Buenos Aires, May 6th 2017

World Journal of Nephrology

Editor-in-Chief

Dear Sir,

I want to thank you and the reviewers for accepting the present manuscript with modifications. Following the requirements of the World Journal of Nephrology, I will refer to each of the 4 reviewers comments in this letter. Moreover, all the modifications have been- as requested- highlighted in the revised manuscript in bold and in 4 different colours, so that it will be easier to identify to which reviewer the modification belongs to. My comments in this letter are in italics.

REVIEWER 00503187 BLUE COLOUR

Page 7: The vast majority of these cells appear to be viable with normal morphology and organelle distribution in the cell body [2,11,12]. *References have been added.*

Page 7: Podocyte apoptosis is also one of the mechanisms of podocyte detachment and loss. However, this is not mentioned in the review. However, Voguelman et al have encountered that in addition to the viable detached podocytes, apoptosis occurred in approximately 50% of the cells [2]. In this regard, Kriz et al state that this discrepancy lies on the method employed to assess podocyturia [11,12]. In any case,…

Reviewer comment: I am wondering about the following two sentences: 'In addition, it has been calculated that around 400 podocytes are lost in the urine every day. Finally, it has been demonstrated that when a glomerulus loses between 20-40% of its podocyte content (around

100-200 podocytes), it renders itself to sclerosis and obliteration [2,4].' The numbers/percentages do not make sense, as based on the above, podocyte loss occurring in one day would be enough to lead to sclerosis. Please, check.

The reviewer is correct. I have deleted this paragraph from the paper.

Page 15. In the sentence: 'The available methods to study podocyturia are time-consuming, expensive when compared to the classical ones, and time-consuming.'

Has been corrected.

Page 15. the spot 'when compared to the classical ones' needs clarification. Has been deleted.

Pages 14 and 15. The review does not touch many diseases or physiological states in which podocyturia has been studied or proposed as a potential method to study the disease state or kidney status, and just Alport and Fabry disease are mentioned. I would like the review to contain a more thorough look-up into literature along these lines. The references are very much concentrated on the papers published by the author himself.

I have added data from other authors and these comments:

Wickman et al. have studied the degree of podocyturia in several gomerulopathies as membranous nephropathy, IgA nephropathy, lupus nephritis, diabetic nephropathy, rapidly progressive glomerulonephritis, severe hypertension, among others [30]. They have shown that there was a significant relationship between proteinuria and podocyturia detachment mainly in advanced diabetic nephropathy, in progressors and in active lupus nephritis or IgA nephropathy. They also demonstrated a significant correlation between the degree of podocyturia and proteinuria for all clinic patients, suggesting that proteinuria is a consequence of glomerular dysfunction. Despite proteinuria is a non-specific marker of

glomerular and kidney injury, they concluded that proteinuria would persist despite effective therapies that reduce the rate of podocyte detachment and that podocyte detachment is not only related to proteinuria, but it also determines the progression process and can provide useful additional information to the nephrologist to guide response to treatment and risk for progression in the clinic [30].

However, as mentioned above, our group suggests that the correlation between podocyturia and proteinuria depends on the stage at which the process is approached. Once the mass of podocytes is low, podocyturia would decrease in contrast to a climb in proteinuria and a parallel decline in kidney function [26]. In addition, podocyturia has been widely studied in preeclampsia. The main conclusion is that podocyturia can be employed as a diagnostic tool of preeclampsia among high risk patients, and correlates with the severity of the clinical case [27]. Finally, we have just published an acute case of early-diagnosed IgA nephropathy with copious podocyturia that significantly decreased with immunosuppression and amiloride was assciated with a decrease in proteinuria and an improvement in kidney function [40].

Figure 1: Even though Figure 1 is a cartoon depicting certain key proteins in podocytes, I would like to suggest to draw the proteins and mark their names as realistically as possible. In several cases it is not easy to recognize which marking represents the specific protein (actin, actinin-4, synaptopodin). In case of CD2AP, the name is outside of the foot process even though the protein is intracellular and drawn there as well. Catenin and cadherin are marked the wrong way round (cadherins are the transmembrane proteins and catenins cytosolic adaptors). Podocin is not an extracellular protein or a transmembrane protein, it is attached to the innermost plasma membrane leaflet and should be imaged correspondingly in the cartoon.

