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ORIGINAL ARTICLE

Mild impairment of renal function (shrunken pore syndrome) is associated with increased risk for future surgery for aortic stenosis

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ABSTRACT

Recently, a new approach was proposed to detect mild impairment in renal function: a reduced ratio between estimated glomerular filtration rate (eGFR) calculated by cystatin C and eGFR calculated by creatinine. We aimed to evaluate if this ratio is associated with aortic stenosis (AS) requiring surgery. We identified 336 patients that first participated in population surveys and later underwent surgery for AS (median age [interquartile range] 59.8 [10.3] years at survey and 68.3 [12.7] at surgery, 48% females). For each patient, two matched referents were allocated. Cystatin C and creatinine were determined in stored plasma. $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ and their ratio were estimated. Conditional logistic regression analyses were used to estimate the risk (odds ratio (OR) with [95% confidence interval (CI)]) related to one (ln) standard deviation increase in the ratio between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$. A high ratio was associated with lower risk for AS requiring surgery (OR [95% CI]) (OR 0.84 [0.73–0.97]), especially in women (0.74 [0.60–0.92] vs. 0.93 [0.76–1.13] in men). After further stratification for coronary artery disease (CAD), the association remained in women with CAD but not in women without CAD (0.60 [0.44–0.83] and 0.89 [0.65–1.23], respectively). In conclusion, a high ratio between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ was associated with lower risk for surgery for AS, especially in women. Mild impairment of renal function is thus associated with future risk for AS requiring surgery.

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Aortic stenosis; valvular replacement; renal insufficiency; creatinine; cystatin C

Introduction

End-stage renal disease is a well-known risk factor for developing ischemic cardiovascular and calcific valvular disease. Also, it increases the progression rate of aortic stenosis (AS) [1,2]. Mild deterioration of renal function has also been associated with increased risk for cardiovascular disease, but it is still unknown if mild impairment of renal function is a risk factor for developing AS. To assess renal function, an estimate of the glomerular filtration rate (GFR) is often used. Accurate estimates of GFR can be obtained from Cr-EDTA or Iohexol clearance [3] – both methods are labour-intensive and expensive. More commonly, GFR is estimated from plasma levels of creatinine ($eGFR_{creatinine}$), and several equations have been presented. Another molecule in the plasma is cystatin C that also can be used for calculating eGFR ($eGFR_{cystatin\ C}$). In most cases, eGFR based on creatinine and cystatin C are similar, but $eGFR_{cystatin\ C}$ has been associated with higher risk estimates for cardiovascular disease compared to $eGFR_{creatinine}$ [4]. The two molecules differ in size, where cystatin C (13,343 Da) is larger than creatinine (113 Da). This may explain the superiority of cystatin C since early changes in kidney function are characterised by shrinking of the pores in the glomerular membranes rather than a decrease in the number of pores; i.e. larger molecules such as cystatin C will be affected first. The discrepancy in filtration estimates based on reduced pore size is expressed as a low ratio between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ and has recently been labelled as the shrunken pore syndrome [5]. This syndrome has been linked to cardiovascular disease and mortality [6–8].

The aim of this study is to test the hypothesis that mild impairment of renal function, reflected by a decreased ratio between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$, is related to increased risk for AS that requires surgery, with and without simultaneous coronary artery disease (CAD).

Methods

Between March 1988 and December 2014, 6691 patients underwent surgery for valvular heart disease and/or disease of the ascending aorta at the Department of Cardiothoracic Surgery, Umeå University Hospital, Umeå, Sweden. Before

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surgery, 708 of these patients had participated in one of three population-based health studies in the Northern Sweden Health and Disease Study. As part of the health study, each patient had donated blood for further research. Altogether 336 of the 708 patients had surgery for AS and were included in the study. From the Västerbotten Intervention Programme (VIP), 237 samples were retrieved as well as 37 samples from MONItoring of trends and determinants in CArdiovascular diseases (MONICA) and 62 samples from the Mammary Screening Program (MSP).

