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ORIGINAL ARTICLE

Reduction in glomerular pore size is not restricted to pregnant women. Evidence for a new syndrome: 'Shrunken pore syndrome'

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Abstract

The plasma levels of cystatin C, β_2-microglobulin, beta-trace protein, retinol binding protein (RBP) and creatinine were determined in plasma samples from 111 randomly selected patients with $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$ and from 55 control patients with $0.9eGFR_{creatinine} \leq eGFR_{cystatin\ C} \leq 1.1eGFR_{creatinine}$ ($eGFR_{cystatin\ C} \approx eGFR_{creatinine}$). The concentration ratios of cystatin C/creatinine, β_2-microglobulin/creatinine, beta-trace protein/creatinine and RBP/creatinine were significantly higher in patients with $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$ than in patients with $eGFR_{cystatin\ C} \approx eGFR_{creatinine}$. When the patients were divided into three groups with different estimated GFR intervals (≤ 40, 40–60 and ≥ 60 mL/min/1.73m^2) the concentration ratios of cystatin C/creatinine, β_2-microglobulin/creatinine, and beta-trace protein/creatinine were significantly higher in patients with $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$ than in patients with $eGFR_{cystatin\ C} \approx eGFR_{creatinine}$ for all GFR intervals. Similar results were obtained when the population without pregnant women was studied as well as the subpopulations of men or of non-pregnant women. Populations of pre-eclamptic women and pregnant women in the third trimester display similar results. Since the production of these four proteins with sizes similar to that of cystatin C is not co-regulated, the most likely explanation for the simultaneous increase of their creatinine-ratios in patients with $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$ is that their elimination by glomerular filtration is decreased. We suggest that this is due to a reduction in pore diameter of the glomerular membrane and propose the designation 'Shrunken pore syndrome' for this pathophysiological state.

Key Words: *Beta-trace protein, creatinine, cystatin C, glomerular filtration rate, kidney diseases, beta 2-microglobulin, retinol-binding proteins*

Introduction

Glomerular filtration rate (GFR) is defined as the volume of glomerular filtrate produced per unit of time and expressed, e.g. as mL/min/1.73m^2. More than 95% of the filtrate consists of water with a molecular mass of 18 Da. GFR is a good general indicator of renal disease and is measured by invasive procedures involving determinations of the urine or plasma clearance of substances like iohexol, ^{51}Cr-EDTA, ^{99m}Tc-diethylenetriaminepentaacetic acid or ^{125}I-iothalamate, which are freely filtered through the glomerular membranes of the kidneys and not absorbed or secreted by the tubular cells [1]. All of these substances have molecular masses below 1000 Da and sieving coefficients close to 1. However, the hypothetical pores of the glomerular membranes of healthy kidneys are also considered to allow substantial filtration of molecules up to about 20,000 Da for which sieving coefficients above 0.5 have been suggested [2–4]. Invasive procedures to determine GFR are expensive, slow and not without risks to the patients, and GFR is therefore most often estimated (eGFR) by use of the plasma concentration of creatinine with a molecular mass of 113 Da. Cystatin C (≈ 13,300 Da) was suggested as a marker of GFR in 1979 [5–7] and there is presently an emerging consensus that cystatin C- and creatinine-based GFR-estimating equations display similar diagnostic performances, if race, sex and age terms supplement creatinine in the creatinine-based equations. We have

