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Podometrics as a potential clinical tool for glomerular disease management

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Abstract

Chronic Kidney Disease culminating in End Stage Kidney Disease is a major public health problem costing in excess of \$40 billion per year with high morbidity and mortality. Current tools for glomerular disease monitoring lack precision and contribute to poor outcome. The podocyte depletion hypothesis describes the major mechanisms underlying progression of glomerular diseases responsible for >80% of ESKD. The question arises whether this new knowledge can be used to improve outcomes and reduce costs. Podocytes have unique characteristics that make them an attractive monitoring tool. Methodologies for estimating podocyte number, size, density, glomerular volume and other parameters in routine kidney biopsies, and the rate of podocyte detachment from glomeruli into urine (“podometrics”), have now been developed and validated. They potentially fill important gaps in the glomerular disease monitoring toolbox. Application of these tools to glomerular disease groups demonstrates good correlation with outcome, although data validating their use for individual decision-making is not yet available. Given the urgency of the clinical problem we argue that the time has come to focus on testing these tools for application to individualized clinical decision-making towards more effective progression prevention.

Keywords

Progression; Kidney Failure; Prevention; Podocyte; Podometrics

Introduction

In this discussion glomerular diseases are viewed from a podocyte perspective with a view to developing concepts and tools that can be used to improve prevention of progression to End Stage Kidney Disease (ESKD).

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Background

Kidney failure requiring renal replacement therapies already costs >\$40 billion per year (7% of the US Medicare budget) as well as high mortality and morbidity for patients and their families (1,2). Improved prevention is therefore a National and International imperative. A multidimensional strategy including public education, reducing prevalence of hypertension and excessive calorie intake (obesity and type 2 diabetes), and more effective prevention and treatments for kidney diseases (particularly glomerular diseases that account for >80% of ESKD) will be required.

A glaring shortcoming in current clinical practice is the inherent weaknesses of the clinical tools needed to identify risk, predict outcome and prevent progression (Table 1A and B). To make progress we must be able to (i) identify those at risk for progression early in the course of disease so they can be treated before extensive nephron loss has occurred, and (ii) be able to monitor treatment efficacy sensitively and specifically so that treatment failure can be identified early and rectified. Such tools would also improve clinical trial efficacy by reducing the length of time and number of patients required to determine efficacy.

The podocyte as a potential marker

The glomerulus is a complex auto-regulating machine in which intrinsic glomerular cells (endothelial cells, mesangial cells, juxta-glomerular cells, parietal epithelial cells and podocytes) cooperate and signal between each other in the context of specialized matrix structures (glomerular basement membrane [GBM], mesangial matrix and Bowman's capsule) renal innervation and blood perfusion under hydrostatic pressure to support the blood filtration necessary for body homeostasis. Within this context the podocyte is a highly specialized long-lived neuron-like cell with unique characteristics that make it an ideal marker for monitoring progression (Table 2).

The podocyte depletion hypothesis for progression is confirmed by three independent lines of evidence

The podocyte depletion hypothesis (3) is based on pathologic concepts of Kriz and colleagues (4-6) and quantitative modeling (7,8). According to this scenario all progressive glomerular diseases can be collectively viewed as “podocytopathies” in which progression is caused by progressive podocyte depletion (3). The central concept is that although there are many different upstream causes of glomerular injury, progression (glomerulosclerosis, nephron loss and ESKD) is driven in large part through a single common podocyte depletion pathway that could be used for monitoring purposes and as a target for prevention strategies. The direct causative and quantitative relationship between the degree of podocyte depletion and the amount of glomerulosclerosis has now been proven (7-10). At the same time this concept is independently confirmed by genetic studies showing that mutations in podocyte-specific genes cause an FSGS phenotype in man (11), and by numerous model systems where podocyte-specific transgene expression and knockouts also demonstrate FSGS-like phenotypes. Finally, and most importantly, observational clinical studies in type 1 and 2 diabetes, IgA nephropathy, hypertension and transplant glomerulopathy all demonstrate a

quantitative relationship between the amount of podocyte depletion and the degree of glomerulosclerosis (12-19).

