Congenital/Infantile Fibrosarcoma of the Colon

Morphologic, Immunohistochemical, Molecular, and Ultrastructural Features of a Relatively Rare Tumor in an Extraordinary Localization

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Summary: Fibrosarcomas diagnosed during the early years of life are called congenital/infantile fibrosarcomas. They differ from adult fibrosarcomas because of their limited aggressive outcome. Congenital/infantile fibrosarcomas occur most frequently on the extremities. This article describes an exceptional case of colonic congenital/infantile fibrosarcoma diagnosed in a 3-day-old baby boy. It is the third intestinal congenital/infantile fibrosarcoma reported in the international literature. The lesion was radically excised. Microscopic examination revealed a densely cellular and poorly circumscribed tumor composed of spindle cells forming interlacing fascicles with herringbone appearance. Necrotic and hemorrhagic areas were appreciable. Mitotic count was 2/10 high-power fields. Immunohistochemistry revealed that the tumor cells were positive for vimentin, focally positive for h-caldesmon, and that they were negative for epithelial markers, muscular markers, S-100 protein, and CD34. The proliferation index (Mib-1) was 15%. Polymerase chain reaction demonstrated the chromosomal translocation t(12;15)(p13;q25). At the ultrastructural level, neoplastic cells had fibroblastic and myofibroblastic features. The patient underwent follow-up without adjuvant therapy. Twelve months after the surgery, he is alive and well. Given the common indolent nature of this tumor, it is important to avoid misdiagnoses with more aggressive tumors. The algorithm for the diagnosis of congenital/infantile fibrosarcoma, especially outside the usual localizations, should comprise morphologic, immunohistochemical, molecular, and ultrastructural studies.

Key Words: congenital fibrosarcoma, infantile fibrosarcoma, intestine, electron microscopy, PCR, ETV6-NTRK3

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Fibrosarcoma is a mesenchymal malignancy of both adults and children representing 10% to 12% of all soft-tissue sarcomas. The term congenital/infantile fibrosarcoma is used to describe the relatively rare fibrosarcomas diagnosed at birth or during the early years of life. Congenital/infantile fibrosarcoma differs from adult fibrosarcoma because of its less aggressive behavior with a metastatic rate of about 10% and a survival rate of more than 90%. It most frequently occurs on the extremities and on the axial regions. Morphologically, congenital/infantile fibrosarcoma may show high cellularity, mitoses, necrosis, and cytologic atypia.[1–4]

Owing to the relatively indolent behavior despite the ominous histology, congenital/infantile fibrosarcoma has to be distinguished from other spindle-cell sarcomas of childhood with more aggressive clinical behavior, such as spindle-cell monophasic fibrous synovial sarcoma, rhabdomyosarcoma, malignant peripheral nerve sheath tumor (MPNST), and leiomyosarcoma. Furthermore, congenital/infantile fibrosarcoma also overlaps some morphologic features of gastrointestinal stromal tumors (GIST) and of infantile myofibromatosis.

Molecular and ultrastructural studies may be useful in the differential diagnosis. The ETV6-NTRK3 gene fusion has been indicated as a molecular mark of congenital fibrosarcoma.[5] Ultrastructurally, neoplastic cells show features of fibroblastic/myofibroblastic differentiation.

In this article, we describe the clinical, morphologic, immunohistochemical, molecular, and ultrastructural features of an extraordinary case of congenital fibrosarcoma that occurred in the right colic flexure.

CLINICAL SUMMARY

An 1800-g 3-day-old baby boy, delivered vaginally and at term, was referred to the Neonatal Intensive Therapy Unit of the Meyer Pediatric Hospital of Florence because of intestinal occlusion and acute abdomen. The presumed diagnosis of intra-abdominal mass causing intestinal perforation was made. The pregnancy was reported as uneventful. The mass had not been detected at the prenatal ultrasound examinations. The baby underwent surgery at the age of 4 days. Surgeons recognized

a 4-cm solid mass in the right colic flexure. Tumor rupture was present. An ileocolonic resection with subsequent anastomosis was made. Surgical resection of the tumor was complete. Postoperative period was uneventful. Because complete surgical resection was performed, the patient underwent follow-up without adjuvant therapy. Twelve months after the surgery, the patient is healthy and disease-free by clinical and radiologic evaluations.

