PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Screening for renal cell carcinoma in renal transplant recipients: a single-center retrospective study
AUTHORS	Yohannan, Binoy; Sridhar, arthi; Kaur, Harmanpreet; DeGolovine, Aleksandra; Maithel, Neha

VERSION 1 – REVIEW

REVIEWER	Montorsi, Francesco Università Vita-Salute San Raffaele, Urology
REVIEW RETURNED	01-Apr-2023

GENERAL COMMENTS	The Authors examined the effectiveness of Renal Cell Carcinoma (RCC) screening in renal transplant (RT) recipients. The study shows a clear kidney cancer specific mortality benefit in patients receiving a screening protocol compared to those that do not. I want to congratulate the Authors because the study is formally well written, and the topic is of interest. The major flaws of the study are: 1) the non randomized retrospective design 2) the size of the population included was not large enough for a direct comparison of the survival rates between those receiving the screening protocol and those who did not. Moreover, some major issues deserve further clarification. SPECIFIC COMMENTS - Major issues 1) No specific screening protocol has been reported in the manuscripts. Who were the patients receiving echography and who were the patients receiving a CT scan? Which was the criteria for deciding the frequency of imaging (once every year vs once every two year)? Please if possible, address this point in the methods section of the manuscript otherwise address this issue in the discussion section 2) It would be interesting to know how many of RT recipients developing RCC had a previous history of pretransplant RCC. Similarly, it would be interesting to know if any of the donors had a previous history of RCC. This could be an important piece of information for deciding in a future scenario which patients should receive screening with higher frequency or should be prioritized compare to others. 3) Given the very wide time-span during which patients underwent renal transplantation (1999-2019), this study is susceptible to “periods effect”, i.e. changes in immunosuppressive regimen, cancer screening practice and treatment have all changed dramatically over time. For this reason, patients with longer follow-up may be less comparable to those with shorter follow-up. Please, address better this limitation at the end of the discussion. 4) Table 1 should be stratified according to the two cohort of patients (receiving screening vs not receiving screening). Please use chi-

	square test and t-test tests to examine the statistical significance in proportions', means' and medians' differences on variables of interest stratifying patients according to screening.

REVIEWER	Jimenez, Rafael Mayo Clinic, Department of Laboratory Medicine and Pathology
REVIEW RETURNED	04-Apr-2023

GENERAL COMMENTS	The authors present a study on the development of renal cell neoplasia in a cohort of patients that underwent renal transplantation, and focus the study around the differences between those who underwent screening protocol for renal cell neoplasia, and those that did not. The manuscript is well written and provides useful information on the incidence of this phenomenon which has not been widely studied. In my opinion, the study would benefit with minor additions/changes: 1. The authors do not perform an analysis based on the type of kidney in which the neoplasm arose. Given the potential management differences between a native kidney and an allograft, it would be useful to present the data between these two different scenarios, particularly, the histology of the tumors, the survival data, and the management received. 2. The authors did not elaborate on the screening protocol that was used at their institution. Was this protocol up previously defined, and why was it used only in a subset of patients, or on the contrary, was up to the decision of the treating clinician? Were the authors able to determine a reason on why patients were placed on the protocol, and could this be introducing a selection bias? The authors can present the clinical rationale to submit patients to a screening protocol, and what was the screening methodology used. 3. From the data, it is clear that there was a difference in the cancer specific survival between the two groups. Was this statistical significant? If not, the reason must be due to the small numbers of events in both subsets. This potentially could also explain the absence of a statistically significant difference in overall survival between the two groups. The authors may elaborate more on the implications of overall survival versus cancer specific survival in this unique clinical scenario. 4. Were the histologies of the tumors revised? New entities, especially entities that arise on end-stage renal disease, have been described since 1999. If this is beyond the scope of the objectives of the author, it should be specified that histologic diagnosis is based on the diagnosis rendered at time of biopsy/resection.

