

## Case Report

# Spontaneous Leiomyomas of the Gastroesophageal Junction in a Chimpanzee (*Pan troglodytes*)

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A 49-y-old, female chimpanzee presented with a history of cardiac failure. Postmortem examination revealed lesions consistent with congestive heart failure and 2 incidental, round, firm, pale-tan intramural nodules (diameter, 2 cm) in the stomach at the gastroesophageal junction (GEJ). Histologically, the GEJ nodules were diagnosed as benign spindle-cell tumors. Immunohistochemical evaluation revealed neoplastic cells diffusely labeled with  $\alpha$ -smooth muscle actin and vimentin, multifocally labeled for desmin, and were negative for c-kit (CD117). Electron microscopy revealed intracytoplasmic bundles of myofilaments with dense bodies, basal lamina, and few pinocytotic vesicles in the neoplastic cells. According to these findings, leiomyomas of the GEJ were diagnosed. Gastrointestinal stromal tumors have been documented to occur in chimpanzees, but there are no reports of GEJ leiomyomas. To our knowledge, this report is the first description of spontaneous leiomyomas of the GEJ in a chimpanzee.

**Abbreviations:** GEJ, gastroesophageal junction; GIST, gastrointestinal stromal tumors.

Nonhuman primates are commonly used as models of human diseases due to their genomic, physiologic and immunologic similarities to humans. Chimpanzees (*Pan troglodytes*) are the nonhuman primates genetically closest to humans, with interspecies sequence differences of approximately 1% to 1.5%.<sup>26,31</sup> Furthermore, most human cancer genes have been documented to occur in the chimpanzee with high conservation.<sup>27</sup> Benign neoplasia is generally not uncommon in chimpanzees.<sup>11</sup> In humans, gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors of the tubular gastrointestinal tract, although mesenchymal tumors of the gastrointestinal tract are less common than are epithelial tumors.<sup>2</sup> GIST in chimpanzees have been reported<sup>8,11,29</sup> however, spontaneous leiomyomas of the gastroesophageal junction (GEJ) have not in this species. In this report, we describe the histologic, immunohistochemical, and ultrastructural characteristics of incidentally discovered spontaneous leiomyomas of the GEJ in a chimpanzee.

## Case Report

A 49-y-old female chimpanzee was socially housed with 5 other female chimpanzees in a large outdoor compound with heated indoor access. All chimpanzees were fed a commercial primate diet (Lab-Fiber Plus, Monkey Diet Jumbo, no. 5050, Purina, Brentwood, MO). The chimpanzee emergently presented to the Yerkes National Primate Research Center Veterinary Service with extreme lethargy and labored breathing. The animal was anesthetized immediately with Telazol (3 mg/kg; Fort Dodge, NY) for examination

and treatment. Physical examination revealed harsh lung sounds bilaterally, and hepatomegaly was palpated in the upper right abdomen. Cardiac auscultation revealed a grade IV/VI systolic heart murmur. Thoracic and abdominal radiographs showed moderate cardiomegaly and hepatomegaly. A CBC count revealed moderate leukocytosis (WBC count,  $21.8 \times 10^3/\mu\text{L}$ ; reference interval,<sup>18</sup> 6.48 to  $16.25 \times 10^3/\mu\text{L}$ ) characterized by moderate neutrophilia ( $20.2 \times 10^3/\mu\text{L}$ ; reference interval,<sup>18</sup> 1.83 to  $11.07 \times 10^3/\mu\text{L}$ ). Remaining hematologic and serum biochemical panel findings were within normal limits, and the animal was involved in a behavioral study that was approved by the IACUC of Emory University. During examination, the chimpanzee developed bradycardia with weak peripheral pulses and went into cardiovascular arrest. Atropine (3.5 mg IV; West-Ward, Eatontown, NJ) followed by lidocaine (138 mg IV; Hospira, Lake Forrest, IL) and cardiopulmonary resuscitation were administered for the bradycardia. Electrocardiography (Portable Multiparameter Patient Monitor, model MEC-1200 VET, Mindray, Hamburg, Germany) revealed ventricular fibrillation, and no pulses were palpated. Due to suspected cardiac failure and poor prognosis, the chimpanzee was euthanized and submitted for a complete necropsy examination.

