Format for ANSWERING REVIEWERS

October , 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 12971-review.doc).

Title: Biomarkers in Chronic Kidney Disease, From Kidney function to Kidney damage

Author: Lopez-Giacoman Salvador, Madero-Rovalo Magdalena

Name of Journal: *World Journal of Nephrology*

ESPS Manuscript NO: 12971

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

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

See below

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Neprhology*

Sincerely yours,

Salvador Lopez MD

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October 2014

Dr. Fang-Fang Ji,
Science Editor, Editorial Office
World Journal of Nephrology

Dear Dr Fang-Fang Ji,

Re: ESPS Manuscript NO: 12971

Please find attached a revised version of our manuscript "**Biomarkers in Chronic Kidney Disease, From Kidney function to Kidney damage**", which we would like to resubmit for publication as a review article in World Journal of Nephrology.

The comments of the reviewers were highly insightful and enabled us to greatly improve the quality of our manuscript. In the following pages are our point-by-point responses to each of the comments of the reviewers.

We hope that the revisions in the manuscript and our accompanying responses will be sufficient to make our manuscript suitable for publication in the World Journal of Nephrology.

We shall look forward to hearing from you at your earliest convenience.

Yours sincerely,
Salvador Lopez MD

Responses to the comments of Reviewer # 503025

Comment: Suggest modify the title to better cover the biomarker of renal function

***Response*:** *We agree that a change in the title is appropriate due to the fact that other reviews talk little about the importance of biomarkers to evaluate renal function. The new title will help emphasize this other important use of biomarkers.*

Biomarkers in Chronic Kidney Disease, From Kidney Function to Kidney Damage

Comment: The authors should also add info on iohexol clearance and methodologies to measure this molecule

Response:

We thank the reviewer for the insightful comment. We have included the following paragraph in the text:

Among these iohexol is the most recent biomarker for mGFR, it is a non-ionic and non radioactive contrast agent, its molecular weight is 821 Da, has a small extra renal clearance and could be measured only as plasma clearance without the need of urine collections [25]. Some of its other advantages are low expense, wide availability, stability in biologic fluids, and rare adverse reactions when given in a small dose (5 ml of 300 mg/ml iodine) [26, 27]. In addition, iohexol does not require a continuous IV infusion and can be given as an intravenous bolus injection. It can be measured by several different techniques, the most used is the high-performance liquid chromatography (HPLC). However, HPLC requires a great deal of effort which limits its usefulness in the clinical setting [28]. Capillary electrophoresis (CE) a technique in which electrophoretic separations are performed in capillary tubes and is easier and faster than HPLC [29]. Shihabi et al. demonstrated that the iohexol determination by CE correlates well with HPLC [30].

Comment: There is almost no info on Retinol Binding Proteins as predictor of proximal tubular function

Response: *we appreciate the reviewers comment; we have now included the following paragraph in the manuscript:*

Urine Retinol-Binding Protein 4 (uRNP4)

Urine Retinol-Binding Protein 4 (uRBP4) is a 21 KDa protein derived of plasma RBP4 (pRBP4), is an integrant of the lipocain family and is produced mainly in the liver but also in the adipose tissue where it performs as an adipokine that has been linked to insulin resistance and obesity [154, 155]. Unlike other biomarkers such as NGAL and KIM-1, uRBP4 is currently the most sensitive functional biomarker of proximal tubule. pRBP4 is filtered at the glomerulus and completely reabsorbed in the

proximal tubule. In addition, it is known that variation levels of pRBP4 (secondary to nutrition, vitamin A levels, liver disease and infection) have small effect on uRBP 4 as a biomarker [156]. Sensitivity for uRBP4 however decreases as kidney function declines due to false positives that occur in the presence of glomerular disease[157]. This marker was been useful in several diseases related with proximal tubule dysfunction, either hereditary, such as Fanconi syndrome, Dent type 1 syndrome and Lowe syndrome [158] , or acquired conditions that directly affect proximal tubule such as drug toxicity in HIV, cadmium toxicity, plasma cell dyscrasias, AKI diagnosis and other renal tubulointerstitial diseases [159]. Amer et al assessed the prognostic value in renal transplantation of a panel of urinary proteins in 221 patients at 1 year post transplant and reported that patients with glomerular lesions had higher albuminuria than patients with normal histology, and in patients with tubulointerstitial disease, uRBP4 has over expressed. In addition, uRBP4 was a risk factor for long term allograft loss and this risk was independent of kidney biopsy histology and albuminuria [160].

Comment: A table could be added to facilitate the perception of comparison among markers, this table could be divided in normal and diseased subjected.