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observed that the plasma level of cystatin C, in contrast to that of creatinine, increases during the third pregnancy trimester, indicating a decrease of GFR [8]. In contrast, invasive measurement of GFR, using the low-molecular-mass substances referred to above, do not indicate a decrease of GFR during the third trimester; neither do creatinine-based GFR-estimating equations [8]. We interpreted these results as reflecting a reduced pore size of the glomerular membranes during the third trimester representing a tentative syndrome of shrunken pores. Further studies of the plasma levels of two other proteins, β_2-microglobulin and beta-trace protein, during pregnancy supported this interpretation [9,10]. Our studies of the plasma levels of cystatin C, β_2-microglobulin ($\approx$11,700 Da) and beta-trace protein (23,000–29,000 Da) in pre-eclampsia showed similar results with still higher levels of these proteins, indicating that an even more pronounced shrinking of pore size is also a feature of pre-eclampsia [11,12]. To study if evidence could be found that the glomerular membrane pore size shrinking process is not unique to pregnant women, we used 1349 consecutive patient samples arriving at the laboratory with a request for eGFR. $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ were calculated using modern GFR-estimating equations [13,14] traceable to international reference materials ('calibrators'). Samples displaying values of $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ within $\pm$10% (i.e. $0.9eGFR_{creatinine} \leq eGFR_{cystatin\ C} \leq 1.1eGFR_{creatinine}$) were compared to samples for which $eGFR_{cystatin\ C}$ was $\leq$60% of $eGFR_{creatinine}$ concerning the levels of β_2-microglobulin, beta-trace protein and retinol-binding protein (RBP $\approx$ 20,600 Da). These proteins were selected because the known factors influencing the production of cystatin C do not generally influence the production of these proteins in the same way. The results indicate that the shrunken pore syndrome noticed in pregnant women is a common condition also in men and non-pregnant women.

Materials

Consecutive plasma samples from 1349 patients, 731 men and 618 women, between 3 and 95 years of age, for which eGFR was requested were either used within 24 h for analysis of cystatin C, creatinine, β_2-microglobulin, beta-trace protein and RBP or frozen and stored at -20°C until analyzed.

Methods

The plasma level of cystatin C was determined by an automated particle-based immunoassay, adjusted to the international reference preparation ERM-DA 471/IFCC [13] and that of creatinine by an enzymatic colorimetric assay with an IDMS-traceable calibrator [14]. Both assays were run on a cobas c-system (Roche Diagnostics, Basel, Switzerland). On a BN-ProSpec Nephelometer (Siemens Healthcare, Erlangen, Germany) the levels of β_2-microglobulin and beta-trace protein were determined by particle-based immunoassays [15,16], whereas that of RBP was determined by immunonephelometry [17]. All assays were performed according to the manufacturers instructions.

Recent GFR-estimating equations, traceable to international reference materials, were used to determine $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ [13,14].

Statistical analysis was performed using StatView (SAS Institute Inc, version 5.0.1). For testing the differences between groups a non-parametric method (Mann-Whitney U test) was used. A p-value < 0.05 was considered significant.

Results

The $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ of the 1349 patients in the study differed less than $\pm$10% (i.e. $0.9eGFR_{creatinine} \leq eGFR_{cystatin\ C} \leq 1.1eGFR_{creatinine}$) for 277 (20.5%) of the patients, while 111 (8.2%) of the patients displayed an $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$. We selected all samples from the 111 patients with $eGFR_{cystatin\ C} \leq 0.6eGFR_{creatinine}$ and 55 samples from randomly chosen control patients with $0.9eGFR_{creatinine} \leq eGFR_{cystatin\ C} \leq 1.1eGFR_{creatinine}$ (here called '$eGFR_{cystatin\ C} \approx eGFR_{creatinine}$') for determinations of their plasma levels of β_2-microglobulin, beta-trace protein and RBP in addition to their levels of cystatin C and creatinine. Table I describes some parameters for these two patient groups.

Table I. Characteristics of the patient groups with $0.9eGFR_{creatinine} \leq eGFR_{cystatin\ C} \leq 1.1eGFR_{creatinine}$ or with $eGFR_{cystatin\ C} \leq 0.6eGFR_{creatinine}$. Data are given as medians (2.5–97.5 percentiles), except for Numbers and Male/Female.

