Editorial Commentary

Inflammatory proteins in diabetic kidney disease—potential as biomarkers and therapeutic targets

Angie S. Xiang[1], Elif I. Ekinci[2,3], Richard J. MacIsaac[3,4]

[1]Central Clinical School, Monash University, Victoria, Australia; [2]Endocrine Center, Austin Health, Melbourne, Victoria, Australia; [3]Department of Medicine, University of Melbourne, Melbourne, Victoria, Australia; [4]Department of Endocrinology & Diabetes, St Vincent's Hospital Melbourne, Victoria, Australia

Correspondence to: Prof. Richard J. MacIsaac, BSc (HONS), PhD, MBBS, FRACP. Department of Endocrinology & Diabetes, St Vincent's Health Melbourne, 4th Floor, Daly Wing, 35 Victoria Parade, PO Box 2900, Fitzroy, Victoria 3065, Australia. Email: r.macisaac@unimelb.edu.au.

Provenance: This is an invited article commissioned by the Section Editor Dr. Linpei Jia, MD, PhD (Department of Nephrology, Xuanwu Hospital of Capital Medical University, Beijing, China).

Comment on: Niewczas MA, Pavkov ME, Skupien J, *et al.* A signature of circulating inflammatory proteins and development of end-stage renal disease in diabetes. Nat Med 2019;25:805-13.

Submitted Jul 25, 2019. Accepted for publication Aug 06, 2019.
doi: 10.21037/atm.2019.08.20
View this article at: http://dx.doi.org/10.21037/atm.2019.08.20

Diabetic kidney disease (DKD) affects approximately 40% of all people with diabetes, and is the leading cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD) (1). With the increasing diabetes prevalence secondary to obesity, as well as improved survival among people with diabetes due to prevention and management of macrovascular complications, the morbidity and mortality form DKD can only be expected to increase in the future (2).

Current diagnosis and monitoring of DKD relies on the detection of increased albuminuria and/or decreased estimated glomerular filtration rate (eGFR) (3). However, identifying people with diabetes at the greatest risk for a progressive decline in renal function using albuminuria and/or eGFR still remains problematic. Albuminuria has long been used to monitor the onset and progression of DKD but the sensitivity and specificity of albuminuria, especially of microalbuminuria, as a marker of progressive DKD has been recently challenged (4). Historically the development of low levels of albuminuria (microalbuminuria) has been considered as a strong predictor of progression to proteinuria which then heralds in renal function decline. However, a remission/regression rather than a progressive trajectory of microalbuminuria is frequently seen in clinical practice and has also been reported in recent studies. Furthermore, microalbuminuria is neither a universal nor a sensitive or specific marker for progressive DKD and recent studies have questioned the previously accepted chronological relationship between albuminuria and GFR decline (3,5). Indeed, a normoalbuminuric phenotype of DKD is now widely recognised (6).

Currently routine methods for determining renal function are also problematic. Creatinine levels from which eGFR is usually derived can remain normal despite renal function loss of over 50% (7). Also, the formulas which are used to calculate creatinine derived eGFR consistently underestimate directly measured renal function (GFR) in the normal range in people with diabetes (8,9). Despite the above, following early trends in eGFR decline is emerging as a useful method for determining a person with diabetes risk of progression to ESRD (10).

The awareness of the discordance between renal function decline and increasing albuminuria suggests other risk markers apart from albuminuria which need to be identified to better determine a person's risk for a progressive decline in GFR. Technological advancements and the rapid embrace of multi-omic approaches have allowed the identification and quantification of several hundred potential protein biomarkers both in urine and in circulation (11-15). With increasing acknowledgement of the key pathogenic role of inflammation in DKD, inflammatory proteins represent some of the most promising novel biomarkers in diabetic and non-diabetic nephropathies alike (7,16,17).

