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Review

Tick-Tock Chimes the Kidney Clock – from Biology of Renal Ageing to Clinical Applications

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Abstract
Ageing of the kidney is a multi-dimensional process that occurs simultaneously at the molecular, cellular, histological, anatomical and physiological level. Nephron number and renal cortical volume decline, renal tubules become atrophic and glomeruli become sclerotic with age. These structural changes are accompanied by a decline in glomerular filtration rate, decreased sodium reabsorption and potassium excretion, reduced urinary concentrating capacity and alterations in the endocrine activity of the kidney. However, the pace of progression of these changes is not identical in everyone - individuals of the same age and seemingly similar clinical profile often exhibit stark differences in the age-related decline in renal health. Thus, chronological age poorly reflects the time-dependent changes that occur in the kidney. An ideal measure of renal vitality is biological kidney age – a measure of the age-related changes in physiological function. Replacing chronological age with biological age could provide numerous clinical benefits including improved prognostic accuracy in renal transplantation, better stratification of risk and identification of those who are on a fast trajectory to an age-related drop in kidney health.

Introduction

The intrinsic measure of biological age has captivated the imagination of the general public for years. The appeal may stem from a desire to quantify one's remaining years, the pursuit of novel treatments to extend lifespan, or as a justification for poor health. For the

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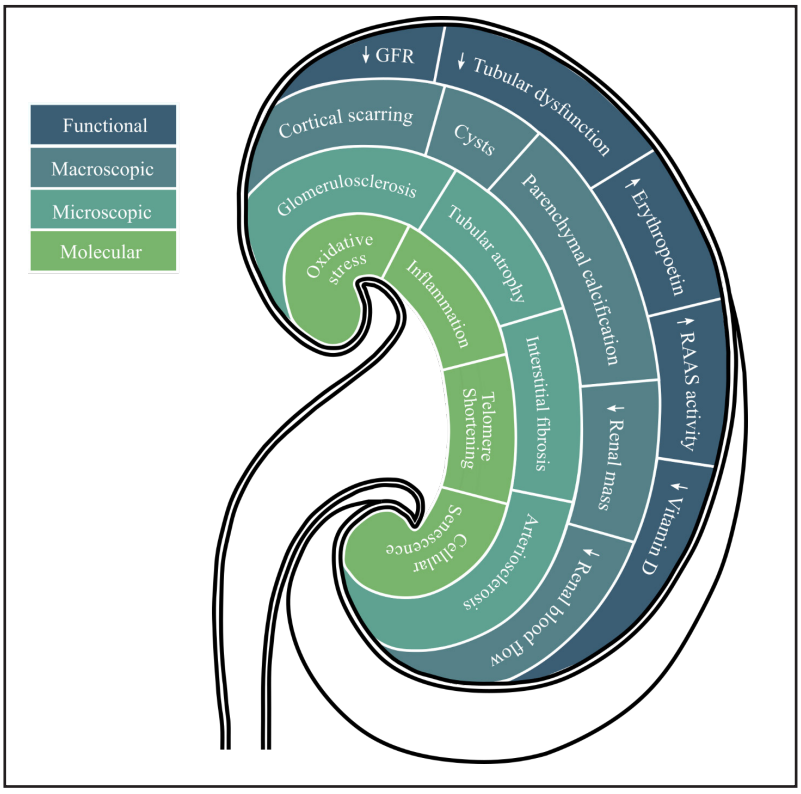

Fig. 1. Molecular, structural (microscopic and macroscopic), and functional dimensions of kidney ageing. Cellular dysfunction due to oxidative stress and the resultant inflammation, in combination with telomere shortening, lead to cellular senescence and apoptosis. Reduced reparative capacity and extracellular matrix dysregulation is associated with microscopic damage known as nephrosclerosis. Sclerotic glomeruli shrivel, leading to reduction in cortical volume. At a macro-anatomical level ageing is associated with cortical scarring and parenchymal calcification. These anatomical changes are accompanied by reductions in glomerular filtration rate, tubular dysfunction and aberrant endocrine activity. Abbreviations: GFR: Glomerular filtration rate, RAAS: Renin-angiotensin-aldosterone system.

clinician, interest in calculating a person's biological age dates back to at least the 1960s, when researchers monitoring the health of survivors from the Hiroshima and Nagasaki bombings sought to quantify their biological age via the amalgamation of predictive biological markers [1].

