Ultrasound and histopathological features of myocardial involvement in HIV infection in children

ANCA MEDA GEORGESCU1), COSMIN MOLDOVAN2), LEONARD AZAMFIREI3), DAN GEORGESCU4)

1) Department of Infectious Diseases, University of Medicine and Pharmacy of Tirgu Mures, Romania
2) Department of Histology, University of Medicine and Pharmacy of Tirgu Mures, Romania
3) Department of Anesthesiology and Intensive Care, University of Medicine and Pharmacy of Tirgu Mures, Romania
4) Department of Internal Medicine, University of Medicine and Pharmacy of Tirgu Mures, Romania

Abstract

Aim: HIV infection in children is an important clinical and pathologic entity, which embraces many forms of presentation and can involve multiple organs and systems. This study aimed at identifying the main forms of cardiovascular involvement in HIV-infected children with horizontally transmitted disease and describing them with the aid of ultrasound and histopathological examinations. Results: We recorded cardiovascular anomalies in 79 (67.52%) patients out of the 117 comprised in the study population, and noted the following prevalence distribution: systolic dysfunction in 49 (41.88%) patients, left ventricular hypertrophy (LVH) in 30 (25.6%) patients, right ventricular hypertrophy (RVH) in 15 (12.82%) patients, and dilated cardiomyopathy (DCM) in 22 (18.8%) patients. We also carried out post-mortem histopathological examinations in five patients, and observed the main modification incurred by the disease. Conclusions: Cardiac involvement during HIV infection differs significantly in different mechanisms of virus transmission, and the horizontal transmission of HIV yields a lower prevalence of this type of pathology. The general diagnostic picture can be significantly improved by adding histopathological examination to the ultrasonographic method of investigation.

Keywords: HIV infection, horizontal transmission, cardiovascular involvement, ultrasound, histopathology.

Introduction

Cardiac involvement has been recognized and considered to be significant and to have an impact on patient morbidity and mortality even since the human immunodeficiency (HIV) virus infection was first described in the 1980’s. During that period, the prevalence of cardiovascular involvement was estimated to be 6–7% of all patients [1, 2].

The advent of highly active antiretroviral therapy (HAART) turned HIV infection into a curable, chronic disease. One of the consequences of its introduction was the alteration of the cardiovascular involvement spectrum, by reducing the incidence of dilated cardiomyopathy (DMC) and pericarditis and, consequently, bringing about a global reduction of its prevalence [3, 4]. On the other hand, cardiovascular morbidity remains relatively high due to the emergence of a pathology associated with metabolic disorders caused by HAART. Also, the more exhaustive the exploration of the cardiovascular system is, the higher the proved morbidity, which in certain studies reaches values of up to 93% [5].

The pathogenetic factors of cardiovascular involvement differ from the classic cardiovascular risk factors, and include the HIV infection, the opportunistic infections and the immunological disorders caused by them. Thus, cardiovascular involvement appears at any age, including HIV-infected children and teenagers [6, 7].

The main goal of this study was to assess pediatric cardiovascular involvement in horizontally transmitted HIV infection by using cardiac ultrasound examinations and by studying histopathological alterations.

Materials and Methods

We carried out a prospective longitudinal cohort study over five years in the Infectious Disease Clinic I, which is the Regional Center for Monitoring HIV infection for the Mureș, Sibiu, Bistrița-Năsăud and Alba Counties, Romania.

The study population comprised children with HIV infection monitored by the aforementioned Center. The inclusion criteria were the presence of HIV infection, horizontal transmission and age under 16 years. The exclusion criteria were the presence of congenital cardiac malformations, vertical transmission of the HIV infection and lack of cooperation for ultrasound examination during the study. After applying these criteria, the study included 117 patients who were examined by ultrasound and monitored over the five-year period.

The diagnosis of the HIV infection was made based upon two-second generation ELISA tests for HIV1 and HIV2 antibodies and confirmed by Western Blot. Viral load was assessed at six-month intervals by Polymerase Chain Reaction (PCR) tests. Clinical and immunological staging, which was needed in order to stratify the study population and assess the correlations between the severity of cardiac involvement and the stage of the HIV infection, was made using the 1993 CDC Atlanta criteria [8]. In order to assess the histological features of cardiac involvement, samples of cardiac muscle tissue were obtained during autopsy, fixed in formalin, embedded in paraffin, sectioned and stained with Hematoxylin and Eosin (HE), following a standard protocol. Histological examination was only carried out in five deceased patients.
who displayed significant cardiac alterations. For technical and ethical reasons, we did not perform endomyocardial biopsies.