I have corrected all the inputs in the figure.

In Figure 2, please mark the tubular cells and podocytes on the image for clarity. Indicate the nature of the blue and green colors on the image.

Figure 2 cells have been marked.

MInor comments:

Page 4: On page 4, it is mentioned that podocyte foot processes self-interdigitate in addition that they interdigitate with the foot processes of the neighboring podocytes; is that so?

I have written:

Adjacent foot processes form the slit diaphragms, structures that determine the quality of the filtered plasma flow[1-3].

page 10, change 'protein chain reaction (PCR)' to 'polymerase chain reaction'

I have written now: Podocytes can also be identified by **polymerase chain reaction** (PCR)

REVIEWER 00503252 RED COLOUR

Reviewer comment:

The authors stated that "It appears that glomerulosclerosis starts to occur when the loss of podocytes per glomerulus is approximately between 20 and 40%. Above this number, the glomerulus starts the point of no return and is destined to obliteration." (P4, L6-8). and "Partial detachments are usually reversible to a certain degree."(P7, L10,11). Appropriate references to each statement should be provided.

Page 4: I have added the references:

When the number of podocytes become critical, matrix expansion and glomerulosclerosis start to take place[4,5]. It appears that glomerulosclerosis starts to occur when the loss of podocytes per glomerulus is approximately between 20 and 40%. Above this number, the

glomerulus starts the point of no return and is destined to obliteration. It is well known that when the number of glomeruli is less than 50%, renal insufficiency ensues[4,5].

And now page 11 (not 10 anymore):

In the adult human kidney, it is estimated that approximately 500 podocytes populate each glomerular tuft, and their turnover rate is very slow [2,4]. As mentioned, it has been demonstrated that when a glomerulus loses between 20-40% of its podocyte content (around 100-200 podocytes), it renders itself to sclerosis and obliteration [2,4,5].

Second comment:

2. This reviewer wonders the sentence "Considering the fact that about 2 million podocytes are distributed in glomeruli in both kidneys, approximately 1 billion podocytes populate both kidneys. In addition, it has been calculated that around 400 podocytes are lost in the urine every day." (P11, L9-12) does not make sense.

As previous reviewer. I have deleted these sentences.

3. misspellings a normal o higher glomerular filtration rate (P13, L3 from last line) disease ore ven at the same stage (P14, L2 from the last line) withou delays (L15, L7)

Have been corrected.

REVIEWER 00503272 GREEN COLOUR

All the modifications made due to this reviewer (mostly type setting errors or grammatical issues) are outlined in the mauscript in bold and in green.

Reviewer's comments 1. Author (Abstract line 10, page 2): '… glomerular filtration barrier: The podocyte.' Reviewer: change to '…. glomerular filtration barrier: the podocyte.'

2. Author (Page 2, line 14): ‘When the loss of podocytes in the urine, or podocyturia, is taken place and its glomerular…’ Reviewer: change ‘taken’ to ‘taking’ in the above line.

3. Author (The podocyte, line 1, page 4): ‘The podocyte mass is small when compared of the whole amount of kidney cells.’ Reviewer: the sentence should be revised to ‘The podocyte mass is small compared to the entire kidney mass.’

4. Author (Page 5, line 9, paragraph 1): ‘… which is critical for the formation of fenestrae in the capillar endothelium, …’ Reviewer: please change ‘capillar’ to ‘capillary.’

5. Author (Page 6, line 22): ‘… and also in the lower quality of these synthesized molecules,a…’ Reviewer: let there be period between ‘molecules,’ and letter ‘a’.

6. Author (Page 9, line 9, paragraph 1): ‘In this regard, our group suggests that a probable…’ Reviewer: change ‘suggests’ to ‘suggested.’