A podocyte zero-sum game approach to glomerular disease monitoring

A zero-sum game in economic or game theory is an arrangement where the gains and deficits add up to zero. Podocytes are long-lived cells essential for normal function whose capacity for replacement is limited (20). Podocytes could therefore in principle be considered as a zero-sum game in which the total podocyte complement in an individual person is a fixed number that has to last for a life-time. We know from model systems that when >80% of podocytes are lost from a glomerulus the “game” is over (global glomerulosclerosis supervenes) (8,9). Since the normal human glomerulus has on average about 500 podocytes (12,21) the podocyte reserve beyond which glomerular failure supervenes is about 400 cells. Therefore by measuring the average number of podocytes left in glomeruli and the rate of loss of podocytes by urine assay it should in principle be possible to derive an estimate for the time to ESKD (predicted progression rate) and response to therapy (Figure 1). Accordingly we have devised methodologies for making the necessary “podometric” measurements in human kidney biopsies and urine (Figure 2).

Biopsy podometrics

A novel method for measuring podocyte parameters in a single formalin-fixed paraffin-embedded archival histologic section has been developed and validated in model systems and man (Figure 2A) (19,21). This simplified method, which could be automated, offers new opportunities for understanding and targeting glomerular disease progression as outlined in Table 2.

Urine podometrics

A simple urine mRNA method built on the urine pellet, as used every day by nephrologists for urine analysis in the clinic, has been developed and validated in model systems and man (Figure 2B) (22,23). The approach uses podocin mRNA as an easily measured sensitive and specific podocyte marker expressed in relation to the urine creatinine concentration, analogous to the urine protein:creatinine ratio. All human glomerular diseases exhibit increased rates of podocyte detachment (23). Those who progress to ESKD over a 4 year period of observation have particularly high levels (average 60 to 100-fold). Disease remission is associated with return of levels to the normal range. Non-glomerular diseases do not have elevated levels.

Table 3 outlines how podometrics could potentially be used to fill gaps in the tools available to the clinician and to offer new opportunities for prevention of progression to ESKD.

Additional quantitative considerations

There are on average about 1 million nephrons (glomeruli) in each of 2 kidneys. The actual measured adult glomerular number per kidney has been reported to vary up to 13-fold (210,000 to 2.7×10^6) although variance is much less in most studies (24). This variance is

related to both genetic and environmental factors and to maternal nutrition and prematurity (24). Approaches to estimating the glomerular density in renal cortex in the kidney biopsy have been reported (25).

The concept of a glomerular life cycle

Podometric analysis has been applied to the normal aging glomerulus to better define its structural biology and to develop age-related ranges for clinical application (26). Important new insights are identified as follows. During normal human aging podocytes are lost from glomeruli at a rate of about 1.7 podocytes per glomerulus per year. Therefore by 80 years of age on average about 25% of the normal podocyte complement will have been lost. At the same time the glomerular volume increases rapidly with normal growth during childhood and adolescence, and continues to increase at a slower rate during adult life (Figure 3A). The podocyte complement in each glomerulus must completely cover the filtration surface with foot processes in order to maintain the filtration barrier. Therefore the combination of reduced podocyte number and increased glomerular volume with age will result in reduced podocyte density (number per volume) with age, necessitating a compensatory hypertrophic podocyte response (Figure 3B and C). By older age the podocyte density approximates a value of 100 per 10^6 um^3 at which time small further decreases in density will require exponential compensatory increases in podocyte hypertrophy inevitably resulting at some point in hypertrophic podocyte stress. Hypertrophic podocyte stress is associated with increased leakage of protein through the glomerular filter. Podocytes attempt to adapt to hypertrophic stress by entering the mitotic cell cycle. The transition of the actin cytoskeleton away from being assigned to maintain foot process integrity and GBM attachment into the mitotic spindle triggers mass podocyte detachment events (“catastrophic podocyte mitotic detachment” as described by Lasagni and colleagues) (27). The glomerulus responds to these mass podocyte detachment events by glomerular tuft collapse and focal global glomerulosclerosis. This sequence of events occurs both during normal aging where it contributes to the increased susceptibility of older people to develop ESKD (2), and in progressive glomerular diseases. (26)