Biological age can be defined as an intrinsic measure of the age-related changes in physiological reserve; that is the capacity for organs to carry out normal physiological function. In the last 50 years, numerous attempts have been made to develop a reliable algorithm to measure biological age [2, 3]. The mechanisms that underlie ageing are more complex than initially expected. Consequently, biological ageing algorithms have grown increasingly complex. Recent evidence suggests that each organ has a unique ageing pattern, indicating that the biological age of each organ should be calculated individually [4].

Herein, we provide an overview of structural and functional changes that occur as the kidney ages (see Fig. 1). We summarise research progress on inter and intra-individual differences in biological age and expound the clinical importance of accurate calculation of an individual's biological kidney age.

The role of genetic, epigenetic and environmental factors in renal ageing

The data on narrow-sense heritability (a proportion of variance explained by additive genetic component) of renal ageing is limited, perhaps due to a lack of clinical measures with sufficient sensitivity and specificity to define renal ageing. Changes in estimated glomerular

filtration rate (eGFR) are commonly used as a measure of age-related decline in kidney health. Our earlier family-based studies documented the heritable nature of eGFR and that the proportion of its variance explained by the heritable additive component is actually higher than that of blood pressure [5]. The estimated heritability for age-related drop in eGFR (h^2= 0.33) is generally less significant than that for eGFR (h^2=0.38-0.75) [5–9], although it appears that monozygotic twins exhibit higher correlation for age-related changes in eGFR than dizygotic twins [10].

Over 60 single nucleotide polymorphisms (SNPs) were associated with eGFR in genome-wide association studies [11, 12]. Similar to other complex polygenic traits, the extent to which these genetic variants explain the proportion of inter-individual variance in the decline in eGFR is minimal [11]. Only 3 SNPs have been associated with eGFR decline so far [8]. Further evidence for the contribution of genes to the development of age-related changes in the kidney come from gene expression studies – hundreds of genes are up- or down-regulated in the human kidney in response to ageing [13]. MicroRNAs (miRNA) - small noncoding RNAs that regulate gene expression post-transcriptionally are also associated with renal ageing. Indeed, at least 18 miRNAs were significantly upregulated and 10 miRNAs were downregulated with ageing in the kidney of the rat [14]. Surprisingly few studies directly addressed the influence of environmental factors on the ageing of the kidney. Dietary factors such as caloric restriction reduce the rate of age-associated autophagy and oxidative stress in the kidney, a process mediated via SIRT1, AMPK and mTOR [15]. Methionine consumption (found in red meat, cheese and nuts) appears to have the opposite effect as documented in experimental models [16].

Key molecular mechanisms of renal ageing

Oxidative stress and inflammation

The free radical theory of ageing proposes that oxidative stress damages cellular constituents, leading to age-related decline. The kidney deploys an arsenal of mechanisms to prevent reactive oxygen species from wreaking havoc. For example, superoxide dismutase 1 and 2 (SOD1 and SOD2) soak up free radicals, preventing organelle and DNA damage. Unfortunately, like many other antioxidants, SOD expression declines with age [17]. As exhibited in knockout (KO) mice, absence of SOD1 leads to glomerulonephritis, nephrocalcinosis and lymphocyte infiltration. Consequently, KO mice have a reduced lifespan [18]. As the kidney ages, damage driven by oxidative stress leads to accumulation of macrophages and lymphocytes in the renal tissue [19]. Infiltrating macrophages release IFNγ, IL-6 and TNFα, which activate key master transcription factors including STAT1, STAT3 and NFκB [20]. Of these transcription factors, NFκB has been studied in extensive detail, with the hope that if able to target it, you could halt the age-associated inflammatory cascade.