Ultrasound examination was always carried out in M (mono-dimensional) and 2D (bi-dimensional) modes. Since our study population consisted of children and was also characterized by an increased prevalence of an important weight deficit, the data obtained were classified as normal or pathologic by comparing them with normal values adjusted for body weight, according to data published by Feigenbaum [9, 10]. Statistical analysis was done with Microsoft Office Excel and GraphPad InStat 2003. A $p$-value $<0.05$ was considered statistically significant.

## Results

Over the duration of the study, ultrasound examination anomalies were recorded in 79 (67.52%) patients, and had the following prevalence distribution: systolic dysfunction in 49 (41.88%) patients, left ventricular hypertrophy (LVH) in 30 (25.6%) patients, right ventricular hypertrophy (RVH) in 15 (12.82%) patients, dilated cardiomyopathy (DCM) in 22 (18.8%) patients, pulmonary hypertension in 43 (36.75%) patients and pericardial collections in four (3.42%) patients. Thirty-eight (32.48%) patients had normal ultrasound features over the entire period of the study, and they were designated as control group.

[I]. We diagnosed left ventricular dysfunction in 24 (16.43%) children. The mean value of the ejection fraction was 42.79±2.31%, compared to 69.88±2.63% in the control group ($p=0.0001$).

The mean age was 15.12±0.55 years. The disorder was more frequent in boys (62.5% vs. 37.5% in girls, $p<0.05$). The clinical stage of the HIV infection was significantly more advanced than in the control group ($p=0.049$): 29.16% of the children were in stage B and 70.83% in stage C. The immunological stage of the HIV infection did not differ significantly from the control group.

The thickness of the walls of the left ventricle displayed important differences between the two groups. The diastolic thickness of the inter-ventricular septum was increased when compared to the systolic one (1.21±0.11 vs. 0.98±0.04, $p=0.0011$). Statistically significant differences between the two groups also appeared in the systolic dimensions of the inter-ventricular septum (1.44±0.07 vs. 1.27±0.06, $p=0.0017$), the diastolic dimensions of the posterior wall of the left ventricle (1.16±0.12 vs. 0.89±0.05, $p=0.0004$) and, respectively, the systolic dimensions of the same wall (1.46±0.1 vs. 1.31±0.1, $p=0.0005$). Data reveals a high prevalence of left ventricular hypertrophy in patients with systolic dysfunction (75%).

The mean value of the mass of the left ventricle was significantly higher in the study group compared to the control group (194.43±34.82 g vs. 143.70±27.51 g, $p=0.029$). It is worth noting that we did not encounter patients with left ventricular hypertrophy who would not display left ventricular dysfunction over the five-year duration of the study.

The morphological background of myocardial hypertrophy and contractile dysfunction was revealed by autopsy studies, which allowed for microscopic examination of the myocardium. On microscopic examination, a hypertrophy of the myocardial cells was apparent, along with disorganized architecture, interstitial edema and a lymphocyte/plasma cell infiltrate (Figure 1).

In some cases, the microscopic appearance is quasinormal, but a careful examination reveals a sparse lymphocytic inflammatory infiltrate, minimal degenerative lesions of the myocardial cells in the proximity of the intercalated discs, which were slightly erased (Figure 2).

The inflammatory lesions are usually limited, but they can be very marked, with the presence of an abundant lymphocyte/plasma cell and polymorphonuclear infiltrate that dislocates myocardial cells and can lead to important degenerative lesions, disorganization of the myocardial architecture and can organize into secondary lymph follicles (Figure 3).

None of the patients with left ventricular dysfunction deceased during the study from cardiac causes. The two deaths recorded were due to cerebral (toxoplasmosis) and respiratory (bronchopneumonia) infectious complications.

The clinical stage of the HIV infection was very advanced in patients with DCM: 27.27% were in stage B and 72.72% in stage C, compared to the control group ($p=0.04$). The immunological stage of the HIV infection was also very advanced: 13.63% of the patients were in stage 2 and 86.36% in stage 3.

