
CD	4.544 (\pm 2.656)	3.636 (\pm 3.876)	0.294

Table 2: Mean value and standard deviation for parameters not correlated to BV/TV. In the third column p-value of the unpaired t-test between the two groups.

Conclusion

In this study femoral trabecular bone specimens obtained from human femoral heads of non-pathologic patients and patients in severe primary OA conditions were analyzed. A correlation matrix showed which parameters correlate to BV/TV and which ones not correlate. The matrix showed that the biggest part of the known structural parameters is correlated to BV/TV. This means that it is not correct to compare structural parameters if BV/TV is not controlled. In fact, although it is not known what the prevailing parameters is, if BV/TV controls the structure or vice versa, they are correlated. So, it is impossible to compare a parameter not considering the other one because it would be statistically incorrect. Structural parameters founded to correlate to BV/TV were analyzed by means of a linear regression analysis. The slopes and the intercepts of the two groups (OA and control) were compared for each parameter. Only Direct Trabecular Thickness was found having different slopes for the two groups, whereas the ash density is near the limit. The other parameters have the same slope and a different intercept. This result underline the difference between the two groups. All the parameters founding to have a dependence on BV/TV presented different regression lows in the two groups. The most of them were different just by a constant (different intercept) but for two of them the difference in the slope suggest a different relation between the parameters that could be ascribed to the pathology.

Only a few parameters are not correlated to BV/TV: the normalizations of the eigenvalues H_1 , H_2 , H_3 , and the CD. A t-test, performed on parameters that not depend on BV/TV, showed a difference in the structure of the OA bone compared to the control bone independently on BV/TV values.

In fact, differences in H_1 e H_2 between the two groups were found, suggesting alterations in the spatial organization of the trabeculae for the OA group. In particular, as the H_1 was found to increase in the OA group, it is reasonable to suppose that the trabeculae are more oriented along the primary direction than the control group and that the distribution of the trabeculae of the latter group was more isotropic. This difference may be related to the advanced state of OA. A less advanced OA may not present the same difference compared to control group [9].

The present results, apart from being in agreement with the literature [22], suggest also the existence of a remodelling activity, if compared to the literature [9], characterized by changes in anisotropy and highlighting a three-dimensional reorientation of the trabecular structure. In fact, since the here reported difference of the structure were not reported in studies about early osteoarthritis[9], the difference of the structure could be related to the advanced level of the pathology. Therefore, it is reasonable to suppose that inflammation process appears earlier of bone remodeling in the pathogenesis of osteoarthritis.

Also differences in gait between non-pathologic and OA patients were analysed in the literature: in particular, there is a reduction in dynamic range of motion of the hip, due to antalgic gait [23]. In fact the patient is led to load his hip in the only position in

which he does not feel pain and this could drive the trabecular framework remodeling along preferred orientations. This could modify the biomechanics of the joint and might explain the more anisotropic specimens found in OA in the present study.

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