Int J Legal Med (2009) 123:47–53
DOI 10.1007/s00414-008-0236-4

CASE REPORT

Histiocytoid cardiomyopathy and ventricular non-compaction in a case of sudden death in a female infant

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Received: 12 November 2007 / Accepted: 28 March 2008 / Published online: 30 April 2008

Abstract A case of sudden infant death with histiocytoid cardiomyopathy and ventricular non-compaction was investigated with immunohistochemical methods. Histiocytoid cardiomyopathy is thought to be a developmental defect of the cardiomyocytes of the conduction system. In contrast to mature cardiomyocytes, the histiocytoid cells showed only weak reactions to desmin and myosin antibodies. They lacked cross-striation but reacted strongly to enolase and myoglobin antibodies. The protein Pax-7, seen only in cells undergoing differentiation, and the proliferation marker Ki-67 were not expressed in the histiocytoid cells. In areas of altered myocardium, clusters of CD4-, CD8-, and CD68-positive inflammatory cells were seen as well an abundance of mast cells. With the TUNEL method, it was found that many of the histiocytoid cells were undergoing apoptosis. Our results confirm that the histiocytoid cells are defective cardiomyocytes. The apoptotic and inflammatory changes point to a degenerative process rather than defective maturation of cardiomyocytes as has been suggested in some earlier studies. Ventricular non-compaction is a developmental defect of the subendocardial tissue with hypertrabeculation and weak development of the papillary muscles. Only one case combined with histiocytoid cardiomyopathy has been described previously. A causal connection between the two conditions cannot be established until more cases have been analyzed.

Keywords Histiocytoid cardiomyopathy · Ventricular non-compaction · Sudden death · Immunohistochemistry

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Introduction

Histiocytoid cardiomyopathy (HC) is characterized by cardiomegaly, ventricular tachycardia, and frequently by sudden death in the first 2 years of life [1]. The macroscopic changes consist of nodular, subendocardial infiltrations that can be very discrete. The histological picture is that of large histiocytoid cells with a centrally located dark nucleus and light finely granular or foamy cytoplasm without cross-striation [2]. By electron microscopy, the cells lack a t-tubule system but show prominent Z-bands and an abundance of defective mitochondria [3]. Immunohistochemical studies have shown positive reactions to antibodies against desmin and actin and negative reactions to histiocyte markers [4]. Therefore, the cells in histiocytoid cardiomyopathy are thought to be transformed myocytes akin to Purkinje cells of the conduction tissue [5, 6, 7], possibly due to a mitochondrial disorder [8, 9]. The female to male ratio is about 4:1, and some cases of affected siblings have been described, suggesting an inherited X-linked etiology [10]. Similar cells in other organs have been reported in a few cases, but the condition is generally limited to the heart. It is not known if the changes are present at birth or develop progressively during

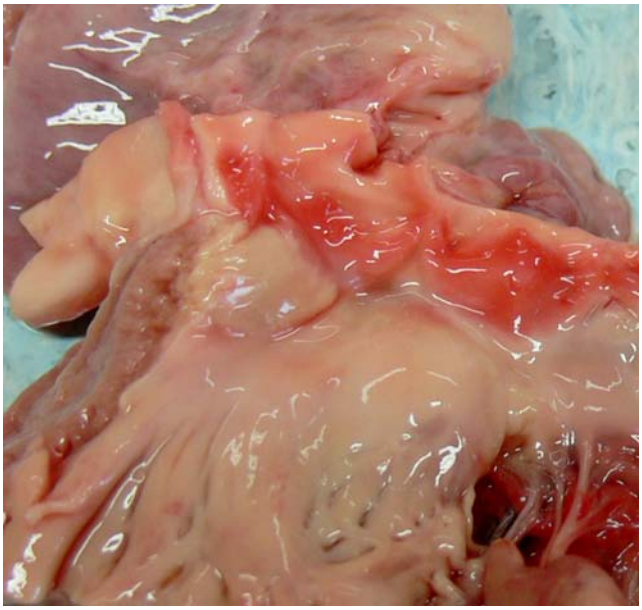

Fig. 1 Thickened endocardium in the upper ventricular septum

the first months of life. Arrhythmogenic nodules that have been removed by radio frequency ablation appear to have cured patients [11].

