Original Paper

Adult Renal Size is Not a Suitable Marker for Nephron Numbers: An Individual Patient Data Meta-Analysis

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Key Words

Renal weight • Renal size • Nephron number • Glomerular number • Nephron endowment

Abstract

Background: Renal size is often used as a marker for nephron numbers as estimation of glomerular numbers is not yet possible in vivo. However, the validity of an association between the two is questionable. As a proper marker for nephron number in an individual is needed in clinical practice, this study was designed to assess the association between renal size and nephron numbers. **Methods:** An individual patient data meta-analysis was performed on data retrieved with a PubMed and Embase search. Only studies were included that described individual human data on kidney size and nephron numbers determined by stereology, the gold standard methodology to estimate nephron numbers. As renal size increases until the end of puberty, and nephron numbers decline after the age of 60 years, only data from individuals aged 18-60 years without renal disease were included. **Results:** Six papers were identified that provided data on renal weight and nephron numbers from 114 individuals. Backward linear regression identified kidney weight and race as the only 2 significant factors explaining nephron numbers (R square 0.085, $p=0.007$). Controlling for race, there was a significant correlation between nephron number and kidney weight ($r=0.231$, $r\text{ square}=0.053$, $p=0.01$). **Conclusion:** These data indicate that only ~5% of the variation in nephron numbers is explained by differences in renal size. Renal size in adulthood should not be used as a marker for nephron numbers in an individual.

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Introduction

Nephron numbers are important in determining renal function and blood pressure. A low nephron endowment leads to glomerular hyperfiltration [1] and is associated with

hypertension [2], glomerular enlargement and glomerulosclerosis [3]. As the number of nephrons is fixed during prenatal kidney development without the possibility of later nephron formation, several perinatal factors, such as intrauterine growth restriction and premature birth, have been shown to influence final nephron numbers [4] and lead to (pre) hypertension in young adults [5].

The gold standard method to determine nephron number is by stereology [6]. Unfortunately, stereological nephron number estimation is currently only possible *ex vivo*, limiting the study of the impact of perinatal insults on kidney development to animal research. To circumvent this problem, renal size is often used as a marker for nephron number. Indeed, 2 studies have shown a statistically significant association between renal weight and nephron number [7, 8], explaining up to 45% of the variation in nephron numbers. In contrast, the landmark study of Keller et al. [2] showed a two-fold difference in nephron numbers between individuals with and without hypertension, but no difference in renal weight.

This illustrates that it is debatable whether renal size or weight can be used to predict nephron endowment in an individual, but it is used that way in an increasing number of papers as a variation in nephron numbers may have clinical consequences.

In order to assess the association between renal weight and nephron numbers in humans, we performed an individual patient data meta-analysis that shows that only 5% of the variation in nephron number is explained by renal size after correction for one other significant factor (race).

Materials and Methods

Search strategy

The PRISMA statement was used for this systematic review [9]. A review protocol was absent before the start of this study.

A PubMed search was conducted for articles published from January 1966 onward that contained the keywords “nephron number” or “glomerular number” in combination with the keywords “stereology” or “stereological” (total hits 134, 4 June 2013, Fig. 1). In addition, an EMBASE search was conducted with the same keywords (total hits 109, 4 June 2013). Two authors screened the title and/or abstract of these records, and potentially eligible papers were read in full. Disagreements between reviewers were resolved by consensus.

After selection, only 5 articles remained. In addition, the “related articles” function in PubMed was used from articles that were considered for inclusion. Also, the publications from two research groups that have the most experience in stereological analysis of the kidney were looked at by searching PubMed with the names of the respective senior researchers (JF Bertram and JR Nyengaard). Finally, reference lists from included publications were searched manually. These strategies provided 1 additional article providing individual data on nephron number and renal size and/or weight (Fig. 1).

