

October 28, 2013

Dear Editor,

Thanks for giving us the opportunity to resubmit our manuscript. We truly appreciate the efforts that the four reviewers have made to provide us with insightful comments. We believe we have addressed most of the reviewers concerns in the current version of the manuscript and we hope that as such the manuscript is now suitable for publication. Please find below a detailed response to each reviewer comments. Also, there is enclosed the edited manuscript in Word format (file name: 4677.doc).

Title: Diabetic Nephropathy: is it time yet for routine kidney biopsy?

Authors: Maria L Gonzalez-Suarez, David B Thomas, Laura Barisoni, Alessia Fornoni

Name of Journal: World Journal of Diabetes

ESPS Manuscript NO: 4677

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

Reviewer #1

The authors are advocates of routine kidney biopsy for patients with diabetes and have written this paper to argue their viewpoint. It is certainly an area which is subject to varied practice patterns and preferences. I have several comments/issues:

1. Under the heading Renal Biopsy; first paragraph: The reference to table 2 does not match the text description. Table 2 lists risk factors for diabetic nephropathy. As such, Table 2 is not referenced in the text.

Thanks for identifying that the reference to Table 2 was missing. We have now modified the text accordingly.

2. In their discussion on safety of renal biopsies, the authors played down the significance of the complications mentioned. It would be prudent to provide some figures to support their argument. As a risk-benefit argument often entails with these decisions, some of the reported complication rates need to be mentioned.

We fully agree with this important point and we have now modified the discussion around the complication issues and we are providing additional references in regard.

3. The information from the reference cited (ref. 36, Preston et al.) is slightly misrepresented. In this retrospective study of 334 elderly patients (mean age 70.5 years), only 10% of patients were diabetic (thus not primarily a study of diabetic patients). Furthermore, in this diabetic subgroup, subjects were biopsied because of "atypical" features, e.g. acute renal failure. Although the other atypical features were not specified, it suggests that the results cannot be generalized to support routine biopsies.

We agree with the reviewer and we have now chosen a new reference where only patients with diabetes are being studied.

4. I'm not sure if table 4 adds anything to the paper as these non-diabetic nephropathies are described in the

text (first paragraph under heading Biopsy findings in patients with diabetes).

We agree with the reviewer and we have now removed Table 4 and modified the table sequence accordingly.

5. With regards to biopsy findings in diabetic patients, the authors report that non-diabetic lesions range from 14-60%. Table 5 would indicate that the range was 14-100%! The large range does raise the question of heterogeneity of the studies and how the study subjects were selected. Were there any studies performing routine/universal biopsies? While Table 5 is useful in highlighting the highly variable results from biopsies, the conclusions are quite limited without further analysis of the data/studies.

We have now selected studies with more homogeneous indications for a renal biopsy and excluded the single study with 100% of the biopsies showing non-diabetes related kidney disease (as the inclusion criteria in this study were clearly sufficient to indicate that the etiology of the proteinuria was of non-diabetic origin). We have also added some additional and more recent studies and the new reported range (14-80%) reflects the studies presented in current Table 4. None of the reported study performed routine/universal biopsies and therefore we cannot exclude a selection bias.

6. The section on minimally invasive diagnostic tools is interesting and worth expanding on. It is currently too brief to enlighten the reader. For example, an adequate description of the ultrasound technique is useful. In the study by Insalaco et al (ref. 90), it is not ultrasound images which are used to differentiate DN from NDRD but the Doppler acquired resistive index. How the resistive index can be used to predict DN should be mentioned (and also its limitations). Similarly, with the proposed circulating biomarkers mentioned: in what way can they be used to predict underlying DN or its progression?

We thank the reviewer for pointing out that it is indeed the quantitative analysis of the resistive indexes that differentiate DN from NDRD. We have modified the text accordingly, and discuss in more details the related limitations. We have also expanded the data about biomarkers but in a limited manner to maintain the focus of this manuscript on the role of a kidney biopsy. We now also mention the potential of a cell based assay we have developed to predict CKD progression in diabetes.

Reviewer #2

In this review Suarez et al. discuss the need of kidney biopsy in estimating the stage of diabetic nephropathy. There are some issues that need correction prior to publication.

1. As the review speaks for the kidney biopsy in patients with diabetes, it would benefit of images showing the structural changes that are characteristic for diabetic nephropathy at different stages of disease progression.

We are now providing images representative of DN at different stages (Figure 1).

2. The expressions 'diabetics type 1', 'type 2 diabetics' and 'diabetic patients/diabetics' could be replaced by 'patients with type 1 diabetes', 'patients with type 2 diabetes', and 'patients with diabetes'.