Left ventricular dilation was revealed in ultrasound examination by an increase of the systolic and diastolic diameters. Right ventricular dilation was assessed by evaluating its diastolic diameter. After confronting the data we obtained to body weight in order to establish whether they were normal or pathologic, patients with DCM had mean diastolic (5.29±0.16 cm) and systolic (4.2±0.2 cm) left ventricular diameter values that were much higher than normal (3.5–5 cm and 2.5–4 cm, respectively) [9, 10]. Similarly, the diastolic diameter of the right ventricle had higher values in the study group compared to the control group (2.58±0.2 cm vs. 1.78±0.12 cm, $p<0.0001$).

The characteristic ultrasound appearance was that of bi-ventricular dilation, in 81.81% of the cases ($n=18$) (Figure 4). Only four of the patients had isolated left ventricular dilation, as these patients were in the incipient stages of DCM, with only a moderate decrease of the ejection fraction.

We also noticed the dilation of the atria in the group with DCM; the mean values of the diameters of the left and right atrium were significantly increased compared to the control group (3.31±0.43 cm vs. 2.81±0.21 cm, $p=0.05$, respectively 4.23±0.3 cm vs. 3.02±0.29 cm, $p<0.0001$).

Ventricular dilation led to important valve alterations and bicuspid, tricuspid and pulmonary valve insufficiency were recorded in percentages that were significantly
higher than in the control group ($p<0.0001$). We did not encounter aortic valve anomalies, as the prevalence of aortic valve insufficiency was not significantly different between the two groups (18.18% vs. 10.52%, $p=0.448$).

Figure 1 – Myocardium, cross-section: interstitial edema, lymphocyte/plasma cell inflammatory infiltrate, hypertrophy, myocardial architecture alteration. HE staining, 200×.

Figure 2 – Myocardium, longitudinal section: slight interstitial edema, vacuolar degenerative lesions of myocardial cells, minimal lymphocytic inflammatory infiltrate. HE staining, 200×.

Figure 3 – Myocardium, longitudinal section: abundant lymphocyte/plasma cell inflammatory infiltrate with lymphoid appearance, myocardial architecture alteration, degenerative lesions. HE staining, 40×.

Figure 4 – Ultrasound examination: dilated cardiomyopathy: important dilation of the four cardiac cavities.

We underline the fact that patients with DCM did not display left ventricular hypertrophy, which was very frequent in patients with systolic dysfunction.

The functional parameters of the left ventricle were severely altered in this group: ejection fraction values were low, and 77.27% of the patients had moderate or severe ventricular dysfunction (EF<49%) ($p<0.0001$); the values of the shortening fraction were below normal in all patients (19.29±3.21% vs. 33.7±2.11%) ($p<0.0001$).

The morphological background of the dilated cardiomyopathy was assessed in autopsy studies.

Grossly, the cardiac cavities were enlarged and the total weight of the heart was increased. Microscopic examination revealed important changes in myocardial architecture, degenerative lesions of the myocardial cells, interstitial edema and polymorphonuclear inflammatory infiltrate (Figure 5).

In some situations, the interstitial edema was very marked ad induced important alteration of the architecture of myocardial cells (Figures 6 and 7).

Microscopic analysis of the inflammatory infiltrate revealed mostly lymphocyte and plasma cells, accompanied by several polymorphonuclears, especially eosinophils (Figure 8).

The inflammatory infiltrate was abundant at times and had a tendency to constitute lymph follicles (Figure 8).

The inflammatory infiltrate invaded myocardial cells, which undergo necrosis and fragmentation, and fibroblasts and interstitial fibrosis appear (Figure 9).

Coronary vasculitis alterations may play an important role in the pathogenesis of cardiac involvement in HIV infection. Microscopic examination revealed a mixed inflammatory infiltrate along with pericoronary fibrinoid necrosis (Figure 10).

Histological data indicate more than just a simple “passage” of the inflammatory cells through the cardiac vessels towards the myocardium, but veritable phenomena of vasculitis, with endothelial lesions and fibrinoid necrosis in the wall of the blood vessel (Figure 11).

Occasionally, the inflammatory infiltrate contained a
high number of neutrophils and eosinophils, suggesting the acute nature of the inflammatory process (Figure 12).

