Peer Review File

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Reviewer Comments

Comment 1: How does PNET differentiate from other diseases? It should be analyzed in combination with clinicopathological and histomorphological characteristics.

Reply 1：

Comment 1, comment 2 and comment 7 are all about how PNET was diagnosed, its pathological and immunohistochemical features, and how our case was diagnosed as PNET and how to differentiate it from other types of rare renal tumors. We would like to answer these three comments together. We have briefly described this issue in the page line of the original manuscript, but as the reviewers said, our discussion on this manuscript is not deep enough.

The diagnosis of ES / PNET mainly depends on the microscopic morphology under HE staining combined with immunohistochemical analysis of pathological tissues. Firstly, under HE staining, PNET showed small and round single tumor cells with sparse cytoplasm, vacuole shape, round to oval nucleus, dark cytoplasm, granular chromatin evenly dispersed, unclear nucleoli and frequent mitosis. Large area necrosis and occasional lymphovascular embolus were also found. For the small round blue tumor cells initially diagnosed as ES / PNET, further diagnosis depends on immunohistochemical staining including chromogranin (CG), neuron specific enolase (NSE), CD99, Wilms tumor (WT1), leukocyte common antigen (LCA), cytokeratin (CK),etc. For the membranous expression with strong CD99 positive and mainly diffuse, NSE can be localized positive, which implies that the tumor is neurogenic; WT1 is negative, which can differentiate it from other Wilms tumors. Other markers include negative LCA (leukocyte common antigen) to differential diagnosis from lymphoma, CK (cytokeratin) to differential diagnosis from synovial sarcoma and so on. In our 7 patients, all patients were positive for CD99, which is a typical

immunohistochemical feature of ES / PNET. In addition, LCA of all patients was negative, which made our specimens well differentiated from lymphoma. In order to identify other epithelial tumors, all our specimens had negative results of CK8 / 18, CK7 or AE1 / AE3.

Changes in the text:

Insert" The patients' specimens were fixed by formalin and embedded in paraffin for HE staining and immunohistochemical analysis. All pathological slices were read by two pathologists, and the results were reviewed by a senior pathologist." at page 4 line 5.

Insert" In our 7 patients, all patients' samples were positive for CD99, which is a typical immunohistochemical feature of ES/PNET. In addition, all patients' samples were negative for LCA, which well differentiates PNET from lymphoma. To identify other epithelial tumors, all our specimens had negative CK8/18, CK7 or AE1/AE3 results." at page 10 line 9.

Comment 2: The detection and research of immunohistochemistry should be increased, which has certain significance for the diagnosis of markers.

Reply 2： See comment 1 reply1.

Comment 3: This study uses telephone follow-up, because the uncertainty of communication is easy to bias the results. The content of written follow-up should be added.

Reply 3：

As reviewers have said, telephone follow-up is very easy to bring potential risk of bias. However, because of our patients come from all over the country and the disease is very rare, there is usually only one patient with the disease in a certain region. It is very difficult for us to follow up the patients and their families with paper-based follow-up forms. Although we used the telephone follow-up method, all the follow-up visits were completed by the same researcher, and the telephone follow-up of each patient's family members was conducted. All the follow-up visits were conducted for the second

time after 1 week. For patients with inconsistent results from the two follow-up visits, we will conduct the third telephone follow-up after a period of time. In addition our telephone follow-up strictly follow the sequence and content of the following questions:

Part I Identification

1. Are you the person or family member of XXX?

2. Did XXX go to the Peking University First Hospital on XXXX(date)and had surgery / puncture?

Part II Survival Situation

3. Is XXX still alive?

4. If the patient died, what did the patient die for? Is it multiple metastasis of tumor or other reasons?

Part III Treatment After Surgery

5. Does the patient receive chemotherapy or radiotherapy after surgery / puncture?

6. If there is chemotherapy or radiotherapy, what is the plan?

Part IV Informed Consent

7. We plan to use the patient's data in a retrospective clinical study to help improve the diagnosis and treatment of the disease in the future. We will keep the patient's name, address, contact information and other personal information confidential, and only use the clinical data of the patient. Would you like to use the clinical data of the patient for this study?

We hope to reduce the potential bias caused by telephone follow-up as far as possible by such a way that only a single researcher is used for follow-up, multiple repeated inquiries, and strict regulations on the order and content of the researcher's questions.

Changes in the text:

Insert" All the follow-up visits were completed by the same researcher, and telephone follow-up of each patient's family members was conducted. All follow-up visits were conducted for a second time 1 week later. For patients with inconsistent results from the two follow-up visits, we conducted a third telephone follow-up after a period of time. In addition, our telephone follow-up strictly followed a set sequence and question content. " at page 5 line 6.