Thanks for this important point. We have modified the text throughout the manuscript accordingly.

3. In the authors' opinion, should the kidney biopsy include both EM- and histopathological analysis?

Thanks for this very interesting query. Yes. The biopsy evaluation needs Histology, Immunofluorescence and Electron Microscopy. Because the occurrence of non-diabetic renal disease (NDRD) in diabetic patients has been increasingly recognized in recent years. Non-diabetic renal disease in diabetes mellitus; varies widely in different regions of the world and is reported to range from 15.7% to 82.9%.

As the thickening of the glomerular basement membrane is the major predictor of outcome at least in patients

with type 1 diabetes, we believe that EM would also be important. PMID: PMID: 23687360

1. Chang TI, Park JT, Kim JK, Kim SJ, Oh HJ, Yoo DE, Han SH, Yoo TH, Kang SW. Renal outcomes in patients with type 2 diabetes with or without coexisting non-diabetic renal disease. *Diabetes Res Clin Pract.* 2011;92(2):198–204. doi: 10.1016/j.diabres.2011.01.017. [PubMed] [Cross Ref]
2. Lee EY, Chung CH, Choi SO. Non-diabetic renal disease in patients with non-insulin dependent diabetes mellitus. *Yonsei Med J.* 1999;40(4):321–326. [PubMed]
3. Soni SS, Gowrishankar S, Kishan AG, Raman A. Non diabetic renal disease in type 2 diabetes mellitus. *Nephrology (Carlton)* 2006;11(6):533–537. doi: 10.1111/j.1440-1797.2006.00681.x. [PubMed] [Cross Ref]
4. Nzerue CM, Hewan-Lowe K, Harvey P, Mohammed D, Furlong B, Oster R. Prevalence of non-diabetic renal disease among African-American patients with type II diabetes mellitus. *Scand J Urol Nephrol.* 2000;34(5):331–335. doi: 10.1080/003655900750048378. [PubMed] [Cross Ref]
5. Jalalah SM. Non-diabetic renal disease in diabetic patients. *Saudi J Kidney Dis Transpl.* 2008;19(5):813–816. [PubMed]
6. Suzuki D, Takano H, Toyoda M, Umezono T, Uehara G, Sakai T, Zhang SY, Mori Y, Yagame M, Endoh M, Sakai H. Evaluation of renal biopsy samples of patients with diabetic nephropathy. *Intern Med.* 2001;40(11):1077–1084. doi: 10.2169/internalmedicine.40.1077. [PubMed] [Cross Ref]
7. Ghani AA, Al Waheeb S, Al Sahow A, Hussain N. Renal biopsy in patients with type 2 diabetes mellitus: indications and nature of the lesions. *Ann Saudi Med.* 2009;29(6):450–453. doi: 10.4103/0256-4947.57167. [PMC free article] [PubMed] [Cross Ref]
8. Huang F, Yang Q, Chen L, Tang S, Liu W, Yu X. Renal pathological change in patients with type 2 diabetes is not always diabetic nephropathy: a report of 52 cases. *Clin Nephrol.* 2007;67(5):293–297. [PubMed]
9. Yaqub S, Kashif W, Hussain SA. Non-diabetic renal disease in patients with type-2 diabetes mellitus. *Saudi J Kidney Dis Transpl.* 2012;23(5):1000–1007. [PubMed]
10. Li H, Li XW, Huang QY, Ye WL, Duan L, Li Y. Non-diabetic renal disease in type II diabetes mellitus. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao.* 2003;25(1):101–104. [PubMed]
11. Zukowska-Szczechowska E, Tomaszewski M. Renal affection in patients with diabetes mellitus is not always caused by diabetic nephropathy. *Rocz Akad Med Bialymst.* 2004;49:185–189. [PubMed]
12. Oh SW, Kim S, Na KY, Chae DW, Kim S, Jin DC, Chin HJ. Clinical implications of pathologic diagnosis and classification for diabetic nephropathy. *Diabetes Res Clin Pract.* 2012;97(3):418–424. doi: 10.1016/j.diabres.2012.03.016. [PubMed] [Cross Ref]
13. Chong YB, Keng TC, Tan LP, Ng KP, Kong WY, Wong CM, Cheah PL, Looi LM, Tan SY. Clinical predictors of non-diabetic renal disease and role of renal biopsy in diabetic patients with renal involvement: a single centre review. *Ren Fail.* 2012;34(3):323–328. doi: 10.3109/0886022X.2011.647302. [PubMed] [Cross Ref]
14. Mou S, Wang Q, Liu J, Che X, Zhang M, Cao L, Zhou W, Ni Z. Prevalence of non-diabetic renal disease in patients with type 2 diabetes. *Diabetes Res Clin Pract.* 2010;87(3):354–359. doi: 10.1016/j.diabres.2009.11.012. [PubMed] [Cross Ref]
15. Lu B, Gong W, Yang Z, Yang Z, Yang Y, Wen J, Zhao N, Zhu X, Hu R. An evaluation of the diabetic kidney disease definition in chinese patients diagnosed with type 2 diabetes mellitus. *J Int Med Res.* 2009;37(5):1493–1500. [PubMed]