PATHOLOGIC FINDINGS

Histologic, immunohistochemical, molecular, and ultrastructural studies was executed in the Department of Human Pathology and Oncology at Florence. The surgical specimen, consisting of a 14-cm intestinal segment, was routinely fixed in 10% buffered neutral formalin. Representative samples were embedded in paraffin. Some of the 5-μm sections of each sample were stained with hematoxylin-eosin for the morphologic evaluation whereas further 5-μm sections of the most representative sample were mounted on electrostatic slides and used for the immunohistochemical study. From the most representative specimen were also obtained samples for the polymerase chain reaction (PCR).

Immunohistochemical stains were performed by standard avidin-biotin complex immunoperoxidase method.

Total RNA was isolated from formalin-fixed, paraffin embedded tissue sections. After dewaxing and proteinase K digestion (1 h at 37°C), the sample was resuspended in 200-μL RNA lysis buffer RTL (RNasy Fibrous Tissue Mini Kit-Qiagen, Hilden, Germany) and homogenized using TissueLyser (Qiagen, Hilden, Germany) in microcentrifuge tubes. Total RNA was isolated using RNasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany) and successively stored at −80°C.

The RNA sample (200 ng) was reverse transcribed to cDNA using iScript Select cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA), according to the manufacturer's protocol. In the real time reaction, negative control (no-RNA) was performed. The PCR assay to detect the ETV6-NTRK3 fusion transcript was performed according to the previously described method.[6] The pair of primers amplify a small 111-bp region flanking the fusion point of the ETV6-NTRK3 gene transcript. In brief, the reactions were carried out in a volume of 50 μL, with 2-mM $MgCl_2$, 0.3-mM dNTPs, 40 pmol of each primers, 1 U of Taq polymerase (Applied Biosystems), and 50 ng of cDNA template. The cycle conditions were— 50 seconds at 94°C, 30 seconds at 60°C, and 60 seconds at 72°C for 45 cycles.

PCR products were analyzed by 2% agarose gel electrophoresis and visualized under ultraviolet illumination. In each PCR reaction, a no-cDNA template control was included.

Tumour tissue fragments for electron microscopy were fixed in 2.5% glutaraldehyde in 0.1-mol/L sodium cacodylate buffer (pH 7.4) for 3 hours and postfixed in 1% OsO_4 in 0.1-mol/L veronal acetate buffer (pH 7.4) for 1 hour. The tissue samples were stained en bloc in 2% uranyl acetate in 50% ethanol, dehydrated in increasing concentrations of ethanol, cleared in propylene oxide, embedded in epoxy resin, and cut, using a diamond knife, with a Leica Ultracut R microtome. Ultrathin serial sections were mounted on formvar-coated Cu/Rh grids, stained with uranyl acetate and lead citrate, and observed with a Philips 410 LS transmission electron microscope.

Microscopic examination revealed a densely cellular and poorly circumscribed tumor in the intestinal wall. The tumor reached the serosal layer. The mucosal layer was not ulcerated and was largely spared with only focal involvement. The tumor was composed of spindle cells forming interlacing fascicles with herringbone appearance and small amounts of interstitial collagen. Giant cells and calcifications were lacking. Necrotic and hemorrhagic areas were appreciable. Mitotic count was 2/10 high-power fields (Figs. 1, 2).

Immunohistochemical stains revealed that the tumor cells were positive for vimentin, focally positive for h-caldesmon, and that they were negative for keratin (AE1/AE3), epithelial membrane antigen, muscle-specific actin (HHF35), desmin, α-smooth muscle actin, S-100 protein, and CD34. The proliferation index, as determined at the immunohistochemistry estimating the percentage of the Mib-1 positive neoplastic cells in the total tumoral cells in the most positive areas, was 15% (Fig. 3).