Podocyte detachment and detectability in urine

For podocytes to be detectable and measurable in urine by the podometric method the cells must remain essentially intact throughout their passage down the urinary tract. The podometric method uses a low speed urine centrifugation step that will not pellet subcellular particles or other small cellular elements. If cells are substantially damaged, apoptotic or necrotic before they are lost from glomeruli then they will disintegrate in their passage down the urinary tract, lose RNA integrity, and not be measurable by the assay. Thus all podocytes that are detectable in urine by the assay can be assumed to have undergone an active biological programmed detachment process analogous to leaves detaching from a tree in the fall.

Whiteside and colleagues predicted on the basis of mathematical modeling that loss of one podocyte would be accompanied by detachment of neighboring podocytes through a cooperative mechanism (28). This prediction has been confirmed experimentally by

Ishikawa and colleagues (“podocyte damage damages podocytes”) (29), by Quaggin and colleagues (“innocent bystander hypothesis”) (30) and by our studies demonstrating that loss of 30% of podocytes from glomeruli triggers progressive loss of the remaining podocytes over time without further insult in an angiotensin II-dependent manner culminating in global glomerulosclerosis (9). In these experiments and in parallel studies using different progression models (5/6 nephrectomy, puromycin aminonucleoside nephropathy and growth-dependent progression) accelerated podocyte detachment is persistently detectable in urine at high levels throughout the progression process until ESKD is present (9,10).

One mechanism for podocyte detachment (“catastrophic podocyte mitosis”) has now been documented in man in association with both aging and progressive glomerular diseases (26,27). Individual glomeruli undergo “mass podocyte detachment events” triggered by critically reduced podocyte density. In this example it can be seen that many podocytes synchronously detach from a single glomerulus in association with tuft collapse and global sclerosis, and that this process is associated with increased detectability of podocytes in urine (26). This is in contrast to a scenario where single podocytes intermittently detach from many glomeruli within a glomerular population under normal or disease conditions. Depending on the pathologic circumstance both scenarios may occur, but as podocyte density reaches criticality (<100 per 10^6 μm^3), and glomerular protein leak increases, mass podocyte detachment events from individual glomeruli within the heterogeneous glomerular population are likely to be the major source of podocytes detectable in urine as measured by the urine podometric method.

Garovic and colleagues have reported an extensive body of work using urine podocyte cell measurements as a sensitive and specific marker for diagnosis and monitoring of pre-eclampsia and its long term consequences (31,32). Other urine assay systems that measure subcellular podocyte fragments (e.g. podocalyxin) (33-36); or differential expression of podocyte products such as the podocin:nephrin ratio where the specific podocyte marker nephrin (mRNA and protein) expression is preferentially down-regulated in comparison to podocin in association with podocyte stress (36,37); or altered systems biology in podocytes captured from urine, will provide different information reflecting altered glomerular biology. These approaches can also be expected to yield useful and clinically relevant information.

Progressive glomerular diseases

As diagrammatically illustrated in Figure 4 all progressive glomerular diseases can be seen to act through the podocyte depletion progression mechanism either (i) by accelerating the rate of podocyte loss (detachment, death or dysfunction), (ii) by glomerular volume increase requiring each podocyte to undergo further compensatory hypertrophy, or (iii) by a combination of these mechanisms. The realization that progression flows through podocyte number and glomerular volume and the interaction between these two parameters opens up new opportunities for both disease monitoring and therapeutic targeting (Table 3).

The importance of podocyte density (reflecting the interaction between podocyte number and glomerular volume) is illustrated in Figure 5. Once podocyte density decreases below a

value of about 100 per 10^6 um^3 the glomerulus begins to become dysfunctional as reflected by it becoming leaky (increasing proteinuria), increasingly glomerulosclerotic (mesangial expansion, adhesions to Bowman's space and segmental glomerulosclerosis) and, as a late event, decreased function (reduced eGFR). The data shown are for kidney allografts, but similar data are reported for diabetic glomerulosclerosis, IgA nephropathy, hypertension and for model systems (9-19).