VIP is an ongoing community intervention programme with the aim of preventing cardiovascular disease and diabetes in the county of Västerbotten [9]. In this programme, all county residents, at the ages of 30 (until 1995), 40, 50 and 60 years, were asked to participate in a health survey and subsequently received health counselling at their primary health care centre. MONICA enrolment involved asking randomly selected individuals in the counties of Västerbotten and Norrbotten to participate in a health survey [10]. Participants were 25–74 years of age. The MSP cohort comprised women that attended routine mammography screenings [11]. Added together, these three surveys included 140,414 participants up to December 2014, with an estimated participation rate of 65–75%.

For each case, we randomly selected two referents (controls) that were matched for sex, age (±2 years), type of survey (MONICA, VIP or MSP), date of health survey (±4 months) and geographic area. We did not exclude referents or cases (patients) with a history of myocardial infarction (MI) or cancer prior to survey. In our cohort, 2.7% of cases and 3.3% of referents had been diagnosed with cancer within 5 years prior to surgery (or the corresponding date for referents). Similarly, 2.4% of cases and 1.3% of referents reported a prior MI at survey.

The study protocol was approved by the Regional Ethics Review Board in Umeå and it complied with the Declaration of Helsinki. All participants provided written informed consent for future use of the data.

Medical records were reviewed and relevant data on the valve disease were retrieved. According to established practice, all except one case (99.7%) underwent a coronary angiogram, and any atheromatosis was classed as CAD (found in 61% of all cases with coronary angiogram). At surgery, $eGFR_{creatinine}$ was (median [interquartile range]) 67 [17] ml/min/1.73 m^2, and 25.6% had an $eGFR_{creatinine}$ below 60.

We have recently described clinical examinations and biochemical analysis performed at baseline in this cohort [12,13]. Participants in VIP and MONICA were asked to complete a health questionnaire regarding their living conditions and cardiovascular risk factors. Subjects were categorised by whether they had smoked tobacco (smokers, including current daily smokers and ex-smokers) or had never smoked tobacco (never-smokers).

An oral glucose tolerance test was performed, and glucose tolerance categories were defined according to WHO guidelines [14]. Glucose intolerance was defined as impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) or diabetes mellitus (DM). Anthropometry and blood pressure measurements were obtained as previously described [12], and hypertension was defined as a systolic blood pressure $\geq$140 mmHg, diastolic blood pressure $\geq$90 mmHg and/or the use of anti-hypertensive medication.

Plasma samples were obtained after fasting for a minimum of 4 h (extended to 8 h after 1992). The samples were stored at −80 °C until analysis. In 2017, Apo A1, Apo B, creatinine, cystatin C and CRP were analysed on a Cobas 8000 modular analyzer, c502 module (Roche Diagnostics, Basel, Switzerland). The reagents employed were Tina-quant apolipoprotein A1 and B (Apo A1 and ApoB) (catalogue nos. 03032566122 and 03032574122, respectively, both version 2), CREP2 (catalogue no. 03263991190), Tina-quant Cystatin C Gen. 2 (catalogue no. 06600239190) and CRPL3 (catalogue no. 04956842190). These were all purchased from Roche Diagnostics (Basel, Switzerland). Apo A1 and Apo B were standardised to reference IFCC SP1-01 and SP3-07, respectively. Creatinine is traceable to isotope dilution mass spectrometry (IDMS) reference measurement procedure. Cystatin C is traceable to the ERMDA471/IFCC standard. CRP is traceable to CRM 470 (CRPL3 2011-01, V3).

The lowest level of detection was 0.03 g/L for both Apo A1 and Apo B, 5 µmol/L for creatinine, 0.4 mg/L for cystatin C and 3 mg/L for CRP. The total coefficients of variation were as follows: Apo A1 3.42% and 2.18% at levels of 0.86 and 1.45 mg/L, respectively; Apo B 1.93% and 2.19% at levels of 1.0 and 1.8 mg/L, respectively; creatinine 3% at both levels of 90 and 500 µmol/L; cystatin C 1.43% and 0.84% at levels of 0.99 and 3.95 mg/L, respectively; and CRP 1.5% and 1.9% at levels of 8 and 47 mg/L, respectively.

The ratio between Apo B and Apo A1 was calculated. The estimated relative glomerular filtration rate (eGFR) was calculated by using the Lund-Malmö-Revised formula for $GFR_{creatinine}$ and the Caucasian, Asian, Paediatric, Adult cohorts (CAPA) formula for $GFR_{cystatin\ C}$ [15,16]. Only 15 cases (4.8%) and 19 referents (2.9%) had an $eGFR_{creatinine}$ below 60 ml/min/1.73 m^2 at survey.

