CrossMark

REVIEW

Quantifying podocyte depletion: theoretical and practical considerations

Victor G. Puelles[1,2] · John F. Bertram[2] · Marcus J. Moeller[1]

Abstract Podocyte depletion is a central event in the pathogenesis of many glomerular diseases. For this reason, methods to quantify podocyte depletion have become increasingly important. Here, we review currently available methods for quantifying podocyte depletion, including the analysis of glomerular cross-sections, the most important and common stereological methods and newer techniques such as whole glomerular analysis in optically cleared samples. Each method has advantages and limitations. We therefore discuss theoretical and practical considerations to assist the selection of the most appropriate method for an individual study.

Keywords Podocyte · Morphometry · Glomerulus · Microscopy · Kidney disease

Introduction

Podocytes are glomerular epithelial cells that play critically important roles in glomerular filtration (Jefferson et al. 2011). A landmark study by Wharram et al. (2005) showed that podocyte depletion is sufficient for the development of glomerular scarring (glomerulosclerosis); both podocyte depletion and glomerular scarring are found in a wide range of glomerular diseases (Wiggins 2007). If a sufficient number of glomeruli become non-functional because of sclerosis, chronic kidney disease and end-stage kidney disease can occur. As a consequence, the podocyte depletion hypothesis has been proposed as a unifying principle of glomerular pathology and is currently the focus of much research.

An important aspect to note is that podocytes are post-mitotic cells that are unable to re-enter the cell cycle and successfully complete cytokinesis (Lasagni et al. 2013). For decades, lost podocytes were believed to be irreplaceable and it was universally accepted that, at the completion of nephrogenesis, kidneys contained their final complement of both nephrons (nephron endowment) and podocytes (podocyte endowment; Puelles et al. 2011). During postnatal life, physiological and pathological stressors have been shown to lead to podocyte depletion in rodents and humans (Puelles and Bertram 2015). These findings have been interpreted as further evidence that podocytes cannot be replaced, although recent studies suggest that podocyte gain may occur in early postnatal life (Berger et al. 2014) and in settings of podocyte depletion (Angelotti et al. 2012; Peired et al. 2013; Pichaiwong et al. 2013; Pippin et al. 2013; Zhang et al. 2013; Wanner et al. 2014; Eng et al. 2015; Lasagni et al. 2015; Roeder et al. 2015).

Given the central role of podocytes in glomerular disease, they are considered potential targets for the development of future therapeutic strategies (Allison 2015), with a focus on reducing or preventing podocyte depletion and/or enhancing podocyte repair/gain. The ability to quantify podocyte depletion reliably is a central factor in the development of therapeutic strategies. To date, no consensus exists concerning the best approach for quantifying podocyte depletion, although all methods involve the counting of podocyte nuclei. Two recent reviews discussed the advantages and disadvantages of most available methodologies (Lemley et al. 2013; Puelles and

✉ Victor G. Puelles
vpuelles@ukaachen.de

[1] Department of Nephrology and Clinical Immunology, University Hospital RWTH Aachen, Pauwelsstrasse 30, 52074 Aachen, Germany

[2] Cardiovascular Program, Monash Biomedicine Discovery Institute and Department of Anatomy and Developmental Biology, School of Biomedical Sciences, Monash University, Melbourne, Australia

Bertram 2015). In this review, we analyse existing methods, highlight new developments and consider practical issues that may influence the choice of method for podocyte quantification.

Definition of podocyte depletion

The current literature describes two forms of podocyte depletion (Puelles and Bertram 2015): (1) absolute podocyte depletion, which involves the loss of podocytes; (2) relative podocyte depletion, which occurs, for example, when glomeruli hypertrophy and a decrease in podocyte density (number per volume) occurs. The initially proposed podocyte depletion hypothesis also considered podocyte phenotypic switch or foot process effacement, which represents a transient loss of functional podocytes, to be a form of podocyte depletion (Wiggins 2007). However, in order to simplify the present analysis, we only focus on absolute and relative podocyte depletion.

A few years ago, Wharram et al. (2005) and Fukuda et al. (2012) showed that both absolute and relative podocyte depletion are sufficient for the development of glomerulosclerosis. In both studies, a threshold of podocyte depletion was found to be required before the development of pathological changes. For example, in the case of absolute podocyte depletion, loss of less than 20 % of podocytes led to mesangial expansion but no changes in renal function, whereas loss of more than 40 % of podocytes led to overt focal segmental glomerulosclerosis and reduced renal function (Wharram et al. 2005). This finding clearly demonstrated the value of being able to quantify podocyte depletion accurately and precisely quantify in order to obtain important insights into glomerular pathophysiology.

Recent studies have also suggested that podocyte size, for example, average podocyte volume, or podocyte nuclear and cytoplasmic volumes reflect settings of absolute and relative podocyte depletion (Venkatareddy et al. 2014; Puelles et al. 2016b). When podocytes are facing stress, for example, following podocyte loss or increases in glomerular volume, increases in podocyte size may reflect an attempt to maintain an intact glomerular filtration barrier and thereby glomerular health (Kriz 2012). For this reason, podocyte volume may serve as an indirect measure of podocyte depletion in at least some circumstances.

