**Conclusion:** The light microscopy observation and immunohistochemical study underscore that it is not easy to obtain information about the level of differentiation of this tumour. The presence of blood-filled lumina and the identification of typical markers of endothelial cells seems to indicate a well-differentiated nature. However, the ultrastructural findings seem to indicate a less differentiated nature.

**Key Words:** Cardiac tumour, Vascular tumour, Sarcoma, Histology, Electron microscopy, Immunohistochemistry.

**Introduction**

While metastatic tumours to the heart occur in about 5% of patients dying of cancer, primary cardiac tumours are rare. Nearly 70% of primary cardiac tumours are benign, the majority of which are represented by myxomas. The most frequent primary cardiac neoplasm is the angiosarcoma that represents 31% of primary cardiac malignant. We report a particular clinical case of cardiac angiosarcoma, its light and transmission electron microscopic aspects and a review of the recent literature.

**Methods:** A 52 years old man died for a severe right ventricle filling deficit caused by an intracavitary tumour originated from the right atrial anterolateral wall. The fragments obtained from autopic tumoral cardiac tissue were processed for light and electron microscopy. The section were stained with haematoxilin-eosin, Masson trichromic and Gomori method. An immunohistochemical study for vimentin, Factor VIII related antigen and peroxidase-conjugated lectin from Ulex Europaeus was also performed using the unlaled peroxidase-antiperoxidase method.

**Results:** The hematoxylin-eosin staining showed that the tumoral mass was composed by a well-differentiated histotype characterized by numerous vascular areas in which neoplastic cells were loosely and irregularly arranged to form incomplete vessels or anastomized blood-filled vascular channels. On the other hand, some less-differentiated solid areas were present and irregularly surrounded the differentiated vascular areas. Results of Ulex Europeaeus Agglutinin I labelling were positive in both solid and vascular areas of the tumour although the positive reaction was less evident in the solid zones Factor VIII related antigen positive cells were less numerous and mainly found in vascular areas. The observation by electron microscopy showed the lack of evident pinocytotic vesicles, the presence of thin and delicate cytoplasmatic processes, Weibel-Palade bodies, and also the disarrangement of the extracellular fibrous matrix.

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turn and ventricular filling\textsuperscript{7,10}. Because of the propensity for pericardial involvement, cardiac haemorrhage and tamponade are frequent\textsuperscript{11}. Metastases are very frequent and up to 75\% of patients with primary cardiac sarcomas can have systemic metastasis particularly to the lungs\textsuperscript{12-14}. The diffusion and the development of new instrumental methods of clinical investigation in regard to the diagnosis of cardiac angiosarcoma have become more frequent, thus permitting to obtain a greater success in the treatment of this highly malignant tumor\textsuperscript{11,15,16}. We report a particular clinical case of cardiac angiosarcoma, its light and transmission electron microscopic aspects and a review of the recent literature.

\textbf{Materials and Methods}

\textbf{Clinical History}

A 52 years old man was admitted in other Hospital of Rome in August 1990 with progressive extensional dyspnea. The two-dimensional echocardiography evidenced an echogenic motionless mass that occupied approximately 80\% of the right atrium; the tricuspid valve and interatrial septum appeared to be normal.

Physical cardiac examination disclosed a grade II/VI sistolic murmur after the apex; normal sinus rhythm, pulse of 80 bpm and blood pressure 140/100 mm Hg. A chest radiograph revealed no evidence of pulmonary lesions, cardiomegaly and pericardial or pleural effusion. Total body computed tomography (CT) showed a right atrial mass and distension (2.5 cm) near right thyroid lobe.

The patient underwent medial sternotomy with parietal pericardiectomy; the examinations revealed a motionless tumoral mass 5 cm in diameter that infiltrated the pericardium, extended on the wall of right atrium and occupied the orifices of superior and inferior vena cavae. Because of its large extension and critical location the tumoral mass was considered unresectable and the patient was discharged on the 12th postoperative day.