Cellular senescence and telomere shortening

Central to the ageing process lies cellular senescence, the irreversible growth arrest that constricts renal regenerative capacity and propagates a pro-inflammatory state termed the senescence-associated secretory phenotype (SASP). In acute senescence e.g. post acute kidney injury (AKI), the SASP coordinates the removal of senescent cells through immune surveillance. However, in ageing, immune system dysfunction prevents effective clearance of senescent cells, leading to persistent SASP factor expression which causes inflammatory and fibrotic damage to surrounding cells [21]. Recently, Baker et al. demonstrated therapeutic clearance of senescent cells using a drug-inducible transgene to initiate apoptosis improved renal function, reduced glomerulosclerosis and ultimately led to increases in mouse lifespan [22]. One of the key instigators of cellular senescence is telomeric shortening. The telomere theory of ageing proposes that lifespan is predetermined by a finite capacity for cellular replication, called the Hayflick limit [23]. There is a documented inverse correlation between

the length of telomeres in the kidney and chronological age [24]. Telomeric attrition was also associated with increased susceptibility to AKI and decreased graft survival post-transplant [25, 26]. However, telomere shortening is not an ideal biomarker of ageing. For one, telomere dysfunction can cause cellular senescence independent of telomere shortening [27]. Moreover, telomere length stops being a useful predictor of age-related morbidity and mortality in those older than 85 [28]. Furthermore, a cell's telomere length is indicative of its' replicative history, and does not necessarily correlate with its' biological age.

Structural and functional changes in renal ageing

Histology

At birth, the human kidney contains approximately 900,000-1 million nephrons [29]. No new nephrons are formed after 36 weeks gestation [29]. Ageing is associated with the depletion of approximately 4,500 nephrons per year [30, 31]. This equates to loss of almost half one's nephrons between early adulthood (18-19) and old age (70-75). Nephron number is proportional to eGFR throughout one's life, except in the elderly age group (70-75) where nephron number drastically drops with minimal consequence to eGFR. One possibility is that compensatory hypertrophy of residual nephrons maintains eGFR in some elderly populations [31]. This could explain (at least to some extent) the inter-individual differences seen in eGFR in elderly populations [32–34].

The drop in nephron numbers with age is accompanied by the changes in renal histology. The glomerular basement membrane thickens, its capillaries shrivel and are replaced by fibrotic tissue (glomerular sclerosis), the renal tubules collapse (tubular atrophy), extracellular matrix components accumulate, expanding the interstitial space (interstitial fibrosis) and arterial walls thicken and lose their elasticity (arteriosclerosis) [35]. This tetrad of abnormalities is termed nephrosclerosis [36]. Pairwise comparison indicates these age-related histological abnormalities are highly correlated with one another [36]. However cumulatively, as measured by nephrosclerosis score; they are not associated with age-related eGFR decline, perhaps due to the involution and eradication of sclerotic glomeruli distorting findings [36].

Nephrosclerosis is the most common pathway for kidney injury in ageing [37]. It has its roots in early life, prior to the development of chronic kidney disease (CKD) [36], but is clinically silent throughout a major part of its natural history; unlike many other renal conditions it does not usually manifest with proteinuria [38]. However, the gradual progression of nephrosclerotic changes leads inevitably to a loss in functional nephron reserve and atrophy increasing the susceptibility of affected patients not only to severe presentations of progressive CKD but also other renal disorders such as acute kidney injury [39]. Nephrosclerosis was also reported to cluster with high mortality and high risk of end-stage renal disease [40]. As a facet of ageing, histological appearance is unique in that it represents the wounds of time, the afflictions faced by an organ.

Macroscopic changes

From the 4-5th decade onwards, renal volume declines [41]. Diminution is largely confined to the renal cortex, as renal medulla volume remains relatively stable perhaps due to tubular hypertrophy or unaccounted increases in renal sinus fat. Loss of renal mass, in combination with atherosclerotic plaque formation and an increase in renal sympathetic tone, results in declining renal blood flow, at a rate of 10% per decade, from the 4-5th decade onwards [39, 42]. Inadequate renal perfusion likely contributes to the age-associated decline in GFR. Furthermore, ageing is associated with increased prevalence of renal cysts, parenchymal calcifications and cortical scars [39].

