Author's response to reviews

Title: Leiomyosarcoma with partial rhabdomyoblastic differentiation: First case report of primary cardiac origin

Authors:

Yoichiro Okubo (yoichiro0207@med.toho-u.ac.jp)
Kazutoshi Shibuya (kaz@med.toho-u.ac.jp)
Atsushi Namiki (namiki@med.toho-u.ac.jp)
Kazuhisa Takamura (kazuhisa.t@med.toho-u.ac.jp)
Noriaki Kameda (nkameda@med.toho-u.ac.jp)
Tetsuo Nemoto (tetsuo.nemoto@med.toho-u.ac.jp)
Aki Mitsuda (akimitsuda@med.toho-u.ac.jp)
Megumi Wakayama (megumi.wakayama@med.toho-u.ac.jp)
Minoru Shinozaki (ushino@med.toho-u.ac.jp)
Nobuyuki Hiruta (.nhr@med.toho-u.ac.jp)
Kanako Kitahara (kit@med.toho-u.ac.jp)
Takao Ishiwatari (takaboo1982@yahoo.co.jp)
Junichi Yamazaki (yamasaki@med.toho-u.ac.jp)

Version: 2 Date: 21 January 2011

Author's response to reviews: see over
Dear Chief Editor

We deeply appreciate for your kindest and valuable suggestions and comments. We have carefully revised the manuscript with following to reviewer’s comments, and are sending following point-to-point response herewith a revised manuscript.

It would be so grateful if you can consider again our manuscript for publication.

For comments of reviewer 1 (Dr. Augusto Orlandi):

Reviewer’s comment:

[Macroscopic and microscopic findings they reported are not confident with the diagnosis of leiomyosarcoma; the overall appearance is that of a solid-myxoid tumor, with no hypercellular and typical spindle cell areas, nor typical nuclear and perinuclear details. Also necrosis and mitosis evidence is lacking. Additional images and new immunohistochemical stainings at low magnification are needed. Since the Authors reported about 70% of a-SMA positive cells, but in the reported images only few cells are a-SMA positive.]
Our response:

First, we apologize for our ambiguous presentation of light microscopic photographs. We carefully observed the surgical specimen again and necrosis was confirmed. Consequently, we revised the sentence as follow from line 11 to line 15 of page 5.

Accordingly, the histological grade of the tumor corresponded to grade-2 (tumor differentiation: score-2; mitotic counts: score-2; tumor necrosis: score-1) following to the French National Federation of Cancer Centers (FNCLCC) grading system (Fig. 3C and D) [12].

Second, we described “The rates of α-SMA and myogenin double negative, α-SMA single positive, myogenin single positive, and α-SMA and myogenin double positive in spindle cells were estimated as 69.1%, 28.8%, 1.1% and 1.0%, respectively” on page 6.

It means about one-third of the spindle cell showed positive reactivity for α-SMA on average.

We apologize for our ambiguous description. We added a new table (table 1) indicating summary of immunohistochemical examination of the identical types of tumor cell in the case to avoid misleading the reader about this description.

Finally, we added and replaced the figures as requested additional images (Figure 3, 4, and 5) of which revised legends are as follow.
Figure 3

Shot title: Photomicrographs showing spindle cells with areas of hypercellularity or necrosis.

Legend: (A) Low-power view of the area with spindle cells proliferation (Hematoxylin and Eosin (HE) double stain, x 40). (B) Spindle cells with positive reactivity for $\alpha$-smooth muscle actin (SMA) comprise approximately 30% area of the tumor (Immunostain, anti-$\alpha$-SMA x 40). (C) There is focus of necrosis in the limited the tumor. Following to the French National Federation of Cancer grading system, presence of tumor necrosis less than half in area corresponds to score-1 (HE double stain, x 100). (D) Spindle cells showed mitosis. On average, twelve mitoses per ten high-power fields were confirmed. Following to the French National Federation of Cancer grading system, mitotic activity of the present case corresponds to score-2 (HE double stain, x 1000).

Figure 4

Short title: Photomicrographs showing spindle cells and their phenotypical expression.

Legend: (A) Spindle cells had an elongated, blunt-ended and hyperchromatic nucleus plus spindle, were fibrillated, and possessed an eosinophilic cytoplasm. Occasional cells have perinuclear vacuoles (Hematoxylin and Eosin double stain, x 400). (B, C, D, E, and F) Photomicrographs of immunostain with desmin (B), $\alpha$-smooth muscle actin (C), myoglobin (D), myogenin (E), and cytokeratin CAM 5.2 (F), respectively (x 400).
Figure 5

Short title: Photomicrographs showing polyhedral cells and their phenotypical expression.

Legend: (A) Polyhedral cells had a hyperchromatic and eccentric nucleus with a polyhedral, large, and eosinophilic cytoplasm (Hematoxylin and Eosin double stain, x 1000). (B, C, D, E, and F) Photomicrographs of immunostain with desmin (B), α-smooth muscle actin (C), myoglobin (D), myogenin (E), and cytokeratin CAM 5.2 (F), respectively (x 1,000).

Reviewer’s comment:

[Moreover, leiomyosarcoma can also express cytokeratins, and this should be verified.]

