Can Renal Biopsy Be Used to Estimate Total Nephron Number?

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With campaigns directed toward the global burden of kidney disease as a focus of the World Congress of Nephrology and the developmental programming of cardiovascular and kidney disease in populations worldwide, we see a surge in the medical literature to describe and validate methods to quantify total nephron number (TNN) (1,2). Why is nephron number important, and how can its quantification aid in the identification and treatment of kidney disease, a priority for global health? The answer is mired in the sordid history of war, famine, and poverty that burden the world as well as the emerging survival of preterm-born individuals who account for up to 10% of the population (2,3). The confluence of the Barker–Brenner Hypotheses is most engaging for nephrologists who have witnessed the rise in kidney disease and the growing numbers of individuals born of low birth weight (LBW) with advanced stages of CKD enter the ESRD programs at an early age (4–6).

Epidemiologic studies across multiple homogeneous populations now clearly show the association of LBW, low nephron endowment, and the propensity toward CKD in later life (7,8). This has been termed the Developmental Origins of Health and Disease hypothesis, which incorporates fetal development of multiple organ systems (9). David Barker, the renowned British epidemiologist, first described increased diabetes, obesity, and cardiovascular and renal disease in young adulthood in individuals who were conceived and delivered after intrauterine growth restriction during the Dutch famine (9,10). Countless other epidemiologic and laboratory animal studies have substantiated these findings and strongly support the concept of developmental programming (11). The Brenner Hypothesis proposes that fewer nephrons per kidney, regardless of cause, result in systemic and individual glomerular hypertension and hyperfiltration characterized by increased glomerular volume (GV), leading to nephron hypertrophy and glomerulosclerosis (2,6). Notably, the lesion of FSGS may be considered a form of renal aging or senescence (12,13). In the case of low nephron endowment from birth, hyperfiltration nephropathy or secondary FSGS may develop, especially if the individuals become obese (14,15). Importantly, this lesion has been associated with both children and adults of LBW and/or prematurity.

Traditionally, in both infants and adults, the assessment of nephron number has relied on the dissector/fractionator technique in autopsied specimens with a wide variability in nephron number per kidney following a physiologic Gaussian distribution from 200,000 to 2 million nephrons per kidney, with an average of 800,000 nephrons per kidney (16,17). The congenital nephron endowment is primarily determined by the genetic code and the intrauterine environment with the full complement of nephrons present at term birth (17). This was substantiated by a report of 15 full-term infants <3 months of age (18). Nephron numbers ranged from 200,000 to over 1.2 million glomeruli per kidney, which were physiologically normally distributed. Moreover, within the narrow range of renal mass, the glomerular density (GD) provided important confirmation of the high GD at the beginning of life (19).

In this issue of the *Clinical Journal of the American Society of Nephrology*, Koike *et al.* (20) provide important observations comparing detailed histomorphometric analyses of renal biopsies from Japanese children of normal birth weight and gestation with analyses of those born prematurely with LBW (LBW<2500 g). The children were of a comparable age of 11 years old with proteinuria secondary to FSGS or minimal change nephrotic syndrome. Distinct differences were shown between the GD and GV of normal birth weight versus LBW children. The decreased GD and increased GV in the biopsy specimens of the LBW children with FSGS were considered an inference for low TNN, with the conclusion that these findings can be used as a surrogate for nephropenia. Although the limitations of the study are the small numbers of patients and the lack of a true assessment of TNN, this is an important demonstration of oligonephropathy and secondary FSGS in young individuals born preterm. With no assessment of renal mass, a true estimate of nephron number is not possible. Nevertheless, this report is unique in that it has provided insight into the life cycle of the kidneys of young children born preterm. There is a descending hyperbolic relationship between GD at birth and renal mass that continues until it reaches a plateau at maturation of renal size by adolescence (19). This is due to a natural decline in GD with growth of the renal interstitium and expansion of GV with maturation of renal function. Conceivably, this mathematical construct should allow nomograms to be developed with the possibility of deriving estimates of TNN from biopsy specimens. Unfortunately, we are far from this goal. The nephron deficit shown in the biopsies of the preadolescent LBW patients

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with FSGS indicates the consequences of a low nephron endowment in early life with accelerated renal senescence.