For histopathologic examination, various tissue samples were fixed in 10% neutral buffered formalin, routinely processed, paraffin-embedded, sectioned at 5  $\mu\text{m}$ , and stained with hematoxylin and eosin. Postmortem examination revealed peritoneal and pleural effusions. The liver was diffusely congested, enlarged, and irregular with an enhanced reticular pattern (nutmeg appearance). The mitral valves had severe endocardiosis, and the aorta contained multifocal mineralized plaques. There were 2 round to oval, firm, pale-tan intramural nodules (diameter, approximately 2 cm) in the stomach at the GEJ (Figure 1). The kidneys were irregular, with many cortical pits. The lungs were moderately edematous. No other significant gross findings were noticed.

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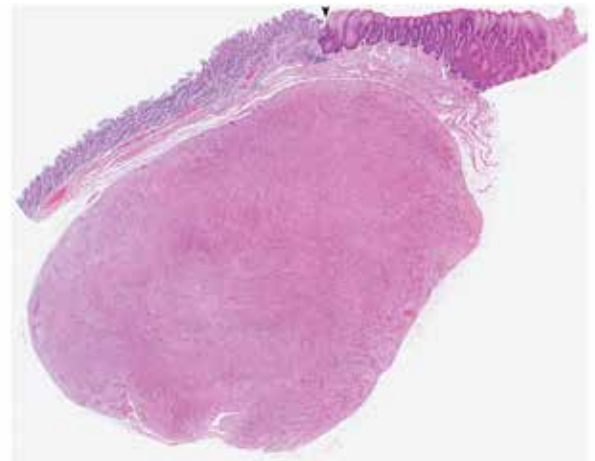


**Figure 1.** The stomach contains 2 round to oval, firm, intramural nodules (diameter, 2 cm) at the GEJ. (Inset) On cut surface (formalin-fixed specimen), the nodules were pale tan.

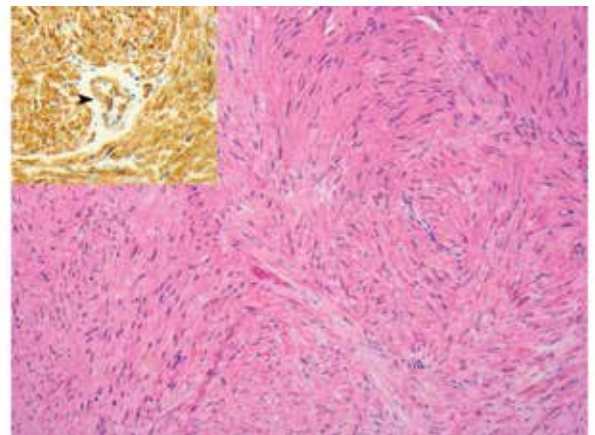
Histologically, the GEJ nodules were unencapsulated, moderately cellular neoplasms that expanded the tunica muscularis and that were composed of spindle cells arranged in interlacing streams and bundles supported by fine fibrovascular stroma (Figure 2). Neoplastic cells were densely packed, with indistinct cell borders and frequently contained moderate amounts of eosinophilic fibrillar cytoplasm (Figure 3). Nuclei were oblong to cigar-shaped, with finely stippled chromatin. Anisocytosis and anisokaryosis were mild. Mitotic figures were not detected in 50 high-power fields of view. The histomorphologic features of the neoplastic cells in the current case were suggestive of leiomyomas or GIST. Other significant microscopic lesions in this case were severe hepatic amyloidosis and chronic passive congestion, moderate splenic amyloidosis, moderate pulmonary edema with numerous hemosiderin-laden macrophages, moderate myocardial fibrosis, severe aortic mineralization, moderate renal interstitial fibrosis, and moderate duodenal Brunner gland hyperplasia. The gross and histologic findings were compatible with congestive heart failure, which caused acute death of this chimpanzee. Aortic calcification has been reported to cause congestive heart failure by eroding compliance and elastance, similar to the situation in the current case.<sup>14</sup>