Response: *We thank the reviewer for this observation; we believe making such a table would be difficult based on the evidence we have to date. Even though it is true that a great deal of research has been done on the biomarkers in kidney disease, the majority of published studies are small, and the largest are directed towards the evaluation of biomarkers in the context of Acute Kidney Injury. Furthermore, almost all the studies of biomarkers in CKD reports the HR o OR, and evaluate different biomarkers in distinct contexts of diseases with different endpoints. For this reasons we believe comparing sensibility/specificity with each other is difficult at this point in time. We have included however a new table that includes the presumptive utility of the biomarkers in different clinical settings.*

Table 3. Utility of New biomarkers in Chronic Kidney Disease.

Biomarker	Origin	Outcome Assessed
Urinary Liver-type fatty acid-binding protein (u-LFABP)	Proximal Tubule	**Diabetic Nephropathy:** Microalbuminuria and mortality
Urinary N-Acetyl-b-O-glucosaminidase (NAG)	Proximal Tubule	**Diabetic Nephropathy:** Albuminuria
Urinary Connective tissue growth factor (CTGF)	Proximal tubule	**Diabetic Nephropathy:** GFR decline
Interleukin-18 (IL-18)	Tubulointerstitial	**Diabetic Nephropathy:** Albuminuria
Apolipoprotein A-IV (ApoA-IV)	Intestinal enterocites	**Chronic Kidney disease:** CKD Progression
Urinary CD14 mononuclear cells		**Polycystic Kidney Disease:** Kidney volume
Neutrophil gelatinase associated lipocalin (N-GAL)	Proximal and distal tubule	**Glomerulonephritis:** GFR and proteinuria **Chronic Kidney Disease:** CKD progression, renal replacement therapy and mortality
Kidney Injury Molecule-1 (KIM-1)	Proximal Tubule	**Chronic Kidney Disease:** CKD progression and renal replacement therapy
Fibroblast growth factor – 23 (FGF-23)	Osteocytes and Osteablast	**Diabetic Nephropathy and others chronic kidney disease:** CKD Progression and mortality
Urinary Retinol Binding Protein 4 (uRBP 4)	Proximal Tubule	**Congenital or acquired tubular dysfunction:** Proximal tubule dysfunction

Comment: In terms of perspectives, the authors could finalize the review with a resume of molecular markers.

Response:

We thank the reviewer for the suggestion, although we consider that the reader should understand the most useful and currently accepted biomarkers, it is also important to mention where the future research would be oriented. New advances in technology have prompted the development of techniques such as proteomics, peptidomics, urinary transcriptomics, and microRNA analysis and this has resulted in new discoveries for novel biomarkers and therapeutic targets that could dramatically change the outcome for patients with CKD in the near future. We have included the following paragraph:

Future Directions.

Advances in technology during the last decade have enlightened our knowledge regarding genetic regulatory pathways. A fast growing arena are the MicroRNAs (miRNAs), the current number of miRNAs in humans are estimated to be between 700 and 1000 , and they have been implicated in several physiological events as well pathologic process, including kidney disease [161]. miRNA have selective expression by different organs, and the kidney expresses mostly miRNA 192, 194, 204, 215 and 216 which

have been implicated in proliferation, migration and structure of renal cells [162, 163]. Little changes in these molecules have implications in kidney function, for instance it is know that deletion of the miRNA 30 family decreases renin cells, affects blood pressure and develop vascular damage and extensive fibrosis [164]. Other miRNAs are related with diverse pathophysologic process, miRNA 155 is associated to blood pressure control through down regulation of type 1 angiotensin II receptor [165, 166] , miRNA 192 and 200 families are related to fibrotic damage in diabetic nephropathy manly by regulation of transforming growth factor beta [167], miRNA 15, 17 and 31 are associated with cystogenesis in polycystic kidney disease [168], and finally miRNA 142, 155 and 223 are increased in acute rejection related to activation of epithelial cells and blood mononuclear cells [169], and can discriminate between acute humoral rejection and cellular rejection [170]. MicroRNA expression pathways have also been evaluated as diagnostic biomarkers in other pathologies. In a study of lupus nephritis patients miRNA 27 and 192 in urine could identified in renal biopsies of lupus patients with nephritis [171] . The knowledge of micro RNA in health and disease remains with several questions concerning its regulation, production and specific target. In addition most studies have measured miRNA in tissue and therefore become cumbersome to measure in clinical practice. Studies evaluating its utility in plasma and urine are urgently needed. Nonetheless this is a rapidly growing field and future research may provide a better understanding of the pathophysiology in kidney disease and may reveal potential diagnosis and therapeutic options.