	$eGFR_{cystatinC} \approx eGFR_{creatinine}$	$eGFR_{cystatinC} \leq 0.6eGFR_{creatinine}$
Numbers	55	111
Age (years)	74 (35–94)	68 (22–94)
Male/Female	23/32	61/50
Cystatin C (mg/L)	1.30 (0.92–2.73)	1.88 (1.23–2.82)
Creatinine (umol/L)	91 (51–211)	83 (44–140)
$eGFR_{mean}$ (mL/min/1.73m^2)	56 (19–102)	45 (22–80)

Since the creatinine and cystatin C concentrations in the GFR-estimating equations used are the dominating terms in deciding the eGFR-values [13,14], the two patient groups will differ in cystatin C/creatinine ratios. Table II shows that the cystatin C/creatinine ratio, expressed as mg/L/μmol/L = mg/μmol, was 0.014 for the group with $eGFR_{cystatin\ C} \approx eGFR_{creatinine}$, whereas it was 0.023 for the group where $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$, a significant statistical difference ($p < 0.0001$). The level of any substance in a system in steady state is decided by its production and clearance rates. If the increase in cystatin C is caused by a decrease in its clearance rate, e.g. caused by shrunken pores in the glomerular membranes, the concentration of substances of similar size as cystatin C will also increase. We therefore determined the concentrations of the proteins β_2-microglobulin, beta-trace protein and RBP in the same samples for which we determined the cystatin C and creatinine levels, and calculated the corresponding protein/creatinine ratios. Table II demonstrates that all three ratios, β_2-microglobulin/creatinine, beta-trace protein/creatinine and RBP/creatinine, were significantly higher in the group with $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$ than in the group with $eGFR_{cystatin\ C} \approx eGFR_{creatinine}$, agreeing with the result for the cystatin C/creatinine ratios. The median creatinine level for the patient group with $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$ was slightly less than that of the patients with $eGFR_{cystatin\ C} \approx eGFR_{creatinine}$ (Table I). To exclude the possibility that this was causing the significant statistical differences between the protein/creatinine-ratios between the two patient groups, the 23 patients with the lowest creatinine levels of the patient group with $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$ were omitted so that the median creatinine levels of the two patient groups became identical. This did not change the statistical outcome (results not shown).

To investigate if similar results were obtained at different levels of GFR, we used the best obtainable estimation of GFR for all patients, which is the mean, $eGFR_{mean}$, of $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ [18–21], and separated the patients into three groups with $eGFR_{mean} \leq 40$, 40–60 and ≥ 60 mL/min/1.73m^2 (Table II). The β_2-microglobulin/creatinine- and beta-trace protein/creatinine-ratios were significantly higher in all groups with $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$ than in all groups with $eGFR_{cystatin\ C} \approx eGFR_{creatinine}$ irrespective of the level of $eGFR_{mean}$ (Table II). The RBP/creatinine-ratios were significantly higher in the group with $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$ than in the group with $eGFR_{cystatin\ C} \approx eGFR_{creatinine}$ for $eGFR_{mean}$ between 40 and 60 mL/min/1.73m^2, but not for $eGFR_{mean} \leq 40$ or ≥ 60 mL/min/1.73m^2 (Table II).

To exclude that the statistically significant differences observed for the protein/creatinine ratios were caused by the presence of pregnant women in the groups with $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$, the results from all possibly pregnant women were omitted before the statistical calculations. This did not change the observed statistical differences (Table III).

In men (Table IV) and in non-pregnant women (Table V) virtually the same differences in protein/creatinine ratios were found as in the unselected population (Table II).

Discussion

The structure of the glomerular filtration barrier (GFB) is complex and there is no generally accepted 3-D model available for its description, although

Table II. Statistical analysis of the relations between the ratios cystatin C/creatinine, β_2-microglobulin/creatinine, beta-trace protein/creatinine and RBP/creatinine for all patients and for all patients stratified for estimated GFR. Medians and *p*-values are given for the protein-creatinine ratios.