Methods to quantify podocyte depletion

Podocyte number per glomerular cross-section

This two-dimensional approach remains the most commonly used method for quantifying podocyte depletion, because it is easy and fast. The method simply requires the counting of the number of podocyte nuclei in a histological section taken through a glomerulus. Unfortunately, it has many major limitations.

First, a histological section through a glomerulus (glomerular cross-section) represents only a small fraction of a whole glomerulus. For example, for a mouse glomerulus (approximately 80 μm in diameter), a 4-μm-thick paraffin section represents ~5 % of the glomerular thickness. Thus, this section may not provide information on the total number of podocytes in the glomerulus. The second limitation is that the likelihood of podocyte nuclei appearing in histological sections may not necessarily be identical to the frequency of the same podocyte nuclei being present in three-dimensional glomerular tissue, because podocyte nuclei may have various sizes and shapes. A third limitation is that larger glomeruli are preferentially sampled compared with smaller glomeruli in single histological sections (Bertram 1995). A fourth limitation is that section thickness influences the number of podocyte nuclei present, with thicker sections having more podocyte nuclei than thinner sections.

The issue of variability in glomerular size and the effects on glomerular sampling in single histological sections deserves further consideration, because heterogeneity in glomerular size is present in normal kidneys and non-uniform hypertrophy of glomeruli is a feature of both normal kidney growth and many kidney diseases (Puelles et al. 2011). To illustrate this problem of glomerular sampling, let us imagine that we have a box containing 10 watermelons and 10 apples. Given that watermelons are much larger and have a different shape from apples, the chance of sampling a watermelon with a single slice (section) through the box is significantly higher than sampling an apple, even though 10 of each are present in the box (Fig. 1a; Bertram 2001). Because podocyte nuclei are large in relation to section thickness, the number of podocyte nuclei present in a glomerular cross-section depends not just on the number of podocyte nuclei in the glomerulus but also on nuclear size, nuclear shape and section thickness (Lemley et al. 2013). Importantly, if glomerular size (reference space) increases but podocyte number remains constant, then the number of sampled nuclei is reduced (Fig. 1b).

Podocytes are not all the same size and shape, especially in the context of glomerular stress (Venkatareddy et al. 2014; Hodgin et al. 2015). Furthermore, glomerular size changes in various pathophysiological settings (Puelles et al. 2011, 2012). These caveats are illustrated by a recent study (Puelles et al. 2016b) comparing three- and two-dimensional data obtained from whole glomeruli of Pod[Cre]iDTR mice (Guo et al. 2012; Wanner et al. 2014). The mice were injected with various doses of diphtheria toxin, which led to different levels of podocyte depletion and showed that two-dimensional quantification of podocyte number did not identify the subtle differences that were detected by three-dimensional methods (Puelles et al. 2016b; Fig. 2).
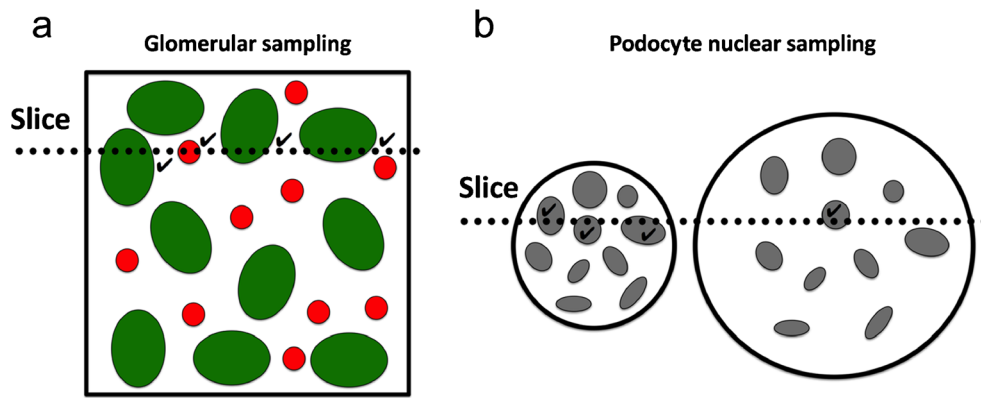
Fig. 1 Problems with two-dimensional sampling. **a** Glomerular sampling: apples (*red*) and watermelons (*green*) in a box. **b** Podocyte nuclear (*grey*) sampling in two reference spaces of differing size; the *dotted lines* and *ticks* represent two-dimensional sampling. In both cases, while the number of apples and watermelons are the same (**a**), and the number of podocyte nuclei is the same in both reference spaces (**b**), two-dimensional sampling favours larger structures (3:1 ratio, when it should be 1:1) and the larger reference space reduces the likelihood of sampling the same number of nuclei (3:1 ratio, when it should be 3:3)

Thus, the reporting of the numbers of podocyte nuclei per glomerular cross-section does not provide an accurate estimation of the total number of podocytes per glomerulus. In this context, values will be influenced by changes in podocyte nuclear dimensions; subtle and important changes in podocyte numbers may therefore be missed. Furthermore, glomerular hypertrophy will result in the preferential sampling of large glomeruli and the undersampling of smaller glomeruli. For these reasons, this approach should be avoided. Fortunately, more reliable methods are now available.