The patient's condition deteriorated rapidly and three months later he was again hospitalized in our Department at the Policlinico Umberto I, University “La Sapienza” of Rome, with recurrent dyspnea, palpitations and sharp pain over the chest.

Physical examination revealed a blood pressure of 110/70 mm Hg, distented neck veins, hepatomegaly and a moderate pitting edema of the lower limb. The electrocardiogram showed a supraventricular tachycardia at a rate of 140 bpm. The chest roentgenograms demonstrated a slightly enlarged cardiac shadow with a prominent right side border and a right hemithoracic effusion involving the inferior lung field. A two-dimensional echocardiography evidenced an echogenic motionless mass in an enlarged right atrium. A nuclear magnetic resonance imaging with a 1.5 Tesla superconducting magnet and using gated spin echo technique (rime to echo: 30 msec) demonstrated dilated cardiac cavities and the presence of a rounded mass in the right atrium. This mass extended across the tricuspid plane into the right ventricle involving also the inferior vena cava and the pericardium. A presumptive diagnosis of a right atrial tumor was established. Biopsy of the mass was performed with a transvenous catheter.

The samples obtained were very small for a light microscopic study; therefore, they were processed for transmission electron microscopy (TEM). That showed spindle-shaped cells having the typical aspects of immature endothelial cells showing an oval indented nuclei with dense heterochromatin and prominent nucleoli; their cytoplasms displayed numerous delicate elongated cytoplasmatic processes and contained some pinocytotic vesicles along cell membrane, dilated cisternae of smooth endoplasmic reticulum, polyribosomes, large mitochondria, many microfilaments and the characteristic Weibel-Palade bodies. Intercellular spaces contained mucoid amorfous material with collagen fibres (Figures 1 A-B).

During the following week the patient became increasingly dyspnoic. A chest radiography showed an increase of the right hemithoracic effusion.

Three subsequent evacuative thoracenteses were performed on the 7th, 10th and 12th days post admission. Each thoracentesis yielded between 1.5 and 3.2 litres of a serohematic fluid (Hb: 3.0-5.0 g/dl). The samples were negative for neoplastic cells.

After removal of the fluid a rapid but temporary relief of the breathing difficulty was
observed. The patient, suffering hypotension, mental obtundation and dyspnea, died on the thirteenth day after admission.

**Autopsy Findings**

Upon opening of the thoracic cavity the right parietal pleura was found thickened and adhering tightly to the adjacent pericardium. The parietal pericardium was observed tightly adhering to the underlying right atrial wall. An intracavitary mass, bossolated and dark red in color, was found originating from the right atrial anterolateral wall. Small nodules containing a blood-stained serous fluid were present on its surface. The tumoral mass occupied almost completely the right atrial cavity and projected through the tricuspid valve into the right ventricle. The orifice of the inferior vena was found almost completely obstructed by the tumour (Figure 2).

**Figure 1.** *A.* Electron micrographs showing sheet of poorly differentiated malignant cells; Weibel-Palade bodies are seen (arrows). In the boxed area, high magnification of a Weibel-Palade body with the characteristic membrane-bound longitudinally striated internal structure (original magnification 60,000 ×). N = nucleolus. *A,* Original magnification 9,000 ×. *B,* Original magnification 20,000 ×. (Courtesy of Prof. Tullio Faraggiana, University of Rome, “La Sapienza”).
The diagnosis was severe right ventricle filling deficit caused by an intracavitary tumour originated from the right atrial anterolateral wall.

Light and Electron Microscopy

The fragments were obtained from autopsic tumoral cardiac tissue.

For light microscopy the samples were fixed in 10% formaldehyde solution and embedded in paraffin. The sections were stained with haematoxilin-eosin, Masson trichromic and Gomori method.

The immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue sections using the unlabelled peroxidase-antiperoxidase (PAP) method. Primary antibodies to vimentin (1/50 dilution, monoclonal, clone V9) and Factor VIII related-antigen (FVIIIIR-Ag) (1/25 dilution, polyclonal) and a peroxidase-conjugated lectin from Ulex Europaeus (1/250 dilution, polyclonal) were obtained commercially (Dako, Glostrup, Denmark).

Incubation times at 4°C were 30 and 60 minutes and 30 minutes for antibodies and 12 hours for peroxidase-conjugated Ulex Europaeus Agglutinin I (UEA-I). Non-trypsinized and trypsinized slides (15 minutes) were used and appropriate positive and negative controls performed.

For transmission electron microscopy the fragments were fixed in 2.5% gluteraldehyde solution on 0.1 M cacodylate buffer at pH 7.3. Post-fixation was carried out in 1.33% osmium tetroxide. The tissues were then dehydrated in increasing concentrations of ethanol and embedded in Epon 812. Ultrathin sections, obtained with a LKB ultratome II, were stained with uranyl acetate and lead citrate and observed with a Zeiss EM 10 electron microscope.

Results

The hematoxylin-eosin staining showed that the tumoral mass was composed by a well-differentiated histotype characterized by numerous vascular areas in which neoplastic cells were loosely and irregularly arranged to form incomplete vessels or anastomized blood-filled vascular channels (Figures 3 A-B). Hemosiderinic deposits were evident throughout these more differentiated portions of the tumour. On the other hand, some less-differentiated solid areas were present and irregularly surrounded the differentiated vascular areas. Transitional areas were not easily seen.

The neoplastic cells in the solid areas had mostly an elongated spindle shape. Cleft-like spaces, often running parallel, were frequently observed in these solid areas (Figure 4 A). Trichromic staining was useful in evidencing the elevated collagen component of this tumor. The collagen compounds were more evident in tissue sections with a predominant solid component but were also seen in the focal solid areas present in the tissue sections with a predominant vascular component. The reticular fibres, evidenced by silver impregnation, were observed in both solid and vascular tumor areas but most typically outlined the anastomosing vascular spaces (Figure 4 B).

Immunoperoxidase staining of vimentin-type intermediate filaments was positive and
almost completely confined to highly vascular areas (Figures 4 C-D). Staining of FVIIIIR-Ag was negative for all trials.

The sections stained with peroxidase-conjugated UEA-I were weakly positive only after 12 hours of incubation on nontrypsinized slides. The change of the concentration of UEA-I did not affect the staining results. The positivity was only observed in vascular areas that likely outlined lumina (Figures 4 C-D).

The observation of Epon semithin sections by contrast-phase microscopy showed many lumina filled up with red blood cells.

Neoplastic cells forming vascular spaces had different shapes; some cells were well-rounded while others were thin and flattened (Figure 4 A).

The sections observed by TEM showed that nuclei were quite varied in shape; all of them had large amounts of peripherally condensed chromatin and many nuclei had multiple nucleoli. The cell cytoplasm was rich in mitochondria and rough endoplasmic reticulum (ER). The ER was compact and densely lined by numerous ribosomes in some areas while it was dilated and smooth in others. Both smooth and granular membranes of the endoplasmic reticulum were amply dilated. Although the majority of dilated ER was clearly void of contents, some portions contained an electron-dense material.

The intracytoplasmic filaments were organized not only in wavy and laminar aggregates but also dispersed randomly. At the cell membrane level we observed desmosome-like junctions and irregular tight junctions. In addition, the extracellular matrix was constituted by banded collagen fibrils which were isolated, bundled or even irregularly arrayed. This fibrous matrix was seen organized in a basement membrane-like manner and forming a wide fibrous interstitium.

Weibel-Palade bodies, pinocytotic vesicles and elongated cytoplasmic processes were present in some neoplastic cells (Figures 5 A-F).

Electron-lucent cavities were evident in numerous nuclei and the alterations of mitochondrial cristae were probably due to artefact fixation performed 48 h after death.