	Gender (male/female)	Cystatin C/ creatinine	β_2-microglobulin/ creatinine	beta-trace protein/ creatinine	RBP/creatinine
$eGFR_{mean}$, all					
$eGFR_{cystatinC} \approx eGFR_{creatinine}$	32/23	0.014	0.028	0.009	0.518
$eGFR_{cystatinC} \leq 0.6 eGFR_{creatinine}$	50/61	0.023	0.049	0.012	0.639
p-value		< 0.0001	< 0.0001	< 0.0001	0.0022
$eGFR_{mean} \leq 40$ mL/min/1.73m^2					
$eGFR_{cystatinC} \approx eGFR_{creatinine}$	11/4	0.012	0.029	0.010	0.448
$eGFR_{cystatinC} \leq 0.6 eGFR_{creatinine}$	19/22	0.020	0.045	0.011	0.525
p-value		< 0.0001	0.0002	0.0117	0.1768
$eGFR_{mean}$ 40–60 mL/min/1.73m^2					
$eGFR_{cystatinC} \approx eGFR_{creatinine}$	7/10	0.015	0.031	0.009	0.557
$eGFR_{cystatinC} \leq 0.6 eGFR_{creatinine}$	22/21	0.024	0.053	0.012	0.704
p-value		< 0.0001	< 0.0001	0.0004	0.0057
$eGFR_{mean} \geq 60$ mL/min/1.73m^2					
$eGFR_{cystatinC} \approx eGFR_{creatinine}$	14/9	0.015	0.025	0.009	0.558
$eGFR_{cystatinC} \leq 0.6 eGFR_{creatinine}$	9/18	0.024	0.049	0.013	0.658
p-value		< 0.0001	< 0.0001	< 0.0001	0.0688

Table III. Statistical analysis of the relations between the ratios cystatin C/creatinine, β_2-microglobulin/creatinine, beta-trace protein/creatinine and RBP/creatinine for all non-pregnant patients (men + women) and for non-pregnant patients stratified for their estimated GFR. Medians and *p*-values are given for the protein-creatinine ratios.

	Gender (male/female)	Cystatin C/ creatinine	β_2-microglobulin/ creatinine	beta-trace protein/ creatinine	RBP/creatinine
$eGFR_{mean,}$ men and non-pregnant women					
$eGFR_{cystatinC} \approx eGFR_{creatinine}$	32/23	0.014	0.028	0.009	0.518
$eGFR_{cystatinC} \leq 0.6eGFR_{creatinine}$	50/48	0.022	0.052	0.012	0.637
p-value		<0.0001	<0.0001	<0.0001	0.0034
$eGFR_{mean} \leq 40$ mL/min/1.73m^2					
$eGFR_{cystatinC} \approx eGFR_{creatinine}$	11/4	0.012	0.029	0.010	0.448
$eGFR_{cystatinC} \leq 0.6eGFR_{creatinine}$	19/22	0.020	0.045	0.011	0.525
p-value		<0.0001	0.0002	0.0117	0.1768
$eGFR_{mean}$ 40–60 mL/min/1.73m^2					
$eGFR_{cystatinC} \approx eGFR_{creatinine}$	7/10	0.015	0.031	0.009	0.557
$eGFR_{cystatinC} \leq 0.6eGFR_{creatinine}$	22/18	0.024	0.053	0.012	0.736
p-value		<0.0001	<0.0001	0.0004	0.0031
$eGFR_{mean} \geq 60$ mL/min/1.73m^2					
$eGFR_{cystatinC} \approx eGFR_{creatinine}$	14/9	0.015	0.025	0.009	0.558
$eGFR_{cystatinC} \leq 0.6eGFR_{creatinine}$	9/8	0.024	0.054	0.013	0.658
p-value		<0.0001	<0.0001	0.0002	0.1937

