

# LIPOFUSCIN, A MARKER OF CELLULAR AGING IN MYOCARDIUM YOUNG PATIENTS AFFECTED BY HIV / AIDS

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## ABSTRACT

Infection with human immunodeficiency virus – HIV is characterized by acquired immunosuppression predisposing the patient to opportunistic infections and malignancies that can affect most organs.

Heart, which initially seemed to escape from this rule, it was showed first necrotic, and then clinical and echocardiographic that is affected from the first phase of HIV infection, when patients do not have clinical symptoms, and the disease was revealed only by positive serological tests.

The functions of all vital organs begin to decline as aging. Age-related changes occur in all cells, tissues and organs, and they affect the functioning of all systems of the body.

With aging, the cells become larger, with less capacity to divide, increasing the amount of pigment, and fatty substances (lipids) within them. Many cells are losing their ability to function, others begin to function abnormally.

Metabolism products accumulate in tissues progressively with aging. A brown pigment called lipofuscin accumulates in many types of tissues together with fat substances.

**Key words:** pigment of wear, aging pigment, lipofuscin, HIV

## INTRODUCTION

HIV infection in the world is expanding impressively smooth. So far, various treatments adopted failed to extend satisfactory the life expectancy of those infected, even if treatment possibilities have been diversified concerning opportunistic infections.

Clinical and pathological determinations of respiratory, gastrointestinal, hematological and nervous system are well characterized in HIV infection. Pathological and clinical aspects affecting the cardiovascular system are being evaluated.

Experimental clinical and anatomic-pathological trials have shown that all heart structures are affected, showing various structural and functional cardiac changes in relation to the progression and severity of disease and risk of death caused by heart failure.

In Romania the first adults case of AIDS in was reported in 1985, and child case in 1989. Systematic investigations concerning patients with HIV/AIDS began only after 1990. With the 5.629 AIDS cases in children up 31.12.2000 – representing more than half of all pediatric cases in Europe, Romania was classified with a special position, because most children were infected during the 1987-1990, especially parenteral, caused by improperly sterilized medical and surgical devices, HIV contaminated.

The measures introduced since 1990, pediatric infection appears to be under control. After 1995 there were further cases of nosocomial infection. In contrast, in adults, infection is worrying upward slope. Ministry of Health identified the priorities concerning this major health issue, consequently the National AIDS Committee was responsible on developing National Anti-AIDS Health Program 1998-2000. (2)

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There are no trails concerning the relationship between HIV/AIDS infection and the heart involvement, on adults, in Romania.

Heart disease or cardiac involvement as a first sign of HIV infection is rarely reported. Obvious, echocardiographic and anatomic-pathological features are more common than clinical signs.

The main reason that led to the choice of this topic is based on real need to obtain data from HIV-infected adult patients regarding the prevalence of cardiac damage in Romania.

## OBJECTIVES

Impact of appearance of lipofuscin-cell aging marker in myocardium of young patients who died from HIV / AIDS.

## METHODS

Pathological changes in cardiac tissue were performed on deceased patients with HIV infection on the Hospital for Infectious Diseases and Tropical Diseases "Dr. Victor Babes", in Bucharest, during 1999-2000.

Includes macroscopic and microscopic anatomic-pathological study made by anatomic-pathological evaluation of 16 fragments of myocardium, post mortem collected at random free from the 16 patients died from HIV infection during 1999-2000.

## RESULTS AND DISCUSSIONS

There were 16 myocardial fragments, histopathologically analyzed, randomly collected from patients who died with HIV infection in 1999-2000 at the Hospital Clinic of Infectious and Tropical Diseases „Dr. V. Babes“, Bucharest.

The quality of is determinant for histopathological diagnosis of cardiac lesions.

The first and most important moment of the histological methods was the histopathological biopsy.

Most important is the rapid sampling to avoid the autolysis. The sampling was made by smooth and sharp devices in order to avoid the crash and damage of the tissues.

The samples were various, representative. The targeted tissues had to respect the following criteria: macroscopic changes of the tissue, tissue located on the border, and cardiac normal tissue.

The collected fragments were less than 1 cm, with smooth and parallel faces. Drying or crushing

was avoided myocardial fragments, quickly fixed in 10% formalin. Formalin fixing goal was to stop the development of cells and tissues in a most close functional status by stopping autolysis.

