ANSWERING REVIEWERS

August 14, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 4583-review.doc).

Title: Silent Diabetic Nephropathy

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The manuscript has been improved according to the suggestions of reviewers:

1. Answers to Reviewer 00503272
   1) Abstract: It has been shortened to one page. Comments about Rapidly progressive kidney injury and Silent DN are explained in other sections of the article. The word 'histological' has been changed to 'histopathological' in every place within the text where it appears.
   2) Introduction Page (page 4) The references for the statements in the first paragraph (lines 12-24) have been provided
   3) Material and methods: The abbreviation '(DN)' and the words '(biopsy proven)' have been deleted. (Page 6) The second paragraph has been completely rewritten (Page 6, Lines 25-26): The presentation of RPKI was considered in those cases where a drop of eGFR higher than 25% was seen between baseline and biopsy, independently of biopsy indication.
   4) Material and methods: The statement (page 7 lines 15-16) 'Endpoint was defined as initiation of chronic dialysis or drop in GFR category accompanied by a 25% drop or greater drop in eGFR from baseline' has been rephrased (Page 6 lines 27-29):'Final end-point was defined as chronic dialysis initiation or progression of CKD according to KDIGO 2012 definition as drop in CKD category and drop of GFR of 25% or more".
   5) Material and methods, Reviewer: Page 8 lines 4 and 5: The sentence 'Was used chi-squared or Fisher exact test and U Mann Whitney test' has to be completed. Authors: (Page 7 Line 22): Association was studied by $\chi 2$ test or Fisher exact test and U Mann Whitney test. The term 'Logrank' has changed by 'Log rank' . Reviewer Page 8 lines 9-11: The statements 'Results are presented with 95% confidence interval. All tests were two-sided and used a significance level of 0.05' should be rephrased. Authors: (Page 7 Line 27-28): All tests were two-tailed and a significance level ≤ 0.05 was considered statistically significant.
   6) Results Reviewer: Paragraph 2, lines 4-8 page 9: the sentence 'In spite of the fact that 66,6% of the patients What the authors are trying to bring out with this statement is unclear. Authors: It has been rephrased (Page 8, lines 14-16): Although 48.8% of patients showed a baseline creatinine $\leq$ 1.4 mg/dl, 68% of them showed a eGFR at time of biopsy < 45 ml/min/1.73 m2 and 15.6% grade 5 eGFR category. Reviewer: category G 5? Uncommon abbreviations should be avoided. Authors: eGFR category G5 has been described in Methods as KDIGO 2012 nomenclature. Reviewer:

Paragraph 3 lines 6-8 page 11: 'Chronic kidney disease diabetic patients with Hb A1c < 7% have a greater renal risk than patients with HbA1c > 7% with a HR 2.9 (1.0-8.4) p=0.054': The statistics does not show that this risk is significant as the 95% CI for the hazard ratio clearly passes through 1 which the p value of 0.054 confirmed (this is also shown in Table 8). The statement by the authors is misleading. This should be revised to reflect this fact. Authors: the statement has been rephrased (Pag 10, Lines 10-13): Although diabetic patients with CKD and HbA1c <7% showed a higher renal progression risk than patients with HbA1c > 7% with a HR 2.9 (1.0-8.4), this effect was not statistically significant, p=0.054.

7) Tables and figures: The range of values have been provided. The spelling errors corrected. Table 2: The abbrevations G1 to G5 and A1 to A3 have been defined as footnotes. The legends for Figures 1 and Figure 2 have been provided.
8) We deeply improved language polishing

2. Answers to Reviewer 00503043
   1) Abstract has been shortened.
   2) Introduction The abbreviation '(DN)' in line 1 paragraph 1 has been changed to diabetic nephropathy (DN).
   3) Material and methods The abbreviation '(DN)' in line 1 paragraph 1 has been deleted
   4) The paper has been changed in accordance with the instructions:
      - Author contributions have been provided in detail
      - Structured abstract has been shortened, specially conclusion
      - Core tip has been added
      - Statistical expressions have been changed
   5) We deeply improved language polishing

3. Answers to Reviewer 00503043

Reviewer:

This is an interesting observational study on the clinical course of DN, focusing in particular on a novel phenotype called silent DN (SDN). Although it is of potential clinical relevance, several points of major concern regarding the methodology followed rather restrict the potentials of the manuscript.

