Annals of Diagnostic Pathology 19 (2015) 409–413

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

Influence of AIDS in collagen deposition and thickness of the bone marrow☆,☆☆

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ARTICLE INFO

Keywords:
AIDS
Collagen
Bone marrow

ABSTRACT

Bone marrow abnormalities are frequently observed in individuals with AIDS. Dysplasia, the most common abnormality, is found in more than 50% of patients infected with the HIV. The aim of this study was to assess trabecular thickness and collagen content as well as cellularity in the bone marrow of patients with AIDS. Sixty bone marrow samples were collected from the sternum of autopsied patients with or without AIDS (n = 30, each). Cellularity and trabecular thickness was assessed by performing hematoxylin-eosin staining; picrosirius staining was used to evaluate collagen content. Morphometric analyses were performed by using a Zeiss KS300 system (Kontron-Zeiss). Patients with AIDS showed a significant reduction in trabecular bone thickness and an increase in collagen deposition. No statistically significant differences were observed in cellularity between the 2 groups. Therefore, reduced thickness and increased collagen deposition were observed in the trabeculae of the bone marrow of patients with AIDS due to possible interaction between cytokines and bone marrow components.

1. Introduction

The AIDS is a systemic disease with multiple complications. Bone marrow abnormalities are observed in all stages of HIV infection, and their frequency increases with disease progression [1]. Low bone mineral density and a decreased bone mass have been reported in male and female HIV-infected patients of several ages [2,3].

HIV infection may compromise hematopoiesis by directly infecting progenitor cells or by causing changes in the bone marrow microenvironment, which may affect its ability to support proliferation and differentiation of progenitor cells [4]. The investigation of the bone marrow healthy is important in patients with peripheral hematological abnormalities. Anemia is present in most of HIV infected symptomatic like normocytic normochromic anemia or anemia of chronic disease [5].

HIV is able to infect a variety of stromal elements of the bone marrow, including monocytes and macrophages, endothelial and myoepithelial cells, and fibroblasts [6–8]. Previous studies have shown that increased bone marrow fibrosis is commonly observed in patients with HIV/AIDS; however, the relationship between this deposition and CD4 T-lymphocyte counts remains controversial [9].

Osteoporosis was three-fold more prevalent in individuals infected with HIV compared to non-infected individuals. The chronic inflammation caused by the virus has been associated with bone resorption and osteoclast activity [3]. Moreover, anti-retroviral therapy has been correlated with changes in bone metabolism, which render patients more susceptible to osteopenia and osteoporosis [10].

Because histopathological studies of bone marrow from autopsied patients with AIDS are rare, the aim of this study was to analyze the trabecular thickness, percentage of collagen in the trabeculae, and bone marrow cellularity in autopsied patients with AIDS. The results of this study provide a scientific basis for understanding this microenvironment better, which will support the development of therapies to reduce bone changes during HIV infection.

2. Materials and methods

This study was approved by the Triângulo Mineiro Federal University Research Ethics Committee, approval number 850.

Sixty bone marrow samples were collected from the middle third of the sternum during autopsies performed at the Clinical Hospital at the Triângulo Mineiro Federal University, Uberaba, Minas Gerais, Brazil, being a retrospective study that occurred between 1983 and 2007.

☆ Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), and Fundação de Ensino e Pesquisa de Uberaba (FUNEPU)

☆☆ We would like to thank Aloísio Costa, Pedro Henrique Ramalho, and Liliane Silvano for their technical support.

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http://dx.doi.org/10.1016/j.anndiagpath.2015.10.003
1092-9134/

These data were collected by 2 pathologists of the General Pathology division of Clinical Hospital at the Triângulo Mineiro Federal University which is responsible for autopsies. From those reports were obtained data such as age, gender, skin color, and body mass index (BMI). The patients were divided into 2 groups: with AIDS (n = 30) or without AIDS (n = 30). The control group was selected with the attempt to pair age, gender, and race with the group of patients with AIDS.

AIDS diagnosis was confirmed by evidence of at least one AIDS-defining disease presented by the patient and by CD4 T lymphocyte count below 200 cells/mm^3 [11,12]. Inclusion criteria for AIDS patients were defined as those who owned at least one of the following defining diseases: invasive cervical cancer, extrapulmonary cryptococcosis, Herpes simplex mucocutaneous, or non-Hodgkin lymphoma [13]. All the patients with AIDS observed in this study died of infection during the post- HAART era. However, not all of them had made use of the therapy, which may be explained by the fact that many of them looked for health services in an advanced stage of the disease. Opportunistic infections were not considered in the selection of patients. There were no exclusion criteria.

Bone marrow fragments were subjected to histological processing, and serial 4-μm thick sections were cut. Cellularity (hypercellularity, normality, or hypocellularity) was assessed by observing hematoxylin-eosin (HE) stained slides under an objective lens with a magnification power of 5× (final magnification ×200). To evaluate trabecular thickness, slides stained with HE were observed under an objective lens with a magnification power of 20× (final magnification ×800); the thickness was expressed in micrometers (μm). To obtain these measures, each histological section was divided into 4 fields, and 5 trabeculae were randomly selected in each field. In each trabecula, 5 minor axis measurements (100 measurements per slide) were performed, determined by the accumulated mean method [14].