Ventricular non-compaction (VNC) is a type of cardiomyopathy seen in both children and adults and denotes the persistence of the trabecular network in the ventricular inner walls which is normally observed only during early embryonal development, before the coronary circulation has been developed after the second month. Isolated cases of VNC are also found in adults. Only when VNC is combined with other developmental defects is it associated with early deaths [12].

With this back ground in mind, a case of simultaneous HC and VNC that showed up at autopsy in our department was investigated.

Case report

The patient was a 3 1/2-month-old female infant who was found dead in bed under circumstances similar to sudden infant death syndrome. The child was born after a normal period of gestation, weighing 3,820 g at birth. Initial electrocardiogram (ECG) recordings showed sinus rhythm with a frequency of about 100 bpm and normal T-wave propagation. However, at the age of about 1 1/2 months, the infant was admitted to hospital for a respiratory infection and was found to have a tachycardia of more than 200 bpm. Surveillance ECG showed no arrhythmic episodes, and C-reactive protein and oxygen saturation were normal. One week before death, the parents brought the child back to the hospital, as they had noted an episode of labored respiration, but examination of the child showed no abnormalities. She was the first-born child, and there was no history of sudden death in the family. As the child was found dead at home, a forensic autopsy was carried out.

External examination showed no injuries or developmental defects. The examination of internal organs revealed cardiomegaly with a heart weight of 45 g (normal at 3 months, 28; SD±4 g [13]), increased trabeculations with invaginations and lack of fully developed papillary musculature, additional, clearly visible nodular and homogenous gray infiltrations of the endocardium and subendocardium, and granular excrescences in the vicinity of the mitral valves (Figs. 1 and 2). The lungs were congested with fresh hemorrhages. Furthermore, the thymic gland was much larger than usual and weighed 43.2 g (normal at 3 months, 9.7; SD±6.9 g) [13]. The left kidney was visibly larger than the right, 24.2 versus 16.0 g, whereas the combined weight was normal for the age. A small non-specific cyst was found in the left ovary.

Routine histological examination revealed changes in the subendocardium consistent with HC (Figs. 3a and b). The changes were extensive and not obviously nodular, encompassing the circumference of both ventricles, the trabecular musculature, and the area around the mitral valves in the left atrium and the atrial septum in the vicinity of the AV node and His bundle (which were not involved). The cells in the altered areas were typically large, pale, and irregular with centrally placed dark nuclei, and no apparent cross-striation in the cytoplasm. Clusters of inflammatory cells were also seen in several places.

Special histological investigation

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded sections from all parts of the heart

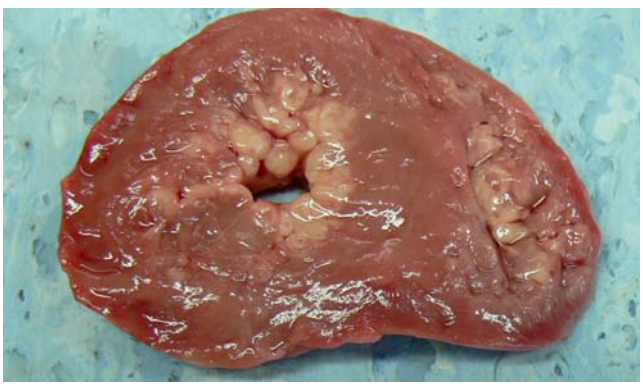

Fig. 2 Transverse section of the heart showing hypertrophied left ventricle and subendocardial yellow-gray discoloration of the myocardium