REVIEWER	Adey, Deborah B. University of California San Francisco
REVIEW RETURNED	04-May-2023

GENERAL COMMENTS	Dear authors: You have addressed an important topic that really is inadequately addressed in the literature, that of screening renal transplant recipients for renal cell carcinoma. The key strength of you manuscript is the finding that rourine screening did diagnosis early RCC in 12 patients. It would be helpful to be more descriptive about the methods: 1. Of the 1998 patients, how many were actually screened every 2 years wih either ultrasound or CT scan? How was it decided who was screened and who was not and how was that decision made? 2. Induction therapy changed after 2003. Do you have any data on cumulative exposure to immunosuppression, ie increased

	immunosuppression for rejection? 3. Were no patients on an mTORi at your center I am not sure you have enough statistical power to make any conclusions regarding screening patients and finding incidental RCC vs those who presented with symptomatic RCC. Clearly the mortality was higher but the n is small, so either you need a different statistical method or you are underpowered to make a statement. How do feel that the information you provided about additional cancers in four patients fits into your study? Do you feel these patients have a more impaired malignancy surveillance system? Lastly, you discuss cancer treatments but this is nor really the intent of your study. This information certainly impacts graft loss as immunotherapy is associated with a high likelihood of rejection and graft loss. This may fit better into the discuss as part of the risk of diagnosis of later stage RCC. For your Table 2 it would be helpfuls to have your headings be Graft survival and Patient survival at the top. Despite the above comments there is value with your findings that no patient who underwent screening had advanced disease.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Prof. Francesco Montorsi, Università Vita-Salute San Raffaele
Comments to the Author:
The Authors examined the effectiveness of Renal Cell Carcinoma (RCC) screening in renal transplant (RT) recipients. The study shows a clear kidney cancer specific mortality benefit in patients receiving a screening protocol compared to those that do not. I want to congratulate the Authors because the study is formally well written, and the topic is of interest. The major flaws of the study are:

1) The nonrandomized retrospective design

2) The size of the population included was not large enough for a direct comparison of the survival rates between those receiving the screening protocol and those who did not.

Response: Thank you for your valuable input. We have highlighted the limitations of this study in our manuscript. Despite the small sample size, our study showed a kidney cancer specific mortality benefit which was statistically significant.

Moreover, some major issues deserve further clarification.

SPECIFIC COMMENTS - Major issues.

1) No specific screening protocol has been reported in the manuscripts. Who were the patient's

receiving ultrasonography and who were the patients receiving a CT scan? Which was the criteria for deciding the frequency of imaging (once every year vs once every two year)? Please, if possible, address this point in the methods section of the manuscript otherwise address this issue in the discussion section.

Response: Thank you for raising this important question. The preference on screening imaging modality (ultrasound vs computerized tomography) and frequency of screening was solely dependent on the discretion of the physician and not based on specific institutional protocol. In addition, some patients who were hospitalized for transplant related complications and received abdominal imaging, were also counted towards screening. We would like to acknowledge that although there is subjectivity and inconsistency in the screening methods, almost all patients who were found to have early stage RCC had yearly or every other year abdominal imaging.

2) It would be interesting to know how many of RT recipients developing RCC had a previous history of pretransplant RCC. Similarly, it would be interesting to know if any of the donors had a previous history of RCC. This could be an important piece of information for deciding in a future scenario which patients should receive screening with higher frequency or should be prioritized compared to others.

Response:

None of the patients had a history of pretransplant RCC prior to their first RT. Among the eight patients who received a second RT, five of them developed RCC after their first transplant. It is unknown if any of the donors had a previous history of RCC.

3) Given the very wide timespan during which patients underwent renal transplantation (1999-2019), this study is susceptible to “periods effect”, i.e. changes in immunosuppressive regimen, cancer screening practice and treatment have all changed dramatically over time. For this reason, patients with longer follow-up may be less comparable to those with shorter follow-up. Please, address this limitation better at the end of the discussion.

Response: Agree. We will mention this limitation in the discussion part.

4) Table 1 should be stratified according to the two cohort of patients (receiving screening vs not receiving screening). Please use chi-square test and t-test tests to examine the statistical significance in proportions’, means’ and medians’ differences on variables of interest stratifying patients according to screening.

