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Age-related changes in bone architecture

Alterações na estrutura óssea relacionadas à idade

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ABSTRACT

Objective*: to evaluate the histologic and morphometric characteristics of bone biopsies of the anterior iliac crest of patients of different age groups.* **Methods***: we studied 30 bone samples from the iliac crest, using brightfield optical microscopy. We divided the samples by donors' age groups in three groups: Group 1 (n = 10), subjects aged between 25 and 39 years; Group 2 (n = 10), subjects aged between 40 and 64 years; Group 3 (n = 10), individuals aged 65 years and over. We randomly divided the samples into two sets with 15 specimens. In the first study segment (n = 15), we used histological to assess the osteogenic property of the graft, through the analysis of cell reserve in the periosteum, the number of osteocytes in the lacunae and the number of Haversian and Volkmann's canals. In the second study segment (n = 15), we investigated the morphology of osteoconductive property of the graft, through quantification of the trabecular meshwork (Vv) and trabecular area (Sv).* **Results***: histologically, we observed degeneration of bone occurring with age, characterized by thinning of the periosteum, with gradual replacement of the steogenic layer by fibrous tissue, small amount of Haversian and Volkmann's canals, osteocyte lacunae voids and fine spongy bone trabeculae, allowing ample medullary space, usually occupied by fat cells and adipocytes. Morphologically, with respect to the quantification of the trabecular meshwork (Vv), we found statistically significant differences between Groups 1 and 3 and between Groups 2 and 3, with reduction of the trabecular meshwork of about 45% in the elderly over 65 years old ; there was no statistically significant difference between Groups 1 and 2. There was also no statistical difference between the Groups regarding Sv.* **Conclusion***: the results of this experiment suggest that, in the elderly (over 65 years old), the osteogenic property of autologous bone graft decreases and the osteoconductive property is compromised.*

Key words: *Bone Development; Bone/biopsy; Bone/anatomy & histology; Bone properties; Ilium; Bone Transplantation*

INTRODUCTION

The use of bone graft procedure is common in current orthopedic practice. Although in our literature there are no data on the number of grafts performed each year, Heppenstall estimated that about 200,000 bone grafting were performed each year in the United States in the early 80's[1]. Clinical conditions such as delayed consolidation, non-union, large bone defects after tumor resection or infections are frequent indications of bone graftsapplication[2-4]. Historically, the use of autografts is the first option in such cases. Their osteogenic (cellularity), osteoinductive (growth factors and bone differentiation) and osteoconductive (extracellular matrix) properties are extremely important in this choice, since to date no existing bone substitute could display all these features[5,6]. Nevertheless, several authors have pointed out disadvantages and complications related to the use of autografts[7,8]. The sources and the amount of grafts are limited and the morbidity in the donor site is frequent, ultimately exerting profound effect on treatment outcome. Furthermore, recent studies have shown reduced osteogenic potential in some clinical situations, such as diabetes mellitus, advanced age and after chronic use of corticosteroids, nicotine and alcohol[9-11].

The purpose of this study was to evaluate the histologic and morphometric characteristics of bone biopsies from the anterior iliac crest of patients of different age groups, using brightfield optical microscopy.

METHODS

In a period of six months, we collected 57 bone fragments of the anterior iliac crest of patients undergo-

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ing orthopedic surgery in the orthopedics service of the lead author. All patients signed an informed consent and the Review Committee of the lead author Institution approved the study.

Of the 57 biopsies performed, we selected the 30 best specimens for microscopic analysis; of these, we randomly selected 15 for histological analysis and 15 for morphometric analysis. We divided the material by donors' age group in three groups: Group 1 (n=10), subjects aged less than 39 years; Group 2 (n=10), subjects aged between 40 and 64 years; Group 3 (n=10), individuals aged 65 years and over. Tables 1 and 2 show the patients' demographic data.