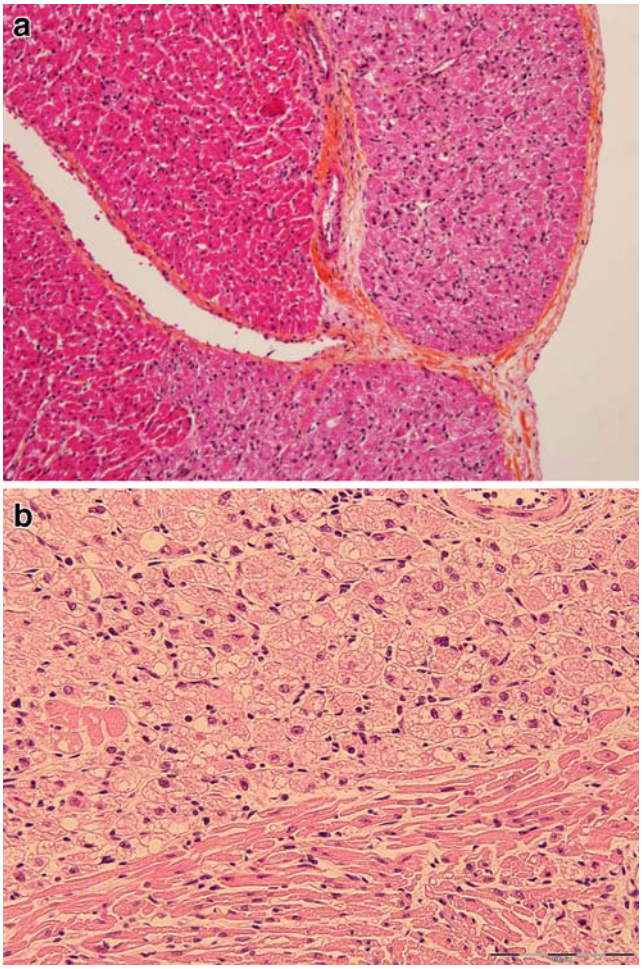

Fig. 3 **a** Left ventricle with altered subendocardial tissue (*pink*) with a sharp border to normal contractile myocardium (hematoxylin–erythrosin–saffron stain, original magnification×20). **b** Transformed histiocytoid cells in the subendocardium with foamy cytoplasm and irregular, centrally located dark nuclei (hematoxylin–erythrosin–saffron stain, original magnification 100×)

including the conduction system. The following monoclonal antibodies were used:

- Complement factor 9 (C9, marker of necrosis; Dako, Copenhagen, Denmark);
- Myoglobin (a cytosol protein of myocytes; Novocastra Labs, Newcastle, UK);
- Macrophage marker (CD68; Dako);
- CD 4 (inflammatory T cells; Novocastra Labs);
- CD8 (cytotoxic T cells; Novocastra Labs);
- Leucocyte common antigen (LCA; Dako);
- Tumor necrosis factor (TNF-α, marker of inflammatory and apoptosis activation; abcam, Cambridge, UK);
- Tryptase (a constituent of mast cell granules; Novocastra Labs);
- Enolase (marker of mitochondrial respiratory activity; Novocastra Visionbiosystem, Newcastle,UK)
- Desmin (an intermediate filament protein of cardiac muscle cells localized at the Z line; abcam);
- Sarcomeric myosin heavy chain (S-MHC; a marker of mature cardiomyocytes; abcam);
- Pax-7 (cardiomyoblasts, marker of differentiation; GeneTex Inc., Quebec, Canada);
- Ki-67 (a marker of proliferation; abcam).

Apoptosis was detected with the TUNEL method. The ApopTag in situ apoptosis detection kit (Chemicon, Temecula, CA, USA) was used according to the manufacturer's recommendations.

Special histochemical stains were used:

- Mallory's PTAH (connective tissue, myocardial contraction bands, and fibrin);
- Weigert's elastin (elastic fibers);
- PAS (mucuous polysaccharides);
- Sudan Black B (lipoproteins) on formalin-fixed and paraffin-embedded sections;
- Osmium tetraoxide (OsO_4, neutral lipids) on formalin-fixed tissue [14].

Results

The results are summarized in Table 1. The histiocytoid cells invariably stained positive with OsO_4 and negative with Sudan Black. PAS was negative. Staining with Mallory's PTAH revealed a discrete collagenous stroma and lack of cross-striations within the histiocytoid cells. Staining for elastic fibers showed deep invaginations of endocardial elastin surrounding islands of ordinary myocardium within the altered areas (Fig. 4).

The histiocytoid cells, like the normal myocytes, contained myoglobin. In many areas, inflammatory infiltrates were found, but necrosis was not observed with C9. The inflammatory infiltrate consisted mainly of T lymphocytes positive for CD 8 and CD 4 and macrophages (CD68). The histiocytoid cells were all negative for the CD antigen markers and contained desmin and S-MHC sparsely in contrast to mature cardiomyocytes (Fig. 5) and failed to stain for the differentiation factor Pax-7. The proliferation marker Ki-67 was negative. Mast cells (MCs) containing tryptase and TNF-α were found in abundance in the interstitium of the altered myocardium (Figs. 6 and 7), but TNF-α could not be seen in the histiocytoid cells.