Selection of articles

All studies in English describing data in humans of both renal size and/or weight together with a stereological estimation of nephron number were considered. As both a young age (for the purpose of this study defined as the age of 18 years) as well as an older age (at which a decline in nephron number can be expected, i.e. 60 years [8]) may influence the association between nephron number and kidney weight, the analysis was performed on individuals aged 18-60 years. To allow for a meta-analysis of individual patient data, such data needs to be presented per individual, rather than an average per group. Only studies using stereology were of interest, as stereology is a bias-free design-based method that provides a reliable estimation of nephron number without any assumption of size and shape [6]. Title and/or abstract of all articles identified were screened, and relevant original studies were read in full. When several articles described (part of) the same cohort, individual data on nephron number, height and body weight were compared to guarantee that each individual was only included once in the analysis. In order to exclude the influence of renal disease on either nephron number or kidney weight, individuals were excluded that were

Fig. 1. Flow diagram of in- and excluded papers for the individual patient data meta-analysis.

known to have chronic renal failure and/or were treated with dialysis or received a renal transplant. In total, 6 articles were included in the meta-analysis. No risk of bias of individual studies or risk of publication bias was assessed.

Data abstraction

Per individual, the following data were collected: age, gender, race (Caucasian vs. African (American)), height, weight, body surface area (BSA), body mass index (BMI), kidney weight, and nephron number. As 4 papers [2, 10-12] did not report BSA, but did report height and weight, BSA was calculated using Mosteller's formula [13]: $BSA = ((\text{height} \times \text{weight}) / 3600)^{1/2}$, using height in cm and weight in kg. For 2 papers [7, 8], information on height and BSA was available, which was used to calculate weight using an adaptation of Mosteller's formula: $\text{weight} = ((BSA^2) \times 3600) / \text{height}$.

Analysis

With these data an individual patient data meta-analysis was performed.

Bivariate correlation analyses between nephron numbers and the other variables (age, gender, race, height, weight, BSA, BMI, and kidney weight) were performed using Pearson's correlation analysis. Backward linear regression (criterion $F \geq 0.10$) was used with all collected data to identify the factors that were significantly associated with nephron number. Using these factors, the association between nephron number and each of these factors was determined after correction for the other significant factor(s) and presented as R or R square. Results are presented as mean (standard deviation, SD) unless otherwise stated. Differences between groups were analyzed by one-way ANOVA. Comparison of two proportions

Table 1. Characteristics of included studies

	Nyengaard 1992 [8]	Bendtsen 1992 [7]	Keller 2003 [2]	McNamara 2008 [10]	McNamara 2009 [11]	Zimanyi 2009 [12]	Cumu- lative	Cumu- lative 18-60yr
Number of patients (n)	37	57 ^a	20	28	24	24	190	114
Country of residence	Denmark	Denmark	Germany	Senegal	Senegal	USA	-	-
Male:female (n:n)	19:18	32:25	18:2	14:14	24:0	24:0	131:59	91:23
Age (yr)	58.1 (19.8)	63.2 (14.7)	46.2 (6.9)	34.9 (19.7)	41.0 (16.3)	45.3 (10.0)	51.2 (18.9)	42.9 (10.7)
Age < 18yr (n)	1	1	0	8	0	0	10	-
18-60yr (n)	17	17	20	16	21	23	114	114
≥ 60yr (n)	19	39	0	4	3	1	66	-
Race Caucasian (n)	37	57	20	-	-	12	126	66
African (American) (n)	-	-	-	28	24	12	64	48
Height (cm)	166.4 (8.5)	166.7 (8.1)	176.9 (8.2)	157.3 (23.1)	168.9 (5.0)	179.4 (6.5)	168.2 (12.9)	172.5 (8.5)
Weight (kg)	63.4 (15.2)	68.1 (12.7)	88.8 (16.4)	64.3 (17.6)	69.7 (11.3)	94.1 (20.7)	72.3 (18.7)	78.1 (18.0)
BSA (m ²)	1.70 (0.23)	1.77 (0.19)	2.08 (0.20)	1.67 (0.33)	1.80 (0.16)	2.16 (0.25)	1.83 (0.28)	1.93 (0.25)
BMI (kg/m ²)	22.7 (4.3)	24.4 (3.7)	28.4 (5.2)	25.9 (4.7)	24.4 (3.9)	29.2 (5.9)	25.3 (4.9)	26.1 (4.9)
Kidney weight (g)	133.0 (35.2)	143.7 (37.6)	180.0 (39.7)	118.8 (35.6)	147.6 (34.6)	199.2 (36.1)	149.3 (43.7)	166.0 (40.8)
Nephron number (n * 1,000)	638.6 (202.3)	612.5 (190.4)	1,074.4 (422.4)	925.5 (225.4)	1,053.4 (306.9)	895.3 (454.7)	803.7 (340.1)	921.5 (351.2)

