**Polar Views in Nephrology**

**Con: Biomarkers in glomerular diseases: putting the cart before the wheel?**

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**ABSTRACT**

Biomarkers have been increasingly sought to improve diagnosis as well as predict prognosis and/or response to therapy. However, they should not replace sound clinical judgment and therapeutic measures. The present article aims to highlight the issues with biomarker research in three selected entities. In focal segmental glomerulosclerosis, many studies fail to differentiate cases of primary versus secondary forms leading to conclusions that are uninterpretable. Biomarkers have also been sought to predict development of diabetic nephropathy but this research should not supersede efforts aimed to optimize treatment of diabetes. Finally, in lupus nephritis (LN), biomarkers so far have failed to prove value in clinical practice. The concept of immunological remission should be added to the concept of clinical remission when judging response to immunosuppressive therapy in LN. In addition, the appropriate time frame for remission to occur should be reconsidered.

**Keywords:** biomarkers, diabetic nephropathy, focal segmental glomerulosclerosis, glomerular, lupus nephritis

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*“The first man I saw had been 8 years upon a project for extracting sunbeams out of cucumbers...He told me he did not doubt in 8 years more he should be able to supply the governor’s gardens with sunshine...”*

Scientist at the Grand Academy of Lagado, talking to Gulliver [1].

**BIOMARKERS IN GLOMERULAR DISEASES**

Kidney biopsy is the gold standard for the diagnosis of glomerular disease and has been used as prognostic indicator as well as to guide treatment. However, kidney biopsy is invasive, associated with risks, and in some cases a clear association between biopsy appearance and prognosis or response to therapy has not been identified. Thus, ‘biomarkers’ have been used to improve diagnosis, more accurately predict prognosis, and enhance therapeutic decision-making. Traditional biomarkers include serum creatinine and estimated glomerular filtration rate, albuminuria and proteinuria. But since these are not specific or sensitive, more sophisticated ‘novel’ biomarkers have been proposed, such as auto-antibodies, modified serum proteins (e.g. galactose deficient IgA1), RNA and its fragments, cytokines, growth factors, urinary IgG/IgM and others [2].

To be useful, a biomarker should be logistically and financially within reach, reproducible, predict clinical course and response to treatment and provide information not given by more readily available tests. In membranous nephropathy (MN), anti-PLA2R antibodies fulfill the above criteria and represent one of the great achievements in glomerular biomarkers [3]. These antibodies are positive in ∼70% of the patients with MN and clearly correlate with disease activity and response to therapy [4, 5]. However, a small number of patients have a negative anti-PLA2R testing in the serum but positive staining on kidney biopsy slides [6]. Thus, a negative serological test does not rule out PLA2R-mediated MN. In addition, ∼30% of patients with MN are PLA2R negative. Therefore, even such an excellent biomarker as PLA2R cannot replace kidney biopsy as the gold standard for the diagnosis of MN.
But before we embark on pursuing novel biomarkers, we need to work on the basics. To epitomize this point, three glomerular diseases will be discussed where great efforts have been conducted in identifying biomarkers but where efforts have been misplaced.

### BIOMARKERS IN FOCAL SEGMENTAL GLOMERULOSCLEROSIS

Primary focal segmental glomerulosclerosis (FSGS) is a common glomerular disease in adults and ranks among the top causes of a primary glomerular disease causing end-stage renal disease (ESRD). A number of circulating and urinary markers have been studied as means to improve diagnosis and predict response to therapy (reviewed in [7]). Examples include serum levels of the soluble form of the urokinase-type plasminogen activator receptor (suPAR), urinary CD80 and microRNAs [8–10]. However, much more could be learned from simple clinical and histological data derived from a proper understanding of the pathogenic process in primary FSGS than from sophisticated biomarkers.