Response: Patients who received screening had early-stage disease when compared to those who were not screened (P=0.001). The mean age in the screening group was 59.25 years compared to 41.38 in the no screening group (p=0.007). The mean duration of dialysis in patients who received screening was 43.5 months compared to 35.8 months in those who were not screened (P=0.62). The mean time from RT to diagnosis of RCC in patients who received screening was 105.3 months compared to 102 months in those who were not screened (P=0.9).

Reviewer: 2
Dr. Rafael Jimenez, Mayo Clinic

Comments to the Author:
The authors present a study on the development of renal cell neoplasia in a cohort of patients that underwent renal transplantation and focus the study on the differences between those who underwent screening protocol for renal cell neoplasia, and those that did not. The manuscript is well written and provides useful information on the incidence of this phenomenon which has not been widely studied. In my opinion, the study would benefit with minor additions/changes:

1. The authors do not perform an analysis based on the type of kidney in which the neoplasm arose. Given the potential management differences between a native kidney and an allograft, it would be useful to present the data between these two different scenarios, particularly, the histology of the tumors, the survival data, and the management received.

Response: Please refer to table 3. There was no difference in RCC related survival comparing patients with native versus allograft RCC (P value = 0.8).

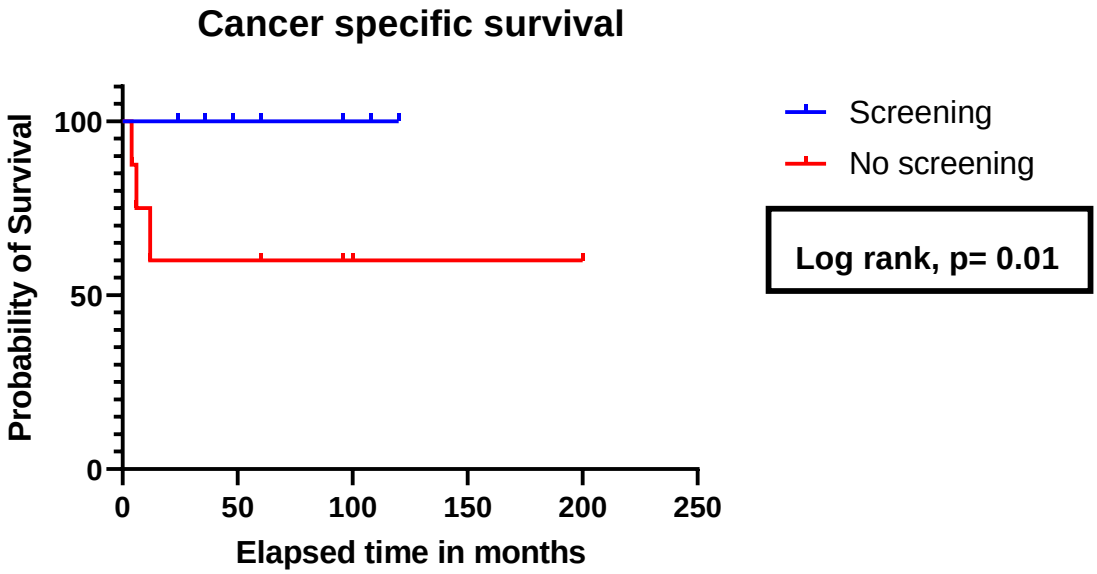
2. The authors did not elaborate on the screening protocol that was used at their institution. Was this protocol previously defined, and why was it used only in a subset of patients, or on the contrary, was up to the decision of the treating clinician? Were the authors able to determine a reason on why patients were placed on the protocol, and could this be introducing a selection bias? The authors can present the clinical rationale to submit patients to a screening protocol, and what was the screening methodology used.

Response: As stated earlier, the screening protocol was not previously defined. The imaging modality and frequency was based on the discretion of the treating physician. We have included this in our methods section.

3. From the data, it is clear that there was a difference in the cancer specific survival between the two groups. Was this statistically significant? If not, the reason must be due to the small numbers of events in both subsets. This potentially could also explain the absence of a statistically significant difference in overall survival between the two groups. The authors may elaborate more on the implications of overall survival versus cancer specific survival in this unique clinical scenario.