Niewczas and colleagues' recent study published in *Nature Medicine* (11) quantified circulating concentrations

Table 1 Effect of baseline levels of TNF-RSF KRIS members on ESRD risk in Joslin and Pima Indian cohorts [HR* (95% CI)]

TNF-RSF protein	Joslin cohorts (T1DM and T2DM) (n=363)	Pima Indian cohort (T2DM) (n=162)
TNF-R1	2.78 (2.02, 3.83)	1.71 (1.07, 2.72)
TNF-R2	1.86 (1.42, 2.43)	2.32 (1.33, 4.06)
DR6	1.38 (1.08, 1.76)	2.24 (1.25, 4.01)
TROY	1.68 (1.31, 2.16)	1.76 (1.09, 2.83)
XEDAR	1.99 (1.59, 2.50)	1.83 (1.17, 2.86)
RELT	1.46 (1.13, 1.88)	2.46 (1.38, 4.40)

*, HRs were calculated using Cox proportional hazard models adjusted for baseline age, GFR, HbA1c, ACR, gender, diabetes duration, systolic blood pressure and BMI. Data from Niewczas *et al.* (11). TNF-RSF, tumour necrosis factor-receptor superfamily; KRIS, kidney risk inflammatory signature; ESRD, end-stage renal disease; T1DM, type 1 diabetes; T2DM, type 2 diabetes.

of 194 inflammatory proteins in three independent and very well characterised diabetic cohorts totalling 525 participants derived from the Joslin Kidney Study and the Pima Indian Study. The three study cohorts consisted of a discovery cohort, 219 Joslin participants with type 1 diabetes (T1DM); a validation cohort, 122 Joslin participants with type 2 diabetes (T2DM); and a replication cohort, 162 Pima Indians with T2DM. Baseline GFRs and urinary albumin to creatinine ratios were 43±1, 49±1 and 155±53 mL/min/1.73 m^2, and 1,262±1,442, 851±1,257, 709±1,772 µg/mg, respectively for the three cohorts. So of note, Joslin patients had starting eGFR values within the CKD stage 3 range (30–59 mL/min/1.73 m^2) whilst Pima Indian participants had a starting GFR values of 155 mL/min/1.73 m^2 as measured by the urinary clearance of iothalamate. The main outcome of interest in the study was the development of new cases of ESRD during 10 years of follow-up with 108 (49%), 35 (24%) and 28 (23%) cases of ESRD occurring in the discovery, validation and replication cohorts, respectively.

Initially, 194 inflammatory proteins were identified using a selection algorithm that included representatives of the major inflammatory classes, interleukins, chemokines, tumour necrosis factor (TNF) and their corresponding receptors, interferons, complement system members and co-stimulatory molecules in plasma samples from all study participants using a custom-designed SCOMA (slow off-rate modified aptamers) scan platform. Of note, aptamers are oligonucleotides or peptides that bind to specific target molecules. Baseline values of 35 of the above proteins were then found to be significantly higher for participants in the discovery (Joslin T1DM) cohort that developed ESRD than those that did not. In the validation (Joslin T2DM) cohort, levels for 17 of 35 proteins were significantly higher in participants that developed ESRD than those that did not. These 17 proteins were collectively termed 'KRIS' (kidney risk inflammatory signature), and predominantly featured members of the tumour necrosis factor-receptor superfamily (TNF-RSF).

The effects of baseline concentrations of 5 members of the KRIS proteins on the time to the development of ESRD were then examined using Cox's regression analysis. For this analysis the two Joslin cohorts were combined. In a fully adjusted model for relevant covariates (including eGFR and albuminuria), the hazard ratios (HR) for the development of ESRD were statistically significant (as assessed as HR per one tertile change in baseline concentration) for 15 of the 17 KRIS proteins. In the Pima Indian cohort, baseline concentrations of 12 of the 17 proteins were significantly associated with the risk of developing ESRD. The effects of the TNF-RSF on the risk of developing ESDR in the above two cohorts is summarised in *Table 1*. Interestingly, although TNF soluble receptors were found to be robust and independent predictors of ESRD, the same relationship was not found for levels of the corresponding ligand for the receptors, namely TNF.

The authors then subsequently performed a number of additional analyses and studies to further investigate the usefulness of the KRIS proteins as markers of renal function loss in diabetes. In summary, regression analyses showed that the baseline concentrations of KRIS proteins were reliably associated with the subsequent rate of renal function decline among the Joslin cohorts, but to a lesser degree in the Pima Indian cohort. When albuminuria was modelled as a mediator of KRIS protein effects in renal function decline, KRIS proteins predicted renal functional decline largely independent of albuminuria. The authors also obtained plasma samples for people with diabetes involved in randomised clinical

trials of various agents with proven or proposed nephron-protective effects. Treatment with the angiotensin receptor blocker blocker (ARB) losartan did not have any effect on KRIS protein profiles. In comparison, the JAK1/2 inhibitor baricitinab reduced levels for 3 out of the 10 KRIS proteins that were measured as part of this sub-study. Although baricitinab did not significantly reduce soluble TNF receptor type 1 (TNF-R1) levels it did reduce soluble TNF receptor type 2 (TNF-R2) levels compared with placebo.

