Inflammation Markers, Chronic Kidney Disease, and Renal Replacement Therapy

Chronic kidney disease (CKD) is associated with a proinflammatory state and an excess of cardiovascular risk. In this work, we describe changes in inflammatory markers—C-reactive protein (CRP), pentraxin 3 (PTX3), serum component of amyloid A (SAA), and procalcitonin (PCT)—in CKD patients compared with a control group of subjects with a normal estimated glomerular filtration rate (eGFR). Blood samples were obtained from 69 healthy individuals (GP) and 70 end-stage CKD patients—25 not yet on dialysis, 22 on peritoneal dialysis (PD), and 23 on hemodialysis (HD). These were the results [median (95% confidence interval)] for the GP, CKD, PD, and HD groups respectively:

- **CRP**: 1.40 mg/L (1.19 – 2.11 mg/L), 6.50 mg/L (3.57 – 8.32 mg/L), 7.60 mg/L (2.19 – 22.10 mg/L), 9.60 mg/L (6.62 – 16.38 mg/L)
- **SAA**: 3.10 mg/L (2.90 – 3.53 mg/L), 7.11 mg/L (3.81 – 15.40 mg/L), 9.69 mg/L (5.07 – 29.47 mg/L), 15.90 mg/L (6.80 – 37.48 mg/L)
- **PCT**: 0.03 ng/mL (0.02 – 0.03 ng/mL), 0.12 ng/mL (0.09 – 0.16 ng/mL), 0.32 ng/mL (0.20 – 0.46 ng/mL), 0.79 ng/mL (0.45 – 0.99 ng/mL)
- **PTX3**: 0.54 ng/mL (0.33 – 0.62 ng/mL), 0.71 ng/mL (0.32 – 1.50 ng/mL), 1.52 ng/mL (0.65 – 2.13 mg/mL), 1.67 ng/mL (1.05 – 2.27 mg/mL)

Compared with levels in the GP group, levels of SAA and CRP (systemic response) were significantly higher in CKD patients on and not on dialysis. Levels of PTX3 were higher only in dialyzed patients, significantly so in those on HD (greatly different from the CRP levels). These differing levels might be related to a local reaction caused by an invasive intervention (PD or HD). As eGFR declines and with the start of renal replacement therapy, PCT increases. Levels of PCT could potentially cause confusion when these patients are being evaluated for the presence of infection, and may also demonstrate some microvascular implications of dialysis therapy.

Key words: Inflammation markers, cardiovascular risk, chronic kidney disease, proinflammatory state, renal replacement therapy

Introduction

More and more studies show that the immune system actively participates in the development of vascular disease. The early stages of atherosclerosis are characterized by infiltration of inflammatory cells into the vascular wall, with the involvement of innate immunity factors (1,2). For that reason, the research effort to elucidate the mechanisms behind these relationships and their consequences is growing.

Among the components involved in the innate immune inflammatory process are found short pentraxins such as C-reactive protein (CRP) and long pentraxins such as pentraxin 3 (PTX3). Some other peptides not structurally related to pentraxins—such as serum component of amyloid A (SAA) and procalcitonin (PCT)—may also be involved in inflammatory states. The systemic acute-phase reactant CRP is released in the liver after induction by interleukin 6. The main functions of PTX3 are closely related to those of CRP, but the PTX3 protein is produced at a locoregional level by many kind of cells (endothelial dendritic cells, smooth muscle cells, fibroblasts, neutrophils, monocytes, and macrophages, and also kidney proximal tubular epithelial and mesangial cells) (3–5). Synthesis and release of PTX3 is orchestrated by inflammatory stimuli such as...
 interleukin 1, tumor necrosis factor α, oxidized low-density lipoprotein cholesterol, and micro-organisms (6,7). Another systemic acute-phase reactant, SAA, is synthesized and released by the liver (8,9). Procalcitonin is a marker of bacterial infection induced in extra-thyroid tissues (kidney, liver, pancreas, brain) by tumor necrosis factor α (10,11); but PCT may also be involved in microvascular inflammatory processes. Some studies of the biocompatibility of hemodialysis (HD) membranes in HD patients having no acute or chronic infection have shown a decline in PTC levels as membranes and filters that are more biocompatible are used (12).