Not only in the area of proteomics (N Gal, KIM-1, etc) and trascriptomics (MicroRNAs) have the kidney markers evolved, the latest piece added to the puzzle corresponds to metabolomics, and as it name points out, is the measure of end products of basic metabolic molecules. These end products could improve the utility of other type of biomarkers [172]. Currently, metabolomics in kidney disease have mainly been studied in uremia, renal cell carcinoma, glomerulonephritis, diabetes mellitus, polycystic kidney disease and drug related nephrotoxicity. For instance in patients with drug related nephrotoxicity, end products from amino acids and simple sugars increase in urine before tissular changes become apparent. The latter has been described with antibiotics [173], and immunosupression therapy, for example, the increase of metabolomic end products during the first month after cyclosporine predicts kidney damage [174]. Similarly metabolomics has been associated to several metabolic profiles (mainly amino acids, derivatives of sugar and phospholipids) that could be useful in the diagnosis and prognosis of different types of renal disease as diabetic nephropathy, IgA nephropathy and other glomerulonephritis, in addition to diagnosis, metabolomics offers a promising future in the area of pharmaco-metabolomics, which could lead to personalized therapeutic targets[175]. At this point metabolomics main limitation is related to problems with specificity and technical variability and is not ready to be implemented in clinical practice.

Responses to the comments of Reviewer # 504373

Comment: English language is not acceptable and needs to be improved.

***Response*:** *I apologize for this, as English is not my native language. The paper was submitted for assistance in English write up and editing. We hope the manuscript was significantly improved after this intervention*

Comment: Page 3 paragraph 2: The True GFR is the most important marker of kidney function, unfortunately GFR cannot be easily measured in most clinical or research setting, please refers to the section in the manuscript in which will discuss this.

***Response*:**
This statement is in the introduction part where we only give a small summary about the content of the body of paper, nevertheless the sentence was modified as follows

Unfortunately GFR cannot be easily measured in most clinical or research settings (see below), and therefore estimating equations are based on filtration markers such as serum creatinine (SCr) and Cystatin C (CysC). Other biomarkers such as albuminuria may precede kidney function decline and have demonstrated to have strong associations with disease progression and outcomes. New potential biomarkers have arisen with the promise of detecting kidney damage prior to the commonly used markers of kidney disease

Comment: Page 5, paragraph 1: eGFR need to be defined the first time the term is used, and in the outlook, the authors should also mention and discuss the CKD BioCon Initiative

***Response*:** *We thank the reviewer for his/her observation, we have changed the term eGFR in the section title of the paragraph where the definition of eGFR appears in parenthesis.*
Regarding the initiative of CKD BioCon, we mentioned in the conclusion that this international consortium has 15 registry researches of different panels of biomarkers for CKD. Although this consortium determines the desirable properties of CKD biomarkers like noninvasiveness, how easy is the access to perform, sensitivity and specificity, biologic plausibility and other characteristic of the biomarkers, we think that delving into this may be beyond the scope of the paper since these are ongoing studies with lack of definitive results. We have included the link of the webpage in the manuscript.
Larger and long term studies are warranted before applying these biomarkers in clinical practice. The CKD Biomarkers Consortium (https://grants.nih.gov/grants/guide/rfa-files/RFA-DK-08-015.html) has

15 ongoing studies with the aim to develop and validate novel biomarkers for CKD.

Comment: The important paper by Fasset et al is not mentioned, What is the value added to what Fasset describes?

Response: *We agree with the reviewer about the elegant paper done by Fasset et al. We believe the main differences with our paper are that Fasset has based his paper in describing the different biomarkers separated by origin of the kidney damage. For instance, regarding kidney damage, he mentions proteinuria, NGAL, KIM-1 and others. For endothelial dysfunction, he talks about ADMA. Concerning inflammation and fibrosis he mentioned PTX3, uIL-18, TGF-b. While for metabolic factor, oxidative stress and others, he suggests other biomarkers.*

He also makes an excellent description of utility of these in the appraisal of cardiovascular risk, but the greatest difference is that our paper has an extensive summary of the evaluation of kidney function, mentioned lightly in the Fasset paper and most papers about this topic in the current literature. We believe that this topic is important because currently in CKD, risk is stratified using GFR estimating equations. The Kidney Disease Improving Global Outcomes (KDIGO) guidelines recommend to classify CKD by eGFR category, albuminuria and by etiology of kidney disease. Large cohorts have demonstrated an independent association of eGFR and albuminuria with mortality and end-stage renal disease. As kidney function declines, there is a higher rate of complications, such as hypertension, anemia, malnutrition and bone disease that ultimately affects the quality of life and increases mortality.

We believe our review adds to the literature as there are currently more than 200 reviews regarding kidney disease and biomarkers but none of them have integrated the use of biomarkers in the context of both kidney damage and kidney function. We hope that with the modifications made, the manuscript satisfies the reviewer´s expectations.