Comment 4: What is the treatment and prognosis of PNET? What is the best treatment now? What is the relationship with heredity?

Reply 4：

For renal PNET， surgery is the main treatment strategy, especially radical nephrectomy, For Ewing sarcoma without further metastasis (not only primary at kidney), VAC (cyclophosphamide + driamycin + vincristine) and IE (ifosfamide + etoposide) are most common postoperative adjuvant chemotherapy ,which can significantly improve the prognosis of patients. However, there is still no high-level evidence-based medicine evidence to answer this question that whether adjuvant or neoadjuvant chemotherapy can improve the poor prognosis of patients with primary renal PNET.

Risi et al summarized the reports of 116 cases of PNET published before 2012. The 2-year survival rate of patients after radical nephrectomy was 80%, and that of patients without radical nephrectomy was 30%(P=0.017). 40% patients had metastasis when diagnosis which median DFS was 5 months and the median survival time was 24 months. In the retrospective study, the 1-year survival rate of patients receiving postoperative chemotherapy or neoadjuvant chemotherapy was 93%, while the 1-year survival rate of patients without chemotherapy was 75%. However, no distinction was made between chemotherapy regimens and neo-adjuvant or adjuvant chemotherapy in this study. Also adjuvant radiotherapy is required for patients with positive margins or late stage. 90% PNET patients show t(11;22)(q24;q12) resulting in EWSR1 – FLI1 gene fusion，which would help diagnosis. In addition, with the further research on Ewing sarcoma family and EWS-FLI1 fusion gene, some drugs aiming at the signal transduction pathways involved in the gene, such as integrin, Wnt, IGF, EGF and PDGF, are also emerging gradually. However, whether these drugs can eventually enter the clinical practice and improve the prognosis of patients is still unknown.

In our 7 patients, the average overall survival time was 12 months (1.9 months to 26.77 months), 5 patients received surgery, 2 patients were unable to perform surgery due to poor general situation, 2 patients received postoperative chemotherapy, and 3

patients did not receive postoperative chemotherapy. The average survival time of patients receiving chemotherapy was 8.3 months (5.4 months to 11.47 months), and the average survival time of patients without chemotherapy was 13.55 months (1.9 months to 26.77 months). The survival situation of our patients was far worse than that reported in the literature.

We analyzed the following reasons: 1. Our patients were basically in the advanced stage, and 6 / 7 patients had distant metastasis when diagnosis which is much higher than 40% in the literature. 2. Due to the bias of literature reports, clinicians are more willing to report some successful cases of their treatment, the operation is more successful and the survival time is longer. For some patients who are also diagnosed with this disease but have poor prognosis, they may not be able to report.

Changes in the text:

Insert" the 2-year survival rate of patients after radical nephrectomy was 80% and that of patients without radical nephrectomy was 30%."at page 3 line 10.

Insert" with a median disease-free survival (DFS) time of 5 months and a median survival time of 24 months." at page 3 line 13.

Insert" In addition, with further research on the Ewing sarcoma family and the EWS-FLI1 fusion gene, some drugs aimed at the signal transduction pathways involved in this gene, such as integrin, Wnt, IGF, EGF and PDGF, are also emerging gradually. However, whether these drugs can eventually enter clinical practice and improve the prognosis of patients is still unknown." at page 11 line 12.

Comment 5: The method description in this study is too simple, please rewrite this part.

Reply 5：We reorganized the method part and wrote a detailed description of the follow-up and data collection process.

Changes in the text:

Insert" The patients' specimens were fixed by formalin and embedded in paraffin for HE staining and immunohistochemical analysis. All pathological slices were read by

two pathologists, and the results were reviewed by a senior pathologist." at page 4 line 15.

Insert" Two researchers collected clinical data," at page 5 line 1.

Insert" After two copies of the data were collected, the third researcher reviewed and collated the data." at page 5 line 3.

Insert" All the follow-up visits were completed by the same researcher, and telephone follow-up of each patient's family members was conducted. All follow-up visits were conducted for a second time 1 week later. For patients with inconsistent results from the two follow-up visits, we conducted a third telephone follow-up after a period of time. In addition, our telephone follow-up strictly followed a set sequence and question content." at page 5 line 6.

Insert" All patients' family members were informed, and all agreed to the use of the patients' clinical data in this retrospective study." at page 5 line 15.

Comment 6: It is recommended to mark the observation position with arrows in Figure 2.

Reply 6：We made a minor modification to Figure2, with a small arrow added as you said.

Comment 7: What are the pathomorphological and immunophenotypic characteristics of PNET? Further analysis and comparison should be carried out in conjunction with the literature.