In pursuit of this methodology, investigators from the Mayo Clinic have developed a clinical protocol in which they coordinate renal biopsy histomorphometry for GD with imaging of the whole-kidney renal cortex to more precisely estimate TNN. In a recent article by Denic *et al.* (21), renal biopsy specimens taken at the time of transplant from healthy kidney donors were analyzed for GD. The cortical volume of the kidneys was determined by computed tomography scans, and the total number of glomeruli was estimated. This technology, applied to healthy kidney donors across a wide age range, has allowed an assessment of normal aging of the renal parenchyma. It is important to highlight that human renal biopsy combined with renal cortical volume studies to estimate nephron number has been done on healthy individuals, not ones with renal disease without a method to validate the results. A similar technique was used in a small study of healthy animals directly comparing magnetic resonance imaging (MRI)–obtained cortical, volumes/renal biopsy with the fractionator method (22). The authors concluded that, overall, there was good agreement between the two techniques but that there was up to a 36% difference between the methods in an individual kidney. The data from the article by Denic *et al.* (21) emphasize not only the wide spectrum of nephron endowment but also, the important effect of senescence with a decrease in both renal mass and nephron number after the age of 40 years old. Preterm birth may predispose an individual to more rapid senescence, which is suggested by the Swedish studies by Crump *et al.* (23) that show increased mortality risk in young adults <40 years of age who were born preterm. This increased mortality is related to cardiorenal disease and inversely associated with gestational age.

Histomorphometry has not been universally adopted in clinical practice due to several reasons. GV can be affected by formalin fixation; therefore, the experts in research laboratories use glycolmethacrylate rather than paraffin embedment to reduce the shrinkage effect. The physical dissector/fractionator technique is also limited due to time and resource consumption. Bertram, a pioneer of renal morphometry, and coworkers (17,18) have published that the individual GV can be reliably determined from as few as nine glomeruli, but these samples were all obtained on *ex vivo* kidneys at the middle of the hilum in contrast to the most often obtained lower-pole samples that often are not perpendicular to the capsule. Even if the numerous biases and technical issues could be addressed to validate the use of a renal biopsy to estimate nephron number, this is not a repeatable technique that could be used in large populations.

Despite the significant advancements in renal imaging techniques, the ability to enumerate the number of nephrons in any living species remains elusive. Noninvasive imaging techniques have failed, even with the best soft tissue resolution and high-field strength magnetic resonance, because the glomeruli cannot be differentiated from the other compartments in the kidney. To address this problem, investigators have been exploring a contrast agent, cationic ferritin (CF), developed to discriminate the glomeruli that are perfused from surrounding tubules and vasculature (24,25). When systemically administered to animals or directly injected to the renal artery of an *ex vivo* human kidney deemed unsuitable for transplant, the CF transiently binds to the glomerular basement membrane. The iron core of the positively charged ferritin is MRI detectable. This method allows for a comprehensive view of the kidney and avoids the biases and estimations inherent in renal biopsy data. Groups have shown how CF-enhanced MRI can be used to enumerate nephron number in living rodents (24,25). This noninvasive method to count and measure the size of glomeruli on a repeatable basis has the potential to transform our understanding of the variability of nephron number and progression of renal disease in LBW infants.

Even if we were able to accurately estimate TNN by renal biopsy, it remains too invasive for clinical practice. For now, it seems imperative that vulnerable populations, especially those born small and/or preterm, be monitored prospectively for renal growth and function to address the risk factors associated with progressive renal disease. The birth weight of an individual (and the gestational age) should become a part of their medical record. Much more research is needed in this regard, but continued advances will provide more focus on early recognition and intervention at a younger age, with the goal to improve quality of life and longevity.

Disclosures

None.

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See related article, "Glomerular Density and Volume in Renal Biopsy Specimens of Children with Proteinuria Relative to Preterm Birth and Gestational Age," on pages 585–590.