The density threshold value below which glomeruli become dysfunctional varies between 125 and 50 per 10^6 um^3 , depending on podocyte hypertrophic capacity and probably also on how much time is available for podocytes to engineer hypertrophic adaptation. In this scenario proteinuria can be seen to be a late marker of podocyte density decrease when podocyte density is already approaching or below 100 per 10^6 um^3 , as opposed to podocyte detachment rate which will be a much earlier marker of progressive podocyte loss detectable early in the progression process.

Primary proteinuric diseases

In some glomerular diseases podocytes become dysfunctional in association with foot process effacement and/or GBM disorganization independent of podocyte density. In the example of Minimal Change Disease proteinuria is a primary result of podocyte dysfunction itself and is not closely related to reduced podocyte density and progression. In this setting reversal of podocyte foot process effacement and dysfunction by glucocorticoids is associated with reduction of the protein leak. In Primary FSGS both podocyte dysfunction and podocyte detachment/depletion are present so that proteinuria is the consequence of at least two different mechanisms. Depending on the degree of foot process effacement and amount of detachment the net level of proteinuria may be wholly, partially, or unresponsive to glucocorticoid treatment. Disorganization of the GBM by immune deposits and other factors also causes a protein leak that will persist in spite of treatment. In these examples glomerulosclerosis and reduced eGFR (progression) are still expected to be directly related to podocyte density, but proteinuria would not be closely related to podocyte density. The rate of podocyte detachment will still provide information about progression. Proteinuria will be a sensitive marker for podocyte dysfunction, but a less reliable marker of podocyte detachment (progression).

Glomerulosclerosis

Glomerulosclerosis occurs when podocytes cannot completely cover the filtration surface either because the podocyte number/size is reduced, or the filtration surface area is increased, or some combination of the above. In both cases the effective podocyte density is reduced ("podocyte depletion") resulting in hypertrophic podocyte stress and eventual pulling apart of podocyte foot processes to leave bare areas of GBM resulting in protein leak into the filtrate that will not be glucocorticoid-responsive (4-6). In younger glomeruli the glomerulus's response to reduced density is (i) to reduce the filtration surface area by expanding the mesangial compartment ("mesangial expansion"), (ii) to seal the leak in peripheral glomerular capillary loops by adhering bare areas to Bowman's capsule via bridging parietal epithelial cells ("adhesions or synechiae"), and (iii) to form matrix plugs to

patch leak sites in order to minimize protein loss (focal and segmental glomerulosclerosis or FSGS). Combinations of these mechanisms in the setting of the biologic variation superimposed by different diseases milieus (e.g. diabetic versus immune-associated growth factors) likely accounts for the typical diagnostic histologic appearances associated with different diseases that form the basis for pathologic classifications. Global glomerulosclerosis occurs either when FSGS becomes increasingly widespread eventually encompassing the whole glomerulus (4-6), or, as outlined above, when mass podocyte detachment events occur cumulating in tuft collapse and superimposed global glomerulosclerosis (26).

Detecting reduced renal function

Although much effort has been expended in attempting to improve sensitivity and specificity of renal function measurements, the fact of the matter is that this approach to monitoring early kidney injury is always going to be obscured by adaptive mechanisms (Table 1a). Waiting to act until renal function is measurably decreased is a recipe for failed prevention because by the time action is taken more than 50% of nephrons have been lost and autonomous progression processes have become set in place and difficult to reverse. By far the best opportunities for prevention occur early in the disease process before renal function is measurably reduced and when interventions with fewer side-effects can be effective at slowing progression and will have much greater impact on time to ESKD.

