Calretinin as a Marker for Cardiac Myxoma

Diagnostic and Histogenetic Considerations

Luigi M. Terracciano, MD, Paulette Mhawech, MD, Katrin Suess, MD, Maria D’Armiento, MD, Frank S. Lehmann, MD, Gernot Jundt, MD, Holger Moch, MD, Guido Sauter, MD, and Michael J. Mihatsch, MD

Key Words: Cardiac myxoma; Calretinin; Immunohistochemistry; Autonomic nervous ganglia

Abstract

To study the usefulness of calretinin as an immunohistochemistry marker in the diagnosis of cardiac myxoma (CM) and the origin of myxoma cells, we examined 24 CMs and 9 fetal hearts with immunohistochemical methods on formalin-fixed paraffin-embedded tissues. We compared 24 CMs with 10 mural thrombi, 6 jaw myxomas, and 2 papillary fibroelastomas. Calretinin expression was identified in 100% of CMs and was negative in all cases of mural thrombi, jaw myxoma, and papillary fibroelastoma. Calretinin expression by the neoplastic cells in CM was strong and diffuse and had a cytoplasmic and a nuclear pattern. Calretinin expression in fetal hearts was found in autonomic ganglia cells in the subepicardial tissue of the atria and atrial appendages, along the interatrial and atrioventricular sulci, and in the atrial septum. Results clearly indicate that calretinin can be used as a marker for the diagnosis of CM and that it is a powerful tool for the differential diagnosis, most importantly with mural myxoid thrombi. Furthermore, the positive expression of calretinin by the autonomic neurons in the fetal heart and CM supports the concept that myxoma cells may originate from endocardial sensory nerve tissue.

Cardiac myxoma (CM) is a benign neoplasm of uncertain origin arising primarily in the atrial septum. The hypothesis that CM could represent organized thrombi has been largely refuted, and its neoplastic nature now is accepted widely. Previous studies trying to identify the cell of origin of CM using immunohistochemistry, ultrastructure, and tissue culture have yielded conflicting results, and its histogenesis remains unclear.

By using different immunohistochemical markers, studies have suggested that myxoma cells could be of endothelial, epithelial, smooth muscle, or neural origin. It is believed that they may develop from primitive, reserve multipotential mesenchymal cells of the subendocardial and endocardial stroma, which are capable of several types of differentiation. The expression of neuroendocrine and Schwann cell differentiation was seen in a significant number of CM cases, which led Krikler et al to suggest that myxoma cells may originate from endocardial sensory nerve tissue. This hypothesis prompted us to use calretinin as an immunohistochemical marker for the diagnosis of CM.

Calretinin, like S-100, is a calcium-binding protein of the EF family. It has been detected in central and peripheral neural tissue, mainly in a specific set of neurons of the sensory pathways, and in intestinal ganglia of the myenteric plexus and the plexus of Meissner. In addition, calretinin has been detected in normal human mesothelium, adipocytes, eccrine glands, convoluted tubules of the kidney, Leydig cells, and Sertoli cells. In neoplastic tissues, it has been found mainly in mesothelioma and neurocytoma and, to a lesser extent, in mastocyctomas and steroid-producing cells of the testis and ovary, and recently in poorly differentiated colon carcinoma. Similar to S-100, the specific function of calretinin in normal tissue and tumor cells is unclear, but it
has been suggested to have a role in intracellular calcium transport.

The purpose of the present study was to determine the usefulness of calretinin as an immunohistochemical marker for the diagnosis of CM and to obtain more insight into the histogenesis of myxoma cells.

**Material and Methods**

We retrieved 24 CMs, 10 atrial mural thrombi, 6 jaw myxomas, and 2 papillary fibroelastomas from the archives of the Department of Pathology, Basel University Hospital, Basel, Switzerland, for review. Nine fetal hearts with gestational age between 14 and 42 weeks were obtained from the Department of Pathology, University of Naples, Naples, Italy.