PCR evaluation demonstrated that the lesion contained the chromosomal translocation t(12;15) (p13;q25) (Fig. 4).

At the ultrastructural level, neoplastic cells had a spindle shape, with oval, often indented nucleus and were embedded in abundant extracellular matrix-containing collagen fibrils. The cytoplasm contained abundant organelles, including rough endoplasmic reticulum cysternae, free ribosomes and mitochondria, and intermediate

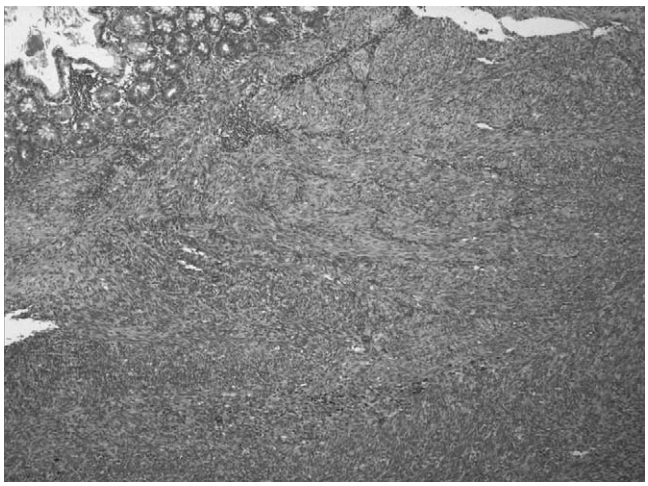

FIGURE 1. Densely cellular tumor in the intestinal wall (H&E, original magnification 25×). H&E indicates hematoxylin and eosin.

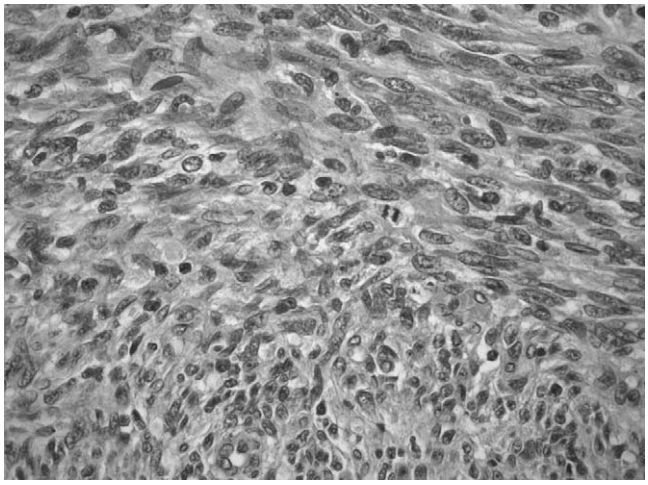

FIGURE 2. The tumor was composed of spindle cells forming interlacing fascicles with herringbone appearance and small amounts of interstitial collagen. Mitoses were appreciable. (H&E, original magnification 200 ×). H&E indicates hematoxylin and eosin.