The example of podometrics applied to transplant glomerulopathy

The half-life of a renal allograft is unexpectedly short at 15 instead of 40 years, with little improvement in spite of better immunosuppression (38). At implantation (2K to 1K transition) the kidney allograft undergoes compensatory hypertrophy by about 25% within a few days (39). Podometric analysis applied to allograft biology (19) shows that glomeruli also enlarge 20% post-implantation so that the filtration surface area increases. Normal podocytes are not able to divide so they adapt by hypertrophy. This causes a 20-25% increase in podocyte volume over the first year, paralleled by a similar reduction in podocyte density. From model systems we would expect that this hypertrophic response would be associated with podocyte stress that would be reflected by an increased rate of podocyte detachment. In fact the measured rate of podocyte detachment in the average allograft is 6-fold above normal in spite of an allograft having half the number of podocytes compared to its 2K control. Recipients with long-standing stable renal function in the normal range have little increase in rate of podocyte detachment compatible with effective adaptation. In contrast recipients who develop transplant glomerulopathy (or recurrent glomerular diseases) have high rates of podocyte detachment and over time develop reduced podocyte density to <100 per 10^6 μm^3 . Figure 5 shows how reduction in podocyte density below 100 per 10^6 μm^3 is associated with development of proteinuria, glomerulosclerosis and reduced eGFR. These data, which are similar to data reported for diabetic glomerulopathy and IgA nephropathy and model systems (12-18), are compatible with the concept that accelerated podocyte detachment due to compensatory hypertrophy and/or immune-driven events can shorten kidney and allograft half-life.

Podometric predictions in relation to epidemiologic data

If the principles outlined above describe a significant component of the progression process, then it should be possible to make predictions that can then be tested by comparison with epidemiologic data.

- i. **Aging is the major driver of progression to ESKD.** Conventional epidemiologic reports have traditionally shown diabetes and hypertension as the two major epidemiologic factors associated with ESKD. Accordingly resources have been assigned to diabetic kidney diseases and hypertension. On the basis of our data and that of others implicating aging in Chronic Kidney Disease (CKD) the United States Renal Data System (USRDS) team (Dr. Rajiv Saran and colleagues) now shows age as an independent variable in the 2014 USRDS data report (2). This demonstrates that older age is by far the most powerful epidemiologic factor associated with contributing more than twice the impact of diabetes or hypertension, an important reality that will need to be recognized in the design and implementation of prevention strategies.
- ii. **Predicted average time to ESKD for glomerular disease groups:** The observed podocyte detachment rate for clinic glomerular disease groups was measured by urine podometric assay and expressed as fold $\times$ normal (23). Since the normal rate of podocyte loss from glomeruli is now known (average 1.7 podocytes per glomerulus per year measured by podometric biopsy assay) (26) this allows an estimation of average rate of loss of podocytes from glomeruli and thus predicted time to ESKD assuming that reduction of podocyte number by 80% is equivalent to ESKD. In a 4 year study a group of progressors who reached ESKD or doubled their serum creatinine within the study period had a high rate of podocyte detachment at 79-fold above normal. These glomeruli can be estimated to have lost approximately $79 \times 1.7 = 153$ podocytes per glomerulus per year. If the total podocyte reserve is 400 podocytes per glomerulus (80% of 500) then average time to ESKD would be predicted to be 2.9 years which is close to the observed value. Similarly, the diabetic kidney disease group had an average 10-fold above normal rate of podocyte detachment that would translate into a predicted 24 years to reach ESKD. Again this is close to the observed value for diabetic progression to ESKD. The kidney allograft group had an average 6-fold increase in the rate of podocyte detachment but only a single kidney with half as many podocytes as normal. Taking into account the reduction in podocyte density occurring post-implantation the projected time to ESKD for allografts would therefore be approximately 15 years, or close to the observed value (38). Therefore by combining data from urine and biopsy podometrics approaches time to ESKD can be predicted within the bounds of the expected ranges.

In summary these data are compatible with, but do not prove, that the zero-sum game approach can work in principle, at least in research group settings.

Novel therapeutic and management opportunities provided by podometrics

The realization that glomerular well-being depends on podocyte density that in turn depends on glomerular volume in relation to podocyte number, size and function opens up new targets for therapy and management as outlined in Table 3.