Formaldehyde fixing mechanism is based on its combination with protein molecules in order to form insoluble macromolecules liquid combination used in histological technique. For this reason, cardiac tissue becomes tough and elastic and can be easily cut. The volume of fixative (formalin 10%) should be 50 times over the volume of tissue fragment at room temperature, within 24 hours.

The proper fixation of the myocardium fragments was followed by their inclusion in paraffin, in order to achieve a proper consistency of the samples to be cut and allow the application of staining techniques, in order to keeping them.

Are required for inclusion in paraffin following stroke: dehydration of the play, clearance, and actual including:

Alcohol cannot be mixed with paraffin it is necessary the clearance procedure, consisting by toluene usage during 24 hours.

Dehydration procedure uses ethylic alcohol, meaning the alcohol passage of the sample. During the procedure, the alcohol concentration increases gradually, until the water is replaced by pure alcohol.

After dehydration and clearance, pieces imbued with toluene were transferred into paraffin baths containing liquid paraffin at a temperature of 54°.

After paraffin inclusion, the pieces were mounted in paraffin blocks, in order to be subsequently sectioned. Sections of 3-6 microns were attached to slides.

The next step was dewaxing and hydration of the sections in order to histological staining.

Special stains were performed for evidence of fibrosis and pathogens (fungi, Koch bacillus, bacteria) present in cardiac tissue.

Stains used were:

- Haematoxylin eosin stain: original was used because it allows an efficient nuclei staining with haematoxylin
- Lie stain
- Ziehl-Nielsen stain
- PAS stain
- van Gieson staining

Histological diagnosis of active or borderline myocarditis was defined according to the Dallas criteria; lymphocyte inflammatory infiltrate was considered present in more than 10 lymphocytes per microscope field (magnification x 400).

Anatomo-pathological processing material harvested from heart of 16 patients deceased with HIV infection conducted in:

- Hospital Pathology Laboratory of Infectious Diseases and Tropical Diseases “Dr. V. Babes” Bucharest
- Pathology Laboratory of Hospital „Th. Burghel“ Bucharest

- National Research – Development of Pathology and Biomedical Sciences; “Dr. V. Babes” Bucharest

Demographic characteristics and anatomopathological diagnosis of the study group (16 people) post-mortem heart fragments samples are shown in Table 1.

**Table 1.** Clinical aspects and anatomopathological diagnosis

Nr.	Name	Gender	Age (years)	Macroscopic diagnose	Microscopic diagnose
1	I-V	M	38	-	Subendocardium fibrosis (+) with recent necrosis areas. Interstitial oedema. Rare microbial colonies. Regenerative type nucleus. Hyperaemia and interstitial oedema.
2	G-P	M	52	Dilatative cardiomyopathy, left ventricle hypertrophy	Diffuse interstitial fibrosis (+++) and arteriosclerosis with narrowing of arteriolar lumen eccentric Regenerative type nucleus.
3	M-M	M	40	Dilatative cardiomyopathy	Diffuse interstitial fibrosis (++) . Arteriosclerosis with narrowing of arteriolar lumen (~30% remaining).
4	C-R	F	36	Dilatative cardiomyopathy	Diffuse interstitial fibrosis (+) and microvascular fibrosis, <b>lipofuscin in myocardium fiber (++)</b>
5	T-V	M	34	Miocarditis Serosus pericarditis	Marked hyperaemia and the presence of red blood cells in rolls. Parietal thrombus with micelles. Without myocardial fibrosis.
6	V-E	F	53	Myocardium dystrophy	Discreet interstitial fibrosis(+). Hyperaemia (+). Large, hiperchrom nucleus, (regenerative aspect). <b>Rich pigment of lipofuscin (+++)</b> . Cryptococcus.
7	C-I	M	39	Left ventricle hypertrophy. Serosus pericarditis	Interstitial moderate fibrosis (+ +) without vascular narrowing. Moderate hyperaemia (++) . Regenerative nuclei. <b>Lipofuscin pigment (+++)</b> .
8	D-C	M	65	-	Massive diffuse interstitial fibrosis (+++), without narrowing the lumen. Discreet lymphoid interstitial inflammatory infiltrate(+). Discrete hyperaemia (+). Large, very numerous hiperchromi nucleus, large. Atrophy of muscle fibers.
9	D-O	F	52	-	Moderate fibrosis (+ +). discreet hyperaemia (+). <b>Lipofuscin (++)</b> . Discrete interstitial inflammatory infiltrate (lymphoid). Cryptococcus. Regenerative nuclei
10	I-C	F	28	-	Fibrosis (+++) with narrowing of the vascular wall. <b>Lipofuscin (+)</b> . Discreet lymphoid interstitial inflammatory infiltrate (+) Regenerative nuclei
11	I-I	F	36	Metastasis in myocardium. Left ventricle hypertrophy	Marked fibrosis without narrowing (+++). Important swelling with narrowing of the fibers; rare hypertrophic nuclei. Rare inflammatory elements. Important hyperaemia. Many hypertrophic nuclei. Fungal thrombus.
12	D-C	M	35	-	Moderate fibrosis (+ +) without vascular damage. Moderate oedema. <b>Lipofuscin (++)</b> ; rare inflammatory elements. Marked hyperaemia. Many hypertrophic nuclei. Fungal thrombus.
13	C-M	F	31	Haemorrhage pericarditis	Fibrosis (+) without vascular damage. Edema and moderate hyperemia. <b>Lipofuscin (++)</b> ; discrete chronic inflammation. Parietal thrombus fungal and fungal interstitial deposits.
14	G-F	M	18	Dilatative cardiomyopathy	Reduced fibrosis (+) without vascular damage. Important oedema. Moderate hyperaemia. <b>Lipofuscin (++)</b> . Moderate chronic inflammation. Small deposits of small venous vessels such fungi. Fibrin thrombi in small vessels.