The pathologic condition of chronic kidney disease (CKD) is associated with a relatively unexplained excess of cardiovascular risk (13,14). For that reason, a study of the impact of these inflammatory and innate immune factors is needed (15). The aim of the present work was therefore to describe changes in these emerging markers of innate immune and inflammatory response (CRP, PTX3, SAA, PCT) in CKD patients on and not on renal replacement therapy [RRT, peritoneal dialysis (PD) or HD] compared with a control group of subjects with a normal estimated glomerular filtration rate (eGFR).

Methods
We obtained serum and EDTA plasma from 69 healthy subjects in the general population (GP) and 70 CKD patients, including 25 in stages IV and V CKD not on dialysis, 22 on PD, and 23 on HD. In the HD group, the samples were taken before a HD session (all patients had been using biocompatible membranes in the immediate past). All of the subjects were associated with the Nephrology Department of University Hospital “Marqués de Valdecilla,” and none had an infection at the time of the blood draw. Samples of EDTA plasma were available only from approximately 15 patients in each group.

In all subjects, these renal function and inflammatory serum markers were quantified: serum creatinine (sCr) by the Jaffe method on a Dimension RXL analyzer (Siemens Healthcare, Mannheim, Germany); standardized CRP, SAA, and cystatin C (CysC) by immunonephelometry (CardioPhase High Sensitivity C-Reactive Protein and N Latex SAA and Cystatin C kits) on a Behring Nephelometer II (Siemens Healthcare); serum PCT by immunoassay (Brahms PCT-sensitive Kryptor assay: Brahms, Hennigsdorf, Germany); and plasma PTX3 by ELISA (Human Pentraxin3/TSG-14 ELISA System: Perseus Proteomics, Tokyo, Japan).

The statistical treatment of data (Mann–Whitney U-test, with significance set at $p < 0.050$) was carried out using the MedCalc software application (MedCalc, Ghent, Belgium). The levels of all inflammatory markers have a nonparametric distribution, and so results are expressed as medians with 95% confidence intervals (CIs).

Results
Table I shows the resulting data.

Serum creatinine and CysC were determined to evaluate the eGFR and evolving renal insufficiency of the subjects.

Compared with the GP group, all CKD patients (on and not on RRT) had higher levels of serum CRP (taking 3 mg/L as the lower reference limit for long-term inflammation). Although CRP tended to increase with eGFR decline, the increase was significant only between the GP group and the CKD groups ($p < 0.050$).

Serum levels of SAA (upper reference limit: 6.40 mg/L) changed in a manner similar to that of the CRP levels. Levels of SAA were elevated in CKD patients and not significantly altered by HD or PD therapy.

Compared with the GP group and with each other, all CKD groups showed differences in PCT levels. This biomarker increased significantly with eGFR decline.

Plasma PTX3 (upper reference limit: 1.18 ng/mL) was higher in the RRT groups than in the GP group. Levels were also different between the patients on HD and those not yet on RRT, but levels in those not yet on RRT were not very different from those in the CP group (Figure 1).

Discussion and conclusions
In the present study, serum CRP was increased in CKD patients, and although it trended slightly higher with RRT, that trend was nonsignificant. Severe CKD therefore seems to bring on a low-level inflammatory state.