Summary

The principles underpinning the podocyte depletion hypothesis for progression have now been proven in models and man. Podometric methodologies have been developed for biopsy and urine that provide a quantitative framework that applied to group data strongly supports the podocyte depletion hypothesis. It is now clear that podometrics could fill important existing gaps in the clinical toolbox that will be essential for effective prevention of progression. Perhaps used in combination with systems biologic approaches, podometrics can substantially change the progression paradigm. In view of the urgency and magnitude of the kidney progression problem we argue that a focused effort on optimization and further development of podometric tools should be facilitated in a prioritized and coordinated manner.

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Figure 1. Panel A. The podocyte depletion paradigm

a. Each glomerulus has a filtration surface that must be completely covered by podocyte foot processes to maintain the filtration barrier. **b.** Podocytes can adapt to glomerular enlargement or podocyte loss by hypertrophy. **c.** If hypertrophic stress exceeds the podocytes' adaptive capacity they detach into the filtrate where they can be measured in urine. **d.** Loss of a critical number of podocytes results in bare areas of GBM that leak protein and become patched by scar tissue (focal sclerosis). **e.** When >80% of podocytes are lost global sclerosis supervenes.

Panel B. Podocyte dynamics as a zero-sum game. Biopsy podometrics can be used to estimate glomerular volume (reflecting the filtration surface area), the number of podocytes remaining per glomerulus (podocyte number) and the average podocyte size (podocyte size). Proteinuria is in part a measure podocyte function and detachment (see text for qualifications). Podocyte replacement capacity (either from *de novo* podocytes or from

podocytes stored on the inner surface of Bowman's Capsule) is very limited in the adult and is probably a minor contributor even in young glomeruli. Urine podometrics noninvasively estimates the rate of podocyte detachment assumed to be directly proportional to the rate of podocyte loss from glomeruli over time. Since podocyte replacement capacity can be assumed to be zero, the net glomerular podocyte complement must be balanced by the rate of podocyte detachment over time. In other words with various assumptions as outlined above podocyte dynamics can be considered as a zero-sum game where the key variables can be directly measured.

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A Biopsy podometrics

Single regular formalin-fixed archival biopsy section

Measure

- Podocyte nuclear mean caliper diameter
- Podocyte nuclear number per cross-section
- Glomerular tuft area
- Glepp1 % area
- Section thickness

Derive estimates for

- Podocyte nuclear volume
- Podocyte nuclear density
- Glomerular tuft volume per podocyte
- Glomerular volume
- Podocyte number per glomerular tuft
- Total podocyte mass per tuft
- Mean podocyte cell volume

B Urine podometrics

Figure 2. Panel A. Biopsy podometrics

The parameters needed to estimate the key podometric parameters can be obtained from a single formalin-fixed paraffin-embedded histologic section that contains at least 8 glomerular profiles as routinely used in renal pathology. Podocyte nuclear number per tuft and size is measured using antibodies to the podocyte transcription factors TLE4 and/or WT1 to identify podocyte nuclei (upper image). Calibrated imaging and Image-Pro software are used to measure podocyte nuclear mean caliper diameter and number. The cover slip is then removed and the same section restained using Glepp1 immunoperoxidase (lower image). Glomeruli are again imaged in the same order as previously. The glomerular tuft area and % of the area occupied by Glepp1 (podocyte) cytoplasm) is measured. Using a downloadable spreadsheet with built-in equations average values can then be derived for the variables shown.

Panel B. Urine podometrics: The centrifuged urine pellet from 50ml of urine is washed in cold phosphate-buffered saline to remove contaminating protein. It is stored in RNA preservative at -80°C for subsequent assay. Following RNA purification, reverse transcription and TaqMan assay for podocin mRNA in relation to cDNA standards the data is expressed per g of urine creatinine measured in the same sample (analogous to the urine protein:creatinine ratio). Data is interpreted as a “rate of podocyte loss” relative to the normal control median value which is stable across ages 4-60 years and between sex and race. Podocyte detachment rate is a relative term that is not necessarily related to cell number since larger cells will have more mRNA. However, since podocyte size can vary substantially measuring podocyte mRNA amount may better reflect the area of GBM served by the detached podocyte.