Stereological methods

The word stereology comes from the Greek roots "stereo" and "logos", which together mean "science of studying solids". Originally, stereology provided practical approaches for the three-dimensional quantification of structures based on two-dimensional measurements. However, in the 1980s and 1990s, stereological methods utilizing thick sections and/or tissue slices were developed and the traditional reliance on two-dimensional measurements was therefore reduced (Gundersen et al. 1988). Stereological methods can be divided

Fig. 2 Glomerular cross-sections versus whole glomerular analysis. **a** Control. Whole mouse glomerulus labelled with p57 (*red* podocyte nuclei) and synaptopodin (*green* podocyte cytoplasm) in which all podocytes (77) were counted by using exhaustive enumeration. **b, b'** Two confocal optical sections through the same glomerulus showing variability in numbers of podocyte nuclei (*red*). **c-e** Quantitative differences between two- and three-dimensional analyses of podocyte depletion. With permission from the Journal of the American Society of Nephrology
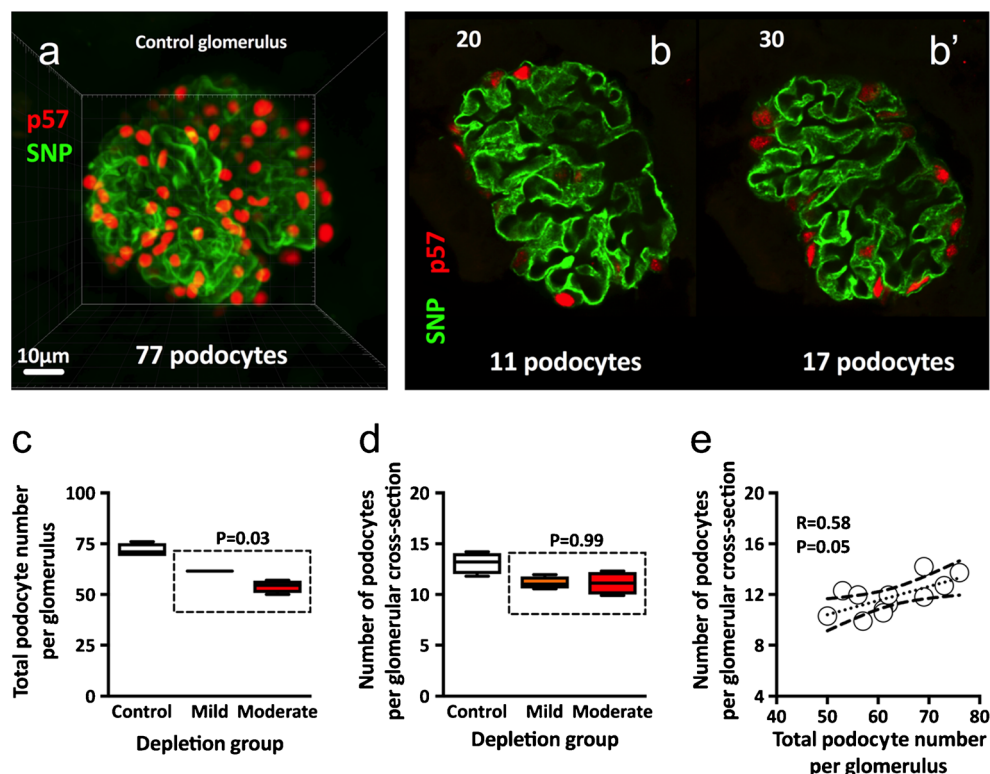
into two broad categories: model-based and design-based. Model-based stereology, as the name implies, requires either knowledge of the geometry of the object of interest or assumptions regarding the geometry. For example, when counting and sizing podocytes with some model-based stereological methods, glomerular size and shape must be known. If these are not known, then assumptions are required, for example, that all glomeruli are spherical and have the same diameter. To the extent that these assumptions are incorrect, the estimates obtained will be biased (Lemley et al. 2013; Puelles and Bertram 2015). In contrast, design-based stereology is based on the application of probes and sampling schemes that are "designed" or "defined a priori", so that the methods are independent of spatial orientation, distribution, size or shape. If applied correctly, these approaches are considered "unbiased", given that their conception minimizes/removes the introduction of assumptions and thus of systematic bias.

Model-based stereology

The most commonly used model-based methods for the determination of podocyte numbers are the methods of Weibel and Gomez (1962) and a modification of the method of Abercrombie (1946) recently described by Venkatareddy et al. (2014). These methods are particularly useful when limited amounts of tissue are available (e.g., renal biopsies). In addition to several measurements on sections, the Weibel and Gomez method (1962) requires knowledge of the podocyte nuclear size distribution and nuclear shape. These values are difficult to obtain and are therefore typically assumed, potentially introducing bias.