Continuous data were checked for normal distributions with formal tests and by visual assessment, and data were transformed to the natural log (ln) scale when needed. The (ln) *Z*-scores were calculated separately for men and women, and as a conservative approach, missing values were replaced with the median value obtained among the referents, calculated separately for men and women. The scores with replaced missing values were used in all models, thus using the entire dataset. Continuous variables were also categorised into quartiles, based on the distribution of the referent values, and they were determined separately for men and women. Missing values were treated as a separate category and were not included in the tables.

Data are presented as (geometric) means with 95% confidence intervals (CIs). Student's *t*-tests were used to analyse differences in the means between cases and referents. Within strata, the cases and referents had the same follow-up times in this nested, matched case-referent study. Therefore, we estimated odds ratios (ORs) and 95% CI with logistic regression analyses (rather than Cox regression) and

the conditional maximum likelihood routine designed for matched analysis. The influence of studied variables on future surgery for AS was tested in univariable and multivariable models. The first model included the ratio between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ (or $eGFR_{cystatin\ C}$, $eGFR_{creatinine}$, cystatin C or creatinine) with the addition of the Apo B/A1 ratio, hypertension (yes/no), glucose intolerance (yes/no) and smoking (present or past/never). In subsequent models, BMI or CRP was added. The analyses were stratified for sex, age at surgery (less than 60 years or 60 years and more), the time between the survey and surgery (less than 5 years or 5 years and more) and the presence of any CAD on the preoperative angiogram. Finally, as a sensitivity analysis, we excluded the MSP cohort since several cardiovascular risk factors were not registered in MSP. All calculations were performed with the statistical program, SPSS version 24 (IBM, Armonk, NY).

Results

Basal characteristics are shown in Table 1. Median age [IQR] was 59.8 [10.3] years at survey and 68.3 [12.7] at surgery; 48% were women. All patients underwent aortic valve replacement (AVR) due to AS as a primary indication in 84%, and in combination with other procedures in 16% (surgery for ascending aorta in 5% and coronary by-pass surgery in 10%). At survey, individuals with future surgery for AS were more obese, had higher systolic and diastolic blood pressure, and had higher total cholesterol levels and higher Apo B/A1 ratio. They also more often had a diagnosis of hypertension and were more often glucose intolerant. At survey, circulating levels of creatinine and cystatin C did not differ between cases and referents, and similarly $eGFR_{creatinine}$ and $eGFR_{cystatin\ C}$ did not differ. In contrast, cases had a lower ratio between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ at survey. This difference remained in women.

The associations between circulating levels of creatinine, cystatin C and corresponding eGFR rates and future AVR were explored, and results of the univariate analysis based on both categorical and continuous analyses are presented in Table 2. Expressed as continuous variables (1 [ln] SD increase of *Z*-scores), no associations were seen between circulating levels of creatinine, cystatin C and corresponding eGFR rates and future AVR.

In contrast, a high ratio between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ was consistently associated with lower risk for surgery (Table 3). The association between 1 (ln) SD increase of the ratio remained significant after adjustment for common cardiovascular risk factors (OR [95% CI]) (0.84 [0.73–0.97]), and also after additional adjustments for BMI and CRP. Exclusion of the MSP cohort did not alter the association. After stratification for sex, the protective effect associated with a high ratio was seen in women but not in men (0.74 [0.60–0.92] and 0.93 [0.76–1.13], respectively). After further stratification for CAD, the association remained significant in women with CAD but not in men with CAD (0.60 [0.44–0.83] and 0.96 [0.75–1.23], respectively). Further adjustments for CRP and BMI or exclusion

Table 1. Characteristics at health survey.

