Introduction of cystatin C as a marker of GFR, step I

Quantitation of γ-trace in human biological fluids: indications for production in the central nervous system

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γ-Trace was purified in large amounts from urine and used for the production of a specific rabbit antiserum. An enzyme immunoassay for quantitation of γ-trace was developed using the pure protein as a primary standard. Its sensitivity was approximately 30 μg/l. An enzyme amplified single radial immunodiffusion was developed as well. Its sensitivity was approximately 0.3 mg/l. These assays allowed quantitation of γ-trace in normal human biological fluids. The following results were obtained (mean ± SD): cerebrospinal fluid: 5.8 ± 2.2 mg/l, plasma: 1.1 ± 0.42 mg/l, saliva: 1.8 ± 0.88 mg/l and urine: 0.095 ± 0.057 mg/l. Plasma samples from patients with advanced renal failure revealed γ-trace values up to 13 times the normal mean plasma value. The results indicate a production of γ-trace in the central nervous system and that the protein is primarily catabolized by the kidney.
Introduction of cystatin C as a marker of GFR, step II

About 3400 publications comparing cystatin C and creatinine as terms in estimating equations for GFR have been published today (2017), using bias and $P_{30}$ (% of eGFR within +/- 30% of mGFR) to evaluate the results.
The general conclusion is that, if the creatinine term is supplemented by terms for age, sex and race in estimating equations, their diagnostic performance (bias, $P_{30}$-value) is often comparable to that of cystatin C-based equations with cystatin C as the only term.
Why is cystatin C or eGFR_{cystatin C} a better indicator of cardiovascular events, hospitalization or death than creatinine or eGFR_{creatinine} in all studies, although it is not always a better marker for GFR than creatinine?
The original articles to show associations between morbidity, mortality and cystatin C


Suggested mechanism to explain the association between cystatin C and mortality

Inflammation (identified by an increase in CRP) causes an increase in cystatin C and can thus explain the superiority of cystatin C over creatinine to predict myocardial infarction, hospitalization, ESRD and death. Knight et al.: Factors influencing serum cystatin C levels .... *Kidney International* 2004: 65: 1416-21.

The proposal has been reiterated hundreds of times, but never challenged.
Dogmas are often bad

As this suggested mechanism was published in a ”high impact” journal and accepted by the kidney nobility, no new alternative hypotheses were suggested and no more research in the area was done.

But the suggested mechanism was wrong!
Ptolemaios ~150 AD: *Megale syntaxis*
Copernicus 1543: *De revolutionibus orbium coelestium*
It was easy to prove that the proposal was wrong:

Grubb A, Björk J, Nyman U, Pollak J, Bengzon J, Östner G, Lindström V:

Changes in plasma levels after surgery in multiples of the preoperative levels
Changes in plasma levels after surgery in multiples of the preoperative levels
A possible explanation for the better prognostic capacity of eGFR_{cystatin C} is that it demonstrates altered glomerular filtration quality, caused by e.g., shrunken pores, in addition to a decrease in GFR. Observe that altered glomerular filtration quality may occur even at normal GFR, i.e., measured GFR using injected substances like $^{51}$Cr-EDTA, $^{125}$I-iothalamate, iohexol etc.
Observations indicating another hypothesis for the mortality associated with cystatin C

A


Diagnosing pre-eclampsia

![Graph showing ROC curves for different markers including Beta-2 microglobulin, Beta Trace Protein, Cystatin C, Urate, and Creatinine.](image-url)
Observations indicating another hypothesis for the mortality associated with cystatin C

B


Functional glomerular pore size

- P-βTP: N
- P-CC: N
- P-Creat.: N
- P-βTP: ↑
- P-CC: ↑
- P-Creat.: N

Time of pregnancy
In disease?
So, if there really is a syndrome of “shrunken pores”, in patients with \( e.g., \) \( \text{eGFR}_{\text{cystatin C}} < 60\% \text{ of eGFR}_{\text{creatinine}} \), not only the cystatin C-creatinine ratio would be increased, but also the creatinine ratios of other low-molecular-mass proteins like \( \beta_2 \)-microglobulin, \( \beta \)-trace protein and RBP.
All patients

\( eGFR_{cc} \approx eGFR_{creat} \)

\( eGFR_{cc} < 0.6 \ eGFR_{creat} \)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystatin C - creatinine ratio</td>
<td>0.014</td>
</tr>
<tr>
<td>( \beta_2 )-microglobulin-creatinine ratio</td>
<td>0.023</td>
</tr>
<tr>
<td>( \beta )-trace protein-creatinine ratio</td>
<td>0.028</td>
</tr>
</tbody>
</table>