First, primary FSGS is a diagnosis of exclusion that is reached after known causes of FSGS, i.e. reduced renal mass, functional adaptation, and infectious (e.g. HIV), drug-induced (e.g. pamidronate, anabolic steroids) and genetic (familial or sporadic) have been ruled out [11]. Secondly, primary FSGS is caused by a putative circulating ‘permeability factor’ which is toxic to the podocyte [12]. The fact that administration of serum from FSGS patients into rats causes proteinuria supports the existence of such a factor [13]. In experimental animal models, chronic administration of the aminonucleoside puromycin, which is toxic to the podocyte, results in the development of proteinuria and FSGS lesions that are similar to the glomerular changes observed in human primary FSGS [14]. The presence of a circulating factor is further supported by cases of recurrent FSGS post-kidney transplantation [15–17], and by remission of proteinuria when a kidney with recurrent FSGS is retransplanted into a normal recipient [18]. In these patients, diffuse foot process effacement followed by massive proteinuria developing within hours to days after the kidney transplant can be observed [19]. With time, the characteristic FSGS lesion develops [14, 20, 21]. Thus, in both animal models and in humans, widespread foot process effacement equally distributed among all glomerular capillary loops seen on EM is the earliest structural change and key initial event in the development of primary FSGS [22]. On the other hand, in models of secondary FSGS, i.e. glomerular hyperfiltration, nephron mass reduction, there is glomerular tuft hypertrophy but podocyte cell numbers do not increase [23]. Rather, podocytes are forced to hyperstretch and stretch to cover a larger surface area. This results in podocyte attenuation, but foot processes are largely preserved [24]. These data show that there are marked differences at the EM level between models of primary versus secondary forms of FSGS. Third, because of the ‘toxic’ nature to the podocyte—reflected by the widespread foot process effacement—there is marked loss of the glomerular permeability barrier. As a result, there is massive proteinuria. As such, adults with primary FSGS commonly present with acute nephrotic syndrome or nephrotic syndrome developing soon after the renal biopsy. In contrast, patients with secondary FSGS are more likely to present with slowly increasing proteinuria that may be in the nephrotic-range, but the patients do not develop nephrotic syndrome (e.g. serum albumin is normal) [25]. It is essential to distinguish nephrotic-range proteinuria from nephrotic syndrome, especially in patients with FSGS or global glomerulosclerosis. This has been recently reviewed and further confirmed by a cohort from the Mayo clinic [26, 27].

However, a number of studies in patients with presumed primary FSGS include patients with sub-nephrotic proteinuria and/or the absence of nephrotic syndrome, provide no EM data, disregard family history of FSGS and ethnicity [28–31]. The failure to appropriately exclude cases of secondary FSGS is of particular relevance in studies evaluating biomarkers in FSGS [9, 32]. The study by Wei et al. that triggered a wave of research in suPAR provided no information on proteinuria, 8 patients had normal serum albumin and albumin levels were not available in 11 of the 23 patients with presumed primary FSGS [9]. No EM data were reported either. A recent study by Zhang et al. proposed evaluating urinary microRNAs as a way of predicting response to corticosteroid therapy in patients with primary FSGS. Urinary miR-196a, miR-30a-5p and miR-490 levels were found to decrease significantly in patients who responded to corticosteroids but not in those who were unresponsive [10]. However, since the decrease in miRNA was commensurate with the decrease in proteinuria, this biomarker has little added value to a simple and cheap measurement of urinary protein excretion. A more useful biomarker would be one that predicts a remission prior to the start of treatment. The validation cohort considered as having active primary FSGS had a mean proteinuria of 5.81 ± 2.98 g/24 h, implying many patients did not have nephrotic syndrome [10]. Similarly, no EM data were reported.

Unless investigators can be precise in the selection of appropriate patients, results of these biomarker studies are likely to be flawed. First, we need to understand the basics: (i) to differentiate nephrotic syndrome from nephrotic-range proteinuria; (ii) to look at a renal biopsy and (iii) to incorporate findings of EM. It is important to consider timing of the renal biopsy in relation to the start of immunosuppressive therapy since EM findings may show improvement of foot process effacement once therapy has been started. These simple things can help to differentiate the large majority of patients with primary from secondary FSGS without the need of biomarkers.

### BIOMARKERS AS PREDICTORS FOR THE DEVELOPMENT OF DIABETIC NEPHROPATHY

Established risk biomarkers for DN include hyperglycemia, hypertension, hyperlipidemia and microalbuminuria. Microalbuminuria has been the traditional biomarker for nephropathy progression but it has a limited predictive value based on its poor correlation with loss of GFR [33, 34]. Some patients have normal serum creatinine without microalbuminuria
Despite advanced DN on renal biopsy [35, 36] while others may develop progressive loss of kidney function before microalbuminuria is diagnosed [37, 38]. As such, additional biomarkers of glomerular damage (e.g. transferrin, nephrin, podocalyxin), oxidative stress, inflammation (e.g. TNF-α), proinflammatory cytokines (e.g. TGF-β), advanced glycation end products, vascular dysfunction (e.g. von Willebrand factor, VCAM-1), tubular biomarkers (e.g. megalin, cubulin, NGAL), activation of the intrarenal renin-angiotensin system, urinary microRNA, urinary proteomics, urinary peptidome, exosomes etc. are evaluated in a quest for the ‘gold’ standard for the diagnosis and to predict progression of DN (recently reviewed by [39, 40]).