	Referents/cases	Referents	Cases	*p*
Age at survey (years)	671/336	56.7 (56.0–57.3)	56.7 (55.8–57.6)	.94
Age at surgery (years)	–/336		67.2 (66.3–68.2)	
Female sex (%)	671/336	48 (44–52)	48 (43–53)	.98
BMI (kg/m^2)	655/322	26.1 (25.8–26.4)	26.9 (26.4–27.4)	**.01**
Apolipoprotein B (g/L)[a]	647/310	1.09 (1.07–1.11)	1.13 (1.10–1.16)	**.05**
Apolipoprotein A1 (g/L)[a]	647/309	1.41 (1.40–1.43)	1.40 (1.37–1.42)	.25
Apolipoprotein B/A1 (ratio)[a]	647/309	0.77 (0.76–0.79)	0.81 (0.78–0.84)	**.01**
Systolic blood pressure (mmHg)	545/270	135 (134–137)	138 (136–141)	**.04**
Diastolic blood pressure (mmHg)	545/269	84 (84–85)	86 (85–87)	**.05**
Total cholesterol (mmol/L)	535/265	6.2 (6.1–6.3)	6.4 (6.2–6.5)	**.05**
Hypertension (%)	545/269	49.2 (45.0–53.4)	61.0 (55.1–66.8)	**.001**
Glucose intolerance (%)	490/242	19.8 (16.3–23.3)	26.4 (20.8–32.0)	**.05**
Smoker (%)	531/258	53.7 (49.4–57.9)	59.7 (53.7–65.7)	.11
Creatinine (μmol/L)[a]	647/310	73.2 (72.1–74.2)	72.3 (70.7–73.8)	.33
Cystatin C (mg/L)[a]	647/309	0.84 (0.83–0.85)	0.85 (0.83–0.87)	.15
$eGFR_{creatinine}$ ($ml/min/1.73\ m^2$)[a]	647/310	79.7 (78.8–80.6)	80.0 (78.6–81.5)	.66
Men		81.4 (80.2–82.6)	81.8 (79.9–83.7)	.76
Women		77.8 (76.5–79.2)	78.3 (76.2–80.5)	.68
No CAD		81.0 (79.6–82.5)	80.9 (78.5–83.2)	.89
CAD		78.8 (77.6–80.0)	79.4 (77.6–81.2)	.60
$eGFR_{cystatin\ C}$ ($ml/min/1.73\ m^2$)[a]	647/309	91.0 (89.6–92.4)	88.9 (86.6–91.4)	.15
Men		91.6 (89.7–93.5)	90.9 (87.6–94.3)	.74
Women		90.4 (88.3–92.5)	87.0 (83.6–90.5)	.09
No CAD		94.1 (91.8–96.5)	92.0 (88.2–96.0)	.34
CAD		89.0 (87.3–90.8)	86.8 (83.8–89.9)	.23
$eGFR_{cystatin\ C}/eGFR_{creatinine}$ (ratio)[a]	647/309	1.14 (1.13–1.16)	1.11 (1.09–1.13)	**.02**
Men		1.12 (1.11–1.14)	1.11 (1.08–1.14)	.48
Women		1.16 (1.14–1.18)	1.11 (1.08–1.14)	**.008**
No CAD		1.16 (1.14–1.18)	1.14 (1.10–1.17)	.24
CAD		1.13 (1.11–1.15)	1.09 (1.06–1.12)	**.04**

BMI: body mass index; glucose intolerance: impaired fasting glucose and/or impaired glucose intolerance or diabetes mellitus; hypertension: systolic blood pressure ≥140 and/or diastolic blood pressure ≥90 and/or antihypertensive treatment; smoker: present or previous smoker.
Values shown are numbers, means ([a]geometric), and proportions with 95% confidence intervals; *p* values were based on the Student *t*-test.
Bold values are p-value ≤ 0.05

Table 2. Creatinine and cystatin C and risk for future AVR.