Enolase showed a discrete positivity throughout the histiocytoid areas, whereas normal myocytes failed to stain.

Apoptotic nuclei were found in great quantities, whereas the nuclei in normal myocytes were never positive in TUNEL (Fig. 8).

Table 1 Histological comparison of histiocytoid and normal cardiomyocytes

Type of staining	Histiocytoid cells	Normal cardiomyocytes
PTAH	Lack of cross-striation	Cross-striation staining
PAS	–	+
OSO4	+	–
Sudan Black	–	–
Weigert's Elastin		
Necrosis	–	–
Apoptotic nuclei	+	–
Myoglobin	+	+
Desmin	(+)	+
S-MHC	(+)	+
Pax9	–	–
Ki67	–	–
Enolase	+	–
CD4+	Increased interstitial infiltration	Occasional sparse interstitial infiltration
CD8+	Increased interstitial infiltration	Occasional sparse interstitial infiltration
Macrophages	Increased interstitial infiltration	Occasional sparse interstitial infiltration
LCA	Increased interstitial infiltration	Occasional sparse interstitial infiltration
Mast cells	Increased interstitial infiltration	Normal residents cells

In the other organs and tissues studied microscopically (brain, thyroid, thymus, lungs, liver, kidneys, pancreatic gland, small and large bowel, ovary, pituitary gland, respiratory mucosa, spleen), no histiocytoid cells were identified.

Discussion

HC and VNC are relatively rare conditions. Up to 1998, no more than 69 known or probable cases of HC had been described in the medical literature [2]. Several clinical studies of VNC [15–18] and one autopsy series of 12 children and two adults with VNC have been published [12]. It can be conceived that several additional cases have not been published, and an unknown number might have been overlooked. It is therefore difficult to estimate the incidence or prevalence of these diseases. Only one case with VNC as well as HC has been reported before the present one [12].

A few cases of HC have been investigated with electron microscopy [2–4, 7, 19, 20–24], immunohistochemical [2, 21–23], and enzymatic methods [6, 8, 22]; also, possible mutations in loci on the X-chromosome have been studied [9, 10]. It is apparent that the term "histiocytoid" is a misnomer, as the cells in question have many features in common with myocytes. The location of the nodules and

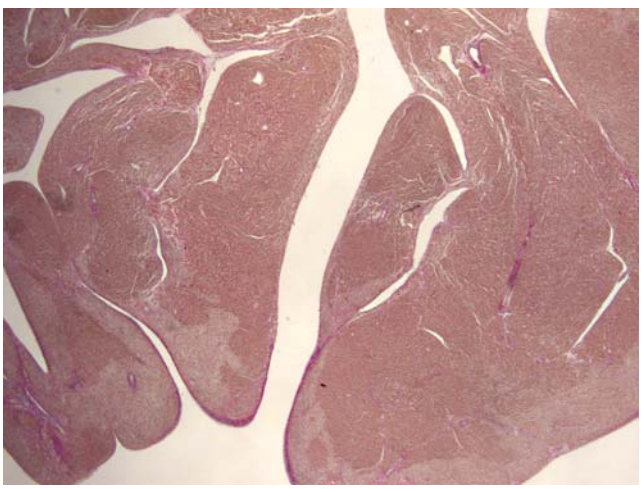

Fig. 4 Multiple trabeculae and invaginations in the non-compacted left ventricular wall with subendocardial histiocytoid areas (Weigert's elastin, original magnification 20×)

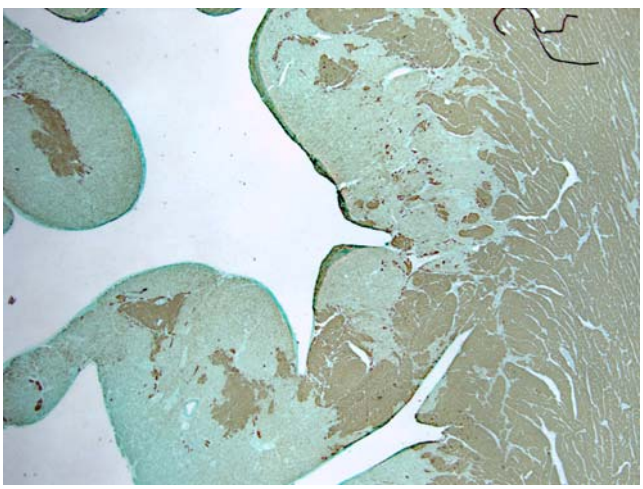

Fig. 5 Antibodies to sarcomeric myosin heavy chain (*S-MHC*) staining normal myocardium but very slightly the histiocytoid cells in the subendocardium (original magnification 40×)