16. Hashim Al-Saedi AJ. Pathology of nondiabetic glomerular disease among adult Iraqi patients from a single center. Saudi J Kidney Dis Transpl. 2009;20(5):858–861. [PubMed]
17. Chawarnkul O, Vareesangthip K, Ongajyooth L, Cheunsuchon B, Parichatikanond P. Non-diabetic glomerular disease in type II DM: 10 years experience. J Med Assoc Thai. 2009;92(Suppl 2):S57–S60. [PubMed]
18. Zhuo L, Ren W, Li W, Zou G, Lu J. Evaluation of renal biopsies in type 2 diabetic patients with kidney disease: a clinicopathological study of 216 cases. Int Urol Nephrol. 2013;45(1):173–179. doi: 10.1007/s11255-012-0164-6. [PubMed] [Cross Ref]

Another reason to do Histology, IF and EM - need it to classify early lesion.

Tervaert et al. Pathologic Classification of Diabetic Nephropathy. J Am Soc Nephrol 21: 556–563, 2010. doi: 10.1681/ASN.2010010010

4. The authors shortly mention the complications associated with kidney biopsy and mention that they are not considered serious medical problems. What are the risk factors associated with the kidney biopsy, especially bleeding? The authors could also shortly discuss what are the main reasons why kidney biopsies are not done routinely.

Although the risk for complications following a kidney biopsy have been dramatically reduced over the past few years (ref. 91), the risks remain not negligible. It is indeed the goal of this review article to stimulate further discussion in the scientific community, so that current guidelines for standard of care may be revised.

5. Shortenings should be written out the first time they appear in the text. Also the same style should be kept throughout the text. For example, Diabetes Mellitus is shortened in the beginning as DM, but later in the text both Diabetes Mellitus and diabetes mellitus are used.

Thank you. We have done this.

6. There are many mistakes in the English language that should be corrected prior to publication.

Thank you. We hope this is now properly addressed.

Reviewer #3

The article is written well and cover most of the points to propose necessity of kidney biopsy in diabetes. Following points should be mentioned to make this proposal more persuasive.

1. Please show latest statics of kidney biopsy case in diabetes patients.

Thank you. We have added new studies as summarized in new Table 4.

2. Please state the statics of complications of kidney biopsy, before and now (it is getting lower?), and whether special care is required for diabetes patients. What is contra-indication associated with diabetes.

We have added a new Figure 6 that demonstrates the rate of complication nowadays. We now also comment on the higher rate for bleeding complication (and/or infection) in patients with diabetes.

3. "Routine" frequently means more than one time. Is biopsy needed more than one time? If so which case? "Routine" refers to the common use of the biopsy rather than the multiple use of the procedure in a given patient. We have therefore elected not to modify this wording.

4. State some examples which can be encountered in routine patients care. For a example, a patient who has 5 years type 2 diabetes, recently found microalbuminuria without retinopathy. Should biopsy be done? If so, what information can be gained to change the course of treatment?

Yes, the biopsy would be indicated in a patient with microalbuminuria and no rethinopathy as suggested by recommendations from ADA and QDOQI. Should the biopsy demonstrate a disease other than DN, then the course of treatment may change. As more studies become available to demonstrate how quantitative histological features may predict the disease course earlier than albuminuria (23687360), our level of confidence to perform universal kidney biopsies in patients with diabetes should increase.

Reviewer #4

1) The article should give the justification for routine use of renal biopsy with evidence as available.

Thank you, we agree. This has been changed trying to clarify our point of view in regards of this matter. Please see conclusions in the manuscript.

“Unfortunately, patients with NDRD are often designated as having DN because of the overlapping features of glomerulopathies[70].. It is important to identify and differentiate these pathologies at an early stage in order to prevent progression and potential complications. There is an overwhelming number of cases where these diagnoses would lead to changes in treatment, ranging from the use of immunosuppression to titration of renin-angiotensin-aldosterone system blockade[108] “

2) The article should be modified as per the second paragraph of the conclusion. It is mentioned there the important reasons why biopsy is required and each point need to be substantiated with evidence or lack of evidence paragraph wise.