		All			No CAD			CAD		
	Ref/case	All	Men	Women	All	Men	Women	All	Men	Women
Creatinine										
Q1	169/95	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Q2	174/81	0.81 (0.56–1.18)	1.17 (0.71–1.94)	**0.53 (0.30–0.93)**	0.77 (0.43–1.37)	1.21 (0.51–2.89)	0.53 (0.23–1.23)	0.86 (0.53–1.39)	1.16 (0.63–2.15)	0.52 (0.23–1.16)
Q3	165/65	0.68 (0.45–1.01)	0.86 (0.49–1.52)	**0.53 (0.30–0.94)**	0.84 (0.46–1.53)	0.98 (0.38–2.54)	0.83 (0.37–1.84)	**0.58 (0.34–0.99)**	0.80 (0.39–1.62)	**0.35 (0.15–0.82)**
Q4	139/69	0.85 (0.57–1.27)	0.83 (0.47–1.47)	0.83 (0.46–1.48)	1.09 (0.57–2.06)	0.77 (0.26–2.32)	1.29 (0.57–2.94)	0.75 (0.45–1.27)	0.90 (0.46–1.76)	0.53 (0.22–1.27)
Cystatin C										
Q1	165/78	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Q2	161/66	0.88 (0.59–1.32)	0.92 (0.52–1.62)	0.85 (0.47–1.51)	0.82 (0.45–1.51)	0.77 (0.30–1.98)	0.87 (0.40–1.91)	0.92 (0.53–1.60)	1.04 (0.51–2.12)	0.79 (0.33–1.87)
Q3	160/72	1.02 (0.69–1.53)	1.00 (0.56–1.80)	1.05 (0.60–1.82)	1.14 (0.61–2.13)	1.16 (0.42–3.19)	1.10 (0.49–2.46)	0.94 (0.56–1.60)	0.92 (0.45–1.90)	0.99 (0.46–2.12)
Q4	161/93	1.32 (0.86–2.01)	1.43 (0.80–2.57)	1.21 (0.66–2.24)	1.19 (0.60–2.39)	1.76 (0.59–5.24)	0.92 (0.37–2.28)	1.42 (0.83–2.43)	1.38 (0.69–2.78)	1.53 (0.66–3.55)
$eGFR_{creatinine}$										
Q1	162/79	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Q2	162/73	0.93 (0.62–1.40)	1.02 (0.55–1.87)	0.84 (0.48–1.47)	0.89 (0.45–1.76)	0.63 (0.19–2.06)	1.08 (0.46–2.54)	0.96 (0.57–1.59)	1.22 (0.60–2.50)	0.72 (0.34–1.53)
Q3	162/72	0.94 (0.62–1.42)	0.82 (0.45–1.49)	1.05 (0.58–1.91)	0.99 (0.51–1.94)	0.77 (0.25–2.38)	1.08 (0.46–2.50)	0.90 (0.52–1.53)	0.82 (0.40–1.67)	1.07 (0.46–2.50)
Q4	161/86	1.13 (0.73–1.73)	1.12 (0.62–2.05)	1.13 (0.60–2.12)	0.97 (0.49–1.90)	0.97 (0.33–2.87)	0.83 (0.34–2.02)	1.24 (0.70–2.19)	1.12 (0.53–2.36)	1.58 (0.63–3.98)
$eGFR_{cystatin\ C}$										
Q1	161/92	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Q2	162/66	0.72 (0.48–1.06)	**0.56 (0.32–0.98)**	0.91 (0.52–1.57)	0.96 (0.51–1.81)	0.49 (0.17–1.43)	1.42 (0.63–3.18)	0.58 (0.35–0.96)	0.54 (0.27–1.07)	0.61 (0.28–1.32)
Q3	163/77	0.78 (0.52–1.18)	0.85 (0.48–1.51)	0.71 (0.39–1.31)	0.84 (0.44–1.62)	0.67 (0.25–1.76)	1.02 (0.42–2.51)	0.75 (0.43–1.28)	0.90 (0.44–1.82)	0.54 (0.23–1.27)
Q4	161/74	0.74 (0.48–1.14)	0.65 (0.36–1.19)	0.85 (0.45–1.61)	0.85 (0.41–1.74)	0.46 (0.15–1.42)	1.31 (0.50–3.40)	0.67 (0.38–1.16)	0.72 (0.35–1.48)	0.59 (0.24–1.42)
Z (ln) creatinine	647/310	0.95 (0.83–1.08)	0.96 (0.80–1.16)	0.93 (0.77–1.13)	1.01 (0.81–1.26)	0.94 (0.66–1.35)	1.05 (0.80–1.39)	0.92 (0.78–1.10)	1.00 (0.80–1.24)	0.82 (0.62–1.10)
Z (ln) cystatin C	647/309	1.12 (0.97–1.29)	1.03 (0.85–1.25)	1.23 (1.00–1.51)	1.14 (0.91–1.43)	1.12 (0.77–1.65)	1.15 (0.87–1.53)	1.11 (0.93–1.33)	1.01 (0.81–1.26)	1.32 (0.98–1.78)
Z (ln) $eGFR_{creatinine}$	647/310	1.05 (0.90–1.23)	1.05 (0.84–1.30)	1.06 (0.85–1.32)	0.99 (0.77–1.27)	1.10 (0.71–1.70)	0.93 (0.68–1.27)	1.08 (0.89–1.31)	1.01 (0.79–1.29)	1.20 (0.86–1.68)
Z (ln) $eGFR_{cystatin\ C}$	647/309	0.89 (0.77–1.02)	0.96 (0.79–1.17)	0.81 (0.65–1.00)	0.87 (0.69–1.11)	0.88 (0.59–1.31)	0.87 (0.65–1.16)	0.89 (0.74–1.07)	0.99 (0.78–1.24)	0.75 (0.55–1.02)