Functional changes

Multiple studies report variable age-associated decline in eGFR from 0.4-2.6 ml/min/year [33, 34, 36, 43, 44]. The rate of decline increases with age. In 20-30-year olds eGFR decreased by 0.82 ml/min/1.73m^2/year in comparison to those over 50 where it decreased by 1.15 ml/min/1.73m^2/year [34]. Ageing is associated with tubular dysfunction including decreased sodium reabsorption, potassium excretion and reduced urinary concentrating capacity [45]. These changes in part account for the increased risk of dehydration and AKI observed in elderly individuals [46]. Ageing also affects the endocrine function of the kidney. Despite decreased renin expression, angiotensin II activity increases with age [47, 48]. This may be due to increases in angiotensin II receptor sensitivity or differential regulation of systemic and intrarenal renin-angiotensin systems [49, 50]. Renal conversion of 25-hydroxyvitamin D into 1,25-dihydroxyvitamin D declines with age, contributing to the vitamin D deficiency commonly seen in the elderly [51]. Commonly used to distinguish age-associated renal dysfunction from chronic kidney disease, elderly individuals exhibit raised levels of erythropoietin, perhaps due to subclinical blood loss, increased erythrocyte turnover or increased insensitivity to the effects of erythropoietin [52].

Intra and inter-individual variation in ageing

A person's chronological age can drastically differ from their underlying biological age. Belsky et al. used an array of biomarkers, including creatinine clearance and blood urea nitrogen, to measure the biological ages of a large cohort of 38-year-olds [2]. Despite being the same chronological age, individual biological ages ranged from 28-61. Some participants aged 3 biological years for every calendar year, whereas others exhibited almost no physiological age-related change in a year.

The pace of renal ageing varies between individuals. Findings by Rule et al. suggest substantial variation in nephrosclerosis scores between healthy individuals within the same age bracket [36]. For example, 10% of individuals aged 60-69 had a nephrosclerosis score of 0, whilst another 10% had a sclerosis score of 4. Our data from the TRANScriptome of renaL humAn TissuE (TRANSLATE) study [53, 54] illustrates how two individuals of the same chronological age and seemingly similar clinical profile exhibit stark contrast in renal histological appearance (see Fig. 2). Longitudinal studies by Linderman, Jiang and Cohen demonstrate 36%, 43% and 15.4% of healthy individuals exhibit no age-related decline in eGFR respectively [32–34]. These findings indicate that age-associated functional decline in healthy kidneys exhibits marked inter-individual variation.

It is not clear at present what offers a protection against age-related decline in the structural integrity and function of the kidney. The differences in the observed phenotypic changes of the kidney can be explained (at least to some extent) by the differences in gene expression profiles between those with faster and slower renal ageing. Indeed, Rodwell et al. evaluated the age-associated changes in gene expression in 74 kidneys [13]. They identified 447 age-regulated genes that form a molecular profile of ageing. In doing so, they noted that certain individuals despite being chronologically younger, had a gene expression profile suggestive of someone more senior (fast agers), and vice versa (slow agers).

Apart from these apparent differences in pace of renal ageing between individuals, variation exists in ageing of different organs from the same individual. For example, comparison of age-associated transcriptomic changes in kidney and muscle, demonstrated minor overlap in age-related gene expression, suggesting discrete molecular ageing mechanisms [13, 55]. Cadaveric studies measuring telomere length in 12 different tissues, including the kidney, demonstrates high variability in the extent of telomeric attrition between organs, suggesting divergent pace of ageing [56]. In addition, different histological