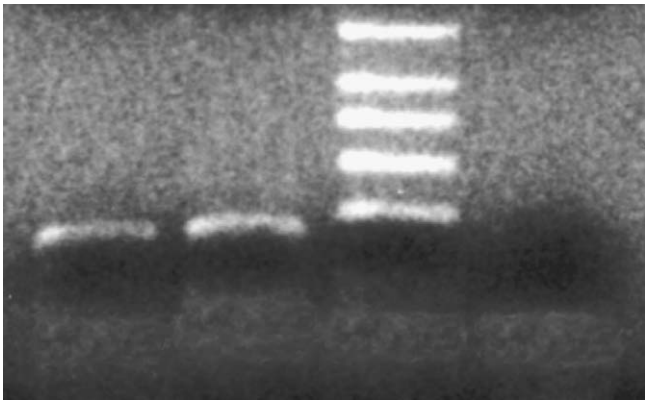

FIGURE 4. RT-PCR detection of the ETV6-NTRK3 chimeric transcript. Ethidium bromide-stained agarose gel of PCR products. Lane 1 indicates patient sample; lane 2, positive control (111 bp) from diagnosed congenital fibrosarcoma; lane 3, size of ladder (700, 500, 400, 300, and 150 bp); lane 4, no-cDNA control. PCR indicates polymerase chain reaction; RT, reverse transcriptase.

filaments. Several neoplastic cells presented peripheral bundles of microfilaments with focal densities and subplasmalemmal attachment plaques (Fig. 5). Adjacent neoplastic cells were joined by immature junctions. Fibronexus junctions were not observed.

Morphologic features along with immunohistochemical, PCR, and ultrastructural results were consistent with the diagnosis of congenital fibrosarcoma.

DISCUSSION

First recognized by Stout in the year 1962,[7] congenital/infantile fibrosarcoma was widely studied by Chung and Enzinger in 1976 and by Soule and Pritchard in 1977.[2,3] All these authors noted that, despite the ominous morphology, congenital/infantile fibrosarcoma had a more favorable behavior than its adult counterpart. Most frequently, this tumor affects the extremities (71% of cases) and the axial regions (29% of cases).[8] Only few cases have been reported outside these regions. Among the unusual localizations, there are the tongue and the oral cavity, the ovary, the retroperitoneum, the

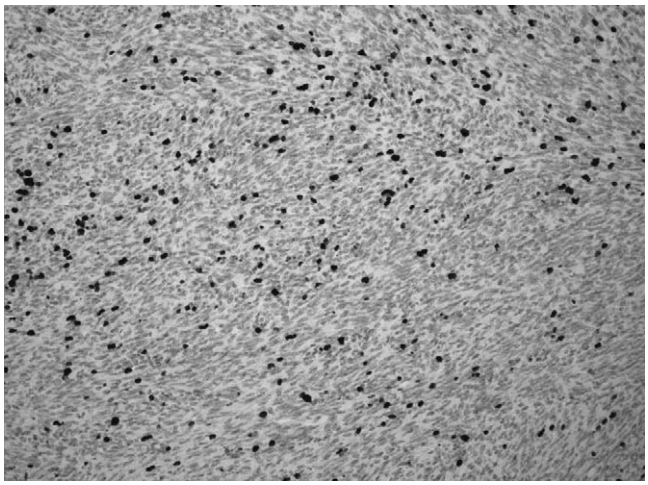

FIGURE 3. The proliferation index as determined by the immunohistochemistry estimating the percentage of the Mib-1 positive neoplastic cells in total tumoral cells in the most positive areas was 15% (Mib-1 immunostain, original magnification 100 ×).

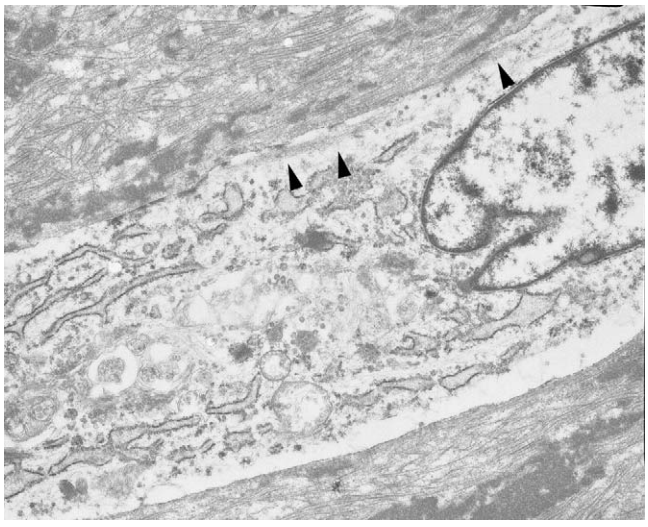

FIGURE 5. Electron Microscopy: neoplastic cell with myofibroblastic features, displaying a cytoplasm rich in RER cysternae, free ribosomes, mitochondria, and intermediate filaments. Peripheral bundles of actin filaments are indicated by arrowheads. The cell is embedded in abundant extracellular collagenous matrix. RER indicates rough endoplasmic reticulum.