However, a shift of efforts to optimizing control of the traditional risk factors and biomarkers for DN (hyperglycemia, hypertension and hyperlipidemia) is bound to yield more tangible results. Poor glycemic control is an established risk for development and DKD and ESRD [41]. Conversely, intensive glycemic control is clearly associated with reduction in the incidence of micro- and macro-albuminuria. It may even slow the decline in GFR and development of ESRD [42, 43]. The benefit can be observed even after proteinuria has developed [45]. In the Action in Diabetes and Vascular Disease: Preterax and Dia-micron Modified Release Controlled Evaluation [ADVANCE] trial, improvement of glycemic control in patients with diabetes mellitus type II (average HbA1c, from 7.3 to 6.5%) resulted in a significant reduction in the risk of ESRD [44]. Similarly, the Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Research Group reported a significantly lower risk for the development of GFR <60 mL/min/1.73 m² in patients treated to achieve tight glycemic control [45]. That normoglycemia can reverse established diabetic nephropathy has been documented by Fioretto et al. showing disappearance of diabetic lesions post-pancreas transplant [46, 47]. Although there has been no defined threshold for HbA1c, the ADVANCE trial showed that there was no significant increase in risks for the development of albuminuria, doubling of serum creatinine level, need for renal replacement therapy, or death due to kidney disease in patients with HbA1c ~6.5% [48].

Blood pressure (BP) is another outstanding (bio)marker— and cheap too. It is well established that lowering BP slows the deterioration in kidney function in diabetes: UKPDS, HOT, IDNT and RENAAAL have all shown a benefit irrespective of the antihypertensive used, even though few of these older studies attained BP targets <130/80 mmHg [49–52]. Current Kidney Disease: Improving Global Outcomes recommendations suggest that 130/80 mmHg should be the treatment goal for all patients with chronic kidney disease [53]. There are limited data to support a lower target of 125/75 mmHg if there is proteinuria [54]. Most of the more recent hypertension trials had excellent control rates—approaching 70% in ALLHAT for example [55]. Even difficult patients in AASK (African-Americans) achieved very good levels—including the ‘intensive’ group aiming for <130 mmHg—overall achieved control rates were ~70% in that study as well. But how well is BP controlled in the general population? Banegas et al. evaluated BP control in over 12 000 hypertensive patients seen in primary care clinics in Spain, using clinic and ambulatory BP monitoring (ABPM) readings. While 24% of the patients had controlled BP based on BP measurements in the clinic, 52% had controlled BP based on ABPM. Using both types of methods, 43.0% of the patients were deemed to have uncontrolled BP [56]. NHANES similarly showed that hypertension was controlled in only 50% of the population [57]. Furthermore, while some surveys indicate that ~10–12% of hypertensives are resistant (meaning uncontrolled with at least three drugs), the incidence of resistant hypertension is much higher in the diabetic population [58–60]. In a cross-sectional analysis of over 68 000 patients, ABPM characteristics were assessed in diabetic (18.5%) versus non-diabetic hypertensive individuals [61]. Compared with non-diabetic hypertensive patients, diabetic hypertensives had higher systolic BP levels in every ABPM period of the day despite the fact that they were being treated with more antihypertensive drugs, confirming previous observations [62]. Furthermore, ABPM shows abnormal circadian variability in BP, linked to kidney disease outcomes, in patients with diabetes [63–65]. A recent study in patients with type 2 diabetes suggested that the ambulatory BP threshold levels that are associated with development of proteinuria were 125/75, 110/65 and 120/75 mmHg for daytime, night-time and 24-h periods, respectively [66].

Nevertheless, BP control can be achieved, as illustrated by the Kaiser Permanente Northern California (KPNC) hypertension program. Using a multifaceted approach to BP control hypertension control within KPNC increased from 44 to 86% in 11 years [67, 68]. The bottom line is that hypertension treatment requires an integrated approach that includes frequent evaluation of medication adherence, and identification of resistant hypertension with the use of ABPM etc. [69].

Finally, hyperlipidemia has been clearly associated with greater risk of proteinuria and decline in GFR, while regression of microalbuminuria has been independently associated with low levels of LDL cholesterol and triglycerides in diabetic patients [70, 71].

Taken together, we have cheap and easily available biomarkers that predict the development of diabetic nephropathy. However, in a large number of patients, we fail to take appropriate measures to shift these biomarkers back into balance and reduce progression to diabetic nephropathy. It seems unlikely that the introduction of sophisticated and expensive biomarkers into clinical management will yield higher success rates. Rather than to pursue these novel biomarkers, we need to invest in patient education, frequent monitoring, achieving targets, even consider financial incentives to the patients—and to their care providers. Investing in prevention will not only save money, but more importantly patient’s lives.
Rituximab (LUNAR) study [76]. In both studies, only ~50% of the patients responded, with the majority having a partial response. Not surprisingly, a number of biomarkers have garnered attention as a means to predict prognosis and response to treatment in LN [77–83]. However, these ‘predictors’ have been unable to differentiate treatment outcomes [84], and are generally positive at the same time as proteinuria, showing that they are confirmatory rather than predictive of damage [80, 85]. Furthermore, none is sensitive or specific for renal involvement in general, or for LN in particular, to be used as a stand-alone clinical test.