We operated all patients on in the supine position. To remove the graft,we performeda curvilinear access on the anterior iliac crest of approximately 5cm by dissection of planes till reaching the periosteum. We withdrew a 1cm³ corticocancellous block, preserving the periosteum. We used electrocautery during the bone biopsy. We placed the material in vials containing 3ml of buffered 10% paraformaldehyde for five days and then sent them for histological and morphometric studies.

Histological Analysis: After fixation, the fragments were decalcified in 5% nitric acid for five days, dehydrated in alcohol, cleared and embedded in paraffin. We used a Spencer® microtome (American Optical, USA) to make 5µmthick sections, sagittal to the longitudinal plane of the bone block. The sections were stained with hematoxylin-eosin (H&E), according to the methodology described by Bancroft and Cook[12]. We used a brightfield optical microscope (Olympus BHs-RFCA, Japan).

The same researcher performed the histological readings, blindly, systematically, and according to a pre-defined script (Table 3).

Morphometric analysis: After fixation, the fragments were decalcified in 5% nitric acid for five

Table 1. *Demographics of the patients used for histological analysis.*

GENDER	AGE (in years)	DIAGNOSIS	SURGERY PERFORMED	COMORBIDITIES
M	35	L ankle arthrosis	Tibio-tarsal arthrodesis	ALC, SMK
M	21	R femur PA	ORIF	-
M	22	R femur PA	ORIF	-
M	26	2nd Metacarpus fracture (R)	ORIF	ALC
M	30	PA of upper arms (R and L)	ORIF	-
M	57	L humerus PA	ORIF	-
F	48	R humerus PA	ORIF	-
F	56	Fracture of proximal third of humerus (R)	ORIF	-
M	48	Fracture of neck of humerus (R)	ORIF	-
F	57	R tibia PA	ORIF	DM, SMK, Hypothyroidism
F	77	R tibial plateau fracture	ORIF	ALC
F	72	Supracondylar fracture of femur (R)	ORIF	ALC
M	84	Fracture of proximal third of humerus (R)	ORIF	HAS
F	73	Fracture of distal third of tibia(L)	ORIF	-
F	72	Diaphyseal fracture of femur (L)	ORIF	-

Source: SOT, 2015
Ledgend: M-male; F-female; L-left; R-right; PA-pseudarthrosis; ORIF-open reduction and internal fixation; ALC-alcoholism; SMK-smoking; DM-diabetes mellitus; HAS-hypertension

Table 2. *Demographic data of the patients used for morphometric analysis.*

GENDER	AGE (in years)	DIAGNOSIS	SURGERY PERFORMED	COMORBIDITIES
F	25	L femur PA	ORIF	-
F	35	L femur PA	ORIF	HAS
M	21	R tibia PA	ORIF	ALC
M	19	R humerus PA	ORIF	-
M	30	Subtrochanteric fracture (R)	ORIF	-
M	57	L humerus PA	ORIF	-
F	56	L tibial plateau fracture	ORIF	-
M	44	R tibial plateau fracture	ORIF	-
M	47	L ulna PA	ORIF	-
M	40	L tibial plateau fracture	ORIF	-
F	74	L radius fracture	ORIF	-
F	65	R tibia PA	EF	HAS, SMK
F	72	R humerus fracture	ORIF	-
F	72	R tibia PA	EF	-
F	73	Fracture of distal third of tibia (L)	ORIF	-

Source: SOT, 2015
Subtitles: M-male; F-female; L-left; R-right; PA-pseudarthrosis; ORIF-open reduction and internal fixation; EF-external fixation; ALC-alcoholism; SMK--smoking; HAS-hypertension

days, dehydrated in alcohol, cleared and embedded in paraffin. We used a Spencer® microtome (American Optical, USA) to make 10μmthick cuts in the cancellous bone, transversely to the longitudinal axis of the bone block. The sections were stained with H&E and studied by brightfield optical microscopy (Olympus® BHs-RFCA, Japan)[12]. The same researcher performed the morphometric analysis, blindly. We calculated the amount of trabecular meshwork (Vv) and the area of the trabecular meshwork (Sv) of cancellous bone according to the method used by Tabor, and we statistically treated the results with significance level □ = 0.05[13]. We used the ANOVA test for comparison between groups and the multiple comparison test of Newman-Keuls for paired comparisons[14-17].