Discussion

Primary cardiac tumours are extremely rare with an incidence of 0.0017% reported by the American Heart Association\textsuperscript{17,18,19}. Malignant tumours account only for 25%\textsuperscript{15,20} and the angiosarcoma is the commonest malignant primary neoplasia\textsuperscript{4,20}. It preferably appears in the right atrium between the 3\textsuperscript{rd} and 5\textsuperscript{th} decades of life\textsuperscript{3,7}. In our case we had a 52 years old patient with a mass that occupied approximately the 80% of the right atrium. We analysed the histological, immunohistochemical and ultrastructural aspects of this tumour.

Our light microscopic and immunohistochemical findings were consistent with the observations already described in other papers\textsuperscript{2,17,21} while the aspects seen with TEM ap-
Figure 4. Immunoperoxidase staining of vimentin type intermediate filaments was positive and almost completely confined to highly vascularized areas. **A**, Original magnification 10 ×. **B**, Original magnification 25 ×. Peroxidase-antiperoxidase stain for UEA-I. Positive cells were mainly found in vascular areas outlining lumina. **C**, Original magnification 10 ×. **D**, Original magnification 25 ×.
peared to be more variable. A micropattern characterized by solid areas of spindle cells irregularly merged into more differentiated areas is typically reported\textsuperscript{7,17,18,21,22}. These vascular areas were characterized by blood-containing channels which were irregularly anastomized and lined by neoplastic cells. Within the tumour the cell population appeared pleomorphic and with a high rate of mitosis\textsuperscript{7,19,23}. The observations made using silver impregnation revealed that reticular fibres mainly surrounded the cells in vascular areas but also in solid and less-differentiated areas. These fibres normally formed a peri-endothe-

\textbf{Figure 5.} A, Semithin sections: tumour tissue forming a luminal space lined by flattened cells. Some lumina contain red-blood cells. (phase contrast blue-metilen stain. Original magnification 40 ×). \textbf{B-E,} Electron micrographs showing immature endothelial cells. N = nucleus; Ni = nucleolus; R = red blood cell; v = micropinocytotic vesicles; m = mitochondria; J = cell junctions; f = collagen fibres; W = Weibel-Palade bodie. \textbf{B,} Original magnification 5.000 ×. \textbf{C,} Original magnification 5.000 ×. \textbf{D,} Original magnification 40.000 ×. \textbf{E,} Original magnification 8.000 ×. \textbf{F,} Original magnification 10.000 ×.)
lial network around the capillaries and seemed to form up the delicate reticular lamina of the basement membrane. This kind of fibres are not normally synthesized by endothelial cells but by overlying fibroblasts. These fibres are also found in normally differentiated mesenchymal tissues and undergo an eventual transformation and agglomeration into typical connective tissue collagen fibres. Our observations by TEM permitted a detailed description of their nature and location in this tumour. In fact, we noticed that reticular fibers formed part of a continuous extracellular matrix which was thinly or amply deposited between neoplastic cells or directly facing the vascular lumina. The characteristics of this extracellular matrix suggested that it was the result of a massive and disarranged synthesis and deposition of fibrous compounds that have no part in the normal structure of the endothelium.

Many Authors performed immunohistochemical studies with two endothelial cell markers, anti-FVIIIR-Ag and UEA-I lectin to confirm the vascular nature of this tumour. Results of UEA-I labelling were positive in both solid and vascular areas of the tumour although the positive reaction was less evident in the solid zones. FVIIIR-Ag positive cells were less numerous and mainly found in vascular areas. These results are in agreement with a study of 27 angiosarcomas in which all tumors were positive for UEA-I labelling. In this study only 74% of tumors were positive for FVIIIR-Ag.