AVR: aortic valve replacement; CAD: coronary artery disease.

Values are the odds ratios with (95% confidence intervals) related to creatinine and cystatin C levels and corresponding estimated glomerular filtration rates (eGFR).

The risk related to quartiles (Q1–Q4) and 1 (ln) SD increase (*Z*-score) is shown. *Z*-scores with missing values replaced were used for all calculations. Fifty missing values for creatinine (and $eGFR_{creatinine}$) and 51 missing values for cystatin C (and $eGFR_{cystatin\ C}$) were replaced with the median values obtained for the referents (sex-specific).

Cut-offs for the quartiles (Q1–Q4) were: creatinine (μmol/L) (men and women): 75.6, 82.3, 88.5 and 71.5, 78.9, 86.7, respectively; cystatin C (mg/L) (men and women): 0.76, 0.83, 0.92 and 0.75, 0.83, 0.94, respectively. $eGFR_{creatinine}$ (ml/min/1.73 m^2) (men and women): 75.6, 82.3, 88.5 and 71.5,78.9, 86.7, respectively; and $eGFR_{cystatin\ C}$ (ml/min/1.73 m^2) (men and women): 82.5, 91.8, 103.5 and 78.6, 91.3, 104.4, respectively.

P values indicating a trend: .33 for creatinine, .24 for cystatin C, .66 for $eGFR_{creatinine}$, and .37 for $eGFR_{cystatin\ C}$.

Table 3. $eGFR_{cystatin\ C}/eGFR_{creatinine}$ ratio and risk for future AVR.

		All			No CAD			CAD		
Quartiles	Case/Ref	All	Men	Women	All	Men	Women	All	Men	Women
Q1	91/160	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Q2	85/163	0.91 (0.61–1.34)	0.98 (0.56–1.70)	0.84 (0.49–1.46)	1.26 (0.66–2.40)	1.91 (0.65–5.55)	1.00 (0.44–2.25)	0.72 (0.44–1.19)	0.72 (0.37–1.41)	0.72 (0.34–1.53)
Q3	71/162	0.75 (0.51–1.10)	0.79 (0.45–1.38)	0.70 (0.41–1.21)	0.76 (0.40–1.45)	0.81 (0.30–2.19)	0.75 (0.32–1.74)	0.77 (0.47–1.27)	0.86 (0.43–1.72)	0.70 (0.34–1.45)
Q4	62/162	**0.62 (0.40–0.95)**	0.73 (0.40–1.32)	**0.52 (0.28–0.97)**	0.79 (0.40–1.57)	0.64 (0.20–2.00)	0.90 (0.38–2.13)	**0.53 (0.31–0.94)**	0.82 (0.40–1.68)	**0.26 (0.10–0.69)**
Z (ln) ratio										
Case/Ref		336/671	175/349	161/322	132/264	54/108	78/156	203/405	120/239	83/166
Univariable		**0.84 (0.73–0.97)**	0.93 (0.77–1.13)	**0.74 (0.60–0.92)**	0.86 (0.69–1.08)	0.85 (0.60–1.19)	0.87 (0.64–1.18)	**0.83 (0.69–0.99)**	0.99 (0.78–1.24)	**0.65 (0.49–0.87)**
Multivariable										
Model 1		**0.84 (0.73–0.97)**	0.93 (0.76–1.13)	**0.74 (0.60–0.92)**	0.89 (0.70–1.13)	0.89 (0.62–1.28)	0.89 (0.65–1.23)	**0.81 (0.67–0.97)**	0.96 (0.75–1.23)	**0.60 (0.44–0.83)**
Model 2		**0.85 (0.73–0.98)**	0.94 (0.77–1.15)	**0.75 (0.60–0.94)**	0.92 (0.72–1.17)	0.96 (0.66–1.42)	0.89 (0.64–1.24)	**0.80 (0.66–0.97)**	0.96 (0.75–1.24)	**0.56 (0.40–0.80)**
Model 3		**0.84 (0.73–0.98)**	0.94 (0.77–1.15)	**0.74 (0.60–0.93)**	0.87 (0.68–1.11)	0.81 (0.56–1.19)	0.89 (0.64–1.24)	**0.82 (0.68–0.99)**	1.00 (0.77–1.29)	**0.60 (0.43–0.84)**
Model 4		**0.80 (0.68–0.95)**	0.93 (0.77–1.13)	**0.53 (0.37–0.75)**	0.77 (0.57–1.03)	0.89 (0.62–1.28)	**0.52 (0.28–0.94)**	0.81 (0.66–1.01)	0.96 (0.75–1.23)	**0.47 (0.29–0.77)**