Picrosirius stained slides were used to quantify collagen expression in the trabecular bones. Histological sections were analyzed under polarized light with an objective lens having a magnification power of 5× (final magnification ×200). Collagen deposition, quantified by the observer, presented as birefringence with orange to red shading. Ten trabeculae from each of the 4 fields per histological section were analyzed, with a total of 40 measurements per slide. Collagen percentage and trabecular thickness were quantified by using a video camera coupled to a common light microscope and an image analysis system (KS300 – Kontron Zeiss). Taking these criteria into account, these measurements were made by a researcher individually.

Statistical analyses were performed by using the SigmaStat software suite. After applying the Kolmogorov-Smirnov test, the Student *t* test was used in case of normal distribution and similar variances; the Mann-Whitney *U* test (T) was used to analyze non-parametric distributions. Correlations were analyzed by using the Pearson (r) and Spearman (rS) tests. Differences in probability (*P*) lower than 5% ($P < .05$) were considered statistically significant.

3. Results

Sixty bone marrow samples were collected from autopsied patients. The average age of patients with or without AIDS was 31.86 ± 7.59 years and 48.73 ± 16.44 years, respectively, and the BMI of patients with and without AIDS was 20.43 ± 4.14 kg/m^2 and 25.21 ± 6.45 kg/m^2, respectively (Table 1). At the AIDS group, 7 patients were female (23.33%) and 23 were male (76.66%); 25 were white (83.33%) and 5 were nonwhite (16.66%). At the without-AIDS group, 11 patients were female (36.66%) and 19 were male (63.33%); 24 were white (80%) and 6 nonwhite (20%).

Patients with AIDS showed significantly reduced trabecular bone thickness (Table 1). A significant positive correlation was observed between the trabecular bone thickness and age of patients with AIDS ($rS = 0.0349$; $P = .006$), and a significant negative correlation was observed between the trabecular thickness and BMI in the same patient group ($rS = -0.156$; $P < .001$).

The percentage of collagen fibers in the trabecular bone was significantly higher in patients with AIDS (Fig. 1, Table 1). A negative and statistically non-significant correlation was observed between the percentage of collagen fibers and the age of patients with AIDS ($rS = -0.0107$; $P = .600$), and a negative and statistically significant correlation was observed between collagen percentage and BMI in the same patient group ($rS = -0.024$; $P < .001$).

No significant differences the bone marrow cellularity of patients between the 2 groups (Fig. 2).

4. Study limitations

As this study was conducted using data collected from autopsy reports, many relevant information for the bone health it cannot be obtained, such as smoking, menopause, use of hormone therapy, use of antiretroviral therapy, CD4 count and viral load at the time of death, among other factors that possibly influence bone formation. A cross-sectional study conducted in São Paulo (Brazil), which included 4.332 women over 40 years who were treated in primary care service, observed the prevalence of osteoporosis (33%) and osteoporotic fractures (11.5%) in these patients [15]. Hypoestrogenism is considered an important risk factor for low bone mineral density and recent studies correlated the occurrence of osteoporosis with the time of menopause. The same reasoning applies to the use of hormone therapy for prevention of osteoporosis. The beneficial effects of estrogen therapy on the preservation of BMI and reduce the risk of fractures are well established in the literature [16]. A World Health Organization study indicated that smoking confers substantial risk for future fracture, regardless of a BMI [17]. Researchers have shown that individuals with low CD4 levels have low bone mineral density; antiretroviral therapy affects the bone structure as leads to a recovery of bone turnover [18].

5. Discussion

The changes of the bone marrow have been the subject of several studies in patients with HIV/AIDS. Its different constituents, such as trabecular bone, collagen fibers, and cellularity show up in the affected syndrome, as shown in Table 2.

In this study, patients with AIDS had significantly reduced trabecular bone thickness. Another study showed that the prevalence of osteopenia and osteoporosis is 3-fold higher in patients infected with

Table 1
Constitutional and morphometric data from bone marrow of autopsied patients with or without AIDS

Group	n (%)	Age[I] (years)	BMI[II] (kg/m^2)	Trabecular thickness[III] (μm)	Collagen fibers[IV](%)
		$X \pm$ SD or Med (Min – Max)			
With AIDS	30 (50)	31.86 ± 7.59	20.43 ± 4.14	71.04 ± 26.32	6.14 ± 3.36
Without AIDS	30 (50)	48.73 ± 16.44	25.21 ± 6.45	88.58 ± 28.77	4.73 ± 3.17
Total	60 (100)				

[I] $t = 5.100$; $P < .0001$.
[II] $t = 3.474$; $P = .001$.
[III] $t = 3328000$; $P < .0001$.
[IV] $t = 507400$; $P < .0001$.