Title: Poor histological lesions in IgA nephropathy may be reflected in blood and urine peptide profiling

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Version: 3 Date: 5 March 2013

Author's response to reviews: see over
Badalona, March 5, 2013

RE: COMMENTS ON MANUSCRIPT Ms. No. 9398799598425947

Dear Dr. Henderson,

Thank you for your valuable and constructive comments. We would appreciate your reconsideration of the revised version of our manuscript, “Poor histological lesions in IgA nephropathy may be reflected in blood and urine peptide profiling.”

We have taken all of the referees’ comments and suggestions and the editorial points into account and produced a second revised version. We hope that these modifications will simplify our manuscript and render it more suitable for publication in BMC Nephrology.

We look forward to hearing from you.

Yours sincerely,

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For easier reference, the points that have been raised by the reviewers are in **bold**, followed by our comments on the changes in the manuscript.

**Reviewer #2 - Minor Essential Revisions**

1. There are still too many paragraphs in discussion (13-15 paragraphs).

   **If possible, put together similar paragraphs and reduce to 6-8 paragraphs.**

   We agree with the reviewer’s comment. Accordingly, we have revised the entire manuscript to unify the manuscript.

   In agreement with the reviewer’s suggestion, we have removed the following paragraphs in ‘Discussion’:

   “IgAN is characterized by high variability in clinical and histological course, which can occur from virtually normal histology by light microscopy to aggressive forms of glomerular/tubular damage, such as severe necrotizing, crescentic glomerulonephritis, and advanced glomerulosclerosis, and tubular atrophy.”

   “We have described a combination of urinary UMOD- and A1AT-derived peptides that accurately categorizes patients with GKD.”

   “In our cohort, all patients showed mesangial hypercellularity at diagnosis; thus, we could not compare subjects with M1 and M0.”

   In addition, we have improved the following paragraph highlighted in yellow under ‘Discussion’ (page 12-13, lines 13-24 and lines 1-6):

   “Recently, new methods have been described to identify biological biomarkers in various fluids using proteomic techniques. In our previous report [38], we demonstrated that MB-based profiling and MALDI-TOF MS identify disparities in urine between GKD patients and controls, suggesting that establishment of a differential peptide profile is the initial step toward classifying GKD. In the current study, the majority of peptides, specifically high
levels of an A1AT-derived peptide \((m/z\ 1945)\) and low levels of a UMOD-derived peptide \((m/z\ 1898)\) and bradykinin \((m/z\ 1063)\), was linked to tubulointerstitial damage.

Alpha-1-antitrypsin protects the extracellular matrix from neutrophil attack through its anti-inflammatory and anti-apoptotic effects. Kwak et al. [40] reported increased levels of A1AT peptides in kidney tissue and urine in IgAN patients compared with healthy subjects. The authors speculated that renal tubular epithelial cells produce A1AT in response to tubulointerstitial damage. Consistently, our results reinforce the suggestion that high levels of A1AT in urine might constitute a response to the inflammatory process in GKD. Likewise A1AT has a function in tubulointerstitial injury; we noted an inverse association between UMOD-derived peptides and tubulointerstitial lesions, consistent with other groups that have described low urinary UMOD levels in association with tubular atrophy and interstitial infiltration in renal biopsies [41].”

Similarly, we have improved the following paragraph highlighted in yellow under 'Discussion' (page 13-14, lines 11-25 and lines 1-13):

"Further, in IgAN, Wu et al. [37] described a urine peptide profile, noting downregulation of peptide that corresponded to UMOD, allowing them to discriminate IgAN from healthy subjects. In the current study, our peptide profile could be a biomarker of histological lesions but is not specific for IgAN.

Rocchetti et al. [23] reported a significant decrease in the urinary excretion of kininogen in IgAN patients, particularly in unresponsive ACEI therapy patients. The authors speculated that this difference reflects the severity of renal damage in IgAN patients. Similarly, we found a decreased in kininogen-derived peptide bradykinin \((m/z\ 1063)\) in plasma from IgAN patients with severe tubulointerstitial damage T2. But, we could not demonstrate this effect because most patients did not receive ACEI or ARB agents at baseline. Thus, we could not compare the effects of these drugs on peptide profiles.

Bradykinin is a nonapeptide that is derived from the kininogen protein, a robust agonist of the bradykinin 2 receptor, enhancing the production of nitric oxide and prostaglandins. Potent renal vasodilator, antithrombotic and antifibrotic effects of kinins have been observed recently in diabetic nephropathy [44]. Furthermore, upregulation of bradykinin
receptor has been described to mediate the progression of focal segmental glomerulosclerosis [45].

In a previous report, Kang et al. [9] observed that segmental glomerulosclerosis and IFTA reflect chronic damage, which can be used to predict the long-term prognosis of patients with IgAN. Our findings suggest that a rise in peptides at $m/z$ 1945, 2392, and 4013 and decline in the peptide at $m/z$ 3389 observed in patients with S1 reflect chronic glomerular injury.

Endocapillary and mesangial hypercellularity lesions were not associated with renal outcome in the original Oxford cohorts, although few reports have reported such findings. In our study, we identified 5 peptides—in serum at $m/z$ 1546, 3264, and 3242 and in plasma at $m/z$ 3242, 8602—of which the plasma and serum peptides at $m/z$ 3242 and plasma peptide at $m/z$ 8602 increased and serum peptides at $m/z$ 1546 and 3264 decreased in the presence of endocapillary hypercellularity.”

Finally, we have improved the following paragraph highlighted in yellow under 'Discussion’ (page 14-15, lines 18-25 and lines 1-2):

“A limitation of this study is that we could not confirm that the urinary peptides of UMOD and A1AT are related specifically to IgAN, because our population was in various stages of kidney disease and degrees of interstitial injury. Interestingly, we found a relationship between peptide profiles in the serum, plasma, and urine of IgAN patients with the doubling of serum creatinine levels—particularly, in the urine samples with UMOD and A1AT peptides. Based on these findings, we propose that this peptide profile is a potential biomarker predictor of renal outcome, but we can not exclude the possibility that these peptides constitute a biomarker of chronic kidney diseases. It is necessary to expand this population study to other types of GKD to mitigate the limitations on its reproducibility and thus obtain a specific peptide profile.”