Thank you, we have now added references to that paragraph.

22. Mazzucco G, Bertani T, Fortunato M, Bernardi M, Leutner M, Boldorini R, Monga G. Different patterns of renal damage in type 2 diabetes mellitus: a multicentric study on 393 biopsies. *Am J Kidney Dis.* 2002 Apr;39(4):713-20. doi: 10.1053/ajkd.2002.31988. PubMed PMID: 11920336.

83. Chong YB, Keng TC, Tan LP, Ng KP, Kong WY, Wong CM, Cheah PL, Looi LM, Tan SY. Clinical predictors of non-diabetic renal disease and role of renal biopsy in diabetic patients with renal involvement: a single centre review. *Ren Fail.* 2012;34(3):323-8. doi: 10.3109/0886022X.2011.647302. Epub 2012 Jan 17. PubMed PMID: 22250665.

110. Li LS, Liu ZH. Epidemiologic data of renal diseases from a single unit in China: analysis based on 13,519 renal biopsies. *Kidney Int.* 2004 Sep;66(3):920-3. doi: 10.1111/j.1523-1755.2004.00837.x. PubMed PMID: 15327382.

111. Haas M, Spargo BH, Wit EJ, Meehan SM. Etiologies and outcome of acute renal insufficiency in older adults: a renal biopsy study of 259 cases. *Am J Kidney Dis.* 2000 Mar; 35(3):433-47. doi: 10.1016/S0272-6386(00)70196-X. PubMed PMID: 10692269.

112. Stratta P, Canavese C, Marengo M, Mesiano P, Besso L, Quaglia M, Bergamo D, Monga G, Mazzucco G, Ciccone G. Risk management of renal biopsy: 1387 cases over 30 years in a single centre. *Eur J Clin Invest.* 2007 Dec;37(12):954-63. doi: 10.1111/j.1365-2362.2007.01885.x. PubMed PMID: 18036029.

3) Introduction & Definitions etc need not be changed.

Thank you.

4) The justification for renal biopsy is missing. Finding additional manifestations / pathologies is not a benefit. What is the clinical implication? Are there any studies to suggest that by doing routine biopsies the renal outcomes improved? The authors need to explain this in detail as this is the major point of the article.

We have now added a new paragraph in the conclusion section, trying to clarify the need of new studies where it is assessed improvement of outcomes by doing routine biopsies in patients with diabetes.

"There is lack of studies in the literature regarding the universal use of kidney biopsy on patients with diabetes. As new studies have become available to demonstrate how quantitative histological features may predict the disease course earlier than albuminuria ^[113], our level of confidence to perform routine kidney biopsies in patients with diabetes should increase. New research studies are required, longitudinal observational clinical trials as well as interventional trials, where the implementation of routine kidney biopsy is evaluated for patients with diabetes at time of diagnosis to evidence improvement in outcomes."

5) Please use the terms T1DM and T2DM instead of IDDM and NIDDM

Thank you, we have changed these terms.

6) Lot of spelling mistakes in the manuscript.

Thank you. We hope this is now properly addressed.

7) References are not matching to the statements. For example Ref 100 deals with patients of heart failure and the authors quoted this as supportive for renal biopsy. The CKD was assessed by eGFR and dipstick proteinuria in the study and not by biopsy. 8) What are the chances of getting a NDRD diagnosed on renal biopsy? What are the implications of the findings? What is the current practice and are there any studies to suggest that the outcomes differ?

Many of NDRD that could be revealed by a kidney biopsy have very different treatment approaches that may require course of immunosuppression such as calcineurin inhibitors and micophenolate mophetyl. As clinical response has been reported in a relatively large subset of patients treated with these drugs, it would be necessary to exclude these diseases which are quite different in their prognosis and clinical course from DN.

9) Proposing a universal requirement of kidney biopsy in all diabetic patients puts a huge stress on the medical system. What are the implications with reference to the complication rates? How many additional problems are you likely to face? What is the protocol to be observed? At what stage do you recommend biopsy? In micralb stage or before that? There has to be a valid reason for subjecting any individual to an invasive test, that too in asymptomatic patient.

We are not proposing that universal kidney biopsies should be performed at this point in all patients with diabetes. We are however strong advocate for the need of additional longitudinal clinical trial where routine kidney biopsies are performed in all patients with type 2 diabetes or in patients with type 1 diabetes and a 5-10 years history of the disease.

Thank you again for publishing our manuscript in the World Journal of Diabetes.

Sincerely yours,

A handwritten signature in black ink, appearing to read 'LDgoley', is positioned above the printed contact information.

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