thoracic wall, the heart, and the intestine.[9–13] To the best of our knowledge, only 2 intestinal congenital/infantile fibrosarcomas have been reported in the medical literature. In the year 1975, Shearburn et al[14] described a 2-month-old baby girl with a duodenal congenital/infantile fibrosarcoma causing duodenal obstruction. In the year 2003, Shima et al[15] reported a case of intestinal perforation with consequent meconium peritonitis, as a result of a congenital fibrosarcoma of the jejunum in utero.

At the intestinal site, congenital/infantile fibrosarcoma needs to be distinguished from other spindle cell tumors, including GIST, leiomyosarcoma, spindle cell rhabdomyosarcoma, MPNST, and myofibromatosis. Overall, no specific immunohistochemical marker has been identified for congenital/infantile fibrosarcoma and therefore, the diagnosis needs the exclusion of these entities with an appropriate panel of markers. Electron microscopy can be a useful complement to demonstrate fibroblastic/myofibroblastic differentiation of tumor cells and to exclude other tumor types characterized by different lines of differentiation.

GIST occurs rarely in children and the reported cases showed mainly a spindle cell morphology, with constant expression of CD117 and CD34,[16,17] both markers being negative in the present case. Leiomyosarcoma is extremely rare in the pediatric age, although cases involving the intestinal wall have been reported.[18,19] Histologically, they show either a spindle or spindle and epithelioid morphology, with strong immunoreactivity for actin.[16] Spindle cell rhabdomyosarcoma may be difficult to distinguish from congenital/infantile fibrosarcoma, but it occurs more frequently in the paratesticular region and in the head and neck, and neoplastic cells are positive for desmin and myogenin. In MPNST, neoplastic cells show wavy nuclear contour, nuclear palisading, and there are distinctly alternating hypercellular and hypocellular zones. Infantile myofibromatosis with multicentric lesions may involve the small and large intestines.[20] Histologically, nodules of infantile myofibromatosis have a typical zonal appearance with central cellular areas with hemangiopericytomatous appearance and peripheral portions with distinct myofibroblastic differentiation.

However, the definitive proof for the diagnosis of congenital/infantile fibrosarcoma comes from molecular analyses proving the ETV6-NTRK3 translocation, which is particularly useful in cases arising at unusual sites, like the intestinal tract. ETV6-NTRK3 is the product of a t(12;15) (p13;q25) translocation that fuses the dimerization domain of a transcriptional regulator (ETV6) with a membrane receptor tyrosine kinase NTRK3. ETV6-NTRK3 fusion protein has in vivo and in vitro transforming activity in many cell lineages. Congenital/infantile fibrosarcoma shares this molecular stigma with some other morphologically different tumors such as congenital mesoblastic nephroma and secretory breast carcinoma.[21–23] On the other hand, tumors morphologically similar to congenital/infantile fibrosarcomas do not have ETV6-NTRK3 gene fusion. However, some authors emphasized that the absence of ETV6-NTRK3 gene fusion does not exclude the diagnosis of congenital/infantile fibrosarcoma and argued that its absence should be considered an unfavorable prognostic factor.[24,25]

In conclusion, given the relatively indolent nature of this disease despite the worrying morphology, it is important to avoid misdiagnoses with more aggressive tumors, particularly in the case of extraordinary localization of the tumor. The diagnostic algorithm for the diagnosis of congenital fibrosarcoma outside the usual localizations should comprise morphologic, immunohistochemical, molecular, and ultrastructural studies.

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