RESULTS

HISTOLOGICAL ANALYSIS

Periosteum

In young subjects (Group 1), the periosteum had become quite thickened and adhered, with a quite visible osteogenic layer, showing great amount of osteogenic cells (pre-osteoblasts and osteoblasts). In its bone para-cortical surface, we observed the basophilic cementing line, with numerous resting surface osteoblasts (line cells) and in some areas the presence of remodeling gaps, with aggregates of highly secreting osteoblasts depositing new osteoid matrix (Figures 1A and 1B). In subjects from Group 2 (between 40 and 64 years of age), the periosteum had become partially detached, irregular and thin.

Table 3. *Histological analysis.*

Periosteum	Cortical bone	Cancellous bone
Cellularity (osteogenic layer)	Cellularity (osteocytes in osteoplasts)	Bone marrow
External cementing line (presence of acid proteoglycans)	Bone thickness	Thickness of bone trabeculae
	Haversian and Volkmann'schannels	

The osteogenic layer was visible. The external cementing line was irregular and basophilic, with various resting surface osteoblasts (Figures 1C and 1D). We hardly observed remodeling gaps. In Group 3 (elderly), we found the periosteum partially detached and thin. The osteogenic layer was extremely small, with few osteogenic cells, with mixed cellularity in some areas (pre-osteoblasts, fibroblasts and osteoblasts). The external cementing line was frankly basophilic, very irregular, with some osteoblasts aggregates that had no sign of being secreting new matrix (Figure 1E and 1F).

Cortical bone

in Group 1 (individuals under 39 years old), the cortical bone was thick, with numerous Haversian channels of large diameter and concentric lamellae. There was a great number of osteocytes in the gaps (average of six per field, H&E, 400X), not counting the empty gaps. There was an average of 1.6 Volkmann'schannels per field (H&E 100X) (Figure 2A). In subjects between 40 and 64 years of age (Group 2), the cortical bone was thinner than in the young (Group 1), occupying a smaller area and demonstrating a smaller diameter and number of Haversian and Volkmann's channels (mean 0.6 per field, H&E, 100X). There were an average of four osteocytes per field (H&E 400X) (Figure 2B). In individuals over 65 years of age (Group 3), the cortical bone was extremely thin, with small amount of Haversian channels. In certain areas,the decrease in collagen matrix was evident. Almost all the gaps were empty, averaging two osteocytes per field (H&E, 400X) (Figure 2C). There was an average of 0.4 Volkmann channels per field (H&E 100X).

Cancellous bone

In the young (Group 1), the bone marrow found was intact, generally presenting small areas of necrosis in the periphery and, less often, bleeding areas. The bone trabeculae were clearly visible, quite thick, making networks with continuity aspect (Figure 3A). There were no osteoclasts. In subjects in Group 2 (between 40 and 64 years), the bone marrow was full, with peripheral areas of necrosis and hemorrhage. The