Data are presented as mean (standard deviation) or as numbers. Individuals with renal failure were excluded from analysis. BSA, Body surface area. BMI, Body mass index. ^a, 7 of the 64 described patients were excluded on basis of reduced renal function that could influence the number of nephrons

of categorical data was done by the chi-square test. Statistical differences were considered significant if $p < 0.05$ (two-tailed). SPSS (version 16.0.2) was used as statistical analysis package.

Results

From the 6 papers included (Fig. 1), 197 individuals were identified. Seven patients were excluded on basis of chronic renal failure. Of the 190 individuals remaining, 10 (5.3%) were aged 17 years or younger, and 66 (34.7%) were aged 60 years or over, leaving 114 (60.0%) 18 to 60-years-old patients (Table 1).

Bivariate correlation analyses between nephron numbers and the other factors (age, gender, race, height, weight, BSA, BMI, and kidney weight) are presented in Table 2. Linear regression including all factors explained only 15.0% of the variation in nephron numbers (R^2 0.150, $p=0.02$). Backward regression identified kidney weight and race as the only 2 significant factors explaining nephron numbers (R^2 0.085, $p=0.007$). Every gram increase in kidney weight was associated with an increase in nephron number of 2,029. Controlling for race, there was a significant correlation between nephron number and kidney weight ($r=0.231$, $p=0.01$), indicating that 5.3% of the variation in nephron numbers is explained by variations in kidney weight. Fig. 2 shows the linear regression between nephron number and kidney weight per race group. The mean number of nephrons showed a trend to be higher in African (American) group (Table 3), whereas kidney weight was higher in the Caucasian group.

Table 2. Correlations between nephron numbers and various parameters

	r	p
Age	-0.076	0.2
Gender	0.216	0.01
Race	0.183	0.03
Height	0.120	0.1
Weight	0.025	0.4
BSA	0.059	0.3
BMI	-0.026	0.4
Kidney weight	0.171	0.03

BSA, Body surface area. BMI, Body mass index

Table 3. Characteristics of included individuals per race group

	Caucasian	African (American)	p
Number of patients (n)	66	48	
Male:female (n:n)	51:15	40:8	0.4
Age (yr)	45.5 (9.6)	39.5 (11.2)	0.003
Height (cm)	173.7 (8.8)	171.0 (8.0)	0.1
Weight (kg)	81.6 (20.6)	73.3 (12.5)	0.01
BSA (m ²)	1.97 (0.27)	1.86 (0.19)	0.02
BMI (kg/m ²)	26.9 (5.7)	25.0 (3.4)	0.04
Kidney weight (g)	175.2 (38.2)	153.4 (41.4)	0.004
Nephron number (n * 1,000)	867.3 (362.7)	996.0 (323.8)	0.05

Data are presented as mean (standard deviation) or as numbers. Individuals with renal failure and age <18 years or >60 years were excluded from analysis

Fig. 2. Association between nephron number and kidney weight per race group. Linear regression between nephron number and kidney weight in Caucasians (open circles, $y=1897x+535,012$; $r=0.200$, $p=0.1$) and African (Americans) (filled circles, $y=2185x+660,823$; $r=0.279$, $p=0.05$) aged 18-60 years.