attempts to create such models have been made [22]. Simple pore models, such as the two-pore model [23], and fiber-matrix models [24] have, however, proven quite useful in describing glomerular permeability under normal and pathophysiological conditions [25,26]. For example, the rapid and dynamic changes in glomerular permeability induced by trauma, sepsis, hyperglycemia or oxidative stress can be functionally ascribed to increases in the normally very low number of functional 'large pores' (radius 110Å) in the GFB, with the 'small pore equivalent' (radius 37–38 Å) remaining largely unaltered [27–30]. Our previous observations concerning the increased plasma levels of cystatin C and several other proteins of similar size (β_2-microglobulin, beta-trace protein) during the third trimester of pregnancy, and even more pronounced at pre-eclampsia, could be interpreted as a decrease in the pore diameters of the functional pores [8–12]. This interpretation was based on the fact that the genes for these proteins are located at different chromosomes [31–34], have different regulation elements and the observations that factors influencing the production of cystatin C do not generally influence the synthesis of β_2-microglobulin, beta-trace protein or RBP in the same way [35–39]. It indicates that the productions of the proteins are not co-regulated and thus cannot explain the concordant increases of their plasma levels. But this concordant increase can be explained if the proteins have a common clearance mechanism. Since proteins below about 20,000 Da in molecular mass (<22 Å in Stokes-Einstein radius) are mainly excreted via glomerular transport [3], a reduction

Table IV. Statistical analysis of the relations between the ratios cystatin C/creatinine, β_2-microglobulin/creatinine, beta-trace protein/creatinine and RBP/creatinine for all men and for men stratified for their estimated GFR. Medians and *p*-values are given for the protein-creatinine ratios.

	Gender (male/female)	Cystatin C/ creatinine	β_2-microglobulin/ creatinine	beta-trace protein/ creatinine	RBP/creatinine
$eGFR_{mean,}$ men					
$eGFR_{cystatinC} \approx eGFR_{creatinine}$	32/0	0.013	0.025	0.008	0.449
$eGFR_{cystatinC} \leq 0.6eGFR_{creatinine}$	50/0	0.020	0.046	0.011	0.538
p-value		<0.0001	<0.0001	<0.0001	0.2078
$eGFR_{mean} \leq 40$ mL/min/1.73m^2					
$eGFR_{cystatinC} \approx eGFR_{creatinine}$	11/0	0.012	0.025	0.009	0.448
$eGFR_{cystatinC} \leq 0.6eGFR_{creatinine}$	19/0	0.019	0.038	0.010	0.511
p-value		<0.0001	0.0004	0.0893	0.9828
$eGFR_{mean}$ 40–60 mL/min/1.73m^2					
$eGFR_{cystatinC} \approx eGFR_{creatinine}$	7/0	0.013	0.027	0.008	0.391
$eGFR_{cystatinC} \leq 0.6eGFR_{creatinine}$	22/0	0.019	0.049	0.011	0.626
p-value		<0.0001	<0.0001	0.0219	0.0665
$eGFR_{mean} \geq 60$ mL/min/1.73m^2					
$eGFR_{cystatinC} \approx eGFR_{creatinine}$	14/0	0.013	0.024	0.008	0.491
$eGFR_{cystatinC} \leq 0.6eGFR_{creatinine}$	9/0	0.020	0.049	0.012	0.398
p-value		0.0002	<0.0001	0.0034	0.8997

Table V. Statistical analysis of the relations between the ratios cystatin C/creatinine, β_2-microglobulin/creatinine, beta-trace protein/creatinine and RBP/creatinine for all non-pregnant women and for non-pregnant women stratified for their estimated GFR. Medians and *p*-values are given for the protein-creatinine ratios.

	Gender (male/female)	Cystatin C/ creatinine	β_2-microglobulin/ creatinine	beta-trace protein/ creatinine	RBP/creatinine
$eGFR_{mean}$, non-pregnant women					
$eGFR_{cystatinC} \approx eGFR_{creatinine}$	0/23	0.016	0.034	0.009	0.567
$eGFR_{cystatinC} \leq 0.6 eGFR_{creatinine}$	0/48	0.025	0.058	0.013	0.798
p-value		<0.0001	<0.0001	<0.0001	0.0040
$eGFR_{mean} \leq 40$ mL/min/1.73m^2					
$eGFR_{cystatinC} \approx eGFR_{creatinine}$	0/4	0.015	0.041	0.011	0.443
$eGFR_{cystatinC} \leq 0.6 eGFR_{creatinine}$	0/22	0.021	0.053	0.013	0.617
p-value		0.0043	0.3934	0.4235	0.1451
$eGFR_{mean}$ 40–60 mL/min/1.73m^2					
$eGFR_{cystatinC} \approx eGFR_{creatinine}$	0/10	0.016	0.034	0.009	0.562
$eGFR_{cystatinC} \leq 0.6 eGFR_{creatinine}$	0/18	0.027	0.058	0.012	0.865
p-value		<0.0001	0.0001	0.0056	0.0030
$eGFR_{mean} \geq 60$ mL/min/1.73m^2					
$eGFR_{cystatinC} \approx eGFR_{creatinine}$	0/9	0.016	0.032	0.009	0.594
$eGFR_{cystatinC} \leq 0.6 eGFR_{creatinine}$	0/8	0.032	0.081	0.014	1.298
p-value		0.0005	0.0017	0.0076	0.0543