Nr.	Name	Gender	Age (years)	Macroscopic diagnose	Microscopic diagnose
15	S-E	F	35	Dilatative cardiomiopathy	Fibrosis (-). Marked hyperaemia and oedema. Chronic subepicardial inflammation with fungi. Vasa vasorum thrombosis, relatively frequent hypertrophic nuclei.
16	F-M	F	35	-	Fibrosis (++) Hyperaemia. Moderate interstitial inflammatory infiltrate. Koch bacillus.

Macroscopic pathological changes were present on 10 cases (table 1). The average age is 39.18 years, 8 cases are men and 8 cases are women.

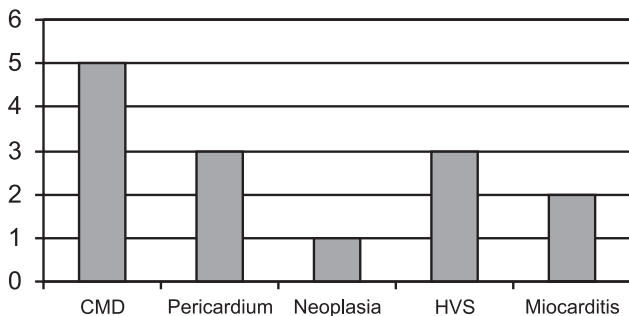


Figure 1. Macroscopic lesions

**The macroscopic described lesions**

Dilatative cardiomyopathy present in 5 cases; 4 cases of dilated cardiomyopathy associated with fibrosis in various degrees.

Pericarditis 3 cases, 1 case pericarditis is associated with myocarditis. Pericardial effusion in low quantity – serocitrin aspect (2 cases) – haemorrhagic aspect (1 case).

Neoplasia: 1 case of cardiac metastasis – 1 case with hypertrophy and left ventricular dilatation.

**The microscopic histopathological lesions**

Present in all 16 cases (100%), including cases in which macroscopically was not described any heart lesion (Figure 2).

Lanjewar (1) and collaborators are describing histopathological changes in 92.30% of subjects. Thus:

- lymphocytic myocarditis is present at 55.76%
- myocardial fibrosis is present at 13.46%
- Cryptococcus neoformans is identified at 3.84%.

The histopatological lesions of our trial are presented in Figure 2.

Thus:

**Pigment-lipofuscin wear** – is described as being present in 8 cases (50%). Pigment wear in all these cases is described as being present in high quantities. 6 of the 8 cases showing lipofuscin are aged between 18-36 years.