(mg/μmol)
mGFR < 60 mL/min/1.73m²

eGFR_{cc} \approx eGFR_{creat}

eGFR_{cc} < 0.6 eGFR_{creat}

cystatin C - creatinine ratio

\beta_2\text{-microglobulin-creatinine ratio}

\beta\text{-traceprotein-creatinine ratio} (mg/\mu mol)
mGFR > 60 mL/min/1.73m²

eGFR_{cc} \approx eGFR_{creat}

eGFR_{cc} < 0.6 eGFR_{creat}

cystatin C - creatinine ratio

\( \beta_2 \)-microglobulin-creatinine ratio

\( \beta \)-traceprotein-creatinine ratio

(mg/µmol)
So, our conclusion is that there is a “Shrunken Pore Syndrome” affecting not only pregnant/preeclamptic persons. It should be observed that the production of cystatin C, $\beta_2$-microglobulin, $\beta$-trace protein and RBP are not co-regulated, whereas they have a common way of elimination determined by GFR.

So what about the clinical consequences of “Shrunken Pore Syndrome”? 
Shrunken Pore Syndrome and mortality (CAPA < 0.6 x LMrev)
Shrunken Pore Syndrome and mortality

\( (\text{CKD-EPI}_{\text{cystatin C}} < 0.6 \times \text{CKD-EPI}_{\text{creatinine}}) \)
Shrunken Pore Syndrome and mortality (CAPA < 0.6 x LMrev)
Shrunken Pore Syndrome and mortality
(CKD-EPI_{cystatin C} < 0.6 \times CKD-EPI_{creatinine})
Shrunken Pore Syndrome and mortality
(CAPA < 0.7 x LMrev)

CAPA-LMrev GFR<60ml/min/1.73m²

Survival

Years after surgery

SPS-  SPS+
405   68   391   59    374   56    268   36    134   20    6     2
Shrunken Pore Syndrome and mortality
(CAPA < 0.7 x LMrev)
In an ongoing study in Lund, samples consequently collected from 2800 patients with measured GFR (iohexol clearance determination), show that patients with “Shrunken Pore Syndrome” has a 5-year mortality of 23%! The lower the eGFR\textsubscript{cystatin C} - eGFR\textsubscript{creatinine} -ratio, the higher the mortality!
Increased shrinking results in increased mortality

<table>
<thead>
<tr>
<th>eGFR\textsubscript{cystatin C} / eGFR\textsubscript{creatinine} - ratio</th>
<th>All-cause mortality, HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.70</td>
<td>3.2 (2.6 – 3.9)</td>
</tr>
<tr>
<td>0.70 – 0.84</td>
<td>2.3 (1.9 – 2.8)</td>
</tr>
<tr>
<td>0.85 – 0.99</td>
<td>1.3 (1.1 – 1.6)</td>
</tr>
<tr>
<td>≥ 1.00</td>
<td>1.0 (Reference)</td>
</tr>
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</table>
Shrunken pore syndrome and mortality and risk of ESRD
A recent publication shows virtually the same results for individuals with “Shrunken Pore Syndrome” in a healthy population as we found in a population of individuals undergoing elective coronary artery bypass grafting.


Shrunken Pore Syndrome: Does it exist in children?

Leion F...... Grubb A: Estimating glomerular filtration rate (GFR) in children. The average between a cystatin C- and a creatinine-based equation improves estimation of GFR in both children and adults and enables diagnosing Shrunken Pore Syndrome

Scand J Clin Lab Invest 2017; (In press)
Shrunken Pore Syndrome: Does it exist in children?


10 % of a childpopulation had \( \text{eGFR}_{\text{cystatin C}} - \text{eGFR}_{\text{creatinine}} \)-ratio < 0.6 and increased ratios of cystatin C/creatinine, \( \beta_2 \)-microg./creatinine and beta-trace protein/creatinine.
Optimal estimation of GFR in the clinical routine:

Determine both cystatin C and creatinine, calculate eGFR\textsubscript{cystatin C}, eGFR\textsubscript{creatinine} and eGFR\textsubscript{mean}.

If eGFR\textsubscript{cystatin C} and eGFR\textsubscript{creatinine} agree: eGFR\textsubscript{mean} is correct. It might be more reliable than an invasive clearance-determination.

If eGFR\textsubscript{cystatin C} and eGFR\textsubscript{creatinine} do not agree: Try to find the cause (low muscle mass, use of a high dose of glucocorticoid). If the cause is found: Use the non-affected eGFR.

If eGFR\textsubscript{cystatin C} and eGFR\textsubscript{creatinine} do not agree and no cause is found: The patient suffers from “Shrunken Pore Syndrome”, at least when eGFR\textsubscript{cystatin C} is less than 60% of eGFR\textsubscript{creatinine}.