in their glomerular filtration rate would result in a simultaneous increase of their plasma levels. The simplest pathophysiological way of interpreting this is that the normally high sieving coefficients of these proteins dropped significantly during the third trimester of pregnancy and even more so at pre-eclampsia. According to the two-pore model of glomerular permeability this can be interpreted as a reduction in the radius of the small pores of the GFB. The explanation that creatinine and other small molecules do not simultaneously increase in concentration is that their sieving coefficients are still close to unity (1) despite the shrunken pores. To illustrate how close to unity the sieving coefficient is, we have calculated glomerular transport in terms of glomerular sieving coefficients for creatinine (SE-radius 3Å) and for an 18 Å radius (small) protein of MW 15kDa for a small pore radius of either 37.5 Å or 32 Å ('shrunken pore'). The relationship between diffusion (AoDx) and convection (GFR) was set as in the review of Venturoli and Rippe [25]. For creatinine, the sieving coefficient fell from 0.9990–0.9986, when the pore radius was reduced (but GFR was maintained constant). For the 15 kDa protein, the decrease was much larger, from 0.852–0.685, i.e. by 20%, under the same assumptions. In fact, due to a higher net transglomerular pressure and GFR during the third trimester of pregnancy, the glomerular elimination of small solutes will even increase.

Our results indicate that a pathophysiological decrease in the radius of the functional small pores ('shrunken pores') is not restricted to the third trimester of pregnancy and pre-eclampsia, but rather is a common phenomenon among both men and non-pregnant women. A 'syndrome' is generally defined as 'A set of symptoms or conditions that occur together and suggest the presence of a certain disease or an increased chance of developing the disease' [40,41]. The term derives from the Greek word σύνδρομον, meaning 'concurrence' [42]. The concurrent increase in plasma levels of several proteins (β_2-microglobulin, beta-trace protein and RBP), with molecular sizes of the same order as that of cystatin C, in a large proportion of patients with significantly lower $eGFR_{cystatin\ C}$ than $eGFR_{creatinine}$ suggests the presence of a syndrome that might be tentatively designated 'Shrunken pore syndrome' as this name offers a possible pathophysiological explanation.

It should be noted that another explanation than the presence of 'shrunken pores' might be offered for patients with $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$, namely that they suffer from abnormally low muscle mass, which means a falsely high value of $eGFR_{creatinine}$. However, the samples were consecutively collected and since the general recommendation in the Skåne Region concerning the use of cystatin C- and creatinine-based eGFR ($eGFR_{mean}$) is to use it in all patients and not only in special patient categories, like intensive care patients or paralytic patients, it is most likely that patient categories with low muscle mass are not dominating parts of the studied population. This was corroborated by studying the wards from which the requests for $eGFR_{mean}$ were sent. A major part of the samples were from primary care centers and very few from intensive care units or units for paralytic patients. Furthermore, when the 23 patients with the lowest creatinine levels of the patient group with $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$ were omitted, so that the median creatinine levels of the patient groups became identical, no changes in the statistical outcomes were observed.