Furthermore, studies were performed to determine if the kidney was the source of circulating KRIS proteins. For this analysis plasma and urine samples were studied from 29 cases (with fast renal function decline) and 26 controls (no or slow renal function decline) from the Joslin T1DM cohort. Cases were found to have increased urinary excretion of KRIS proteins many years before the onset of ESRD. Also, in kidney biopsy specimens obtained from 56 of the Pima Indian cohort members, there were no strong correlations between serum levels and the tissue expression of KRIS proteins. These results suggest that systemic overproduction of KRIS proteins rather than retention because of reduced renal clearance is the cause for elevated levels of these proteins in those at risk of progression to ESRD. Of relevance to this finding, we have recently shown that over an 8-year period, an early decline in renal function in people with T1DM or T2DM is associated with a progressive increase in serum levels of the soluble TNR-R1 (18).

The decision to identify the KRIS proteins by examining the three cohorts independently rather than collectively as a pooled cohort is a point of interest. The design of the present study could lead to a number of key protein biomarkers of progressive DKD being missed due to specificity for one phenotype of diabetes or the loss of statistical power resulting from the analysis of smaller groups. The authors argued that the study design was chosen to allow for the identification of shared inflammatory processes, which current evidence suggests underpins the development and progression of DKD in both T1DM and T2DM. Whilst oxidative stress and the upregulation of inflammatory cytokines secondary glucotoxicity and lipotoxicity appear to be universal drivers in both types of diabetes, the differing diabetes natural history, onset and rate of renal function decline and incidence of ESRD amongst the three cohorts suggests that the disease processes between study groups are not identical.

Although 15 out of the 17 proteins were significantly correlated with DKD in all three cohorts, their relative effect sizes and order and order of importance as predictors of ESRD differed. This could be due to the occurrence of different pathophysiological processes or perhaps similar processes occurring, but to different degrees, that drive DKD progression between the different groups of participants in this study. It should be noted that the KRIS proteins were most poorly correlated with kidney disease in the Pima Indian cohort who in fact showed the most dramatic decline in renal function. However, in comparison to the Joslin cohort, these participants had starting GFR levels in the hyperfiltering and not the renal impairment range, as mentioned above, had the greatest decline in renal function. Notably, the Pima Indian cohort of hyperfiltering participants appropriately had appropriately had renal function measured directly rather that estimated from a creatinine-based equation. This is an important consideration as creatinine-based eGFR eqautions are known to significantly under estimate true GFR values in the hyperfiltering range (3).

The identification of a number of inflammatory proteins associated with DKD progression aligns closely with the increased recognition of the pathophysiological role of inflammation in diabetic nephropathy (7,16,17). In particular, the incorporation of six members of the TNF-RSF among the KRIS proteins supports the growing body of evidence from previous studies showing that elevated levels of the TNF-RSF members are associated with progressive DKD in both T1DM and T2DM (19-21). Interestingly, Niewczas *et al.*'s study did not find any correlation between ligands of the TSF-RSF KRIS proteins and renal function decline, including TNF-α. This is an interesting finding as TNF-α is the upstream ligand of TNF-R1 (reported as TNF-RSF1A) and TNF-R2 (reported as TNF-RSF1B), the two most strongly correlated TNF-RSF proteins in the Joslin cohorts (11), and have both been previously been associated with renal function decline in both T1DM (19) and T2DM (20,21). The lack of association between KRIS protein ligands and genes in general corresponds with the limited knowledge regarding the exact pathophysiological mechanisms connecting TNF and its receptors with renal disease (22), and represents an area for further study.

An ongoing issue for all 'omic' studies seeking to identify disease biomarkers is the panel of metabolites for which the study will attempt to quantify. The breadth of this panel could be restricted due to technological limitations, but could also be a conscious decision at the hands of the researchers. Whilst the inclusion of more proteins or peptides may lead to the discovery to a greater number of potentially relevant markers for subsequent investigation, it may conversely also lead to interpretational difficulties due

to increased false positives and the identification of species for which there is limited pre-existing knowledge.