AVR: aortic valve replacement; CAD: coronary artery disease.
Values are numbers and the odds ratios with (95% confidence intervals) for 1 (ln) SD increase (*Z*-score) in the $eGFR_{cystatin\ C}/eGFR_{creatinine}$ ratio. *Z*-scores with missing values replaced were used for all calculations; 51 missing values were replaced with the median values obtained for the referents (sex-specific).
Cut-offs for the quartiles (Q1–Q4, $eGFR_{cystatin\ C}/eGFR_{creatinine}$ ratio) were (men and women): 1.02, 1.13, 1.25 and 1.03, 1.17, 1.30, respectively. *P* value indicating a trend was .03 for the ratio.
Model 1 includes, in addition to the $eGFR_{cystatin\ C}/eGFR_{creatinine}$ ratio, apolipoprotein B/A1 ratio (*Z*-score), glucose intolerance (yes/no), hypertension (yes/no) and smoking (present or past/never). Model 2 includes model 1 plus BMI, and model 3 includes model 1 plus hsCRP. Model 4 is identical to model 1 but all individuals from the MSP survey were excluded.

of the MSP cohort did not change this association in those with CAD. In women with CAD, the association remained in those aged 60 years or more at surgery and if surgery was performed more than 5 years after survey (0.62 [0.45–0.85] and 0.59 [0.41–0.84], respectively).

Similarly, the highest quartile of the ratio (cut-offs 1.25 and 1.30 in men and women, respectively) was associated with reduced risk for AVR in all women and in women with CAD, and the linear trend indicated a significant association (p=.03).

Discussion

In the present study with a nested-case referent design, we show that a high ratio between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$, indicating a normal renal function is associated with reduced risk for future surgery for AS in women but less or not at all in men, and in particular in women with concomitant CAD. It is known that end-stage renal disease is associated with the development of AS and a rapid progression of the disease [2]; however, whether mild impairment of renal function associates with future risk for AS has not been studied, to our knowledge, in a prospective manner. Several of the traditional cardiovascular risk factors have been attributed to the development of AS but most of the early studies were small, cross-sectional and biased regarding inclusion criteria as stated by Stewart et al. [17]. Recently, we reported an association between traditional cardiovascular risk factors (hypertension, diabetes, hypercholesterolemia and smoking) and the risk for future surgery for AS in the present cohort, and this risk was mainly seen in those with concomitant CAD [13]. This was recently confirmed in a large Canadian study [18] of elderly individuals, not stratified for CAD.

Several biomarkers have also been discussed as markers of AS severity, and that potentially might identify subjects that benefit from early AVR [19]. However, most of these biomarkers have not gained acceptance and usage. Only B-type natriuretic peptide (BNP) has been incorporated in current guidelines [20].