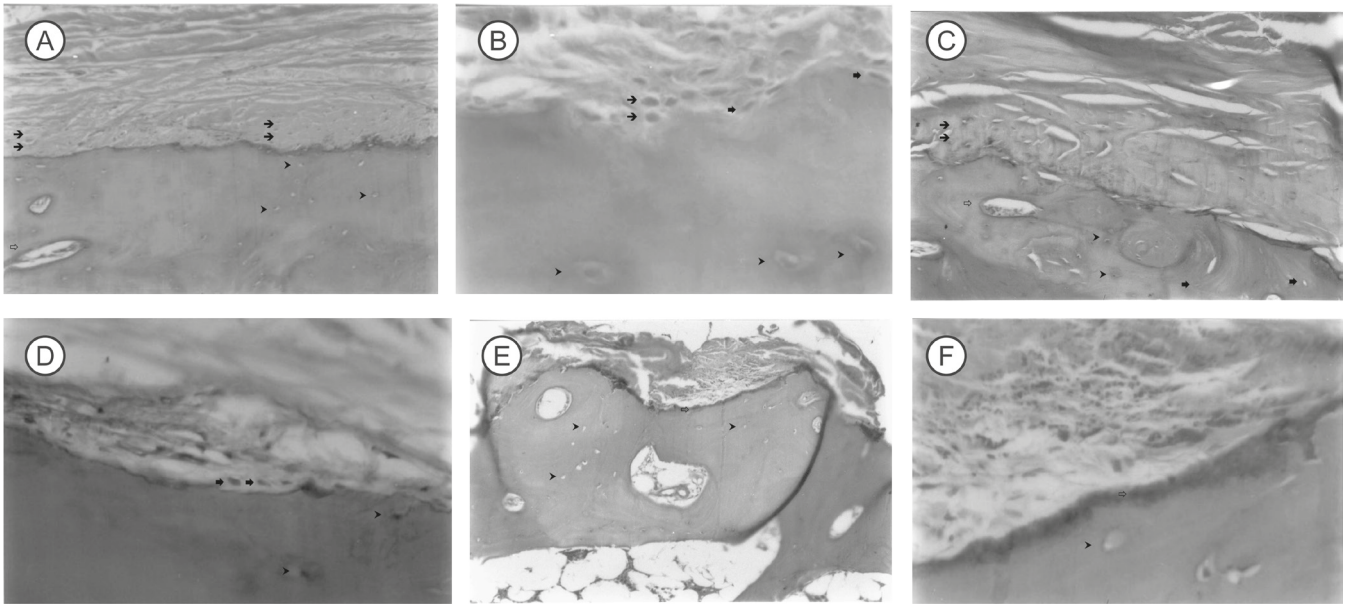

Figure 1. *Photomicrograph of histological biopsies of the anterior iliac crest of adults – PERIOSTEUM. A and B (Group 1) – thick periosteum adhered to the cortical bone with a well defined osteogenic layer, rich in osteogenic cells (➔). basophilic and cortical cementing line with numerous gaps filled by osteocytes (➤). Note Volkmann's channel (⇨) in lower magnification and remodeling gap with secreting osteoblasts (➔), resting surface osteoblasts (➡) and osteocytes in lacunae (➤) in higher magnification (H&E 100X / H&E 400X); C and D (Group 2) –Thinned periosteum, partially detached from cortical bone, with osteogenic layer rich in osteogenic cells (➔). Little basophilic cementing line and cortical with a mixture of gaps filled by osteocytes (➤) and empty gaps (➡). Note Haversian channel in formation (⇨) in lower magnification and resting surface osteoblasts (➡) and osteocytes in lacunae (➤) in higher magnification (H&E 100X / H&E 400X); E and F (Group 3) – Fine periosteum, completely detached from the cortical bone, with thin osteogenic layer and mixed cellularity. Frankly basophilic cementing line (⇨) and cortex with most osteocyte gaps empty (➤) (H&E 100X / H&E 400X).*

