Author's response to reviews

Title: Urinary Transforming Growth Factor-beta 1 (TGF-b1) as a marker of response to immunosuppressive treatment in patients with crescentic nephritis.

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Version: 3 Date: 31 August 2005

Author's response to reviews: see over
Dear Sirs,

We would like to thank you for the prompt review of the manuscript entitled “Urinary Transforming Growth Factor-beta 1 as a marker of response to immunosuppressive treatment in patients with crescentic nephritis” (MS: 1644171414593956).

We believe that the reviewers’ comments helped as to improve this manuscript and enhance its importance. In the revised manuscript, we have generally addressed all the suggestions and criticisms done by the reviewers.

Revisions were incorporated in the manuscript.

Below is a point-by-point response to the reviewers’ comments:

**Reviewer: M. Negata**

*Immunostaining of TGF –beta is still insufficient quality and the data is not essential for the content of this manuscript. I think it would be better to omit this figure, then the concept of this paper become clearer.*

We agree that the data coming from the immunohistochemistry is not so essential for the content of the manuscript. The subject of the manuscript is focused on the value of urinary TGF –beta levels as a marker of response to immunosuppressive treatment in patients with crescentic nephritis. The figure of the localization of TGF –beta in the kidney has been added as a complementary data. We also kept the figure in the manuscript for one more reason.
There is a discrepancy in the comments of the reviewers concerning this figure since we have been asked to add one more figure at a lower magnification by the following one.

**Reviewer: Maria Pia Rastaldi**

*Although the manuscript is improved, the number of patients has been minimally increased. I understand the rarity of the disease, but I feel the overall numbers are still limited to provide good statistical analysis.*

*Table 2 has been added. But it misses very important data such as interstitial infiltration and fibrosis, where the higher levels of serum creatinine in the non-responder group seem to indicate differences in these parameters.*

*I also requested a low magnification picture that has not been provided. In case the problem is the number of pictures, the authors can compose one multi-panel figure showing low and high magnification images.*

Crescentic nephritis is a rare type of renal disease. However, we believe that the number of patients used is adequate and since the results of the statistical analysis are clear (strong predictive value of urinary TGF-beta levels for final serum creatinine and significant difference between TGF-beta levels in responder and non-responder patients, p<0.01).

The data about interstitial infiltration and fibrosis in all patients as well as in responder and non-responder groups has been added in tables 1 and 2.

In the manuscript there are two pictures, one for TGF–beta immunostaining in the kidney and a negative control according to a previous suggestion of this reviewer. Although the subject of the manuscript is focused on the value of urinary TGF–beta levels as a marker of response to immunosuppressive treatment in patients with crescentic nephritis the existing picture shows the localization of TGF–beta in the kidney. We kept the existing pictures in the manuscript for one more reason. There is a discrepancy in the general comments of the reviewers concerning this picture since we have been asked to omit it by the previous reviewer.
Reviewer: Meguid El Nahas

This is an interesting observation highlighting links between TGF–beta1 and crescentic glomerulonephritis. The research is conducted by a group of good reputation and expertise in the field. The study limitations include:

1. The small number of patients which makes statistical analysis subject to potential error.
2. The fact that the authors use parametric analysis of the data when the data are not always normally distributed; data on urinary TGF–beta1 shows very large SD.
3. The immunohistochemical analysis should be solely qualitative and not quantitative as human percutaneous biopsy material’s size precludes accurate quantitation of the immunostaining. This may also explain the lack of correlations between tissue and urinary TGF-beta1 levels.
4. The authors refer to correlation analyses without giving the data.
5. The authors should also attempt to give predictive values (R2) for baseline urinary TGF-beta1 values and changes in renal function in responders.
6. Clinically, the test would add little to the known prognostic parameters such as sCr at presentation and histological changes as confirmed by the authors.

1. Crescentic nephritis is a rare type of renal disease. However, we believe that the number of patients used is adequate and since the results of the statistical analysis are clear (strong predictive value of urinary TGF-beta levels for final serum creatinine and significant difference between TGF-beta levels in responder and non-responder patients, p<0.01).
2. The statistical analysis has been done with both parametric and non-parametric tests for comparison of means with SD and median values respectively for the reasons described by the reviewer (statistical analysis). No difference with both tests was found in the urinary TGF-beta levels.
3. The quantitation of immunostaining was done according to the reviewer suggestion in the previous review of the manuscript. The qualitative estimation of immunostaining as suggested is better for human percutaneous material. However, in this study neither the extent (quantitative estimation) nor the intensity (qualitative estimation) showed any difference between patients with or without response to treatment and with urinary TGF–beta1 levels.
4. The only significant correlation referred to the manuscript is that of TGF–beta immunostaining with fibrocellular crescents (r=0.531, p<0.05). Data of this correlation is not shown since we did not consider that as a very important result of this work which is focusing
on the value of urinary TGF-beta levels as a marker of response to immunosuppressive therapy.

5.6. The predictive value (R2) of baseline urinary TGF-beta1 levels for the final serum creatinine (after immunosuppressive treatment) has been added in the Results. The predictive value of urinary TGF-beta is stronger than that of baseline creatinine and number of glomeruli with crescents. This finding suggests that baseline urinary TGF-beta levels may be proved as an important marker of prognosis and/or response to treatment for patients with crescentic nephritis.

Hoping that the revised manuscript fulfills the reviewers’ standards, we are looking forward for your answer.

Sincerely yours

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