Niewczas *et al.*'s study examined for a panel of 194 known inflammatory proteins and proteins previously studied in the context of DKD, however it should be noted that broader proteomic studies have been previously undertaken leading to the identification of hundreds of proteins associated with DKD (11-15). Although these proteins lack the aetiological background of the KRIS proteins, it is possible that they may outperform the KRIS proteins in predicting renal decline or progression to ESRD in patients with diabetes either individually or combined within a select panel. Furthermore, whilst a focus on inflammatory proteins theoretically allows earlier disease detection by identifying pathology that may precede the development of renal dysfunction, elucidating more general biomarkers of kidney disease may result in broader clinical utility including the application to non-DKDs.

Before any novel biomarkers can be implemented within routine clinical practice, they must outperform current tests of albuminuria and eGFR, as well as existing tests that could be repurposed to predict and diagnose DKD (16). It should be noted that predictive analyses from multiple studies suggest have suggested that some of the novel biomarkers studied by Niewczas *et al.* do not perform as well when evaluated in prospective studies (12,23). Furthermore, not only would these new proposed biomarkers need to show superior disease prediction, they also need to show that through their monitoring and targeting with novel interventions, improved clinical outcomes are possible. The latter will depend on the development of novel efficacious therapeutic agents for DKD, which is a rapidly evolving field.

Currently, there is a focus on the reno-protective effects of sodium-glucose transporter 2 (SGLT2) inhibitors, renin-angiotensin-system inhibitors, and systemic anti-inflammatory agents (24,25). Previously treatment with the irbesartan for two years improved the urinary proteome among individuals with T2DM and DKD towards a 'healthier' profile in line with reduced albuminuria (15). The change in urinary proteome in the above study suggested a mechanism of reduced collagen breakdown and fibrosis rather than anti-inflammation. However, Niewczas and colleagues' inflammatory KRIS panel was unchanged following treatment with the ARB losartan among a subset of Pima Indians with T2DM, although participants in this study were only treated for a minimum of six months and it is also unclear as to whether participants followed a subsequent rapid decline or stable pattern of renal function (11).

Niewczas *et al.*'s study did however report a statistically significant reduction in four of the KRIS proteins among patients with advanced DKD that were treated with barcitinib, a JAK1/2 inhibitor, for 24 weeks compared with placebo (11). Of relevance to this study, the JAK-STAT signalling pathway represents a key intracellular mediator for inflammatory pathways (24). The significance of this finding in terms of its potential relationship with amelioration of renal function decline in people with diabetes still remains to be defined. In the future, there is also potential for discovered biomarkers to reveal new therapeutic targets of DKD, or categorise multiple phenotypes of DKD requiring different treatment regimens in line with the rise of personalised medicine.

In summary, this study of Niewczas *et al.* has contributed to elucidating the inflammatory proteome associated with the development and progression of DKD. It furthers our understanding of the pathophysiological role of inflammation in the development of DKD, and may reveal novel diagnostic tests allowing earlier and improved disease detection. However, the clinical utility of these proteins is yet to be proven and must be evaluated through studies that assess their impact and additive benefit in combination with currently available clinical markers. This includes assessment of the cost-effectiveness of employing novel markers in routine clinical practice and their utility for improving outcomes. Efforts to identify novel biomarkers must therefore be complemented with a similar vigour to develop therapeutic strategies to attenuate DKD onset and progression. Identified protein biomarkers of DKD should be explored as potential therapeutic targets, and could lead to the development of novel classes of pharmacological agents.