Other Authors used the antibody anti CD-31 and anti CD-34 to characterize the angiosarcoma. The antibody against CD-31 stains well differentiated angiogenetic structures and poorly differentiated solid areas while the antibody against CD-34 does not stain undifferentiated malignant cells from solid areas. The antibody anti CD-31 and UEA-I seems to be the most sensitive marker staining well differentiated vasiformative structures and poorly differentiated solid areas. In contrast, non-vascular tumours do not express CD-31 and FVIIIR-Ag.

However, the ability of these immunohistochemical markers to define the endothelial nature of this tumour seems secure and the immunohistochemistry is very important to confirm the diagnosis of angiosarcoma. In fact, a general histological characteristic of all cardiac sarcomas is the presence of many vessels inside the tumours that, without immunohistochemistry, may be confused with the vascular space of angiosarcomas.

On the other hand, the use of these markers to define if these cells derived from blood or lymphatic vessels or from a primitive mesenchymal cell remains unaccomplished and other markers have been recently found.

The light microscopy observation and immunohistochemical study underscore that is not easy to obtain information about the level of differentiation of this tumour. The presence of blood-filled lumina and the identification of typical markers of endothelial cells seems to indicate a well-differentiated nature. However, the ultrastructural findings seem to indicate a less differentiated nature.

The ultrastructural studies of angiosarcomas varied in details and were often made in reference to a wide TEM study by Yang et al. The most described characteristics of these tumours are the presence of neoplastic cells bound by tight junctions, forming vascular lumina and containing micropinocytotic vesicles and intracytoplasmatic filaments. The presence of Weibel-Palade bodies and basement membrane has been reported only in one case. An evident dilatation and vesiculation of the rough ER has been reported in 5 cases apart from ours. The coalescence of the smaller vesicles has been frequently reported as forming the larger intracytoplasmatic cavities. There is a regrettable tendency to refer to these features as “intracytoplasmatic lumina” and as evidence of angiogenesis. The term “intracytoplasmatic lumina” refers principally to membrane bound cystic spaces bearing microvilli formed by an invagination of the cell membrane. These are typically found in the normal gastric parietal cells and in the cells of different adenocarcinomas. “Dilated ER” and/or “vesiculated ER” are recommended terms for these ultrastructural features of angiosarcoma tumor cells. The use of the term “intracytoplasmatic lumina” can lead to the erroneous assumption that these features play a defined role in angiogenesis. Whatever we call them, these features do not seem to have a role in angiogenesis at all; in fact, they have been inconstantly reported in angiosarcoma.
comas and are not reported in benign vascular lesions or in the developing capillaries of early human placenta or of regenerating tissues

The observations by TEM demonstrated a "morphological distance" between neoplastic cells of this vascular tumour and the typical cells of the normal endothelium. In our case, for example, the lack of evident pinocytotic vesicles, the presence of thin and delicate cytoplasmatic processes, Weibel-Palade bodies, and also the disarrangement of the extracellular fibrous matrix, define the neoplastic and undifferentiated nature of the tumour.

Antemortem identification of intracardiac tumours by clinical and electrocardiographic findings did not occur until 1934. It was only in the late 1970s when echocardiography, CT and magnetic resonance imaging became available that antemortem diagnosis become more feasible. The clinical presentation of this neoplasia depends on the site of the tumour and the degree of the involvement of the myocardium and the pericardium: when the angiosarcoma involves the pericardium it can produce pericardial effusion; when it is intramycardial in turn, it can cause arrhythmias or congestive heart failure; and finally, when it is intracavitary, it can mimic valvular lesions. Some tumours produce no symptoms and are found incidentally.

The method most frequently used for the diagnosis of this pathology are echocardiography and especially the transesophageal technique. Besides, the diagnosis can be performed also by CT or nuclear magnetic resonance.

In the end, the treatment of angiosarcomas is controversial due to the early and rapid local and systemic dissemination. Surgical resection could be performed only when there is no evidence of metastasis and when there is the possibility to do a curative resection. There is no evidence of the utility of chemotherapy and radiation therapy.

References