Plasma PTX3 increased only in end-stage CKD patients on HD (greatly different from the CRP levels). These differing levels might be related to a systemic proinflammatory state caused by CKD, followed by
**TABLE I** Features of the general, chronic kidney disease (CKD), peritoneal dialysis, and hemodialysis populations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>General</th>
<th>CKD</th>
<th>Peritoneal dialysis</th>
<th>Hemodialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>69</td>
<td>25</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>sCr (mg/dL)</td>
<td>0.91±0.04</td>
<td>3.95±0.46</td>
<td>7.46±1.06</td>
<td>8.15±0.82</td>
</tr>
<tr>
<td>CysC (mg/L)</td>
<td>0.81±0.05</td>
<td>3.16±0.28</td>
<td>5.34±0.69</td>
<td>5.56±0.50</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.40 (1.19 to 2.11)</td>
<td>6.50 (3.57 to 8.32)b</td>
<td>7.60 (2.19 to 22.10)b</td>
<td>9.60 (6.62 to 16.38)b</td>
</tr>
<tr>
<td>SAA (mg/L)</td>
<td>3.10 (2.90 to 3.53)</td>
<td>7.11 (3.81 to 15.40)b</td>
<td>9.69 (5.07 to 29.47)b</td>
<td>15.90 (6.80 to 37.48)b</td>
</tr>
<tr>
<td>PCT (ng/mL)</td>
<td>0.03 (0.02 to 0.03)</td>
<td>0.12 (0.09 to 0.16)b,d,e</td>
<td>0.32 (0.20 to 0.46)b,c,e</td>
<td>0.79 (0.45 to 0.99)b,c,d</td>
</tr>
<tr>
<td>PTX3 (ng/mL)b</td>
<td>0.54 (0.33 to 0.62)</td>
<td>0.71 (0.32 to 1.50)c</td>
<td>1.52 (0.65 to 2.13)b</td>
<td>1.67 (1.05 to 2.27)b,c,e</td>
</tr>
</tbody>
</table>

* Data shown as mean ± standard deviation, or median (95% confidence interval) for variables with a non-normal distribution.

* p < 0.050 compared with the general population.

* p < 0.050 compared with the CKD population.

* p < 0.050 compared with the PD population.

* p < 0.050 compared with the hemodialysis population.

* Sample sizes are different because of difficulties in collecting another EDTA tube.

sCr = serum creatinine; CysC = cystatin-C; CRP = C-reactive protein; SAA = serum component of amyloid A; PCT = procalcitonin; PTX3 = pentraxin 3.

**FIGURE 1** Number of times (n) that each determination exceeded the upper reference limit (URL). The URLs are, for C-reactive protein (CRP), 10.00 mg/L (acute inflammation); for pentraxin-3 (PTX3), 1.18 ng/mL; for serum component of amyloid A (SAA), 6.40 mg/L; and for procalcitonin (PCT), 0.200 ng/mL (local infection). GP = general population; CKD = chronic kidney disease; PD = peritoneal dialysis; HD = hemodialysis.
a local (microvascular) reaction (release of PTX3) in the case of invasive intervention (HD) (12).

Similarly, we observed that levels of serum SAA, an acute-phase reactant with a structure different from the pentraxins, changed in the same way that the levels of C-reactive protein did (systemic inflammatory response).

Of particular relevance was the progressive increase in serum PCT as eGFR declined. The increase in PCT was especially marked in the PD and HD groups (invasive RRTs) and could potentially cause confusion when such patients are being evaluated for the presence of infection. Those levels may also demonstrate certain microvascular implications of RRT.

We observed that PTX3 and PCT increased in parallel in patients on RRT. These increases might be a result of local stimulation of peritoneum by dialysis fluids and of blood polymorphonuclear cells by dialysis membranes.

Recent reports have provided evidence for the efficacy and safety of the combination of ezetimibe and simvastatin for lowering low-density lipoprotein cholesterol among a wide range of patients with CKD not on RRT, but not among CKD patients on RRT (16,17).

Taken together, these data showing elevations in innate immune and inflammatory markers suggest that, because of the decline in eGFR, CKD patients are in a proinflammatory state that should be intensively treated to prevent undesirable cardiovascular events. In the case of CKD patients on RRT, the traditional therapy (statins) is insufficient to handle the excessive proinflammatory state because of the chronic proinflammatory stimuli induced locally by RRT. We therefore think that conventional therapy should be combined with new therapies to combat the inflammatory state and lower the cardiovascular risk in CKD patients on RRT.

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Disclosures
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References


17 Canadian Heart Research Centre (CHRC), SHARP Collaborative Group. Results: question and answers about the SHARP trial (web page). Toronto, ON: CHRC; n.d. [Available online at: www.chrc.net/LDLINCKD/results.html; cited June 27, 2011]

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