To characterize the nature of the GEJ nodules, immunohistochemical staining and transmission electron-microscopic examination were performed. The formalin-fixed, paraffin-embedded sections of GEJ nodules were labeled with biotinylated monoclonal mouse antibodies to human  $\alpha$ -smooth muscle actin (clone 1A4), human c-KIT (CD117; clone T595), human desmin (clone D33), and human vimentin (clone V9) by using the streptavidin-biotin complex peroxidase method as described by the manufacturer (EnVision+ System-HRP Labeled Polymer, Dako, Carpinteria, CA). The positive control tissues were sections of human tissue previously assayed for reaction with the primary antibodies (not shown). The negative controls were prepared by substituting the specific primary antibody with antibody diluent. Neoplastic cells labeled diffusely positive for  $\alpha$ -smooth muscle actin (Figure 3) and vimentin, multifocally positive for desmin, and negative for c-KIT.

The tissue sections from GEJ nodules were fixed in 10% neutral buffered formalin prior to processing for electron microscopy. For transmission electron-microscopic examination, 1-mm<sup>3</sup> frag-

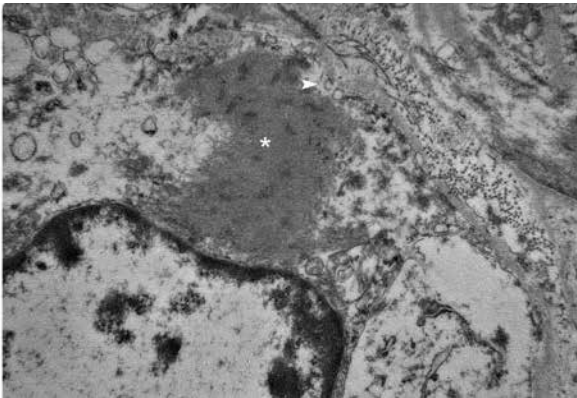


**Figure 2.** The GEJ nodule is an unencapsulated, moderately cellular neoplasm expanding the tunica muscularis and composed of spindle cells. Hematoxylin and eosin stain; magnification, 5 $\times$ .



**Figure 3.** The GEJ neoplasms are composed of tightly packed spindle cells arranged in multiple interlacing streams and bundles. Hematoxylin and eosin stain; magnification, 100 $\times$ . (Inset) The neoplastic cells intensely immunolabeled with  $\alpha$ -smooth muscle actin. Internal positive control, vascular smooth muscle cells (arrowhead). Immunohistochemical staining for  $\alpha$ -smooth muscle actin; magnification, 100 $\times$ .

ments of the affected areas of GEJ nodules were fixed in 3% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in liquid epoxy resin (Embed-812, Electron Microscopy Sciences, Hatfield, PA). The ultrathin sections (70 to 90 nm) were stained with lead citrate and uranyl acetate and examined with an electron microscope (JEM-1011, JEOL, Tokyo, Japan). Ultrastructurally, neoplastic cells had intracytoplasmic bundles of myofilaments with dense bodies, basal lamina, and few pinocytotic vesicles (Figure 4); these features are characteristic of smooth muscle differentiation.<sup>5</sup> Neoplastic cells contained moderate numbers of mitochondria and profiles of rough endoplasmic reticulum. Nuclei were elongated or contracted and contained coarsely stippled prominent heterochromatin or, in some cases, were margined at the inner nuclear membrane. The histomorphologic, immunohistochemical, and ultrastructural features of the neoplastic cells in the current case were most consistent with leiomyoma.



**Figure 4.** Ultrastructural view of neoplastic cells in the GEJ neoplasms demonstrates the presence of bundles of myofilaments with dense bodies (asterisk), basal lamina, and few pinocytotic vesicles (arrowhead). Lead citrate and uranyl acetate stain; magnification: 12,000 $\times$ .