The specimens were fixed in 4% buffered formaldehyde (pH 7) and embedded in paraffin. Five-micrometer sections were used for conventional light microscopy and immunohistochemistry. For light microscopy, slides were stained with H&E and alcian blue–periodic acid–Schiff reagent. To study calretinin expression, rabbit anticalretinin serum (Zymed Laboratories, San Francisco, CA) was used at a dilution of 1:50. Immunostaining was enhanced by treating sections in a microwave oven in a 10-mmol/L concentration of citrate buffer (pH 6.0) at 90°C for 60 minutes. The avidin-biotin-peroxidase complex method (Vectastain, Burlingame, CA) was used as the detection technique as described by the manufacturer. Appropriate positive and negative controls were used in every staining run.

**Results**

We evaluated 24 cases of CM. The patients were 16 women and 8 men (female/male ratio, 2:1), and their ages ranged from 18 to 82 years (median, 48.7 years). Nineteen cases had the primary tumor in the left atrium and 5 in the right atrium. One patient with left atrial myxoma, who had a very aggressive clinical course, died 2 weeks after admission. In this case, an autopsy was performed and showed myxoma of the left atrium with extensive myxoma emboli to cerebral, coronary, and renal arteries.

All 24 CMs revealed the typical histologic features of this tumor, ie, classic myxoma cells with an oval nucleus and delicate dispersed chromatin, sometimes appearing as short branching cords or syncytia embedded in a myxoid matrix. In addition, characteristic multilayered rings of myxoma cells around blood vessels were observed frequently. The stroma was rich in neutral and acid mucopolysaccharide, as demonstrated by alcian blue–periodic acid–Schiff stain. Myxoma cells within a myxoid background also were seen in the lumen of the coronary, cerebral, and renal arteries. In 1 case, the tumor showed a widespread glandular pattern characterized by the presence of numerous glandular spaces arranged in the background of loose myxoid matrix and lined by columnar cells and interspersed goblet cells. Four of 6 mural thrombi were characterized histologically by spindle mesenchymal cells entrapped in fibrous tissue or embedded in myxoid matrix. In the 2 remaining cases, a clear-cut distinction between CM and mural thrombus was not possible on H&E-stained slides.
because of the presence of scattered myxoma-like cells within a widespread background of myxoid substance Image 3. All jaw myxomas had a similar histologic finding and displayed a poorly circumscribed myxoma-like mass with focal dense cellularity and slight cellular pleomorphism Image 4. The 2 papillary fibroelastomas showed the typical microscopic findings of avascular fronds lined by endothelial cells, and the matrix was composed of elastic fibers and rare spindle cells.

Immunohistochemically, the neoplastic cells in all 24 CMs showed strong and diffuse expression of calretinin in a nuclear and cytoplasmic pattern Image 5A, Image 5B, and Image 5C. Calretinin expression by myxoma cells was independent of the histologic pattern, vascular channels, endocardial surface, degenerative changes, and the degree of myxomatous background. Calretinin also was positive in the myxoma emboli of the cerebral, coronary, and renal arteries and had the same pattern of expression as seen in the primary myxoma tumor Image 5D. In all cases, vascular endothelium, stroma cells, and the epithelial component were negative for calretinin (Image 5C). Calretinin did not stain any of the mural thrombi, jaw myxomas, or papillary fibroelastomas.

In fetal hearts, calretinin expression was found in several cells of autonomic ganglia Image 6. These ganglia were located in the subepicardial tissue of the atria and atrial appendages, along the interatrial and atrioventricular sulci, and in the atrial septum. They consisted mainly of clusters of nerve cells and nerve fibers and supporting elements, such as ensheathing satellite cells, fibroblast-like partitioning cells, and vascular cells. Apart from ganglion cells, we also found immunoreactivity in the flattened mesothelial cells of the pericardium. Other type of cells, such as cardiac myocytes, endothelial cells of the endocardium, smooth muscle cells of the vascular wall, and lymphocytes, were all negative. Finally, there was no correlation between gestational age and intensity of staining and/or the number of positive ganglion cells.