Discussion

Based on this individual patient data meta-analysis, there is a significant association between renal weight and nephron numbers. However, renal weight in adulthood should not be used as a marker for nephron endowment, as variations in weight only explain about 5% of nephron numbers.

In clinical practice, renal size is estimated by ultrasound using the formula for an ellipsoid. Unfortunately, these renal size estimations are a poor predictor of true renal size and show an average underestimation of 19-25% and a poor repeatability [14-16]. Estimations using CT scans seem to perform better (correlation coefficient 0.79, $p<0.01$) [17], but still explain only 62% of variation in renal size. Using optimal MRI settings improves this further [18], resulting in the greatest accuracy (at least in vitro) [15]. As true renal size is a poor predictor of nephron endowment, and in vivo renal size estimation using any radiological modality is a poor predictor of true renal size, it is doubtful that renal size estimation is helpful in predicting nephron endowment. However, many papers still use renal size estimation as a marker for nephron numbers.

Even though renal size is not a proper marker for nephron numbers in adults, there have been several studies that show an influence of renal size on functional markers. For instance, transplant survival is better in large kidneys when compared with the smaller kidneys [19-21]. Furthermore, premature birth (i.e. before termination of nephrogenesis) has been shown in animal models to negatively influence nephrogenesis [4]. In a cohort of

20-year-old individuals born very prematurely, kidneys were significantly smaller in length and volume than kidneys from term born controls with a normal birth weight [22]. Such studies highlight that differences may be found, just as an association between renal weight and nephron numbers was described in some populations [7, 8], but this may be due to chance as this was not a consistent phenomenon.

As stated previously, estimation of nephron numbers is currently only possible *ex vivo*. However, it has recently been shown possible to estimate nephron numbers quite reliably by the use of a 9.4 Tesla MRI in embedded kidneys, which shows that techniques are progressing towards nephron number estimation *in vivo* [23].

Publication bias is a frequently observed phenomenon that prohibits proper meta-analysis, for which it may be vital to retrieve non-published data or cohorts. The association between renal size and nephron numbers has not been a subject of research, but is rather a by-product of studies describing nephron number of size variations in populations. We feel that this makes it unlikely that publication bias has been of influence on the results and therefore did not contact authors of potentially relevant studies.

This individual patient data meta-analysis has some limitations. First, only 6 cohorts were available that described adults in whom nephron numbers were estimated using stereology. As stereology is the only gold standard and bias-free method to estimate nephron numbers [6], this criterion was essential to allow for pooling of the data. But even with stereology, there may be an inter-observer variability in estimating nephron numbers that has an impact on the current meta-analysis. Second, as stated previously, only adult data were included, which indicates that no conclusion on the merit of renal size in childhood can be provided. Third, most papers have used the right kidney predominantly [24]. A study that did use kidneys from both sides [7] did not find any difference in nephron numbers between left and right (JR Nyengaard, personal communication), but differences between the sides in congenital anomalies are well noted [25]. Any impact of such a potential difference between the sides cannot be excluded with the data available. Finally, nephrons are lost in the course of life, which was the reason to exclude individuals over 60 years of age from the current analysis. However, it may be that nephrons are lost at a higher rate before that age, which may have had an impact on the analysis. Keller et al. specifically describe that no large numbers of sclerotic glomeruli were found [2], which would argue against a potential influence of aging on the data, but other studies did not report such data on glomerulosclerosis. Based on a re-analysis of the data in individuals between the ages of 18 and 40 that showed similar outcomes (partial correlation between nephron number and kidney weight, controlled for race: $r=0.273$, $p=0.08$), we feel that early loss of nephrons does not have a significant effect on the results.

Conclusion

This individual patient data meta-analysis shows that renal weight, and thereby renal size estimations, in adulthood should not be used as a marker for nephron numbers.

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