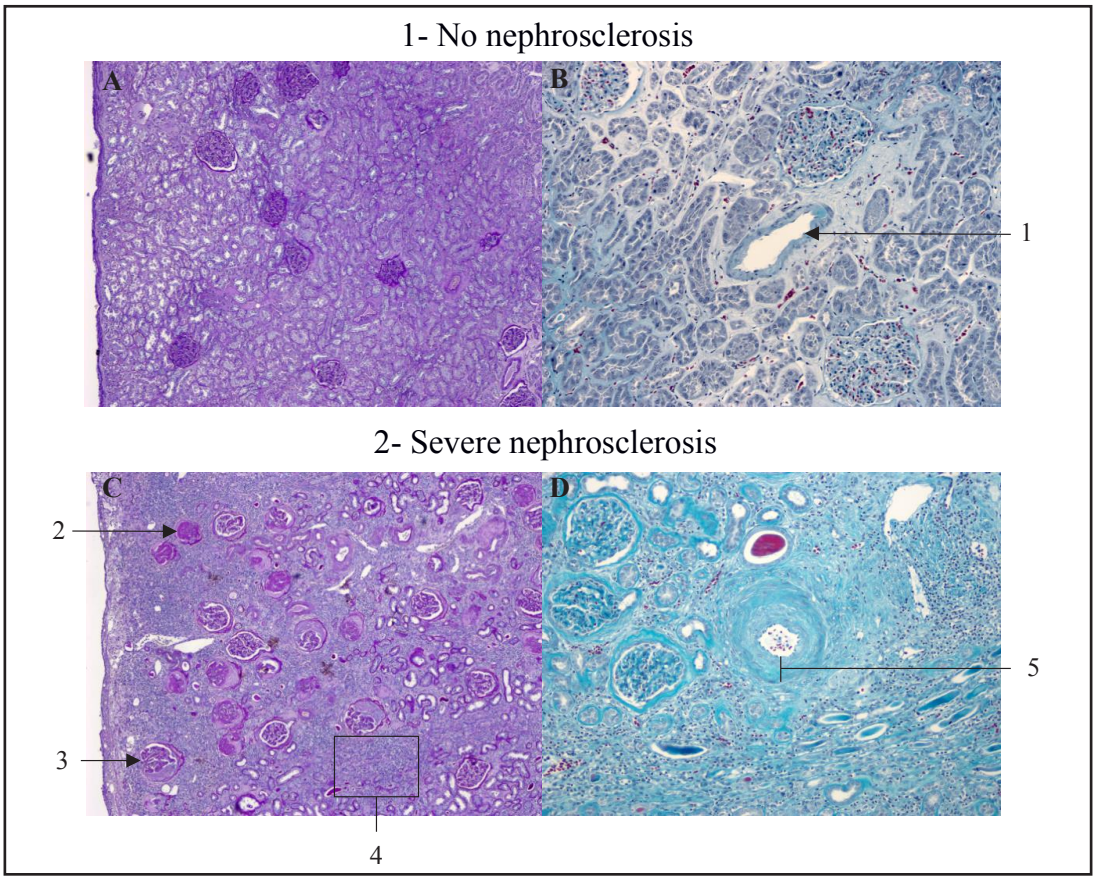

Fig. 2. Inter-individual variation in nephrosclerosis. Histology images from two TRANScriptome of renaL humAn TissuE (TRANSLATE) study patients (1 and 2) with a seemingly comparable clinical profile; both 62-year-old men with hypertension and obesity. Despite similar clinical histories the two patients exhibit dramatically different nephrosclerotic changes. 1. Normal interlobular artery. 2. Global glomerulosclerosis. 3. Glomerular collapse. 4. Tubular atrophy and interstitial fibrosis with inflammation. 5. Interlobular artery with fibrointimal thickening. A and C = Periodic acid–Schiff, x40. B and D = Masson's trichrome, x100.

components of the same organ from the same individual respond differently to ageing. Within the kidney, Rodwell et al. demonstrate little overlap in expression of ageing-associated genes between the cortex and the medulla [13]. Likewise, Melk et al. observed a faster rate of telomeric attrition in the renal cortex, than the renal medulla [24].