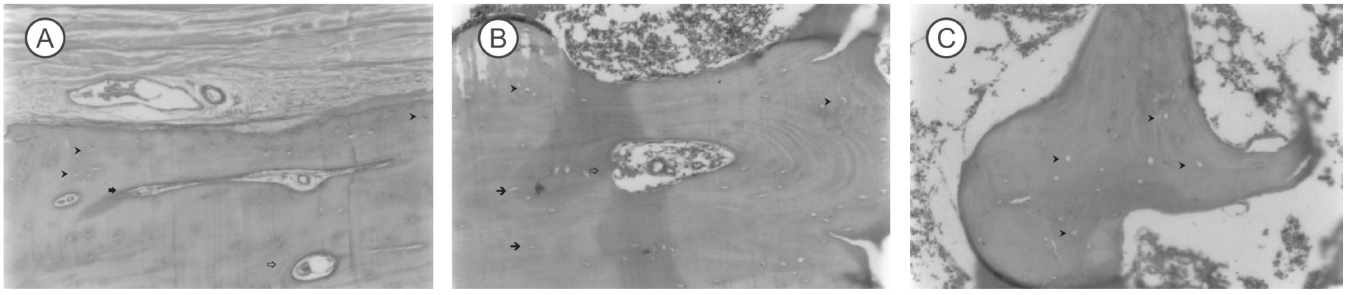

Figure 2. *Photomicrograph of histological sections of the anterior iliac crest biopsies of adults – CORTICAL BONE. A (Group 1) – Mostly filled osteoplasts (➤). Note the presence of Haversian (⇨) and Volkmann's (➡) channels (H&E, 100X); B (Group 2) – filled osteoplasts (➤) and empty osteoplasts (➔). Note Haversian channel in the center (⇨) (H&E, 100X); C (Group 3) – Mostly empty osteoplasts (➤) (H&E, 100X).*

bone trabeculae were thinner than in the young (Group 1), generally parallel, but still with a net aspect (Figure 3B). In one biopsy, we observed the presence of osteoclasts (MLCC, 57 years old). In the elderly (Group 3), the bone trabeculae were very thin, forming a network, allowing large medullary spaces occupied by fat cells and adipocytes (Figure 3C). The bone marrow was scarce and there were loads of necrotic areas on the periphery. There were no osteoclasts.

MORPHOMETRIC ANALYSIS

The amount of trabecular meshwork (Vv) was 52.2 ±5.0 (mean ± SD) in Group 1, 52.1 ± 13.3 in Group 2 and 2.9 ± 28.9 in Group 3. There was significant loss of Vv between the groups ($p < 0.05$, ANOVA), with the elderly population (Group 3) displaying a reduction of 45% of the trabecular meshwork in the cancellous bone ($p < 0.01$, Newman-Keuls). We observed no statistically significant difference with respect to Vv between Groups 1 and 2 was ($p>0.05$, Newman-Keuls) (Figure 4).

The area of the trabecular meshwork (Sv) was 6.35 ± 0.82 (mean ± SD) in Group 1, 5.96 ± 0.62 in Group 2 and 6.69 ± 0.98 in Group 3. There was no statistical difference between the groups ($p > 0.05$, ANOVA and Newman-Keuls) (Figure 5).

DISCUSSION

The development of new biomaterials to mimic the characteristics of autologous bone graft has advanced in recent years. In vitro and clinical investigations have suggested that some of these bone substitutes may actually stimulate consolidation[18,19]. However, the great diversity among the biomaterials available and poor understanding of the mechanisms by which these substances participate in the bone repair process limit their application[2,18]. Even today, the use of the autograft is the best solution for reconstruction of large bone defects and osteogenic stimulating bone healing[2-5]. Its unique structure provides an excellent mechanism of self-regulation and functional adaptability. Its