**Myocardial fibrosis** in varying degrees-formation is detected in a total of 14 cases (87.5%). Depending on the degree of fibrosis, is described:

- reduced fibrosis, described in 5 cases
- moderate fibrosis described in 5 cases
- pronounced fibrosis described in 4 cases
- **Myocarditis lymphocytic interstitial** described in 3 cases (18.75%).
- **Interstitial oedema** is present in 7 cases (43.75%)
- **Large nuclei**, hypertrophic hiperchromi with regenerative aspect are present in 9 cases.
- **Candida albicans** is described to histopathological in a total of 7 cases (43.75%)
- **Cryptococcus neoformans** is described in 1 case (6.25%)

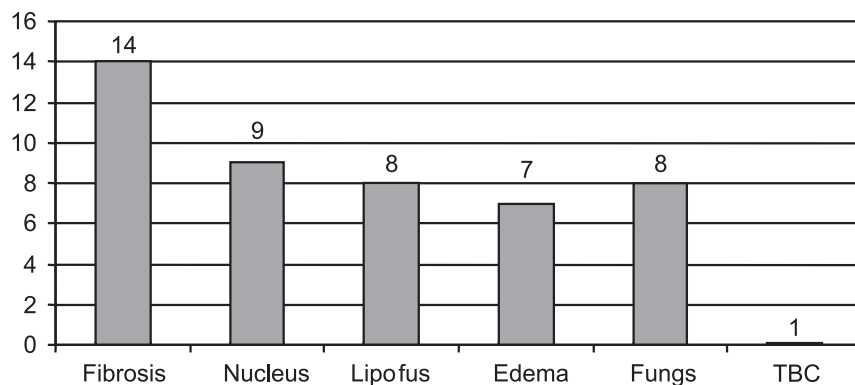
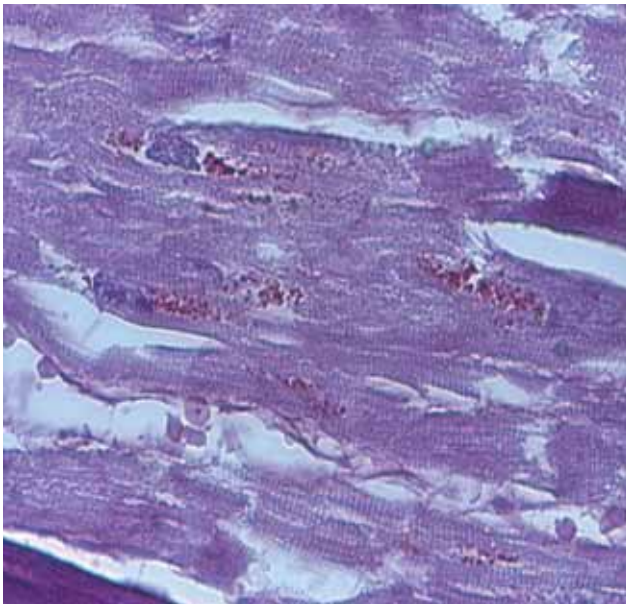


Figure 2. Histopathological lesions

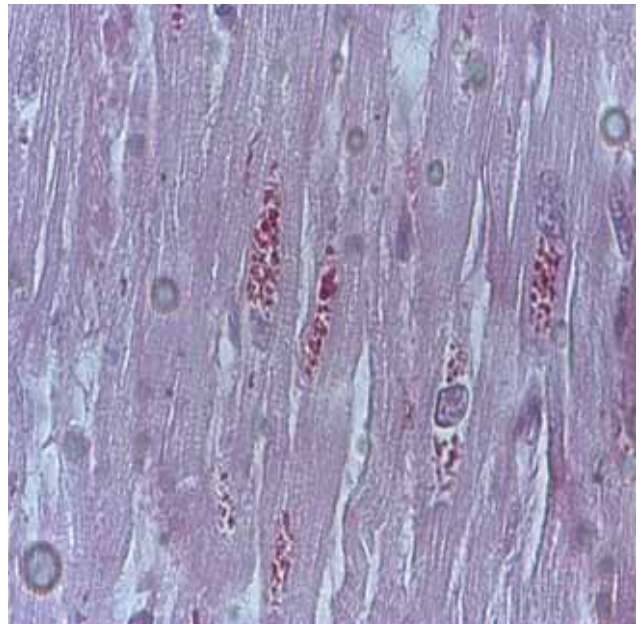
- **Myocardial tuberculosis**, Koch bacillus, is viewed histologically in 1 case (6.25%)
- **Atherosclerotic ischemic lesions** are present in 2 cases (12.5%)
- **Myocardial necrosis** is present in 2 cases (12.5%).