We consider that it is worth noting the hypertrophic alterations of the tunica media, appearing especially in the small coronary arteries (Figure 13). This hypertrophy may cause ischemic disorders and is most likely the consequence of the action of inflammation mediators and inflammatory viral proteins.

The general mortality associated with DCM was 18.18% over the five-year duration of the study and determined the global cardiac mortality within the study, representing 2.05% of the entire study population.

Figure 5 – Myocardial architecture alteration, interstitial edema, lymphocyte/plasma cell inflammatory infiltrate. HE staining, 100×.

Figure 6 – Important interstitial edema with myocardial cell dissociation. HE staining, 20×.

Figure 7 – Important interstitial edema, inflammatory infiltrate dislocating myocardial cells. HE staining, 200×.

Figure 8 – Abundant myocardial inflammatory infiltrate consisting of small and large lymphocytes, plasma cells, histiocytes, few eosinophils. HE staining, 200×.

Figure 9 – The inflammatory infiltrate destroys myocardial cells, which are very fragmented; free nuclei can be seen; some of the myocardial cells retain their myofibrils. HE staining, 200×.

Figure 10 – Two coronary arterioles in longitudinal and transversal sections, with an abundant perivascular inflammatory infiltrate. HE staining, 100×.
Discussion

The study population has some epidemiological characteristics that render it unique and justify some of the differences between our results and data published in the literature. The patients belong to a pediatric “cohort” with horizontal transmission of the HIV infection, which was acquired at the end of the 1980’s following an epidemiologic accident. Another particularity is that this population was infected with the F1 subtype of HIV, which represents less than 1% of the circulating strains worldwide [11–14].

Ultrasound cardiac examination in children with HIV infection reveals pathological aspects in 67.52% of cases. This elevated prevalence of cardiac anomalies had been previously suggested by a radiological study carried out on the same population, but that method has the disadvantage of low sensitivity and specificity [15–17]; thus, ultrasound examination was the “gold standard” in diagnosing morphological and functional cardiac modifications. Data published in the literature confirms the central role of ultrasound examination in the cardiologic evaluation of HIV-infected patients [18–20].

The analysis of the morphological parameters of the left ventricle indicates an important prevalence of ventricular hypertrophy in patients with systolic dysfunction from the study group (75%). The data are similar to those in the literature. Shah et al. found a 38.5% prevalence of left ventricular hypertrophy in a group of 26 children [21], Lipschultz et al. 48.38% in 31 children [18, 19], and the P2C2 (Pediatric Pulmonary and Cardiac Complications in HIV) study reported a prevalence of 29% [22]. The differences among the data published can be explained by the different characteristics of the study groups and the variable accuracy of the measurements [18, 22, 23].

Comparative analysis of the left ventricular mass in patients with systolic dysfunction indicates a statistically significant increase compared to the control group (p=0.029), which is concordant with the P2C2 study [24]; however, this study included both children with left ventricular systolic dysfunction and children with DCM, whereas our study analyzed these two categories separately.

Dilated cardiomyopathy had a prevalence of 18.8% in our study group. Data from other studies indicate similar percentages: Diógenes et al., 12.2% in a group of children perinatally exposed to HIV infection [25], Miller et al., 29% in teenagers with vertically acquired HIV infection [26], Okoromah et al., 33.7% in children with vertically acquired HIV infection [27], Shah et al. 38.5% [21] and, respectively, Singh et al., only 2% in a group of children between one and 18-year-old [28]. The highest values of the pre-valence of dilative alterations are published in studies that assess uni- or bi-cameral dilation isolation, reaching values of 85% in a pediatric group [29]. The P2C2 study reported a 17.15% prevalence of DCM [22].

We could not find hypertrophic alterations in the group with DCM. During our study, none of the patients with hypertrophic alterations and systolic dysfunction evolved towards cardiac dilation, indicating different and independent pathogenetic mechanisms for dilative pathology. We consider that the first alteration was dilatation and contractile insufficiency. It is likely that cardiac dilatation renders hypertrophic alterations undetectable by ultrasound examination; the P2C2 study pleads for the same pathogenetic hypothesis, indicating a decrease in thickness of initially hypertrophic cardiac walls, as the alterations progress towards cardiac dilatation and cardiac insufficiency [22, 24].
The hemodynamic consequences of DCM were severe and determined a high mortality in the study group (18.18%). This value is lower than 52.5%, as reported by the P2C2 study over five years in a pediatric group with DCM [22]. The relative risk of death in seropositive children with DCM is 2.8, compared to children without cardiovascular disorders [6].