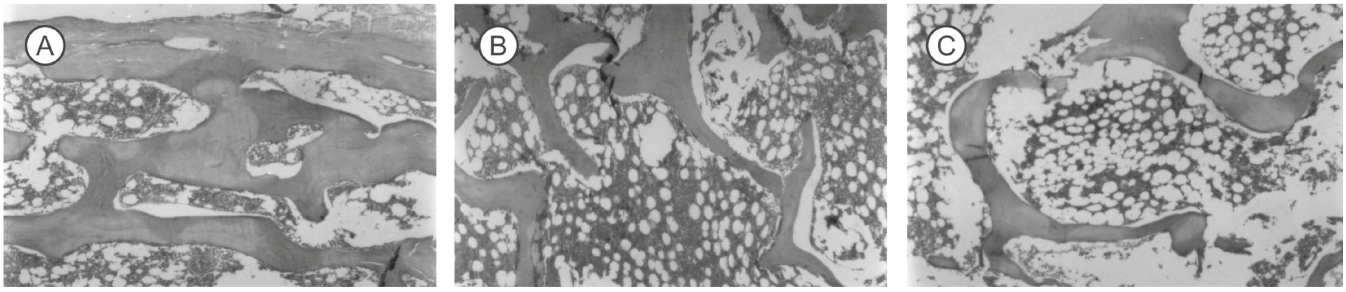

Figure 3. *Photomicrograph of histological sections of the anterior iliac crest biopsies of adults – CANCELLOUS BONE. A (Group 1) – thick bone trabeculae forming networks with small medullary space (H&E, 100X); B (Group 2) thinner bone trabeculae, but still forming networks with increased medullary space compared with Group 1 (H&E, 100X); C (Group 3) – thin and broken bone trabeculae, with large medullary space filled with fat cells and adipocytes (H&E, 100X).*

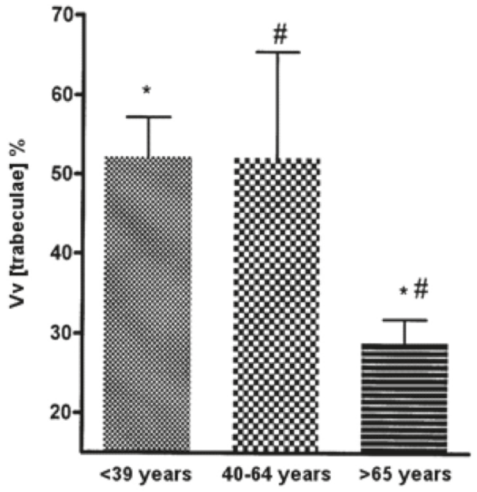

Figure 4. *Amount of trabecular meshwork (Vv) – There was a significant loss of Vv between the groups (p<0.05, ANOVA), the elderly population (Group 3) displaying a reduction of 45% of the trabecular meshwork in the cancellous bone (p<0.01, Newman-Keuls). There was no statistically significant difference with respect to Vv between Groups 1 and 2 (p>0.05, Newman-Keuls).*

solid matrix facilitates the exchange of biomechanical, biochemical and electromechanical signals, endogenous and exogenous of system to which it is exposed to,to the cells responsible for bone modeling[6,20]. The sum of these interactions determines the success or failure of the grafting procedure. In general, the less biologically active is the graft, the more dependent on the receptor site it becomes[2,6]. This aspect of bone physiology gains more importance from the moment that recent studies have shown that complex changes occur in the skeletal microarchitecture throughout life, with reduction mainly of the volume and density of the trabecular bone and hematopoietic tissue[21-24] .

Birkenhäger-Frenkel et al.used electron microscopy to investigateiliac biopsies of 94 human specimens between 20 and 80 years of age[21]. They noted that both the trabecular bone area and the number of trabeculae decrease with age in areas commonly used as donor sites for bone graft. Burkhardt et al.used optical microscopy to retrospectively analyze 81 biopsies of the iliac crest and 400 samples of iliac crest, sternum, lumbar vertebra, calcaneus and radius distal third taken between two and 12 hours postmortem of 79 normal individuals of different age groups (one at 96 years old)[22]. They observed that the volumes of trabecular bone, osteoid matrix and hematopoietic tissues and the cell number are contingent on age, with a gradual decrease in older individuals. Their results indicate a possible role of microcirculation in the genesis of these changes, since the reduction in the number of sinusoids is common in the geriatric population, always accompanied by aplasia of hematopoietic marrow and increase in the number of fat cells. Rehman et al. applied semi-automated analysis to ileum biopsies images of 234 subjects between 16 and 100 years of age. They found that the trabecular bone volume decreases with age in both genders, reaching statistically significant values between 61 and 70 years in women (56% reduction) and between 81 and 90 years in men (34% reduction)[24].