Discussion

CM is the most common primary tumor of the heart, accounting for approximately 50% of all primary cardiac tumors. About 75% are located in the left atrium, and 25% are located in the right atrium. A small proportion of CMs are multicentric or intraventricular.1,2 CM is considered a benign neoplasm, and the existence of its malignant counterpart is controversial.20 However, recurrence after surgical excision is reported to occur in 2% of cases.

The histologic features of CM often are characterized by the presence of typical myxoma cells forming multilayered rings around blood vessels and/or short cords and syncytia within a myxoid background. Areas with increased cellularity and few mitotic figures can be found, but there is no histologic finding that can predict the clinical outcome. Secondary changes, such as fibrosis, calcification, and thrombosis, rarely may obscure the underlying nature of the lesion, and, in these circumstances, the presence of rare myxoma-like cells entrapped in fibrous tissue could be the only histologic feature suggesting the diagnosis of CM. The most challenging differential diagnosis is with mural thrombi showing myxoid changes and mesenchymal cell proliferation, and, for those particular cases, the use of a descriptive diagnosis has been suggested.2
In the present study, calretinin expression by the neoplastic cells was strongly and diffusely positive in all 24 CMs and was negative in all other cases: mural thrombi, jaw myxomas, and papillary fibroelastomas. In addition, the autopsy case was a strong example of the usefulness of calretinin for differentiating mural thrombus from myxoma emboli; it is negative in the former and clearly positive in the latter. As for the 2 cases of mural thrombi in which the differential diagnosis with CM was not possible based on H&E staining alone, the negative immunostaining for calretinin allowed us to reach the diagnosis of organized myxoid thrombi.

The histogenesis and the relation of CM to mural thrombus is still a subject of debate, and observations based on chromosomal abnormalities, tissue culture, and DNA ploidy pattern strongly support the concept that CM is a true neoplasm and not a thrombus. However, some authors still consider them as reactive rather than neoplastic. Furthermore, despite many immunohistochemical studies, the controversy about the cellular origin of CM has not been resolved. Several reports described the expression of myxoma cells with cytokeratin, vimentin, endothelial, neuroendocrine, or smooth muscle markers. Thus, the cell of origin of CM was assumed to be a multipotent endocardial “vasoformative reserve cell” capable of differentiating into several cell types. Based on recent studies indicating that myxoma cells may express neural antigens,
such as protein gene product 9.5, S-100, and neuron-specific enolase and, therefore, suggesting a neural origin from endocardial sensory nerve tissue, we used calretinin as a marker of neuronal differentiation.

The marked and diffuse positivity of calretinin in all CMs is intriguing. We agree that the identification of tumor origin by specific antigen expression may be misleading, because the phenotypic expression in neoplasia can be variable and does not necessarily reflect its origin. However, the positivity of calretinin in CMs, as well as in ganglion cells of fetal hearts, clearly suggests neuronal origin. This concept also could explain the rare association of CM and other extracardiac nervous tissue tumors such as neurofibromas, psammomatous melanotic schwannoma, and/or nontumoral manifestations such as cutaneous lentiginosis. Furthermore, glandular differentiation, as occasionally observed in CMs, also has been described in peripheral nervous tissue tumors.

Nerve terminals immunoreactive for neuropeptide Y, calretinin, and calbindin were detected in rat cardiovascular pathways of the sympathetic nervous system, and further studies to elucidate whether a sympathetic cardiac pathway with a similar “chemical coding” also is present in human heart could be of interest.

The present study showed that calretinin is a reliable marker for the diagnosis of CM and is useful for the differential diagnosis with the most challenging entities, such as mural thrombus with myxoid changes. In addition, calretinin expression by the autonomic neurons in the fetal heart supports the concept that myxoma cells can originate from endocardial sensory nerve tissue.

From the Institute for Pathology and Department of Internal Medicine, University Hospital of Basel, Basel, Switzerland; and the Institute of Pathology, University “Federico II,” Naples, Italy.
References