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Figure 3. Podocyte density decreases with age creating podocyte hypertrophic stress

Panel A. Glomerular volume increases with age. Points derived from both normal kidneys (obtained at kidney transplantation from living and deceased donors and normal pole of nephrectomy samples for kidney cancer) and kidney biopsies performed for glomerular diseases particularly at younger ages (FSGS, Minimal change disease and Alport Syndrome). 95% confidence limits are shown.

Panel B. Podocyte nuclear density (nuclear number per tuft volume) decreases with age reaching a value of 100 per $10^6 \mu\text{m}^3$ by older age.

Panel C. Podocyte volume has to increase in inverse proportion to reduced density in order to completely cover the filtration surface area. As density approaches 100 per $10^6 \mu\text{m}^3$ at older age any further decrease in density requires exponentially increased podocyte volume adaptation inevitably leading to hypertrophic stress. Every glomerulus has to obey these

rules. Falling off this curve results in development of a protein leak and glomerulosclerosis. 95% confidence limits are shown. Data in Panels B and C modified from reference 26.

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Figure 4. Diagrammatic representation of factors driving progression emphasizing the key role of aging

In this scenario age (representing the effect of time in relation to genetic, epigenetic and environmental interactions) is the dominant underlying major pathway. All progressive glomerular diseases impact progression through effects on podocyte number or glomerular volume or both. The net effect is to drive progressive reduction in podocyte density which triggers further podocyte detachment, tuft collapse and glomerulosclerosis. These processes including downstream proteinuria drive tubule-interstitial inflammation and scarring. Parallel age-associated NFκB-driven processes (arteriosclerosis and glomerular aging) further amplify these processes (40,41). According to this scenario the central elements common to all glomerular disease progression (glomerular volume and podocyte number) will be determinative of outcome and represent major unrecognized opportunities for therapeutic intervention.

A. Proteinuria vs podocyte density

B. Glomerulosclerosis vs podocyte density

C. eGFR vs podocyte density

Figure 5. Podocyte density in relation to clinical parameters

Proteinuria (Panel A), glomerulosclerosis (Panel B) and reduced renal function (Panel C) all become increasingly prevalent at podocyte densities below 100 per 10^6 um^3 . Data are modified from reference 19.

Table 1A
Clinical tools and their limitations

1	Renal function tests. Estimated glomerular filtration rate (eGFR) is a measure of the efficiency of clearance of marker substances from blood by the kidney. Since the kidney will always compensate for lost filtration units (nephrons) to the extent possible this functional assessment is not designed to achieve, and does not reflect, an accurate measure of the number of remaining filtering units. Instead eGFR is a measure of the extent to which remaining filtering units have been able to compensate for lost units. This is useful information, but it is not the information needed for optimal prevention purposes. This is because the normal kidney can readily compensate for loss of >50% of nephrons whose loss is thereby in large part hidden from an eGFR functional assessment.
2	Proteinuria. Kidney dysfunction at many levels results in increased amount of albumin or protein appearing in urine. These include (i) high levels of small proteins in blood, (ii) hemodynamic factors, (iii) glomerular endothelial injury, (iv) glomerular basement membrane disorganization, (v) podocyte dysfunction and/or detachment, (vi) defective tubular reuptake of filtered proteins, and (vi) combinations of the above. Normal urine contains at baseline quite large amounts of proteins, some of which are made in the kidney itself. Therefore proteinuria (or albuminuria) is a moderately sensitive but non-specific marker of diverse kidney dysfunctions and injury.
3	Renal biopsy. A kidney biopsy represents about 0.002% of kidney mass. Renal pathologic reports are usually non-quantitative or semi-quantitative descriptors defining characteristic patterns of injury that reflect the underlying disease process together with degree of scarring reflecting the net impact of injury that has occurred over the life time of that kidney. Major changes in podocyte density can occur with no major alteration in histologic appearance. Pathologic reports are often descriptive of many kidney compartments providing non-quantitative information whose significance and actionability is uncertain.

