Trimestral variations of C-reactive protein, interleukin-6 and tumour necrosis factor-α are similarly associated with survival in haemodialysis patients

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Abstract
Background. The impact of intra-individual changes of inflammatory markers [other than C-reactive protein (CRP)] on mortality in haemodialysis (HD) patients is unknown. We therefore studied survival in relation to trimestral variations of CRP, interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α).

Methods. In 201 prevalent HD patients from the Mapping of Inflammatory Markers in Chronic Kidney Disease cohort, serum CRP, IL-6 and TNF-α were measured 3 months apart and survival was assessed during follow-up. Based on fluctuations along tertiles of distribution, four patterns were defined for each inflammatory marker: stable low, decrease, increase and stable high. Hazard ratios were
Patients with persistently elevated CRP values had the worst mortality in crude [HR 2.98 (95% CI 1.71–5.20)] and adjusted [2.79 (1.58–4.94)] Cox models, together with those who increased in their CRP levels [crude 3.27 (1.91–5.60); adjusted 3.13 (1.79–5.45)]. Similar survival patterns were observed for IL-6 and TNF-α variation categories. Correlations among these changes were, however, not strong. In the replication cohort, individuals with persistently elevated CRP values also showed the highest mortality risk [crude 3.8 (2.31–4.94); adjusted 2.33 (1.58–3.45)].

Conclusions. Trimestral variations of TNF-α, IL-6, and CRP are similarly associated with survival in HD patients. The agreement between changes of these biomarkers was low, suggesting that different pathways may trigger each of these markers.

Keywords: chronic kidney disease; C-reactive protein; interleukin-6; tumour necrosis factor-alpha; variability

Introduction

Patients with advanced chronic kidney disease (CKD) are at an increased mortality risk [1]. Understanding the pathophysiology of this excess mortality may contribute to adequate risk profiling and encourage clinical interventions. Recent attention has focused on the role of increased inflammation among advanced CKD patients in promoting progression of underlying comorbid illnesses and acting as a catalyst for other risk factors, as a consequence leading to increased mortality [2].

The robust evidence concerning single measurements of various inflammatory biomarkers as independent predictors of comorbidities and mortality in CKD patients has justified their use for identification of patients at increased risk [3–5]. However, few studies have until now addressed the relationship between longitudinal inflammatory variation and mortality risk [6–9]. Clinically, this translates into an uncertainty regarding how to interpret the monitoring of the inflammatory response. Moreover, the few studies available on this topic have only focused on C-reactive protein (CRP) variation. Hence, we aimed at studying the association between the pattern of changes over a 3-month period of CRP, interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) and survival in two well-characterized cohorts of haemodialysis (HD) patients.

Materials and methods

Subjects

This study comprises individuals from two independent patient cohorts. The first one corresponds to the Mapping of Inflammatory Markers in Chronic Kidney Disease (MIMICK) cohort, the protocol of which has been described elsewhere in more detail [10]. This cohort includes prevalent patients (n = 228) on maintenance HD therapy recruited during the period of October 2003 to September 2004 in six dialysis units in the Stockholm-Uppsala (Sweden) region. Patients were observed for a period of 3 months and inflammatory biomarkers were measured at the beginning and after 3 months, with subsequent follow-up for survival analyses. During the 3-month observational period, 3 patients died and 24 additional individuals had at least one missing value in one of the inflammatory markers studied. Therefore, 201 patients with complete data for all inflammatory markers at both time points were included. From inclusion on forward, events of death were recorded, with no loss to follow-up. The Ethics Committee of Karolinska Institutet, Stockholm, Sweden approved the protocol, and informed consent was obtained from each patient. To replicate findings, a sample from the Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD) study was included [11]. Briefly, NECOSAD is a prospective follow-up study including all incident dialysis patients from 38 dialysis centres in the Netherlands between 1997 and 2002. This study includes 472 patients with serum CRP assessed at 3 and 6 months after start of HD therapy. The NECOSAD protocol was approved by the ethics committees of all participating centres and after being informed, all patients consented. In both cohorts, comorbidity was classified according to Davies et al. [12] on a seven-point scale which was later simplified into three risk categories (low, medium and high comorbidity risk). Nutritional status was quantified by means of the subjective global assessment (SGA) which, for reasons of simplicity, was trans-calculated to a three-point scale [13]. Body mass index (BMI) was calculated as the body weight (kg) divided by the squared height (m²).