Quantifying the biological age of the kidney

There are a number of potential surrogates of biological renal age (Table 1) [3, 27-28, 32-33, 36, 57-58]. Telomere length and eGFR have previously been utilised to predict biological age [2], however, they have their own limitations as discussed earlier. Molecular markers such as Klotho may have a potential role in renal ageing. Indeed, overexpression of Klotho has been shown to extend the lifespan of mice by 20-30% [59]. In the kidney, Klotho is involved in the prevention of cellular senescence, regulation of interstitial fibrosis, and suppression of inflammation [59–61]. Urinary and serum Klotho levels have already been utilised as prognostic markers of CKD [62, 63]. Nephrosclerosis is a potential proxy, having been closely correlated with age [36], age-associated kidney gene expression [13] and renal transplant outcome [64]. However, obtaining histological samples via renal biopsy is not feasible in patients without clear clinical indication i.e. evidence of overt nephropathy. The

Table 1. Surrogates of renal biological age – past, present and future

Signature	Source	Limitations	References
eGFR	Blood	Significant inter-individual differences. Indirect measure based on serum levels of creatinine. Poor correlation with histological measures of age-related kidney damage.	Linderman et al [32] Jiang et al [33]
Telomere length	Blood/Tissue sample	Mostly measured in leukocytes from peripheral blood. Poor correlation with biochemical measures of kidney function. Cell replication ≠ biological cell age.	Arai et al [27] Martin-Ruiz et al [28]
Klotho expression	Urine/Blood	Reliability of urinary/serum measurement questionable.	Akimoto et al [57]
Nephrosclerosis	Tissue sample	Measurement requires invasive procedure.	Rule et al [36]
Transcriptomic signatures	Urine/Blood/Tissue sample	Limited evidence in relation to kidney ageing.	Suthanthiran et al [58]
Epigenetic signatures	Urine/Blood/Tissue sample	Limited evidence in relation to kidney ageing.	Horvath et al [3]

successful identification of robust signatures of kidney-specific biological age will require exploiting new molecular strategies such as transcriptomics, epigenomics, proteomics and metabonomics. Each of them offers an unbiased systematic insight into thousands of genes and molecules many of which are the key determinants of the individual trajectories of kidney ageing. A particularly promising strategy is transcriptomic profiling of cells harvested from urine samples [58]. Additional strategies, including the measurement of circulating levels of cell-free DNA have shown promise in the prediction of outcomes in renal transplantation [65, 66], and this could be potentially further exploited in studies on renal ageing. Next-generation RNA-sequencing-based profiling of epigenetic master regulators, such as small (miRNA, small interfering RNA, piwi-interacting RNA) and long non-coding RNAs may shed an insight into these ageing signatures [67, 68]. Horvath's DNA methylation-based measure of biological age has already shown the potential of exploiting human epigenome in search of signatures of tissue ageing [3]. Ideally, the use of "omics" should be integrated with the objective and direct measures of age-related kidney damage such as histologically confirmed nephrosclerosis. The availability of resources where both histologically-confirmed measures of age-related kidney damage together with biological materials suitable for omics-type profiling (i.e. TRANSLATE Study) [53, 54] brings us closer to finding the multi-marker signatures of biological kidney age. We believe that the future of a kidney-specific ageing signature lies in systems biology – combining genetics, transcriptomics, epigenetics, proteomics, clinical and histological data. Such efforts are currently underway as part of our TRANSLATE Study [53, 54] and in the NEPTUNE Study [69].

Clinical prediction of biological kidney age

The attractiveness of determining the biological age of the kidney lies in its diagnostic and predictive potential. The shortage of donor kidneys has led to increased use of suboptimal organs, often from elderly donors. In Great Britain, the UK kidney donor risk-index scoring system is commonly used to screen the allograft quality. It incorporates donor age, which is known to predict poor outcome after transplant [70]. Biological kidney age could supersede chronological age, as a more accurate predictor of prognosis. Thus, kidney donors rejected due to their old age, may now be able to donate if found to be younger than their expected biological age, increasing the pool of available kidneys. Conversely, organs of apparent optimal suitability for renal transplantation based on chronological age, may require more careful monitoring upon transplantation should their biological age be much older than their chronological age. The value of incorporating markers of ageing into prognostic algorithms in renal transplantation is receiving increasing attention due to its potential to improve outcome in recipients [71]. For example, two non-coding RNAs, miR-217 and miR125b were shown to predict delayed graft function with a 61% sensitivity and 91% specificity.