We present some aspects of aging pigment – lipofuscin present in the myocardium of patients infected and deceased from HIV / AIDS at young ages. Images no. 4, 5, 6 reveal the presence of myocardial fiber sarcoplasm wear pigment – lipofuscin in high quantity.

Fragments were taken from a woman of 28 years.



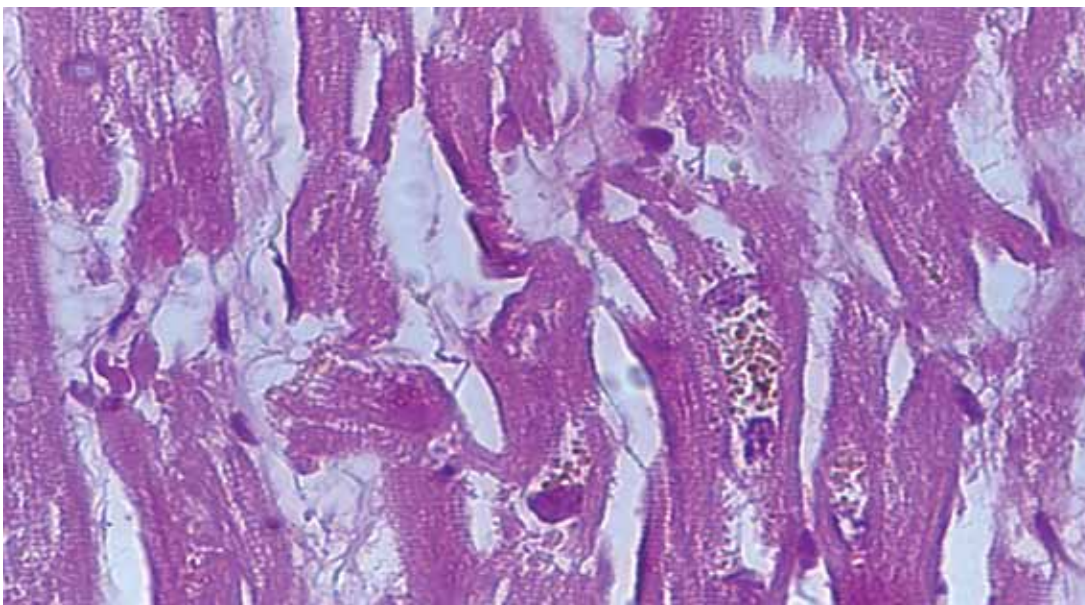
**Figure 3.** PAS coloration, OB 40X



**Figure 4.** PAS coloration, OB 20X

## CONCLUSIONS

We did not find this comment in the literature. Support the assumption that human immunodeficiency virus – HIV is that which would cause wear pigment accumulation in myocardial fibers of young patients (3-5) where normally there should not be. Currently it is known (8) that lipofuscin accumulation in the nervous system results in a functional and structural damage to neurons. This seems to be true for the formation and accumulation of lipofuscin deposits in myocardis at the young ages.



**Figure 5.** PAS coloration Ob 40X High levels of lipofuscin in miocardic fibers

The result is a functional and structural damage of myocytes. (6,7).

It is likely that the accumulation of lipofuscin pigment in myocardial fibers is harmful phenomenon. It raises the suspicion that human immunodeficiency virus – HIV is the pigment that determines the accumulation of wear in myocardial fibers. (6,7). In the literature we found no references or joint regarding on the presence of pigment in the myocytes of the patients deceased by HIV infection.

It seems that HIV could induct lipofuscin accumulation and formation of deposits in myocardial fibers.

The presence of lipofuscin in myocardium cells suggests the direct action of myocardial infection with HIV virus and multiple extrinsic factors (inflammation, catecholamine, and adrenergic sympathetic activity), myocardial cells undergo autophagy and apoptosis involved in ventricular remodeling and heart failure development.

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