The knowledge of the clinical consequences of the suggested pathophysiology of the 'Shrunken pore syndrome' is obviously scanty and warrants epidemiological studies of a large number of patients suffering from the 'Shrunken pore syndrome' and with

varying established other diagnoses and adequate control patients without the syndrome. However, it is interesting that it repeatedly has been observed that a decrease in $eGFR_{cystatin\ C}$ is associated with a much higher risk for end-stage renal disease, hospitalization, myocardial infarction and premature death than a decrease in $eGFR_{creatinine}$ [43,44]. This has been suggested to be due to inflammation, causing an increase in the cystatin C level, in addition to cystatin C being a marker for GFR [45]. However, it has been distinctly shown that inflammation *per se* does not cause an increase in the cystatin C level [46]. It is therefore tempting to suggest that the increased association of $eGFR_{cystatin\ C}$ with end-stage renal disease, hospitalization, myocardial infarction and premature death is due to its capacity to, in contrast to that of $eGFR_{creatinine}$, identify the 'Shrunken pore syndrome'. It is also possible that some of the pathophysiological and clinical consequences of pre-eclampsia/eclampsia, also called 'toxemia of pregnancy', are caused by shrunken glomerular pores. For if the 'Shrunken pore syndrome' means increased levels of cystatin C, β_2-microglobulin, beta-trace protein and RBP caused by a decreased glomerular filtration of these, it will also most probably cause abnormally high levels of signalling proteins/peptides similar in size to these proteins, including cytokines, hormones and growth factors. Increased levels of some of these, e.g. IL-6, are known to be associated with cardiovascular disease.

The observations in this study, and earlier similar observations in pregnancy and pre-eclampsia, indicate in our opinion the existence of a 'Shrunken pore syndrome'. But to be able to study this syndrome and its clinical consequences in more detail, a more rigorous definition is required. We suggest that a tentative operational definition of 'Shrunken pore syndrome' should be the presence in a patient of an $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$ and a simultaneously raised plasma level of β_2-microglobulin. This definition will probably erroneously identify a 'Shrunken pore syndrome' in a few patients suffering from other conditions. For example, in patients with myeloma, vigorously treated with glucocorticoids, the malignant cells generally have a raised production of β_2-microglobulin and the glucocorticoid treatment increases the production of cystatin C [47–49] and the above criteria of 'Shrunken pore syndrome' might then be fulfilled in the absence of such a syndrome. Addition of a raised plasma level of beta-trace protein to the suggested criteria for 'Shrunken pore syndrome' would reduce the risk of erroneous identification of the syndrome, *inter alia* because glucocorticoids downregulate the synthesis of beta-trace protein [49,50], but these more extended criteria would also probably mean that a significant number of patients with the 'Shrunken pore syndrome' will not be identified.

The criterion that $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$ is also arbitrary, but is tentatively chosen because $eGFR_{cystatin\ C}$ in the third trimester indicates a reduction of GFR of 30–40% compared to the $eGFR_{cystatin\ C}$ of non-pregnant women or women in their first trimester [8–10]. It is possible that different degrees of the 'Shrunken pore syndrome' can be defined by selecting smaller or greater differences in $eGFR_{cystatin\ C}$ and $eGFR_{creatinine}$ as a diagnostic criterion for 'Shrunken pore syndrome'. It is, as a matter of fact, likely that 'Shrunken pore syndrome' generally indicates a process of continuously shrinking pores and not only a state in which the pores have shrunken to a certain degree and then stopped shrinking.

It would be possible to more rigorously define a shrunken pore syndrome by using invasive procedures in which substances, only being excreted by glomerular filtration, and of different molecular sizes, were injected and their plasma or renal clearance determined. The polysaccharides dextran and Ficoll of great polydispersity have been used in animals, but the more reliable ('rigid') probe Ficoll has been only rarely tried in humans due to its toxicity in man [25]. We have found only one report of the use of dextran (which in itself is hyperpermeable across the GFB) in pregnancy [51], where the results are compatible with our interpretation of the results for cystatin C and other proteins of similar size as indicating a reduced small pore radius in the third trimester. Like dextran and Ficoll, polydisperse preparations of inulin have been described and might also theoretically be used in man to rigorously define the shrunken pore syndrome.

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