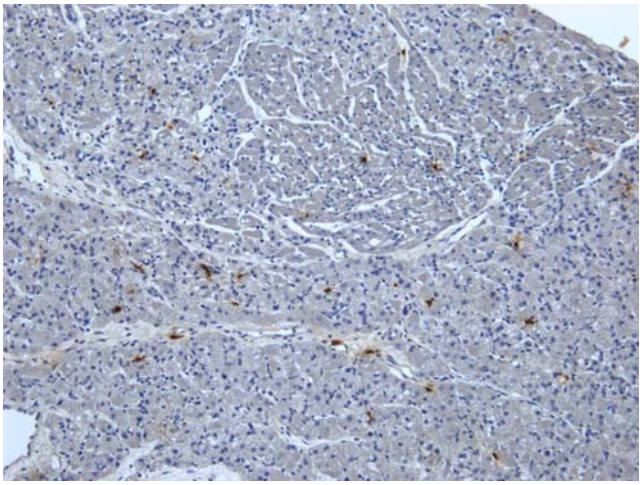

Fig. 6 Cells within the altered histiocytoid areas identified as mast cells with antibodies to tryptase (original magnification 100×)

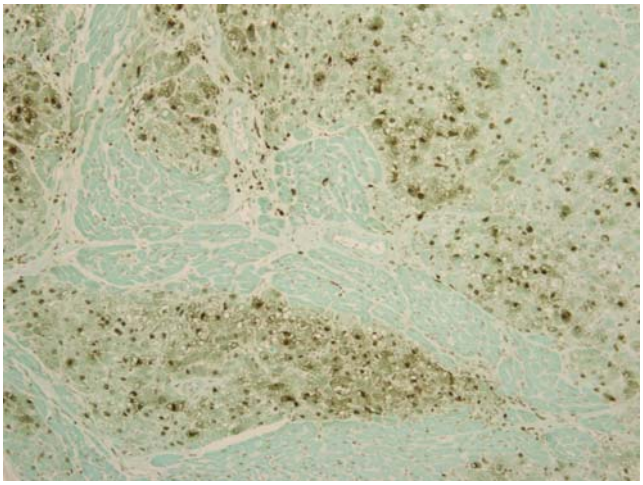

Fig. 8 Abundance of histiocytoid cells with apoptotic dark brown nuclei within the altered myocardium as shown with the TUNEL method (original magnification 100×)

their ultrastructural features point to a possible kinship with the Purkinjecells of the conduction system [5–7].

The present case has features that are in agreement with previous findings, namely that the histiocytoid cells are not histiocytes and our results support the hypothesis that they are cardiomyocytes. They contain sparse quantities of proteins found in mature myocytes such as desmin, myoglobin, and S-MHC. They also contain enolase, an enzyme of the mitochondrial respiratory chain, probably reflecting the abundance of mitochondria seen in these cells under the electron microscope [4]. This is consistent with the view that they have differentiated towards Purkinje cells of the conduction system, which might also explain their arrhythmogenic potential. The lack of Pax-7, only seen in immature cells undergoing differentiation, however, does not support that hypothesis.

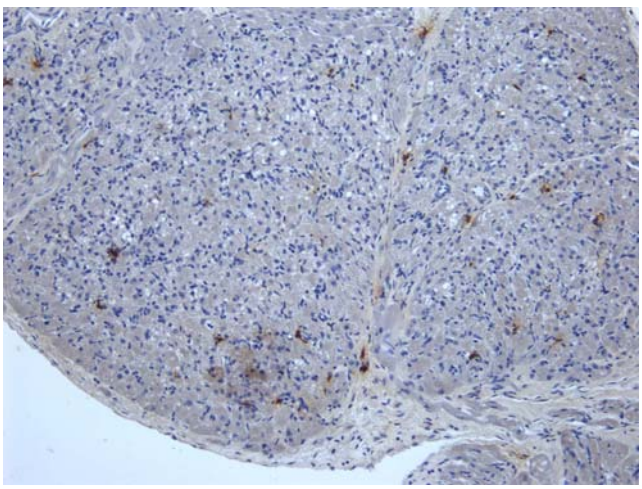

Fig. 7 Cells in the histiocytoid areas containing TNF-α of which the majority are in the same locations as the mast cells (immunohistochemical stain using monoclonal TNF-α antibodies. Original magnification 100×)