1) Reviewer: Specifically, the major problem with this paper is the definition of SDN itself, which is totally arbitrary and includes widely divergent conditions which are themselves largely arbitrarily defined, such as the so called RPKI.

   Authors: There are recognized limitations in defining rapid progression of kidney disease. KDIGO 20121 defines rapid progression as *ä sustained decline in eGFR of more than 5 ml/min/1.73 m2/year. They estimated two indices of change in eGFR: the absolute annual rate of change (categorized as: increase, stable and -1, -2, -3, -4, and Z -5 ml/min/1.73 m2/year decline); and the annual percentage change (categorized as: increase, stable, -1 to -2, -3 to -4, -5 to -6, and -7 percent decline/year). ……..The risk of ESRD increased almost two-fold for every 1 ml/yr decline in eGFR, when adjusted for covariates and eGFR at the time of the first eGFR measurement. The risk remained significant, but was less pronounced, when adjustments were performed at the time of the last eGFR measurement. Similar results were obtained when change in eGFR was defined by a percentage. For an Absolute rate of change (over a median of 3.5 years) -7%/year or more, the Adjusted ESRD risk associated for eGFR at first creatinine measurement HR (95% CI) =11.30 (8.53–14.97); Adjusted eGFR at last creatinine*

measurement HR (95% CI)= 2.17 (1.60–2.93) ¨

Considering that the average time between baseline and biopsy GFR was 7.3 ± 5.2 [1.1-24.1] months, we believe that a decrease in GFR> 25% in this period could be fairly regarded as RPKI (Material and Methods Page 7, line 1): "The presentation of RPKI was considered in those cases where a drop of eGFR higher than 25% was seen between baseline and biopsy, independently of biopsy indication".

(Material and Methods Page 7, line 6): Silent diabetic nephropathy variable was defined for cases that showed an atypical disease pattern or where DN was not suspected. This variable grouped patients with RPKI without significant proteinuria (<0.5g/day) and/or a duration of DM of less than 5 years and/or need of starting RRT in less than 1.5 years from renal biopsy.

2) Reviewer: Furthermore, another point that renders the findings of the study of questionable credibility and, therefore, restricts the clinical value of the study. Is the fact that the selection criteria for inclusion in the study per se, ie indication for biopsy, renders the study amenable to significant patient selection bias.

Authors: (Material and Methods page 6 line 1): In all cases, the nephrologist was the specialist who recommended the biopsy considering all the available data. We classified the indications for renal biopsy in three groups: nephrotic proteinuria with or without nephrotic syndrome, Rapidly Progressive Kidney Injury (RPKI) and CKD.

(Discussion page 11 line 3): "Although our study presents a selection bias which is the clinical indication for renal biopsy, our series includes only cases of DN in which other causes of renal damage have been excluded. Therefore our findings even if they are not extrapolated to all of the patients with DN they can help to understand why some patients with diabetes have an atypical clinical course and are diagnosed in advanced stages of renal disease with minimal therapeutic possibilities. Even though the majority of patients had been medically followed-up before biopsy this fact did not avoid its bad evolution and a late diagnosis of the illness. The RPKI presentation form, predominant in 33% of the patients, was associated with bad renal prognosis although it behaved as a confusing factor, not as an independent risk factor.

(Discussion Page 10, line 14) It has been shown [18] that, in DN, relatively small elevations in serum creatinine can significantly underestimate the degree of renal damage and these elevations are unpredictable most of the time. Without a doubt, this fact has contributed to the high proportion of patients in our series that we have classified as silent diabetic nephropathy, that is, cases that have been unnoticed until the advanced stages. These patients had a shorter diabetes evolution time; they presented a higher frequency of RPKI, a major loss of renal function at the moment of the biopsy and had a higher proportion of renal events. Although they had more cardiovascular diseases when compared to the rest of the group, this difference was not statistically significant.

Thank you again for inviting us to publish our manuscript in the *World Journal of Nephrology*.

Sincerely yours,

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[1] Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney inter., Suppl. 2013; 3: 63-72.