## Discussion

Neoplasia in nonhuman primates is becoming increasingly common due to current emphasis on aging research.<sup>10-12,20</sup> Given the limited number of chimpanzees used in a research setting, there are rare comprehensive studies documenting neoplasia in this species. A recent survey on the incidence of neoplasia in chimpanzees at the Southwest National Primate Research Center and Yerkes National Primate Research Center reported the urogenital system as the most commonly affected, followed by the integumentary, endocrine, alimentary, respiratory, musculoskeletal, and cardiovascular systems in female chimpanzees. Furthermore, cervical, uterine, and vaginal leiomyomas were the most common tumors seen in female chimpanzees.<sup>11</sup> In male chimpanzees, the alimentary system was the most commonly affected, followed by the endocrine, cardiovascular, integumentary, respiratory, hematopoietic, and urogenital systems.<sup>11</sup> Although GIST of the stomach, colon, and rectum have been diagnosed, gastrointestinal leiomyomas in chimpanzees have not been reported. Neoplasms in the gastrointestinal system were more likely to be malignant than are those of other organ systems.<sup>11</sup> Spontaneous intestinal adenocarcinomas are commonly diagnosed in nonhuman primates, whereas leiomyomas of the esophagus, stomach, cecum, or colon are rare.<sup>10,17,30</sup>

Colonic leiomyomas found in a dwarf galago and cotton-top tamarin were 0.2 cm in diameter.<sup>10</sup> A recent study described a larger (3  $\times$  5  $\times$  3 cm) small-bowel leiomyoma in a black-crested macaque.<sup>6</sup> In humans, leiomyomas have ranged from 0.2 to 12 mm with a mean diameter of 1.7 mm, and all were diffusely positive for  $\alpha$ -smooth muscle actin but negative for c-KIT (CD117).<sup>2</sup> Giant leiomyomas of the GEJ, with sizes varying from 4 to 12 cm in diameter, has been described in humans.<sup>9</sup> The tumors at the GEJ in the current case (2 cm in diameter) were discovered as an incidental finding; GIST was considered as a differential diagnosis during the necropsy examination. Transmission electron-microscopic examination was performed in the current case because GIST histologically resemble smooth muscle tumors. Ultrastructurally, GIST in rhesus macaques showed features of primitive mesenchyme or Schwann cells.<sup>7</sup> In addition, c-KIT expression is considered an important factor in diagnosing GIST in nonhuman primates and humans.<sup>8,22,29</sup> However, no such transmission electron-microscopic or immunohistochemical changes were

apparent in the present case. Neoplastic cells in this chimpanzee labeled strongly with the smooth-muscle marker ( $\alpha$ -smooth muscle actin) and ultrastructurally showed myofilaments with intermediate densities, pinocytotic vesicles, and basal lamina. These are all features of smooth muscle tumors.

In canines, esophageal tumors account for less than 0.5% of all neoplasms, and leiomyomas are found most commonly in stomach, particularly at the GEJ; some of these masses are incidental findings.<sup>13,28</sup> However, a recent retrospective study in canines revealed leiomyomas predominantly localized in the stomach (76%), followed by esophagus (14%) and intestine (10%); only one tumor was detected at GEJ.<sup>15</sup> In humans, gastric leiomyoma is the most common smooth-muscle tumor and accounts for 80% of all gastric stromal tumors. Approximately 1/3 of these tumors are located at the GEJ and often are asymptomatic.<sup>16,19</sup> In addition, esophageal leiomyomas are diagnosed more frequently than are GIST in humans.<sup>23</sup> GIST and leiomyomas usually are detected either because of clinical symptoms ranging from gastrointestinal bleeding<sup>22,24,25</sup> to abdominal pain or because they are incidentally discovered during surgical procedures for other reasons.<sup>2,32</sup> The term 'seedling leiomyomas' was used to describe incidentally discovered, small (< 7 mm), esophageal leiomyomas.<sup>32</sup> Among 342 patients, 7.9% had GEJ leiomyomas<sup>32</sup> a higher incidence (22%) was seen in another study of patients ( $n = 77$ ) undergoing surgical resections for esophageal carcinoma.<sup>3</sup> A recent study showed higher incidence of seedling leiomyomas (47%) in comparison to incidental GIST (10%) in patients ( $n = 150$ ) undergoing surgery for esophageal carcinoma.<sup>2</sup> Leiomyomas of the gastrointestinal tract are predominantly found in male patients and occur most often in the colon, rectum, and by esophagus.<sup>14</sup> Various X-chromosomal abnormalities, including collagen type IV  $\alpha 5$  and  $\alpha 6$ , have been proposed to account for the male predominance of these neoplasms.<sup>21</sup> Here, we report the incidental discovery of GEJ leiomyomas in a female chimpanzee.

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