7. Author (Page 10, paragraph 3, line 4): ‘In addition, it has beencalculated that around 400…’ Reviewer: put a period between ‘been’ and ‘calculated.’

8. Author (Page 12, paragraph 2, line 11): ‘a different regenerative potential at distinct stages of life,exhibiting the highest…’ Reviewer: put a period between ‘life,’ and ‘exhibiting.’

9. Author (Page 13, lines 9-12, paragraph 1): ‘We and others have assessed podocyturia in a wide variety of glomerulopathies, as well as in transplantation and in entities where the glomerulus is not primarily affected, as in polycystic kidney disease….’ Reviewer: Remove ‘We and others’ and revise to read ‘Podocyturia has been assessed in a wide….’

10. Author (Page 13, line 13, paragraph 1): ‘Podocyturia is ideally employed in hereditary kidney disorders like Alport of Fabry disease.’ Reviewer: What is the meaning of ‘Alport of Fabry disease’ in the above statement?

11. Author (Conclusion): ‘Podocytes are HIGHLY DIFFERENTIATED CELLS UNABLE TO REPLICATE under normal conditions. DESPITE PHYSIOLOGIC PODOCYTURIA EXISTS, any insult received by the podocyte either directly or indirectly, would trigger contractile mechanisms DESTINED TO RETAIN THEM ATTACHED to the glomerular basement membrane, DESPITE RESIGNING THEIR CAPACITY TO AVOID PROTEIN LOSS in the urine. Podocytes attach to the glomerular basement membrane via integrins, that anchor cells.’ Review: What is the meaning of each of the statements in upper case in the above lines in your conclusion? Please, make them clear.

REVIEWER 02888410 GOLDEN COLOUR

Reviewer comments:

The manuscript is too condensed. To separate paragraphs and introducing subtitles would help to a better text understanding.

I have divided the long paragraphs into shorter ones.

A figure on the pathogenesis of GSFS after podocyte destruction would be welcome.

Not performed. I humbly believe a figure like this would not contribute to the essence of this manuscript, that is podocyturia and not glomerulosclerosis.

The phrase "Thus, podocyturia would be useful in clinical grounds when employed routinely for screening purposes in any patient" would be better placed in "Conclusions" rather than in limitations.

Done in page 17: Podocyturia has been employed as a diagnostic tool in a wide variety of gomerular disorders. Unravelling the mechanisms of podocyte detachment may lead to targeted therapeutic interventions that could delay the progression of chronic kidney diseases, as shown by the decrease of podocyte loss when amiloride is employed.

In any case, a comment on the cost of such measure for screening purposes must be added (this is obviously the limitation).

Done in page 16: Finally, the cost of the study of loss podocytes is another major determinant that at this times positions this method in the research field and not also in the clinical ground.

Conclusions: Erase " Podocytes attach to the glomerular basement membrane via integrins, that anchor cells to laminin and other membrane components". This is not a conclusion.

Done.

The authors discussed the diagnostic use of podocyturia, and this should be included in the conclusions.

Done in Conclusions.

The last phrase: "Unravelling the mechanisms of podocyte detachment could lead to targeted therapeutic interventions that could delay the progression of chronic kidney diseases" is logical but it is not supported by the matter discussed in the text.

Due to this comment, I have added this content in pages 14 and 15 to support it:

In this regard, we have reported that in several glomerular diseases the addition of amiloride contributed to the decrease in the degree of podocyturia, probably due to its interaction with uPAR, and consequently with the atachment of podocytes to the glomerular basement membrane via integrins, as explained above [16,23,38-41]. Zhang et al have shown that amiloride reduces uPAR expression, inhibtis uPAR mRNA and protein

synthesis in podocytes. Noteworthy, uPAR is highly expressed on cell the surface of diseased podocytes, but only scarcely on normal podocytes[41].

I have to have fulfilled the requirements by the World Journal of Nephrology and to have the manuscript published in the near future.

Kind regards,

Dr Hernán Trimarchi