The method described by Venkatareddy et al. (2014) relies on the direct measurement of the mean calliper diameter of podocyte nuclei, which is used to determine a correction factor for section thickness. This represents an important development because, previously, the mean calliper diameter was usually assumed rather than measured, providing a likely source of bias. Importantly, the method of Venkatareddy et al. (2014) also allows an estimation of podocyte size, including nuclear and cytoplasmic volumes. This method represents a significant advance in the field of podometrics, because it requires no geometric assumptions for podocyte nuclei and only requires a single section, not multiple sections or serial sections, as is the case with some other methods (see below). However, despite its many advantages, this method only provides an estimate of the average number of podocytes per glomerulus in a kidney. This is an important limitation given that podocyte depletion is focal in multiple disease settings; moreover, the use of a mean value per kidney does not provide any indication of the variation in podocyte numbers between glomeruli.

Reports of the reliability of model-based methods to estimate podocyte numbers acurately differ between studies. Steffes et al. (2001) and White and Bilous (2004) showed that podocyte numbers obtained by using model-based and design-based stereology in human biopsies are similar, especially when electron microscopy is used to identify and count podocytes. In whole mouse glomeruli, Basgen et al. (2006) found that the Weibel and Gomez method overestimates glomerular cell number and densities by 10 % compared with exhaustive enumeration (see below) and design-based methods. In contrast, Nichols et al. (2011) reported that model-based stereology overestimates podocyte number by 57 % and glomerular volume by 17 % in mice. Similar to the method of Venkatareddy et al. (2014), the method of Weibel and Gomez (1962) is easy to establish and reproduce but can provide data on individual glomeruli, thereby allowing the assessment of the variability between glomeruli within a specimen for the detection of focal injury. This is a potential advantage that should be taken into account.

Although model-based stereological methods have limitations, they can provide reliable and reproducible quantitative data and are especially useful when dealing with limited amounts of tissue. The direct measurement of the mean nuclear diameter of podocytes in the method of Venkatareddy et al. (2014) represents an important development. Indeed, recent reports from the Wiggins laboratory on podocyte depletion in human disease and ageing clearly demonstrate the immediate impact that this method is having on the field (Hodgin et al. 2015; Wickman et al. 2016).

We recommend that an effort to minimize the introduction of systematic bias should always be made when using model-based stereological methods, e.g., Venkatareddy et al. (2014) measured mean calliper diameter rather than relying on assumptions. Limitations and potential sources of bias should be identified, acknowledged and discussed.

Design-based stereology

Unlike model-based stereological methods, design-based stereological methods do not require knowledge or assumptions of the geometry of the objects of interest (Bertram 1995; Nyengaard 1999). However, these methods are often time-consuming, complex and difficult to implement.

We will use the disector/fractionator method as an example. Disector refers to the analysis of "two sections". A disector can be "physical" when two histological sections are compared or "optical" when two optical sections (possibly confocal sections), typically from one thick section, are compared (Puelles and Bertram 2015). The fractionator principle relies on the systematic sampling of events (e.g., podocyte nuclei) based on a known fraction of the tissue (Nyengaard 1999). A disector/fractionator method for estimating podocyte number has recently been described for the analysis of human autopsy tissue (Puelles et al. 2014) and combines serial sectioning of paraffin-embedded tissue, immunofluorescence labelling for podocyte identification,

confocal microscopy, optical disectors for podocyte counting (Fig. 3; Sterio 1984) and the Cavalieri estimator for estimating glomerular volume (Gundersen 1986). This approach provides estimates of the total numbers of podocytes per glomerulus, individual glomerular volumes and podocyte density per glomerulus, without any geometric assumptions. This method has been used to identify higher podocyte numbers in adults than children (Puelles et al. 2015) and mild podocyte depletion in association with older age and hypertension (Puelles et al. 2016a).