Given that chronological age is the major risk factor for AKI, it is tempting to speculate that the prediction of risk and monitoring of clinical outcomes in patients with AKI can be

improved by knowledge of their biological kidney age. This is particularly relevant to AKI due to drug nephrotoxicity (approximately 20% of AKI) [72] as clinical guidelines recommend careful monitoring and increased caution when prescribing high-risk medications to the elderly (based on their chronological age) [73]. Information on biological kidney age could more accurately inform the decision-making process. For example, the Mehran contrast-induced AKI risk score advocated by the Kidney disease: Improving global outcomes (KDIGO) guidelines advises that those chronologically older than 75 are at increased risk of contrast nephropathy [74]. If an individual chronologically younger than 75 was found to have a biological age greater than 75, it would be sensible to assume increased risk and if appropriate, modify their management plan in accordance with best practice guidelines.

Perceived biological age currently plays a role in surgical decision making, often conveyed in the notes as "remarkably fit for 88" or "a rather old 71-year-old". With respect to the kidney, eGFR is typically the only measure of renal vitality used in pre-operative assessments. Reduced eGFR prior to surgery is associated with increased mortality, independent of AKI [75]. However, eGFR is a suboptimal measure of overall renal health. As previously noted, eGFR only measures one aspect of renal function, it does not reflect the age-related kidney change in a significant cohort of the population [32–34], and it poorly correlates with other markers of renal biological age e.g. nephrosclerosis score [36]. Thus, pre-operative appraisal of biological kidney age in addition to eGFR could provide more accurate prognostic information.

Advanced chronological age is independently associated with poor prognosis in IgA nephropathy [76] and renal malignancy [77]. Conversely, increased chronological age at disease onset in autosomal recessive polycystic kidney disease is associated with improved prognosis [78]. In these conditions, biological age could replace chronological age as a prognostic indicator. Other kidney diseases with a less well-defined role of age on outcome include diabetic nephropathy [79], focal segmental glomerulosclerosis [80] and membranous nephropathy [81].

Ageing is associated with increased prevalence of chronic kidney disease (CKD), indeed, almost half of elderly individuals fulfil the current diagnostic criteria for CKD [82, 83]. Elderly patients with CKD have a greater adjusted risk of death [83] and exhibit lower functional kidney reserve when they present with CKD [84]. Current diagnostic criterion relies on a fixed threshold to identify CKD, with little consideration for age-related decline in eGFR, with some suggesting this leads to over-diagnosis of otherwise healthy individuals [85, 86]. One possible solution is incorporation of biological kidney age into diagnostic criteria in this group of patients.

For the healthy individual, whose baseline eGFR is otherwise normal, biological kidney age could be used to identify those with accelerated renal ageing. For example, Rule et al. demonstrate in a population of healthy living kidney donors, 15% of those aged 30-39 exhibit substantial age-related nephrosclerotic change (indicating accelerated renal ageing), yet all these individuals have a measured GFR >75 ml/min/1.73m^2 [36]. It is reasonable to assume these fast renal agers are more susceptible to age-related renal disease (e.g. chronic kidney disease). Thus, an increased rate of kidney ageing could warrant prophylactic measures i.e. prescription of prospective anti-ageing therapies [87].

Conclusion

Renal ageing is associated with a progressive decline in eGFR and structural disfigurement at a microscopic and macroscopic level. At a molecular level, this is accompanied by cellular senescence, telomere shortening, apoptosis and fibrosis. There are numerous gaps in knowledge on how molecular changes influence renal histology and how histological changes translate into a decline in renal function. Furthermore, little is known about the genetic and environmental factors that influence individual rates of age-related decline. Intra-individual

differences in organ ageing advocate the development of a kidney-specific ageing signature. This successful identification of such signatures relies on combining "omics", clinical and histological data. This will have several potential benefits for clinical nephrology including improved prognostics and identification of high-risk patients.

Disclosure statement

The authors of this review have no conflicts of interest.

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