Reply 7：see comment 1 reply1

Comment 8: There are many uncertainties in retrospective research, which increase the deviation of research results. How to explain and solve this problem?

Reply 8：As the reviewers have said, the bias and uncertainty of retrospective study are greatly increased compared with prospective study. In order to solve this problem as much as possible, we first strictly control the collection process of retrospective medical records. We arranged two researchers to collect clinical data, including patient's age and

gender, preoperative test results, pathological HE staining results and immunohistochemical results. After two copies of the data were collected respectively, the third researcher reviewed and collated the date. In the process of telephone follow-up, all follow-up were completed by the same researcher, and the telephone follow-up of each patient's family members was conducted. All the follow-up visits were conducted for the second time after 1 week. For patients with inconsistent results, we will conduct the third telephone follow-up after a period of time, and strictly follow the fixed order and content of questions. The clinical data at the time of diagnosis are relatively reliable because of the detailed records of electronic medical records. However, most of the patients were not treated in our center after surgery, and all patients died when we followed up by telephone. Therefore, there is a memory bias in this part of the data. It also wrote in our manuscript at page line that although two patients received chemotherapy after surgery, their families could not recall the specific plan and implementation cycle of chemotherapy due to too long time. This is a very big defect of our article. In order to make up for this regret, we also discussed the prognosis of existing case reports in the discussion section. For such a rare type of renal tumor, it is difficult to carry out a prospective clinical study. We believe that reporting the clinical information as far as possible can also contribute to the research of this rare disease.

Changes in the text:

Insert" Two researchers collected clinical data," at page 5 line 1.

Insert" After two copies of the data were collected, the third researcher reviewed and collated the data." at page 5 line 3.

Insert" All the follow-up visits were completed by the same researcher, and telephone follow-up of each patient's family members was conducted. All follow-up visits were conducted for a second time 1 week later. For patients with inconsistent results from the two follow-up visits, we conducted a third telephone follow-up after a period of time. In addition, our telephone follow-up strictly followed a set sequence and question content." at page 5 line 6.

Insert" And due to the existence of literature bias, clinicians are often more willing to report successful treatment of their patients, with more successful operations and

longer survival times. Therefore, some patients who are also diagnosed with this disease but have poor prognosis may not be reported. These factors create a possible bias in the literature. All of these factors led to the overall survival time of our patients being lower than that reported in the previous literature. ”at page 12 line 7.

Insert” There are several limitations in our study: 1. This is a retrospective study, and the number of cases is low. This creates a potential bias, although for such a rare type of renal tumor, a prospective study is very difficult, and prospective clinical trials are undoubtedly of greater significance for diagnosis and treatment. 2. Because our patients come from all over the country and the disease is very rare, there is usually only one patient with the disease in a certain region. It is very difficult for us to follow up with patients and their families with paper-based follow-up forms. Therefore, we chose telephone follow-up, which creates a potential risk of bias. 3. Since postoperative treatment was not conducted in our hospital, and it had been some time since the death of the patients when the patients' family members were contacted, the family members of the only two patients who received postoperative chemotherapy could not recall the chemotherapy plan and cycle administered to the patients.” at page 12 line 13.

Comment 9: The introduction is too simple. Many researches on PNET are not involved in the introduction of this paper. It is suggested to supplement relevant information.

Reply 9： We re-wrote the introduction part, and reviewed some literatures that had not been included before.

Changes in the text:

Insert” and fewer than 200 cases have been reported thus far. Renal PNET is more common in young adults and older children (median age: 29 years old, 4-61 years old), and the male-to-female incidence ratio is 2:1 ~ 3:1.”at page 3 line 7.

Insert” the 2-year survival rate of patients after radical nephrectomy was 80% and that of patients without radical nephrectomy was 30%.” at page 3 line 10.

Insert” with a median disease-free survival (DFS) time of 5 months and a median survival time of 24 months.” at page 3 line 13.

Insert” Renal PNET also has no specificity in imaging examinations; it often manifests as a single, large mass without obvious boundaries in CT, MRI or ultrasonography.4 The diagnosis of PNET is still based on the histological and immunohistochemical staining of biopsy or surgical specimens; 90% of patients with PNET had a specific translocation gene, t (11:22) (q24; q12), and the fusion gene EWS-Fli-1, which could be found by fluorescence in situ hybridization (FISH) and reverse transcription polymerase chain reaction (RT-PCR), which can help in diagnosis.5 However, there is still no consensus on the best treatment for such a rare disease. Surgery is currently the main treatment strategy, especially radical nephrectomy. Adjuvant radiotherapy is also required for patients with positive margins or late-stage disease, and neoadjuvant and adjuvant chemotherapy may prolong survival in patients with metastases.” at page 3 line 15.