Based on these authors' findings[21-24], in this experiment we studied histologic and morphometric characteristics of bone biopsies from the anterior iliac crest. We divided patients into three groups of different age groups. With regard to Groups 1 and 2, we set the division based on the hormonal decline and consequent deterioration of bone tissue microstructure that occur around 40 years of age, especially in women. There is a direct relationship between low bone density and pathologic fractures[25]. The inclusion of Group 3 followed the guidelines of the World Health Organization, which considers "elderly" individuals aged 65 years and over[26].

Histologically, we observed that bone tissue degeneration occurs with age. In Groups 1 (age under

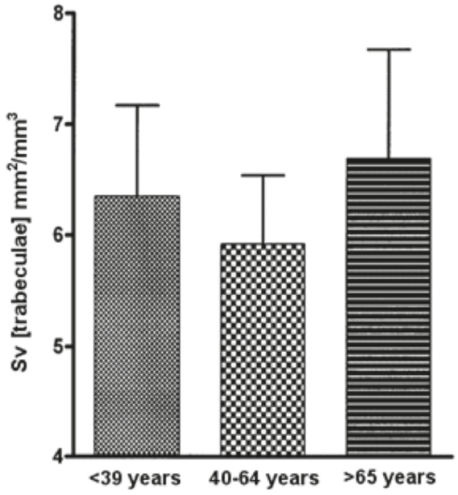

Figure 5. *Area of the trabecular meshwork (Sv) – There was no statistical difference between the groups (p>0.05, ANOVA and Newman-Keuls).*

40 years) and 2 (between 40 and 64 years of age), the osteogenic periosteum was very thickened, rich in bone lineage cells (pre-osteoblasts and osteoblasts). In the cortical layer, there were loads of nutritious (Haversian) and connecting (Volkmann's) channels and most osteoblasts were occupied. The trabeculae of the cancellous bone were clearly visible, thick (which became more evident in the Group 1) and arranged like a net. In Group 3 (over 65 years), the periosteum was thin, low in osteogenic cells, though displaying intense basophilia in the cementing line, reflecting increased synthesis of acid proteoglycans. Gradual replacement of the osteogenic layer of fibrous tissue, bone turnover imbalance and reduction of osteoblastic activity may be associated with the presence of mixed cellularity and decreased collagen matrix observed in these individuals[27]. In the cortical bone, there was a small amount of Haversian and Volkmann's channels and almost all osteoblasts were empty. Several authors have observed that the number of occupied osteoblasts falls from 95% at ten years of age to about 70% at 40 years[28-30]. Parfitt showed that the number of osteocytes significantly reduced with age, with decline in overall density and in the ratio of occupied gaps, particularly in the deeper layers of the cortical bone[30]. Ultimately, the osteocytes deficiency may contribute to the observed bone fragility of the elderly[31]. Finally, in the cancellous bone the trabeculae were thin, allowing ample medullary space, often occupied by fat cells and adipocytes.

Since the structure of the cancellous bone is determinant of bone strength, the adoption of procedures for quantitation of trabecular bone has been classically proposed[32-39]. Croucher et al. showed a strong correlation between different rates of assessment of bone structure, such as analysis of the ultrastructure, starring volume and pattern of bone trabecular factor[32]. Kubik et al. confirmed the value of such methods, especially in the description of age-related changes in the trabecular bone in individuals over 50 years[34]. Vesterby showed increased starry medullary space volume in the iliac crest and the first lumbar vertebra in ten human cadavers aged 27 to 87 years, suggesting that the reduction in trabecular bone occurs in all bony structures of the elderly[39]. In this experiment, we used the amount of trabecular meshwork (Vv) and the area of trabecular meshwork (Sv). We observed a statistically significant difference in Groups 1 and 2 with respect to Group 3 as for Vv, with reduction of 45% in the elderly, but not between Groups 1 and 2. There was no statistical difference between the groups regarding the Sv. The interpretation of these results clearly shows that the resorption of trabecular bone occurs with age, manifesting itself clinically by increased fracture risk in older individuals[40].