For example, urinary monocyte chemoattractant protein-1 (uMCP-1) has been proposed as an earlier predictor/diagnostic of renal flare [86, 87]. Since the level of uMCP-1 correlates with proteinuria, it has no added value to simply checking albuminuria. In addition, the great overlap in uMCP-1 levels between LN in remission and active LN makes the test of little help when evaluating an individual patient. More recently, patients with active LN were found to have significantly elevated serum levels of anti-histone 3 and anti-α-enolase IgG2 [88]. The levels correlated with the degree of proteinuria and renal failure. However, since patients were only studied at the time of biopsy these markers cannot be interpreted as predictors. Only a prospective study in patients with SLE is able to establish whether they can work as predictors.

Part of the problem in interpreting the low complete remission rates in LN may be the fact that current definitions of ‘complete remission’ in LN usually imply the reduction of proteinuria to <0.5 g/24 h after 6–12 months of treatment [53, 89, 90]. Approximately 50% of patients with LN show improvement in renal parameters after 6 months of treatment, but the proportion of responders increases to 65–80% after 12 and 24 months of treatment [74, 91]. This is similar to observations in MN, where the majority of clinical responses are partial remissions. Median proteinuria may decline during up to 24 months or even persist despite the absence of immunological disease (i.e. disappearance of anti-PLA2R antibodies) in the majority of patients [92]. Thus, the decline in proteinuria lags behind that of anti-PLA2R antibodies [4]. Secondary changes from basement membrane and podocyte remodeling, focal sclerosis or interstitial damage from prolonged disease may lead to low-level proteinuria indefinitely, despite the absence of an ongoing immunological response. Thus, similar to observations in MN, there appears to be a lag between immunological changes and renal response in LN. Therefore, a proteinuria endpoint at 12 months may be too soon to evaluate the true effect of treatment in LN, let alone the even shorter 24-week time frame in ALMS [81]. In patients with MN, we have argued that this endpoint should not be earlier than 18 months from the start of treatment [93]. Exploratory data in LUNAR support this argument: the proportion of patients who received RTX and had a >50% reduction in proteinuria increased from 10% at week 52 to 17% at week 78, a difference that reached statistical significance [76]. As such, the response rate is critically determined by where we decide to draw the time line for success.

This disconnect between immunological improvement and proteinuria status is very clinically relevant and suggests that the concept of immunological remission should be added to the concept of clinical remission and that both are used when judging immunosuppressive treatment in MN as well as in LN. In patients who continue to have sub-nephrotic proteinuria despite the absence of immunological/histological disease activity, further immunosuppression is unnecessary and potentially harmful, and management should be conservative. Proteinuria has to be interpreted in the context of other biomarkers such as hemoglobin, serum creatinine, C3/C4 complement and anti-double stranded DNA levels, and hematuria. Indeed, normalization of complement C3 and/or C4 are a strong predictor of renal response [81]. Thus, in the presence of normal complement levels, recovery of anemia, improved/normalized creatinine, disappearance of hematuria and negative anti-double stranded DNA, persistence of proteinuria equals damage. To demonstrate this requires a repeat renal biopsy but the same standard should apply to any potential biomarker. In other words, biomarker investigators need to prove that in a cohort of LN patients with isolated proteinuria, that undergoes repeat biopsy, biomarkers outperform traditional markers of activity.

CONCLUSION

To the question, are novel biomarkers any better than the conventional ones in glomerular disease? The answer is a categorical NO! They have no additional value when treating patients with FSGS, DN or LN. All ‘Novel Biomarkers’ are plagued by lack of sensitivity, specificity, reproducibility, high cost etc., to be useful in clinical practice. Before further pursuing biomarkers, we need to: (i) know how to properly identify disease, e.g. primary FSGS. (ii) Appropriately use the information provided by simple risk predictors in patient management, e.g. hyperglycemia, hypertension, dyslipidemia in DN; show us a significant number of patients with diabetes that have all these three risk factors controlled and still develop DN and we would concede. (iii) Correctly define response to treatment and the appropriate time frame for remission to occur in LN. It is not by providing a group of biomarkers that the treatment of LN will be improved. To the biomarkers community we say, you are putting the cart before the wheel!

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CONFLICT OF INTEREST STATEMENT

The authors have no potential conflict of interest relevant to this article.

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