Response:

There was a difference in RCC specific survival in patients who were screened (P=0.01) and it was statistically significant. There was no overall survival difference. The benefit from screening is best represented by cancer specific survival as there are competing nonmalignant comorbid factors that contribute to mortality and thus leads to underestimation of the value of screening.



4. Were the histologies of the tumors revised? New entities, especially entities that arise on end-stage renal disease, have been described since 1999. If this is beyond the scope of the objectives of the author, it should be specified that histologic diagnosis is based on the diagnosis rendered at time of biopsy/resection.

Response: Histologies were not revised, and it was based on the diagnosis at the time of biopsy/resection. We have included this in our manuscript.

Reviewer: 3
Dr. Deborah B. Adey, University of California San Francisco
Comments to the Author:
Dear authors: You have addressed an important topic that really is inadequately addressed in the literature, that of screening renal transplant recipients for renal cell carcinoma. The key strength of your manuscript is the finding that routine screening did diagnosis early RCC in 12 patients. It would be helpful to be more descriptive about the methods:

1. Of the 1998 patients, how many were actually screened every 2 years with either ultrasound or CT scan? How was it decided who was screened and who was not and how was that decision made?

Response: Among the 12 patients who received regular screening, 9 had screening every year whereas 3 patients were screened every 2 years.

2. Induction therapy changed after 2003. Do you have any data on cumulative exposure to immunosuppression, ie increased immunosuppression for rejection?

Response: We do not have data on cumulative exposure to immunosuppression. A few patients received pulse doses of glucocorticoids for treating acute rejection episodes. We have included the immunosuppressive agents used at any point in the management of these patients in Table3.

3. Were no patients on an mTORi at your center?

Response: Yes. Six patients received sirolimus as part of the immunosuppressive regimen. Please refer to table 3.

4. I am not sure you have enough statistical power to make any conclusions regarding screening patients and finding incidental RCC vs those who presented with symptomatic RCC. Clearly the mortality was higher, but the n is small, so either you need a different statistical method, or you are underpowered to make a statement.

Response: Given the small sample size, it will be difficult to compare screening patient's vs finding incidental RCC vs those who presented with symptomatic RCC. However, there is a statistically significant RCC specific survival benefit comparing patients those who were screened vs not.

5. How do feel that the information you provided about additional cancers in four patients fits into your study? Do you feel these patients have a more impaired malignancy surveillance system?

Response: It is not a surprise to see a higher incidence of additional cancers with longer follow up as solid organ transplant recipients have a significantly higher risk given chronic immunosuppression and poor immune surveillance.

6. Lastly, you discuss cancer treatments, but this is not really the intent of your study. This information certainly impacts graft loss as immunotherapy is associated with a high likelihood of rejection and graft loss. This may fit better into the discussion as part of the risk of diagnosis of later stage RCC.

Response: Agree and it has been included in the discussion part.

7. For your Table 2 it would be helpful to have your headings be Graft survival and Patient survival at the top.

Response: Correction made to manuscript.

VERSION 2 – REVIEW

REVIEWER	Montorsi, Francesco Università Vita-Salute San Raffaele, Urology
REVIEW RETURNED	25-Jul-2023

GENERAL COMMENTS	GENERAL COMMENT: The authors examined the effectiveness of Renal Cell Carcinoma (RCC) screening in renal transplant (RT) recipients. The study shows a clear kidney cancer specific mortality benefit in patients receiving a screening protocol compared to those that do not. SPECIFIC COMMENT: Despite the small size of the population included and the retrospective design, the topic is extremely novel and of interest. The manuscript is well written, complete in each part. TITLE The title is correct. ABSTRACT The abstract is very informative and summarises all the main sections of the manuscript. It includes a specific paragraph of

	"Strengths and limitations of the study". INTRODUCTION Epidemiology data are up-to-date and correct. Considerations upon risk factors for post-RT RCC and screening are appropriate and well described. MATERIALS AND METHODS Inclusion and exclusion criteria of patients' enrolment are extensively and correctly defined. An important limitation of the study is the lack of a precise screening protocol: the authors underline that the imaging modality and frequency was based on the discretion of the treating physician. Statistical analyses are correctly described. RESULTS Results are exhaustively reported. DISCUSSION Results are properly discussed. It is important to focus on three main topics: the influence of different immunosuppression regimens (data was collected for a period of 20 years), the use of immunotherapy relative to the risk of graft rejection and the annual screening recommendation for early detection of RCC. CONCLUSION No changes suggested.