Site with tools to do this: www.egfr.se
<table>
<thead>
<tr>
<th>Laboratoriets anteckningar</th>
<th>Ys</th>
<th>Si</th>
<th>Lu</th>
<th>La</th>
<th>He</th>
<th>Än</th>
<th>Kr</th>
<th>Hä</th>
<th>Ma</th>
<th>Tr</th>
<th>SI</th>
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<td>Hä</td>
<td>Ma</td>
<td>Tr</td>
<td>SI</td>
<td>Ba</td>
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<tr>
<td>Meddelande till laboratoriet / provtagarens telefonnummer</td>
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By what mechanism would altered glomerular filtration quality cause cardiovascular events, hospitalization or death?
GFR/filtration quality regulated levels of LMW-proteins/peptides

<table>
<thead>
<tr>
<th>Protein</th>
<th>MW (kDa)</th>
</tr>
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<tbody>
<tr>
<td>Glucagon</td>
<td>3.5</td>
</tr>
<tr>
<td>Insulin</td>
<td>6</td>
</tr>
<tr>
<td>β₂-microglobulin</td>
<td>11</td>
</tr>
<tr>
<td>ProBNP</td>
<td>12</td>
</tr>
<tr>
<td>IL1-β</td>
<td>17</td>
</tr>
<tr>
<td>TNF-α</td>
<td>17</td>
</tr>
<tr>
<td>FGF-2</td>
<td>18</td>
</tr>
<tr>
<td>Growth Hormone</td>
<td>22</td>
</tr>
<tr>
<td>Light Ig-chains</td>
<td>23</td>
</tr>
<tr>
<td>TGF-β</td>
<td>25</td>
</tr>
<tr>
<td>IL-6</td>
<td>26</td>
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</table>
Cytokines known to promote atherosclerosis

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Molecular Weight (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1-β</td>
<td>17</td>
</tr>
<tr>
<td>IL-18</td>
<td>18</td>
</tr>
<tr>
<td>MIF</td>
<td>12</td>
</tr>
<tr>
<td>TNF-α</td>
<td>17</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>19</td>
</tr>
</tbody>
</table>
We studied the plasma levels of 177 peptides/proteins (cytokines, chemokines etc) in four groups of patients with known GFR to learn, if there are peptide patterns specific for SPS and/or reduced GFR

1 patients with SPS and with normal GFR
2 patients without SPS and with normal GFR
3 patients with SPS and reduced GFR
4 patients without SPS and reduced GFR
If the changes in peptide levels between
1 patients with SPS and with normal GFR
and
2 patients without SPS and with normal GFR
are similar/identical
to the changes in peptide levels between
3 patients with SPS and with reduced GFR
and
4 patients without SPS and with reduced GFR

there is a “specific” SPS-pattern
If the changes in peptide levels between 1 patients with reduced GFR and without SPS and 2 patients with normal GFR and without SPS are similar/identical to the changes in peptide levels between 3 patients with reduced GFR and with SPS and 4 patients with normal GFR and with SPS there is a “specific” reduced GFR-pattern
Changes in peptide levels in Shrunken Pore Syndrome (SPS)

- Comparison of peptide/protein levels in patients with SPS and normal GFR with the levels in patients without SPS and normal GFR
- Comparison of peptide/protein levels in patients with SPS and reduced GFR with the levels in patients without SPS and reduced GFR
Changes in peptide levels when GFR is reduced

---: Comparison of peptide/protein levels in patients with reduced GFR without SPS with the levels in patients with normal GFR without SPS

---: Comparison of peptide/protein levels in patients with reduced GFR with SPS with the levels in patients with normal GFR with SPS
If the peptide levels were compared between patients with both reduced GFR and SPS and patients with normal GFR and without SPS, all differences between patients with normal and reduced GFR and between patients with and without SPS should be found to verify the two distinct patterns for SPS and reduced GFR.
The changes in peptide levels between patients with both reduced GFR and SPS compared to patients with normal GFR and no SPS are virtually the sum of the changes specific for SPS and for reduced GFR.
Good or stupid ideas that might deserve testing, despite that they are disliked by the kidney nobility?

1 The etiology of ESRD (requirement of hemodialysis when GFR is very reduced) is not only accumulation of toxic substances, but also the appearance of chaotic signaling.

2 Some of these disturbances might be treated without use of hemodialysis, *e.g.*, by use of monoclonal antibodies.

3 Shrunken Pore Syndrome is characterized by a different type of signaling chaos and might also be treated with monoclonal antibodies.
What is the duration between an interesting clinical observation and its acceptance by the health authorities? In my case, one generation!