Design-based stereology is considered to be the gold standard for estimating total nephron number in the kidney (Puelles et al. 2011; Puelles and Bertram 2015). Similarly, the disector/fractionator method has been identified as the preferred method for the evaluation of podocyte depletion when sufficient tissue is available for analysis (Lemley et al. 2013; Puelles and Bertram 2015). Unfortunately, the use of this approach has many disadvantages, including the need for serial sectioning of thick paraffin sections (approximately 14-μm-thick sections), extensive use of confocal microscopy
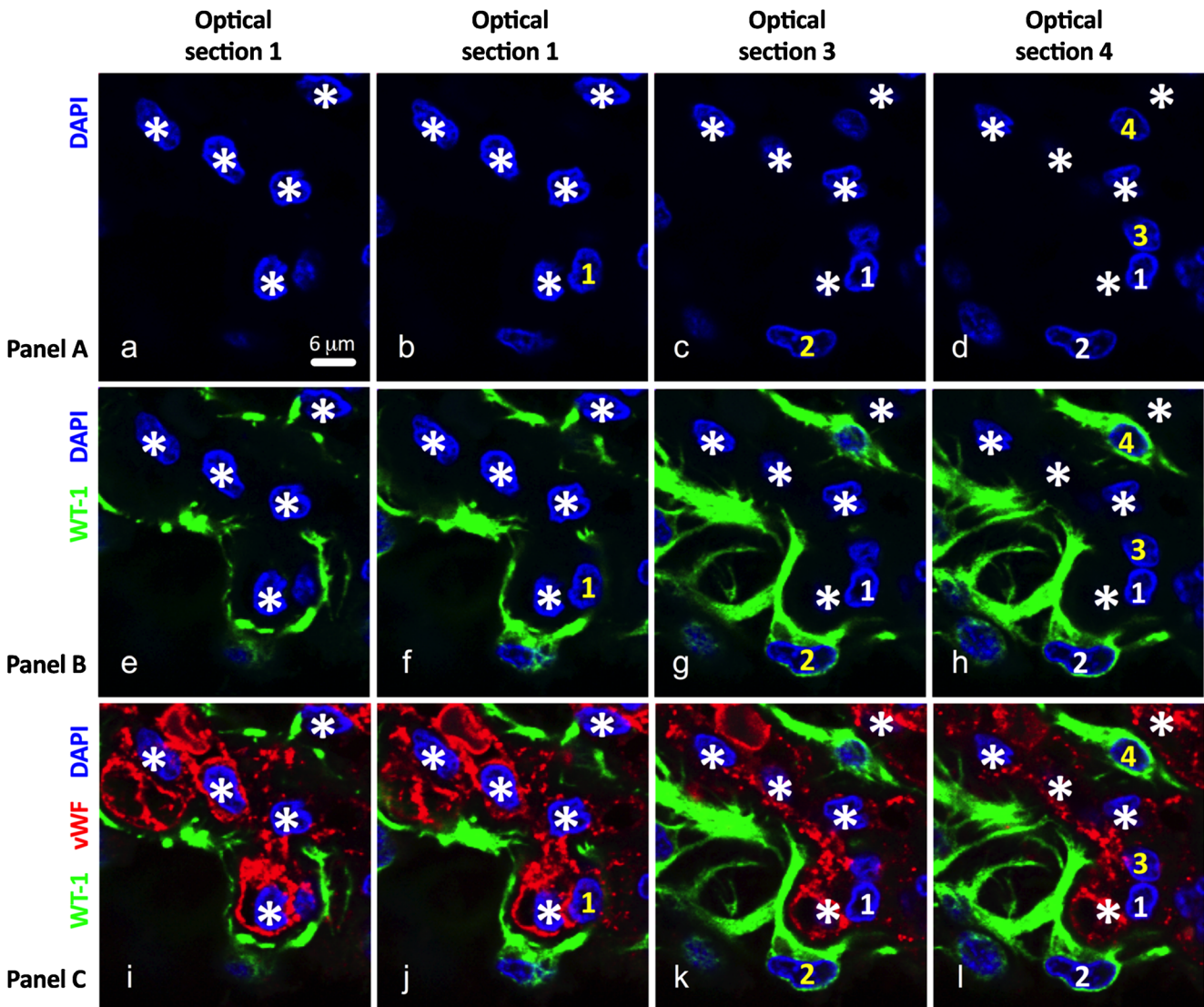

Fig. 3 Use of the optical disector method to identify and estimate podocyte number. When cell nuclei are counted with optical disectors, they are counted at a unique moment (i.e., when they first "come into focus"). We start counting on *Panel A* (**a–d**). The nuclear stain 4′,6-diamidino-2-phenylindole (*DAPI*) was used to label all nuclei *blue*. *Optical section 1* is used first to identify those cell nuclei that are in focus (*stars*). These nuclei are excluded from further analysis. Consecutive sections 1 μm apart (*Optical sections 2, 3* and *4*) are then used to identify nuclei that "come into focus": these are the nuclei that are counted. In this case, we identified four nuclei that "come into focus" in optical sections 2–4; the *numbers in yellow* indicate the moment when the nucleus comes into focus, i.e., the moment when it is counted. The second step is to "turn on" the fluorescent channels to define whether these "new nuclei" are podocytes defined as Wilms' Tumour 1 (*WT-1*)-positive cells and appearing in *green* in *Panel B* (**e-h**) or as other glomerular cells, e.g., endothelial cells that express von Willebrand Factor (*vWF*) and appear in *red* in *Panel C* (**i-l**). In this sequence, we identified only two podocytes (cells with nuclei *2* and *4*). All images are shown at the same magnification with **a** as the reference. With permission from Annals of Anatomy

and significant time for counting performed by a highly trained observer.

Whole glomerular analysis

So far, we have considered methods that provide "estimates" of podocyte depletion by using single or multiple sections through glomeruli. An alternative approach is exhaustive enumeration (Basgen et al. 2006; Lemley et al. 2013) in which every podocyte nucleus in whole glomeruli is counted. Traditionally, this method has involved the serial sectioning (at a thickness of 1–2 μm) of glomeruli embedded in Epon-araldite. Podocytes are identified based on their morphology and location outside capillaries, with each podocyte being seen in multiple sections further assisting their identification (Basgen et al. 2006; Nicholas et al. 2011). This method requires a high level of technical expertise and resources.

Significant progress has been achieved recently with development of a new podocyte counting method that employs a combination of optical clearing, immunolabelling and confocal microscopy to determine podocyte loss in whole glomeruli (Puelles et al. 2016b). Unlike exhaustive enumeration, which relies on physical sections, optical clearing enables the use of serial optical sections obtained by confocal microscopy to identify and count every podocyte nucleus in whole glomeruli. In addition to podocyte counts, this method provides estimates of average podocyte volume per glomerulus, podocyte nuclear and cytoplasmic volumes and an analysis of spatial relationships between podocyte nuclei within each glomerulus. Major advantages of this method include data accuracy and precision and the high speed of analysis. However, this method requires the significant employment of a confocal or two-photon microscope and the use of optical clearing in combination with immunofluorescence of thick slides of tissue (approximately 800 μm). A recent review is available on the use of optical clearing in kidney morphometry (Puelles et al. 2017).