REVIEWER	Jimenez, Rafael Mayo Clinic, Department of Laboratory Medicine and Pathology
REVIEW RETURNED	24-Jul-2023

GENERAL COMMENTS	Much improved manuscript with much more complete information. In my opinion, paragraph 1 of the results section can be structured differently for clarity. I would talk about first the 1998 patients, then mention the 16 that developed RCC, and finally add the 4 patients that were only followed at the institution. Also, some of the details annotated of the 1998 patients do not seem relevant to the paper.

REVIEWER	Adey, Deborah B. University of California San Francisco
REVIEW RETURNED	27-Jul-2023

GENERAL COMMENTS	Dear authors. Your manuscript reads well and though limited potentially by some inherent biases, addresses this topic as best possible. My only question for you is in the conclusion in the recommendation of both ultrasound and CT scanning? In the absence of limiting factors such as body habitus, would ultrasound be adequate screening? Also, could you mention whether the CT scans were performed with contrast. In general RCC would be more reliably detected with contrast, but on the other hand the risk associated with contrast exposure warrants some comment.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1
Prof. Francesco Montorsi, Università Vita-Salute San Raffaele
Comments to the Author:
GENERAL COMMENT: The authors examined the effectiveness of Renal Cell Carcinoma (RCC) screening in renal transplant (RT) recipients. The study shows a clear kidney cancer specific mortality

benefit in patients receiving a screening protocol compared to those that do not.
SPECIFIC COMMENT: Despite the small size of the population included and the retrospective design, the topic is extremely novel and of interest. The manuscript is well written, complete in each part.
TITLE
The title is correct.
ABSTRACT
The abstract is very informative and summarises all the main sections of the manuscript. It includes a specific paragraph of “Strengths and limitations of the study”.
INTRODUCTION
Epidemiology data are up-to-date and correct. Considerations upon risk factors for post-RT RCC and screening are appropriate and well described.
MATERIALS AND METHODS
Inclusion and exclusion criteria of patients’ enrolment are extensively and correctly defined. An important limitation of the study is the lack of a precise screening protocol: the authors underline that the imaging modality and frequency was based on the discretion of the treating physician. Statistical analyses are correctly described.
RESULTS
Results are exhaustively reported.
DISCUSSION
Results are properly discussed. It is important to focus on three main topics: the influence of different immunosuppression regimens (data was collected for a period of 20 years), the use of immunotherapy relative to the risk of graft rejection and the annual screening recommendation for early detection of RCC.
CONCLUSION
No changes suggested.

Response: completed

Reviewer: 3
Dr. Deborah B. Adey, University of California San Francisco
Comments to the Author:
Dear authors. Your manuscript reads well and though limited potentially by some inherent biases, addresses this topic as best possible. My only question for you is in the conclusion in the recommndation of both ultrasound and CT scanning? In the absence of limiting factors such as body habitus, would ultrasound be adequate screening? Also, could you mention whether the CT scans were performed with contrast. In general, RCC would be more reliably detected with contrast, but on the other hand the risk associated with contrast exposure warrants some comment.

Response: Screening in the form of ultrasonography and/or CT every year or every 2 years appears to be an effective tool for early detection of RCC. As highlighted in the paper, ultrasound is operator dependent and may not be sensitive enough to detect early lesions, especially in obese patients and those with acquired cystic kidney disease. In our study, patients received
CT scans with or without contrast depending on the renal function. Contrast enhanced CT scan is the preferred imaging modality but in patients with renal insufficiency a non-contrast study is a reasonable alternative due to risk of contrast nephropathy.

VERSION 3 – REVIEW

REVIEWER	Adey, Deborah B. University of California San Francisco
REVIEW RETURNED	19-Aug-2023

GENERAL COMMENTS	Dear authors: Your manuscript addresses an important topic that is under appreciated but quite important. Your revisions have addressed any and all questions I had.