A low ratio between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ has recently been suggested as representing a new syndrome, named shrunken pore syndrome [5], and has been shown to be a risk marker for cardiovascular disease and mortality [6,7,21,22], including subjectively healthy elderly [8]. An increased retention of 5–40 kDa molecules compared to small molecules, like creatinine and water, has been described in this condition. These larger molecules include atherosclerosis-promoting proteins [5,21]. A recent study showed that the lower the ratio between the $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ used to identify the syndrome, the higher was the mortality associated with it [22].

Our findings indicate that the development of AS is a part of a general vascular disorder, and that the low ratio between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ indicates early vascular damage, i.e. endothelial dysfunction. Endothelial dysfunction is an early indicator of cardiovascular disease and renal dysfunction [23], and our findings of the predictive

value of a decreased ratio 10 years before AVR suggest that the process leading to AVR starts early, that is supported by recent observations on the accumulation of atherosclerosis-promoting proteins in shrunken pore syndrome [21].

Microalbuminuria has been found to be associated with cardiovascular disease and endothelial dysfunction, e.g. in CAD [24] and cystatin C is correlated with microalbuminuria [25]. Cystatin C in serum has also been found to be associated with the metabolic syndrome [26]. In a recent study, $eGFR_{cystatin\ C}$, or a combination of creatine and cystatin C was associated with HOMA-IR (homeostatic model assessment of insulin resistance) among men, whereas $eGFR_{creatinine}$ only was not [27]. The causality has not been proven, as genes causing elevated cystatin C does not increase risk for metabolic syndrome [28].

The suggestion of a sex-related difference in the association between a low ratio and AVR is intriguing and has not been described previously as related to other cardiovascular outcomes and mortality. We found no sex-related differences related to traditional and biochemical risk-markers (lipoprotein(a), Apo B and Apo A1) in our previous reports based on the same cohort [12,13]. There may be sex-related differences in the vulnerability for traditional risk-markers [29], and we and others have shown differences related to circulating levels and prognostic ability of the adipokine leptin, which also affects endothelial function and relates to AS [30,31].

One limitation of this study is that only patients who underwent surgery for AS are included, thereby leaving those who are recommended a non-surgical strategy due to comorbidities such as renal dysfunction, older age and pulmonary diseases. Therefore, the data are not automatically transferable to more severe renal disease. Notably, only one person had a ratio below 0.6, 10 years prior to surgery. Furthermore, those with mild and moderate states of AS are not included (except those with CAD as a primary indication for intervention). Second, the inclusion criteria for participating in VIP and MSP studies are related to age and sex. In VIP, only those who are 30, 40, 50 and 60 years of age are asked to participate, and MSP includes women in the age span of 40–74. These limitations of inclusion might have caused an underrepresentation of younger individuals. Furthermore, health surveys tend to attract the healthier part of the population. Last, there were no urine samples donated in the cohort, thus we could not measure albuminuria, which is an early marker for renal impairment and a predictor of cardiovascular disease.

Discrepancies in filtration estimates derived from cystatin C and creatinine can also arise from conditions affecting circulating creatinine levels such as total muscle mass and protein catabolism, or conditions affecting circulating cystatin C levels such as thyroid disease or steroid administration. We do not believe such conditions play any significant role in the present study. Notably, circulating levels of cystatin C are unrelated to inflammation [32].

Even if there are limitations, the study has several strengths. First, the cohort is large and is thoroughly evaluated. Furthermore, the vast majority of all surgeries for AS in Northern Sweden are performed at the Umeå University Hospital, and all AS cases that require AVR in the three cohorts are probably identified, thus minimising selection bias. Other strengths are the standardised procedure of blood sample collection and that all blood samples were analysed at the same laboratory at a defined period of time, and that the cases and their matched referents were analysed in fixed triplets at the same run. These procedures reduce both pre-analytical and analytical biases as are often encountered in large prospective studies.

In conclusion, in this prospective study approach, we found that a high ratio between $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$, indicating normal kidney function and the absence of the shrunken pore syndrome, is associated with a reduced risk for AS requiring surgery, and in particular in women. These intriguing and new findings encourage studies focusing on sex-related differences in risk factors for AS.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability

The datasets generated during the current study are not publicly available as the dataset contains identifiable patient data but are available from the corresponding author on reasonable request.

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