Practical suggestions

Major points for deciding which of the above-mentioned methods to use are presented below. Problems to consider include the amount of kidney tissue available and access to imaging hardware and software. However, before we consider these practical issues, we need to contemplate the most important point when counting podocytes, namely their unambiguous identification.

Unambiguous identification of podocytes

Podocytes can be identified by using several approaches including morphology, e.g., light and electron microscopy (Bertram et al. 1992; White and Bilous 2004),

immunolabelling, e.g., Wilms' Tumour 1 (WT-1; Puelles et al. 2015), TLE-4 (Venkatareddy et al. 2014) and p57 (Eng et al. 2015) and constitutive/inducible genetic labelling, e.g., podocyte-specific reporter lines (Berger et al. 2014; Wanner et al. 2014; Lasagni et al. 2015). All of these approaches have potential limitations.

As discussed above, the ability to identify podocytes with a high level of certainty also relies on section thickness and the imaging technique employed. Transmission electron microscopy enables easy podocyte identification, at least in normal glomeruli (Bertram et al. 1992). Confocal microscopy allows the visualization of thin optical sections without the need for physical sectioning and the use of multiple cell-specific markers (Richardson and Lichtman 2015). Furthermore, confocal microscopy facilitates the analysis of multiple, perfectly aligned, serial optical sections through each podocyte; this assists cell identification and also facilitates the generation of three-dimensional datasets, again enhancing our capacity to identify podocytes (Puelles et al. 2014).

Detecting mild levels of depletion

A recent study showed that conventional two-dimensional approaches are able to detect moderate/large levels of podocyte depletion but may miss mild levels of depletion (Puelles et al. 2016b). This illustrates an important point: the decision as to which method to use relies heavily on the experimental model (expected level of podocyte depletion) and potential impact of interventions (expected level of prevention or recovery). In our opinion, when high levels of accuracy and precision are essential in order to answer the research question, methods that minimize or negate the introduction of systematic bias should be used, including model-based stereology (e.g., Venkatareddy et al. 2014) and design-based stereology (e.g., disector/fractionator; Puelles et al. 2014) or whole glomerular analysis (Puelles et al. 2016b).

Available resources

Most laboratories physically cut 3–4 μm paraffin sections. However, cutting serial sections with a defined or even greater than normal thickness (14 μm) requires significant technical expertise. This favours the use of glomerular cross-sections and model-based stereology, whereas design-based stereology becomes more tedious and less appealing. The use of thick kidney slices (1–2 mm) bypasses the need for serial physical sectioning, which makes optical clearing an exciting possibility.

A critical point is access to imaging hardware and software. Whereas model-based stereological methods can usually be implemented with standard laboratory equipment, design-based and optical clearing methods may require access to expensive imaging hardware and software. Model and

design-based stereological methods can be implemented by using light, electron, confocal and two-photon microscopy. The newly developed optical clearing approaches may require more specialized equipment (i.e., BABB compatible objective lens; Puelles et al. 2016b).

Towards automated quantitation of podocyte depletion

We are perhaps not too far from automating, or at least semi-automating, assessments of podocyte depletion. Image segmentation algorithms can now detect, count and size immunolabelled nuclei in histological sections (Venkatareddy et al. 2014). With the novel clearing/immunolabelling/confocal strategy that we developed to count podocyte nuclei in whole mouse glomeruli (Puelles et al. 2016b), we manually counted all podocytes in a single glomerulus within about 2 min. The use of commercially available three-dimensional–rendering software also allows the semi-automated quantification of podocyte number per glomerulus with remarkably similar results. A recent study by Klingberg et al. (2016) provided an example of the way in which current data analysis can be used for the automation of large datasets and the automation of morphological quantification (e.g., total glomerular number) in a whole kidney. Further refinement and development in this area will accelerate experimental studies and perhaps have utility in the analysis of renal biopsies for diagnostic purposes.

Conclusions

Podocytes have emerged as a crucial cell type in the pathogenesis of glomerular diseases. Several methods are available for quantifying podocyte depletion. Careful considerations should be made when deciding which method to utilize based on their theoretical and practical advantages and disadvantages. In the near future, automated podocyte counting may accelerate experimental studies and allow the inclusion of podocyte quantification in renal biopsies for diagnostic proposes. Finally, we encourage researchers to use proper terminology when referring to the analysed parameters in order to facilitate reproducibility and comparisons between studies.