Morphological alterations of the right ventricle were dilative, encountered in 81.18% of the children with DCM, who had bi-ventricular modifications. Other studies analyzing right ventricular dilation as an isolated alteration indicate prevalences between 44%, as uni-camer d dilation, and 7% within the context of bi-ventricular dilation [29].

Myocarditis and consecutive DCM characterized by left ventricular dysfunction are the dominant manifestation of cardiovascular involvement in HIV infection, ultrasound studies carried out prior to the introduction of HAART indicating a global prevalence of the latter of 30% [6, 30, 31]. The largest study concerning cardiovascular involvement in vertically HIV-infected children, P2C2, reports a cumulative incidence of cardiomypathy of 28% over five years. This study found decrease in contractility in 42%, cardiomegaly in 13.7%, increase of the left ventricular afterload in 20%, dilatedcardiomyopathy in 10%, left ventricular hypertrophy in 25%, and congestive heart insuficiency in 7% of the children over a five-year period. The relative risk of cardiac death was 8.5–14.6 times higher than in the control group; it was higher in higher stages of HIV infection [22, 31].

Cumulative mortality during our study was 2.05%, much lower than reported in other studies. In the P2C2 study, the mortality was higher, reaching 75% over five years in children with important left ventricular hypertrophy [24].

The gross appearance of the heart in DCM shows atrial and ventricular dilation with a certain degree of left ventricular hypertrophy, which was not seen in ultrasonography in our study. On cross section, minimal myocardial fibrosis is found and the myocardium has several paler areas. The myocardium is flaccid at palpation. Occasionally, intracavitary thrombi are present, either free or adherent to the walls. In myocarditis without dilation, the weight of the heart is normal, the consistency is less flaccid and gross modifications are minimal [32, 33].

Microscopically, inflammatory and degenerative myocardial lesions are seen, without particularities suggestive for HIV etiology. Myocardial cells may display varying degrees of hypertrophy, enlarged hyperchromatic nuclei, diffuse or focal vacular degeneration, myxoid degeneration, interstitial edema, and diffuse and interstitial fibrosis. Myocardial inflammatory alterations are frequent but moderate, and are represented by a focal or diffuse lymphocyte/plasma cell infiltrate that can surround bundles of myocardial cells, composed mostly of CD8 T-lymphocytes. In numerous cases published in the literature there was no myocardial inflammatory infiltrate present. Occasionally, the number of cardiac dendritic cells increases [20, 32]. Half of the patients display lipofuscin deposits, a marker of cell aging [34].

The conduction system is often invaded by lymphocyte/plasma cell infiltrate and fibrosis can be present. At this level, calcifications and vacuolated Purkinje cells can be observed. Thrombosis and cell infiltrates in the vessels that supply the conduction system are also frequent.

The morphological background of cardiac involvement consists of myocardial inflammatory infiltrate, inflammatory and dystrophic lesions of the myocardial cells, cardiac hypertrophy, modifications of the architecture of the myocardium, modifications encountered in our study, which are concordant with data in the literature [33–35]. Approximately 50% of the patients who die of HIV infection display modifications that are characteristic for myocarditis at autopsy [36].

Conclusions

The systolic dysfunction of the left ventricle has a morphological background that can be observed early by ultrasound examination. Ultrasonography provides information on the nature and the extent of the cardiovascular involvement in HIV-infected children. Cardiac involvement during HIV infection differs significantly in different mechanisms of virus transmission, and the horizontal transmission of HIV yields a lower prevalence of this type of pathology. The general diagnostic picture can be significantly improved by adding histopathological examination to the ultrasonographic method of investigation.

Acknowledgments

The authors express their gratitude to Dr. Carmen Caraşca and Prof. Dr. Anca Sin who contributed to this study by conducting and interpreting the histological examinations and also to Assoc. Prof. Dr. Amalia Făgărăşan for performing echocardiographic examination.

References