Acknowledgments

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Alicic RZ, Rooney MT, Tuttle KR. Diabetic Kidney Disease: Challenges, Progress, and Possibilities. Clin J Am Soc Nephrol 2017;12:2032-45.
2. GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 2017;390:1211-59.
3. Macisaac RJ, Ekinci EI, Jerums G. Markers of and risk factors for the development and progression of diabetic kidney disease. Am J Kidney Dis 2014;63:S39-62.
4. Bakris GL, Molitch M. Microalbuminuria as a risk predictor in diabetes: the continuing saga. Diabetes Care 2014;37:867-75.
5. MacIsaac RJ, Ekinci EI, Jerums G. Progressive diabetic nephropathy. How useful is microalbuminuria?: contra. Kidney Int 2014;86:50-7.
6. Macisaac RJ, Jerums G. Diabetic kidney disease with and without albuminuria. Curr Opin Nephrol Hypertens 2011;20:246-57.
7. Mischak H, Delles C, Vlahou A, et al. Proteomic biomarkers in kidney disease: issues in development and implementation. Nat Rev Nephrol 2015;11:221-32.
8. MacIsaac RJ, Ekinci EI, Premaratne E, et al. The Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation does not improve the underestimation of Glomerular Filtration Rate (GFR) in people with diabetes and preserved renal function. BMC Nephrol 2015;16:198.
9. Wood AJ, Churilov L, Perera N, et al. Estimating glomerular filtration rate: Performance of the CKD-EPI equation over time in patients with type 2 diabetes. J Diabetes Complications 2016;30:49-54.
10. Krolewski AS, Niewczas MA, Skupien J, et al. Early progressive renal decline precedes the onset of microalbuminuria and its progression to macroalbuminuria. Diabetes Care 2014;37:226-34.
11. Niewczas MA, Pavkov ME, Skupien J, et al. A signature of circulating inflammatory proteins and development of end-stage renal disease in diabetes. Nat Med 2019;25:805-13.
12. Heinzel A, Kammer M, Mayer G, et al. Validation of Plasma Biomarker Candidates for the Prediction of eGFR Decline in Patients With Type 2 Diabetes. Diabetes Care 2018;41:1947-54.
13. Rao PV, Lu X, Standley M, et al. Proteomic identification of urinary biomarkers of diabetic nephropathy. Diabetes Care 2007;30:629-37.
14. Rossing K, Mischak H, Dakna M, et al. Urinary proteomics in diabetes and CKD. J Am Soc Nephrol 2008;19:1283-90.
15. Andersen S, Mischak H, Zurbig P, et al. Urinary proteome analysis enables assessment of renoprotective treatment in type 2 diabetic patients with microalbuminuria. BMC Nephrol 2010;11:29.
16. Winter L, Wong LA, Jerums G, et al. Use of Readily Accessible Inflammatory Markers to Predict Diabetic Kidney Disease. Front Endocrinol (Lausanne) 2018;9:225.
17. Teodoro JS, Nunes S, Rolo AP, et al. Therapeutic Options Targeting Oxidative Stress, Mitochondrial Dysfunction and Inflammation to Hinder the Progression of Vascular Complications of Diabetes. Front Physiol 2019;9:1857.
18. MacIsaac RJ, Farag M, Obeyesekere V, et al. Changes in soluble tumor necrosis factor receptor type 1 levels and early renal function decline in patients with diabetes. J Diabetes Investig 2019. [Epub ahead of print].
19. Gohda T, Niewczas MA, Ficociello LH, et al. Circulating TNF receptors 1 and 2 predict stage 3 CKD in type 1 diabetes. J Am Soc Nephrol 2012;23:516-24.
20. Niewczas MA, Gohda T, Skupien J, et al. Circulating TNF receptors 1 and 2 predict ESRD in type 2 diabetes. J Am Soc Nephrol 2012;23:507-15.
21. Barr EL, Barzi F, Hughes JT, et al. High Baseline Levels of Tumor Necrosis Factor Receptor 1 Are Associated With Progression of Kidney Disease in Indigenous Australians With Diabetes: The eGFR Follow-up Study. Diabetes Care 2018;41:739-47.
22. Al-Lamki RS, Mayadas TN. TNF receptors: signaling pathways and contribution to renal dysfunction. Kidney Int 2015;87:281-96.
23. Colombo M, Valo E, McGurnaghan SJ, et al. Biomarker panels associated with progression of renal disease in type 1 diabetes. Diabetologia 2019;62:1616-27.
24. Heerspink HJ, De Zeeuw D. Novel anti-inflammatory drugs for the treatment of diabetic kidney disease. Diabetologia 2016;59:1621-3.
25. Cherney DZI, Bakris GL. Novel therapies for diabetic kidney disease. Kidney Int Suppl (2011) 2018;8:18-25.

Cite this article as: Xiang AS, Ekinci EI, MacIsaac RJ. Inflammatory proteins in diabetic kidney disease—potential as biomarkers and therapeutic targets. Ann Transl Med 2019;7(Suppl 6):S243. doi: 10.21037/atm.2019.08.20