References

Abercrombie M (1946) Estimation of nuclear population from microtome sections. Anat Rec 94:239–247

Allison SJ (2015) Chronic kidney disease: actin cytoskeleton alterations in podocytes: a therapeutic target for chronic kidney disease. Nat Rev Nephrol 11:385

Angelotti ML, Ronconi E, Ballerini L, Peired A, Mazzinghi B, Sagrinati C, Parente E, Gacci M, Carini M, Rotondi M, Fogo AB, Lazzeri E, Lasagni L, Romagnani P (2012) Characterization of renal progenitors committed toward tubular lineage and their regenerative potential in renal tubular injury. Stem Cells 30:1714–1725

Basgen JM, Nicholas SB, Mauer M, Rozen S, Nyengaard JR (2006) Comparison of methods for counting cells in the mouse glomerulus. Nephron Exp Nephrol 103:e139–e148

Berger K, Schulte K, Boor P, Kuppe C, van Kuppevelt TH, Floege J, Smeets B, Moeller MJ (2014) The regenerative potential of parietal epithelial cells in adult mice. J Am Soc Nephrol 25:693–705

Bertram JF (1995) Analyzing renal glomeruli with the new stereology. Int Rev Cytol 161:111–172

Bertram JF (2001) Counting in the kidney. Kidney Int 59:792–796

Bertram JF, Soosaipillai MC, Ricardo SD, Ryan GB (1992) Total numbers of glomeruli and individual glomerular cell types in the normal rat kidney. Cell Tissue Res 270:37–45

Eng DG, Sunseri MW, Kaverina NV et al (2015) Glomerular parietal epithelial cells contribute to adult podocyte regeneration in experimental focal segmental glomerulosclerosis. Kidney Int 88:999–1012

Fukuda A, Chowdhury MA, Venkatareddy MP, Wang SQ, Nishizono R, Suzuki T, Wickman LT, Wiggins JE, Muchayi T, Fingar D, Shedden KA, Inoki K, Wiggins RC (2012) Growth-dependent podocyte failure causes glomerulosclerosis. J Am Soc Nephrol 23:1351–1363

Gundersen HJ (1986) Stereology of arbitrary particles. A review of unbiased number and size estimators and the presentation of some new ones, in memory of William R. Thompson. J Microsc 143:3–45

Gundersen HJ, Bagger P, Bendtsen TF, Evans SM, Korbo L, Marcussen N, Møller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sørensen FB, Vesterby A, West MJ (1988) The new stereological tools: disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. APMIS 96:857–881

Guo JK, Marlier A, Shi H, Shan A, Ardito TA, Du ZP, Kashgarian M, Krause DS, Biemesderfer D, Cantley LG (2012) Increased tubular proliferation as an adaptive response to glomerular albuminuria. J Am Soc Nephrol 23:429–437

Hodgin JB, Bitzer M, Wickman L, Afshinnia F, Wang SQ, O'Connor C, Yang Y, Meadowbrooke C, Chowdhury M, Kikuchi M, Wiggins JE, Wiggins RC (2015) Glomerular aging and focal global glomerulosclerosis: a podometric perspective. J Am Soc Nephrol 26:3162–3178

Jefferson JA, Alpers CE, Shankland SJ (2011) Podocyte biology for the bedside. Am J Kidney Dis 58:835–845

Klingberg A, Hasenberg A, Ludwig-Portugall I, Medyukhina A, Männ L, Brenzel A, Engel DR, Figge MT, Kurts C, Gunzer M (2016) Fully automated evaluation of total glomerular number and capillary tuft size in nephritic kidneys using lightsheet microscopy. J Am Soc Nephrol 28:452–459

Kriz W (2012) Glomerular diseases: podocyte hypertrophy mismatch and glomerular disease. Nat Rev Nephrol 8:618–619

Lasagni L, Lazzeri E, Shankland SJ, Anders HJ, Romagnani P (2013) Podocyte mitosis—a catastrophe. Curr Mol Med 13:13–23

Lasagni L, Angelotti ML, Ronconi E, Lombardi D, Nardi S, Peired A, Becherucci F, Mazzinghi B, Sisti A, Romoli S, Burger A, Schaefer B, Buccoliero A, Lazzeri E, Romagnani P (2015) Podocyte regeneration driven by renal progenitors determines glomerular disease

remission and can be pharmacologically enhanced. Stem Cell Reports 5:248–263

Lemley KV, Bertram JF, Nicholas SB, White K (2013) Estimation of glomerular podocyte number: a selection of valid methods. J Am Soc Nephrol 24:1193–1202

Nicholas SB, Basgen JM, Sinha S (2011) Using stereologic techniques for podocyte counting in the mouse: shifting the paradigm. Am J Nephrol 33 (Suppl 1):1–7

Nyengaard JR (1999) Stereologic methods and their application in kidney research. J Am Soc Nephrol 10:1100–1123

Peired A, Angelotti ML, Ronconi E, la Marca G, Mazzinghi B, Sisti A, Lombardi D, Giocaliere E, Della Bona M, Villanelli F, Parente E, Ballerini L, Sagrinati C, Wanner N, Huber TB, Liapis H, Lazzeri E, Lasagni L, Romagnani P (2013) Proteinuria impairs podocyte regeneration by sequestering retinoic acid. J Am Soc Nephrol 24: 1756–1768

Pichaiwong W, Hudkins KL, Wietecha T, Nguyen TQ, Tachaudomdach C, Li W, Askari B, Kobayashi T, O'Brien KD, Pippin JW, Shankland SJ, Alpers CE (2013) Reversibility of structural and functional damage in a model of advanced diabetic nephropathy. J Am Soc Nephrol 24:1088–1102