We can extrapolate theseresults for the quality of the bone tissue taken from the anterior iliac crest and its use as a graft in situations where there is a need for structural support (e.g. in tibial plateau or vertebral body fractures) or osteogenic stimulus (such as delayed union or avascular pseudarthrosis) in the elderly population. At least two of its fundamental properties, i.e., osteogenic and osteoconductive, are definitely committed in this age group. The reduction in the trabeculae thickness, the increase in the intertrabecular distance and the osteocytes numerical reduction potentially make the elderly patients' iliac bones less resistant and of low quality. Thus, we believe that the bone graft taken from the iliac crest should be avoided in the elderly, and other donor sources be considered. Papavero and Santin demonstrated that the removal of autologous bone graft from the distal third of the femur and proximal third of the tibia is a good option for these individuals[5]. Another good alternative is the use of the Reamer Irrigator Aspirator (RIA) system in the long bones of the lower limbs[41-43]. Henrich et al. showed that, compared with the graft from the iliac crest, the aspirate obtained from the femur using RIA has a higher concentration of CD34+ lineage osteogenerating cells and mesenchymal stem cells[43].

The findings of this experiment suggest that the osteogenic property of autologous bone graft decreases with age and is characterized by the reduction of cell population and function and nutritious and bone connecting tubules; the osteoconductive property is impaired in the elderly, since the graft ability to provide structural support is reduced; Osteoforming activity, although diminished, continues in the elderly; the removal of bone graft from the anterior iliac crest should be avoided in the elderly, and one should think of another donor source.

RESUMO

Objetivo: avaliar as características histológicas e morfométricas de biópsias ósseas da região anterior da crista ilíaca de pacientes de diferentes faixas etárias. **Métodos**: foram estudadas 30 amostras de osso da crista ilíaca, utilizando-se microscopia óptica de campo claro. As amostras foram divididas pela faixa etária dos doadores em três grupos: Grupo 1 (n = 10), indivíduos com idade entre 25 e 39 anos; Grupo 2 (n = 10), indivíduos com idade entre 40 e 64 anos; Grupo 3 (n = 10), indivíduos com idade igual ou superior a 65 anos. As amostras foram separadas aleatoriamente em dois conjuntos com 15 peças. No primeiro segmento do estudo (n = 15), foi avaliada histologicamente a propriedade osteogênica do enxerto, através da análise da reserva celular no periósteo, do número de osteócitos nas lacunas e da quantidade de canais de Havers e de Volkmann. No segundo segmento do estudo (n = 15), investigou-se morfologicamente a propriedade osteocondutora do enxerto, através da quantificação da rede trabecular (Vv) e da área trabecular (Sv). **Resultados**: histologicamente, observou-se que ocorre degeneração do tecido ósseo com a idade, caracterizada pelo adelgaçamento do periósteo, com substituição gradual da camada osteogênica por tecido fibroso, pequena quantidade de canais de Havers e de Volkmann, osteoplastos vazios e trabéculas finas de osso esponjoso, permitindo amplo espaço medular, em geral ocupado por células lipídicas e adipócitos. Morfologicamente, com relação à quantificação da rede trabecular (Vv), foi observada diferença estatisticamente significante entre os Grupos 1 e 3 e entre os Grupos 2 e 3, com redução da rede trabecular de cerca de 45% no idoso acima de 65 anos de idade; não foi observada diferença estatisticamente significante entre os Grupos 1 e 2. Não foi observada diferença estatística entre os grupos quanto à Sv. **Conclusão**: os achados do presente experimento sugerem que nos indivíduos idosos (acima de 65 anos de idade), a propriedade osteogênica do enxerto ósseo autólogo diminui e a propriedade osteocondutora está comprometida.

Descritores: Desenvolvimento Ósseo; Osso/biópsia; Osso/anatomia & histologia; Propriedades do osso; Ílio; Transplante ósseo.

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