Ancona,
27th July 2017

Reviewer's comments.

Liver nested epithelial stromal tumor is a rare primary hepatic tumor with growth of spindle cell and epithelial cell and it is very difficult to confirm differential diagnosis. The reported number of this non-hepatic and non-biliary tumor is very limited. Accumulation of clinical knowledge is also small. Usually this rare tumor develops in young female and less aggressive. This case is 30's male and the character of tumor is very aggressive. Experience of chemotherapy is also important.

1. To clarify the tumor cell, interpret the result of immunohistochemistry.
2. In this case, the character of tumor cell is quite different from that of reported ones. Do you think how the tumor cells acquire the malignant potentials?
3. Is there any predisposing factor for the increased the risk of tumor?
4. Is there any similarity between this tumor and hepatoblastoma?

We thank the reviewer for the comment.

Point-by-point reply to reviewer's comments.

1. The cellular nests showed positive staining for markers of epithelial differentiation such as citokeratins CAM 5.2, AE1/AE3, EMA, and for β-catenin, WT1, GPC3, and CD56.

   The negativity for chromogranin allow to rule out a neuroendocrine tumor, while the negativity for hepatocyte antigen EpPar1 and for CK7 and CK19 exclude a hepatocellular tumor and/or an adenocarcinoma.

   The stroma surrounding the cellular nests showed a myofibroblastic nature, with positive staining for smooth muscle actin (ACTML) and negative for citokeratins and EpPar1.

   <u>As we reported in the revised manuscript</u> : "The cellular nests showed positive staining for markers of epithelial differentiation positive for CAM 5.2, AE1/AE3, EMA (focal), β-catenin (both membrane and

nuclear), WT1 (both membrane and cytoplasmatic), GPC3 (focally cytoplasmatic) and CD56 (diffuse) and negativity for hepatocyte antigen EpPar1 (hepatocyte paraffin 1), CK7, CK19, CD34, CD99, cromogranin and desmin ."

2. Compared to the literature[4], the neoplasm in this report showed a higher mitotic rate, foci of necrosis and most importantly, a more invasive pattern with respect to the surrounding hepatic parenchyma and the peri-hepatic soft tissue. Moreover a vascular invasion was observed macroscopically. The morphology appearance and the immunohistochemical results are comparable with the other ones described; tumoral cells did no showed significant atypia. We assumed that necrosis, high mitotic rate, invasion of the surrounding parenchyma and vascular invasion are the features defining the malignant potential and so the aggressive behavior of this rare neoplasm.

   As we reported in the revised manuscript: "Necrosis, high mitotic rate, invasion of the surrounding parenchyma and vascular invasion are the features that might explain the malignant potential and so the aggressive behavior of this rare neoplasm."

3. As far as we know, there are no predisposing factors increasing the risk of occurrence of this rare type of tumor. In the few cases reported in the literature, in two cases it occurs in children with multiple abnormalieties, one with omphalocele, renal abnormalities, and mental retardation suggestive of Beckwith-Wiedemann syndrome and another who was subsequently diagnosed with perilobar nephroblastomatosis and Wilms tumor. [4] Furthermore in literature was describe a few cases of NEST associated with Cushing syndrome at diagnosis. In this cases after excision of the tumors the Cushing syndrome was abated but the correlation remain unknown.

   As we reported in the revised manuscript: "As far as we know, there are

no predisposing factors increasing the risk of occurrence of this rare type of tumor. In literature was describe a few cases of NEST associated with Cushing syndrome at diagnosis. In this cases after excision of the tumors the Cushing syndrome was abated, but the correlation remain unknown"

4. The main differential diagnosis for this tumors is hepatoblastoma. At first glance, areas predominantly composed of epithelioid nests may resemble islands of fetal hepatoblastoma cells. At closer inspection, the epithelioid components of NSET are discohesive, and lack the homogeneity and cord-like organization of fetal hepatoblastoma cells. Hepatoblastomas may have mesenchymal components with the formation of osteoid like the neoplasms in this report but lacks the stromal architecture of NSET. Moreover, both component, epithelioid and stromal cells, were negative for the hepatocyte antigen EpPar1.

   As we reported in the revised manuscript: "Areas predominantly composed of epithelioid nests may resemble islands of fetal hepatoblastoma cells."