Pippin JW, Sparks MA, Glenn ST, Buitrago S, Coffman TM, Duffield JS, Gross KW, Shankland SJ (2013) Cells of renin lineage are progenitors of podocytes and parietal epithelial cells in experimental glomerular disease. Am J Pathol 183:542–557

Puelles VG, Bertram JF (2015) Counting glomeruli and podocytes: rationale and methodologies. Curr Opin Nephrol Hypertens 24:224–230

Puelles VG, Hoy WE, Hughson MD, Diouf B, Douglas-Denton RN, Bertram JF (2011) Glomerular number and size variability and risk for kidney disease. Curr Opin Nephrol Hypertens 20:7–15

Puelles VG, Zimanyi MA, Samuel T, Hughson MD, Douglas-Denton RN, Bertram JF, Armitage JA (2012) Estimating individual glomerular volume in the human kidney: clinical perspectives. Nephrol Dial Transplant 27:1880–1888

Puelles VG, Douglas-Denton RN, Cullen-McEwen L, McNamara BJ, Salih F, Li J, Hughson MD, Hoy WE, Nyengaard JR, Bertram JF (2014) Design-based stereological methods for estimating numbers of glomerular podocytes. Ann Anat 196:48–56

Puelles VG, Douglas-Denton RN, Cullen-McEwen LA, Li J, Hughson MD, Hoy WE, Kerr PG, Bertram JF (2015) Podocyte number in children and adults: associations with glomerular size and numbers of other glomerular resident cells. J Am Soc Nephrol 26:2277–2288

Puelles VG, Cullen-McEwen LA, Taylor GE, Li J, Hughson MD, Kerr PG, Hoy WE, Bertram JF (2016a) Human podocyte depletion in association with older age and hypertension. Am J Physiol Renal Physiol 310:F656–F668. doi:10.1152/ajprenal.00497.2015

Puelles VG, van der Wolde JW, Schulze KE, Short KM, Wong MN, Bensley JG, Cullen-McEwen LA, Caruana G, Hokke SN, Li J, Firth SD, Harper IS, Nikolic-Paterson DJ, Bertram JF (2016b) Validation of a three-dimensional method for counting and sizing podocytes in whole glomeruli. J Am Soc Nephrol 27:3093–3104

Puelles VG, Moeller MJ, Bertram JF (2017) We can see clearly now: optical clearing and kidney morphometrics. Curr Opin Nephrol Hypertens 26:179–186

Richardson DS, Lichtman JW (2015) Clarifying tissue clearing. Cell 162: 246–257

Roeder SS, Stefanska A, Eng DG, Kaverina N, Sunseri MW, McNicholas BA, Rabinovitch P, Engel FB, Daniel C, Amann K, Lichtnekert J, Pippin JW, Shankland SJ (2015) Changes in glomerular parietal epithelial cells in mouse kidneys with advanced age. Am J Physiol Renal Physiol 309:F164–F178

Steffes MW, Schmidt D, McCrery R, Basgen JM, International Diabetic Nephropathy Study Group (2001) Glomerular cell number in normal subjects and in type 1 diabetic patients. Kidney Int 59:2104–2113

Sterio DC (1984) The unbiased estimation of number and sizes of arbitrary particles using the disector. J Microsc 134:127–136

Venkatareddy M, Wang S, Yang Y, Patel S, Wickman L, Nishizono R, Chowdhury M, Hodgin J, Wiggins PA, Wiggins RC (2014) Estimating podocyte number and density using a single histologic section. J Am Soc Nephrol 25:1118–1129

Wanner N, Hartleben B, Herbach N, Goedel M, Stickel N, Zeiser R, Walz G, Moeller MJ, Grahammer F, Huber TB (2014) Unraveling the role of podocyte turnover in glomerular aging and injury. J Am Soc Nephrol 25:707–716

Weibel ER, Gomez DM (1962) A principle for counting tissue structures on random sections. J Appl Physiol 17:343–348

Wharram BL, Goyal M, Wiggins JE, Sanden SK, Hussain S, Filipiak WE, Saunders TL, Dysko RC, Kohno K, Holzman LB, Wiggins RC (2005) Podocyte depletion causes glomerulosclerosis: diphtheria toxin-induced podocyte depletion in rats expressing human diphtheria toxin receptor transgene. J Am Soc Nephrol 16:2941–2952

White KE, Bilous RW (2004) Estimation of podocyte number: a comparison of methods. Kidney Int 66:663–667

Wickman L, Hodgin JB, Wang SQ, Afshinnia F, Kershaw D, Wiggins RC (2016) Podocyte depletion in thin GBM and Alport syndrome. PLoS One 11:e0155255

Wiggins RC (2007) The spectrum of podocytopathies: a unifying view of glomerular diseases. Kidney Int 71:1205–1214

Zhang J, Pippin JW, Krofft RD, Naito S, Liu ZH, Shankland SJ (2013) Podocyte repopulation by renal progenitor cells following glucocorticoids treatment in experimental FSGS. Am J Physiol Renal Physiol 304:F1375–F1389