Our response:

We appreciate reviewer’s important advice. To follow the comment, we performed immunohistochemical examination using four clones of anti-cytokeratin (CK) antibodies, including CK AE1/AE3, 34 β-E 12, 5/6, and CAM 5.2. As a result, the spindle cell showed focal positive reactivity for only CK CAM5.2 and we added following sentence from line 17 to line 25 of page 7.

Meanwhile, it is interesting to note that the spindle cell showed positive reactivity for CK CAM 5.2.

Although, it has been well known that leiomyosarcoma usually showed negative reactivity for
epithelial markers [15], some investigators described that a part of leiomyosarcoma shows positive reactivity for CK [15-17]. Therefore, CK CAM 5.2 expression in the present case may support a diagnosis of leiomyosarcoma.

Reviewer’s comment:

[As presented here, my preferred diagnosis for this case is undifferentiated sarcoma with some rhabdomyoblastic features (that are quite limited in the documented evidences), that are sometimes reported in this type of cardiac tumors. In that sense, the differential diagnosis in the discussion is clearly incomplete. The Authors should also consider and discuss the differential diagnosis with other a rare rhabdomyosarcoma and/or rhabdomyoma of the heart.]

Our response:

We would like to thank you for your important comment. To discuss the differential diagnosis of the tumor, we added the following sentence from line 25 of page 7 to line 29 of page 9.

However, to make diagnosis of leiomyosarcoma with rhabdomyoblastic differentiation, we should refer three important tumors and deny them, respectively, which comprise undifferentiated pleomorphic sarcoma (UPS), rhabdomyosarcoma, and rhabdomyoma. Cardiac UPS usually occurring at the left atrium, histopathologically comprises a mixture of spindle cells in a storiform
pattern with polyhedral cells [5]. Furthermore, high-grade undifferentiated sarcomas can exhibit focal $\alpha$-SMA expression [15]. These findings are similar to the present case. However, the spindle cell, a major component of the present tumor, had an elongated, blunt-ended, and hyperchromatic nucleus plus spindle, fibrillated, and eosinophilic cytoplasm. In addition, the cell showed positive reactivity both for $\alpha$-SMA and desmin, focally, by immunohistochemical examination. These findings allowed disclosing the smooth muscle differentiation. Furthermore, some of the spindle cell also showed positive reactivity for CK CAM 5.2, of which positive ratio has been reported ranging from 22.2% (2/9) to 35.0% (14/40) in leiomyosarcoma [16, 17]. Although it still remains a difficulty for decision, we made the diagnosis of leiomyosarcoma rather than UPS. On the other hand, since rhabdomyosarcoma has been know as the second most common primary cardiac malignant tumor [2], that should also be considered as a disease for differential diagnosis. Especially, embryonal rhabdomyosarcoma usually shows similar morphologic findings of the present case, such as varying degrees of cellularity containing hypercellular and loosely textured myxoid areas, hyperchromatic and round or spindle-shaped nucleus, and eosinophilic cytoplasm [18]. However, embryonal rhabdomyosarcoma is uncommon in patients older than 40 years of age [18] and neither cross-striation nor myoglobin expression was proven in the present case. Furthermore, a large body of spindle cells showed negative reactivity for myogenin (only 2.1 % of them showed positive reactivity) that has been largely accepted as a sensitive and specific immunohistochemical marker.
for rhabdomyosarcoma or other tumors with rhabdomyoblastic differentiation [14]. According to our immunohistochemical examinations, we were able to deny typical rhabdomyosarcoma. As for rhabdomyoma, the most common subtype of cardiac origin has been known as cardiac rhabdomyoma, but it occurs almost exclusively in the hearts of infants and young children and composes predominantly large polygonal vacuolated spider cells [19]. Therefore, the adult type of rhabdomyoma should be considered as differential diagnosis which is usually composed of tightly polygonal cells which had peripherally placed nucleus plus acidophilic, finely granular, and vacuolated cytoplasm. However, mitotic figures are nearly absent, cross-striations can be discerned, and show positive reactivity for rhabdomyogenic markers immunohistochemically in these two subtypes of rhabdomyoma [19]. These results were different from the findings extracted from the present case.

On the other hand, only one case of sarcoma arisen from myocardium with rhabdomyoblastic differentiation has been reported by Kabir et al. [20] who described a malignant peripheral nerve sheath tumor indicated an area of rhabdomyoblastic differentiation in part. In their report, a little information of immunohistochemical examinations was described which simply comprised positive reactivity for s-100 protein and focal for desmin. These results were different from these of the present case, but comparative discussion could not be completed in detail.
Reviewer’s comment:

[A clinical image should be added to document the involvement of left atrium.]

Our response:

We added cardiac ultrasonography as figure 1. Figure legend is as follow:

Short title: Photograph showing cardiac ultrasonography.

Legend: (A) Transthoracic cardiac ultrasonography performed in our hospital showed showing a club-shaped tumor of 34 mm in diameter inside the left atrial cavity in a four-cavities tomogram.

(B) Transesophageal cardiac ultrasonography showed showing a broad-based, gigantic, and multilocular tumor occupying almost the entire left atrium.