List of Responses

Dear Editors and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "Primary squamous cell carcinoma with sarcomatoid differentiation of the kidney associated with ureteral stone obstruction: A case report

" (ID: 78555). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked in red in the paper. The main corrections in the paper and the responds to the reviewer's comments are as flowing:

Responds to the reviewer's comments:

Reviewer #1:

1. Response to comment: (Creatine in serum was 80 umol/l. Kindly confirm the reading)

Response: We are very sorry for our negligence of the creatine in serum. We have revised it as the patient's serum urea and creatinine levels were 3.37mmol/l and 67μmol/l

2. Response to comment: (Elaborate on the other published case of SRCC. Compare features with the present report)

Response: Considering the Reviewer's suggestion, we have added the following paragraph:

This case was a 55 years old female,presented with flank pain and an abdominal mass , CT scan demonstrated gross right renal hydronephrosis with renal parenchymal atrophy and multiple right renal calculi. She accepted open nephrectomy.The pathology suggested that the macroscopic examination showed hydronephrosis with severe pyonephritis, obvious cortical atrophy and obstructive kidney stones at PUJ. Histological examination unexpectedly revealed squamous metaplasia of pelvic urothelial epithelium with free debris of squamous cell carcinoma in situ (SCC) and sarcomatoid tumors.The five epithelial markers of sarcomatoid tumors were

all negative, and only p63 was positive. Two months after the operation, a large mass was found in the original renal fossa. The needle biopsy considered squamous cell carcinoma with sarcomatoid transformation. Then she received palliative radiotherapy and died 3 months after nephrectomy. Our case is similar to this case, the difference is that our case can find obvious tumors on CT.

3. Response to comment: (Add a table summarizing the few reported cases)

Response: As other reports are all reports of renal squamous cell carcinoma, and some of the data are incomplete, a complete table cannot be formed.We are very sorry for this outcome.

4. Response to comment: Differential diagnosis at both clinical, radiographical and histopathological levels must be elaborated.

Response: We have re-written this part according to the Reviewer's suggestion

As Reviewer suggested

Clinically, SCC or sRCC occurs in middle-aged patients with symptoms including flank pain, hematuria, and an abdominal mass, and is often associated with chronic inflammation, hydronephrosis, and squamous metaplasia[11]. In 18–100% of cases, urolithiasis is a main risk factor[2]. Therefore, it is difficult to differentiate the disease clinically.

Radiologically, primary SCC of the renal pelvis may appear as a solid mass, with hydronephrosis, calcifications, or as arenal pelvic infiltrative lesion without evidence of a distinct mass[12].The radiologic differential diagnosis includes primary and secondary renal neoplasms and xanthogranulomatous pyelonephritis (XGP) associated with renal calculi. XGP is an uncommon form of chronic pyelonephritis, typically occurring as a result of chronic obstruction, which leads hydronephrosis, causing destruction of renal parenchyma. XGP is commonly associated with lithiasis however, rarely

causes keratinizing squamous metaplasia and its manifestations closely mimic renal neoplasm, leading to misdiagnosis of malignancy[13].The non-specific clinical and radiologic features in renal SCC may cause diagnostic confusion and histopathology is needed for confirmation[14].

Histologically, SCC shows extensive squamous differentiation and keratin pearls [15]. sRCCs usually is large (median size ~10 cm) , the section is white or grayish white,and tough texture[16].Primary squamous cell carcinoma (SCC) of the renal parenchyma is a very unusual entity which needs to be differentiated from primary SCC of renal pelvis, SCC fromanother primary site, and urothelial carcinoma with extensive squamous differentiation.In the presence of an identifiable urothelial dysplastic element including urothelial CIS (carcinoma in situ), the tumor should be classified as primary urothelial carcinoma with squamous differentiation. However, the conspicuous presence of keratinizing squamous metaplasia of the adjacent flattened urothelium, especially if associated with dysplasia, supports a diagnosis of primary SCC of the renal pelvis which is rare. No such dysplastic urothelial component or metaplastic and/or dysplastic squamous lining of urothelium was found in this case[17].

5Response to comment: When the kidney present with renal masses and long-standing urinary calculi and massive hydronephrosis. ---------Seems like sentence is incomplete

Response:We are very sorry for our negligence and complete the sentence:In patients with renal masses, long-standing urinary calculi and massive hydronephrosis,should be alert to the possibility of renal squamous cell carcinoma.

Special thanks to you for your good comments.

Reviewer #2:

1. Response to comment:(briefly discuss the evolving scenario of the classifications)

Response :Considering the Reviewer's suggestion, we have briefly discuss the classifications: Lee et al in their study classified these tumors into two groups,according to localisation of the tumors as central and peripheral. Central renal cell carcinoma presents more Intraluminal components and is usually associated with lymph node metastasis whereas peripheral renal squamous cell carcinoma presents with prominent renal parenchymal thickening and might invade the perirenal fat tissue before lymph node or distant metastasis could be identified[8]. Based on these criteria the present case classified as central renal squamous cell carcinoma.

2. Response to comment:(briefly discuss the role of immunoistochemistry in renal cancer)

Response :Considering the Reviewer's suggestion, we have add the paragraph:

Immune checkpoint inhibitors may have some therapeutic effects in the treatment of sRCCs.In pathological conditions,activation of the PD-1/PD-L1 signaling pathway may block immune cell activation, a mechanism exploited by tumor cells to evade the antitumor immune control. Targeting the PD-1/PD-L1 axis has represented a major breakthrough in cancer treatment.Indeed, the success of PD-1 blockade immunotherapies represents an unprecedented success in the treatment of different cancer types[22].Future clinical trials should focus on therapies such as anti-CTLA4, anti-PDL1, anti-PDL1, and combinations with targeted therapy agents that have been shown to augment the cytotoxic tumor immune microenvironment to improve progression and survival outcomes in patients with sRCCs[23-24].

3. Response to comment:(briefly discuss the role of heterogeneity in renal cancer)

Response :Considering the Reviewer's suggestion, we have add the sentence:

Due to the heterogeneity of tumors, 3D fusion sampling can better grade and stage tumors, better guide clinical adjuvant treatment and evaluate prognosis[20]

4. Response to comment:(briefly expand the concept of PD1-PDL1 role)

Response:Considering the Reviewer's suggestion, we have add the sentence:

In pathological conditions,activation of the PD-1/PD-L1 signaling pathway may block immune cell activation, a mechanism exploited by tumor cells to evade the antitumor immune control. Targeting the PD-1/PD-L1 axis has represented a major breakthrough in cancer treatment.Indeed, the success of PD-1 blockade immunotherapies represents an unprecedented success in the treatment of different cancer types[22].

5.Response to comment:(can you show adding a new figure the immunoistochemical positivity of the markers p40 and p63?)

Response:Considering the Reviewer's suggestion, we have add new figure the immunoistochemical positivity of the markers p40 and p63.

Special thanks to you for your good comments.

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper.

We appreciate for Editors/Reviewers' warm work earnestly, and hope that the correction will meet with approval.

Once again, thank you very much for your comments and suggestions.