Table 1B: Consequences of tool limitations

1	Uncertainty of whether disease will be progressive or not in the face of significant drug side-effects results in delayed treatment decision-making until progression is unequivocally established which in turn means that treatments are started late in the course of disease when they cannot be optimally effective.
2	Determination of whether or not a treatment is effective in an individual is uncertain so that decision-making on modifying treatment is delayed until measurable changes in function have occurred often resulting in more lost function and unnecessary exposure to more drug side-effects.
3	Tool limitations mean that management strategies are dictated by short-term decision-making (e.g. whether eGFR changed measurably in the last 3 months) giving rise to unrecognized and untreated longer term progression.
4	Clinical trials require large numbers of patients and long follow-up (>5 years) to see measurable effects. This translates to prohibitively high cost resulting in fewer trials and therefore limited information on treatment efficacy.
5	Heterogeneity of glomerular diseases lumped together under oversimplified non-quantitative descriptive pathological classifications means that treatment efficacy for some individuals within the context of a large group is masked in those trials that are performed.

Table 2
Characteristics that make the podocyte suitable to serve as a marker

1	The podocyte is arguably the determinant of outcome for all glomerular diseases. Therefore measuring podocytes (or podocyte products) will reflect the most critical determinant of outcome rather than a less relevant downstream epiphenomenon such as function or proteinuria.
2	The podocyte is a highly specialized cell with unique proteins and mRNAs that can serve as sensitive and specific cell markers.
3	The podocyte is resident on the urinary space side of the GBM so that when the cell or its products detach they can be measured in urine.
4	The podocyte is a long-lived cell with very low turnover so that the podocyte complement can be treated as a “zero sum game” in which a known number of podocytes per glomerulus at a point in time can be directly related to the rate of podocyte loss to predict outcome.

Table 3
Potential opportunities provided by podometrics

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i.	Initial evaluation using podometrics requires both a recent biopsy (to determine podocyte number, density and glomerular volume) as well as the current rate of podocyte loss. These two measures together will define progression risk and time to ESKD. For subsequent decision-making to determine treatment efficacy the urine podocyte loss rate alone would usually be adequate.
ii.	Glomerular volume (GV): GV enlargement is a major driver of progression. GV is impacted by nutrition and growth factors. If reduced podocyte density is due to increased glomerular volume, then reducing the growth factor/nutrient milieu by diet, medications, bariatric surgery and related strategies (targeting BMI) would be projected to effectively prevent progression. Exposing patients to steroids would be expected to be counterproductive. Systematic implementation of this strategy is projected to be a major largely unrecognized opportunity to reduce progression prevalence.
iii.	Reduced podocyte number: If podocyte number per tuft is decreased and urine assay shows that the rate of podocyte detachment is increased then that patient will be projected to continue to progress unless effective treatment to reduce further podocyte detachment is implemented. On the other hand if the urine assay shows that the rate of podocyte detachment has returned to baseline then management can focus on determining what therapeutic strategies are needed to maintain a normal rate of podocyte detachment.
iv.	Podocytes too small: New strategies for facilitating podocyte adaption to hypertrophic stress will need to be developed and tested.
v.	Podocytes are dysfunctional: The most common dysfunctional phenotype is effaced foot processes that predominate in Minimal Change Disease but are also present to lesser extents in other glomerular diseases where foot process effacement may account for some proportion of the total proteinuria. Steroid therapy can be expected to return effaced foot processes to the mature phenotype. Podocyte dysfunctional phenotypes may also occur in genetic glomerular diseases, but in each case podocyte number, size and density should be evaluated to determine the most effective therapeutic approach.
vi.	Projected time to ESKD: As described above rate of podocyte detachment data (expressed as fold $\times$ normal) combined with estimation of the remaining podocyte number per glomerulus can potentially be used to project the time to ESKD (80% podocyte loss) for an individual person (if treatment was not modified). Further studies are required to validate this concept for individual people.
vii.	Progression risk: If the rate of podocyte detachment is increased above the normal median then the risk for progression is expected to be directly related to the degree of increased rate of podocyte detachment. On the other hand if the rate of podocyte detachment is not increased then that person will not be at risk for progression, even if they have abnormal eGFR.