The abundance of morphologically abnormal mitochondria has led to the hypothesis that HC may be a manifestation of a mitochondrial disease. Deficiency of mitochondrial cytochrome b has been demonstrated in one previous case [9]. A mutation in the A8344G mitochondrial DNA gene with subsequent lack of reduced co-enzyme Q-cytochrome c reductase was demonstrated in one case study [25], and in another study, an infant with muscular hypotonia showed decreased activity cytochrome c oxidase [26]. Yet, another case had changes in the heart similar to HC together with so-called MERRF (myoclonic epilepsy with ragged red fibers); absence of cytochrome c oxidase was demonstrated [22].

If HC were a genetic disease caused by an inherited mutation, abnormal cells would be seen regularly also in organs other than the heart, but they have actually only occasionally been observed elsewhere [27–29]. If there were a mutation causing HC, it would require a mutation during early embryonal development in a local cell clone in the primitive heart. In cases where extracardiac histiocytoid cells have been observed, a common origin of these cells and of those in the heart has never been proven.

The finding of concomitant HC and VNC in the present case, which is the second case described in the medical literature, could be a random coincidence as suggested by previous authors [12]. It seems to be equally likely that HC, if sufficiently extensive, may have caused VNC, but a causal connection cannot be ascertained until more cases have been analyzed.

HC has been seen in conjunction with various kinds of developmental defects [2], but only a few cases of microphtalmia with linear skin defects [10] and endocardial fibroelastosis [4, 20, 30] have been observed, and there

have been no consistency. Concomitant with VNC thymic hyperplasia and a large difference in size between the kidneys were found, features that have not been previously described.

Infiltration of inflammatory cells has been observed in many cases of HC. In our case, we found T cells, MCs, and macrophages in abundance, but no signs of necrosis. The abundance of inflammatory cells points to an immunological reaction. MCs containing tryptase, chymase, and TNF-α are seen in small numbers in the connective tissue and around vessels in the normal myocardium in both infants and adults, and their numbers increase in disorders such as atherosclerosis and allergic disease [31]. Mast cell proteases are thought to be involved in, for example, apoptosis and cell proliferation, angiogenesis, and tissue remodeling. The increased presence of MCs in HC might be related to those functions, but as the MCs contain numerous preformed enzymes and are capable of rapid synthesis of others, many other possibilities, known or unknown, remain.

A large proportion of the histiocytoid cells had apoptotic nuclei, indicating a high degree of cell death. For the pathological cell population to be maintained or to progress with time would require a high turnover rate. In a previous immunohistochemical study of HC, the proliferation antigens Ki-67 and MiB1 could not be detected [4]. Ki-67 was negative also in our case, indicating that these cells were not proliferating. The presence of a large number of mast cells producing TNF-alpha, an apoptosis inducer, indicates alternative pathogenic mechanisms behind HC: (1) the histiocytoid cells might be derived from previously normal cardiomyocytes undergoing degeneration (by an unknown hereditary X-linked mechanism). The lack of proliferation markers, the great number of inflammatory T cells and macrophages, and intracellular fat accumulation give additional support to that hypothesis. That risk for arrhythmias and sudden death develop progressively during the first months of life is another supportive observation. (2) The apoptotic activity might be caused by acute ischemia during periods of ventricular tachycardia preceding sudden death if the histiocytoid cells are especially vulnerable to anoxia. This hypothesis is supported by the observed apoptosis, but is contradicted by the observed lack of necrosis in the histiocytoid areas.

In conclusion, the investigations in this case of HC confirm that the histiocytoid cells are altered cardiomyocytes. The cells showed no proliferative activity nor did they seem to undergo differentiation towards mature cardiomyocytes. Thus, our results do not support the hypothesis of defective maturation of cardiomyocytes of the Purkinje system [2, 5, 6, 25]. On the other hand, we found that the altered cells were apoptotic, i.e., dead cells, pointing towards a degenerative acute or chronic process.

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