Biochemical methods

In both cohorts, venous blood was obtained before the dialysis session at each time point. Plasma was separated, and samples were kept frozen at −80°C if not analysed immediately. CRP in the NECOSAD was analysed with an immunoturbidimetric assay with a detection limit of 3 mg/L. In the MIMICK cohort, high-sensitivity CRP levels were measured by an immunometric assay (detection limit 0.1 mg/L) on an Immulite Analyser (Immulite, DPC, Siemens, CA). Serum albumin levels were measured with bromocresol purple in the MIMICK cohort, whereas immunonephelometry was used in the NECOSAD.

Statistical methods

At each time point, patients were grouped according to each biomarker’s tertiles of distribution (low, middle, high). The change in inflammatory marker was categorized according to the fluctuation between the tertiles.
Table 1. Baseline characteristics of patients included in the study and after stratification according to CRP tertile variation categories in MIMICK subjects

<table>
<thead>
<tr>
<th>Characteristics during follow-up</th>
<th>All patients</th>
<th>Stable low</th>
<th>Decrease</th>
<th>Increase</th>
<th>Stable high</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of deaths</td>
<td>n = 201</td>
<td>n = 82</td>
<td>n = 40</td>
<td>n = 42</td>
<td>n = 57</td>
<td></td>
</tr>
<tr>
<td>Time till death, months</td>
<td>17.3 (8.4–28.1)</td>
<td>18.0 (10.6–30.8)</td>
<td>21.5 (8.3–31.7)</td>
<td>13.5 (8.3–23.0)</td>
<td>18.3 (6.6–28.9)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 2. Baseline characteristics of patients included in the study and after stratification according to CRP tertile variation categories in NECOSAD subjects

<table>
<thead>
<tr>
<th>Characteristics during follow-up</th>
<th>All patients</th>
<th>Stable low</th>
<th>Decrease</th>
<th>Increase</th>
<th>Stable high</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of deaths</td>
<td>n = 472</td>
<td>n = 179</td>
<td>n = 115</td>
<td>n = 95</td>
<td>n = 83</td>
<td></td>
</tr>
<tr>
<td>Time till death, months</td>
<td>20.1 (8.6–32.7)</td>
<td>28.0 (15.5–39.8)</td>
<td>19.3 (5.3–23.2)</td>
<td>20.4 (7.7–39.4)</td>
<td>12.8 (5.4–23.1)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
inflammatory levels, the logarithmic transformed value was included in crude as well as in multivariate analyses. Since NECOSAD is composed of incident patients with identical preceding time on dialysis, dialysis vintage was not included as covariate in the Cox models for this cohort. All variables satisfied the proportional hazards assumption. As sensitivity analyses, different cut-off values (p40 and p80) were used to form the different groups. Also, in both cohorts, HRs were recalculated using one cut-off value for CRP (10 mg/L) on both time points to define the different groups.

To assess the correspondence between changes of the different inflammatory markers, we used Pearson correlation tests. For all statistical tests, SPSS version 16.0 (SPSS Inc., Chicago, USA) was used. For all HRs, 95% confidence intervals (95% CI) not including 1 and for all other tests, a P-value < 0.05 was considered to be statistically significant. Kaplan–Meier figures were created using Prism 5.02 (Graphpad, 1992).

**Results**

In the MIMICK cohort, the 33rd and 66th percentiles of CRP distribution were 3.6 and 14 mg/L for the first measurement and 3.6 and 12 mg/L for the second measurement, respectively. In the NECOSAD cohort, these values were 3.0 and 12.0 mg/L plus 4.0 and 12.0 mg/L for the two consecutive measurements correspondingly.

Tables 1 and 2 depict the baseline characteristics of the patients included in this study according to the four CRP variation categories for the MIMICK and NECOSAD cohorts, respectively. Also, the characteristics of follow-up are presented. In the MIMICK cohort, no difference was observed among the groups except for the severity of co-morbidities and dialysis vintage: patients with increasing CRP levels having more frequently a comorbidity ≥2 (high comorbidity risk) and a lower dialysis vintage. In addition, baseline values and changes of IL-6 and TNF-α were also present in the MIMICK cohort (see Materials and methods) served as the reference category.

Table 4. Correlation matrix of changes in inflammatory markers

<table>
<thead>
<tr>
<th></th>
<th>ΔCRP</th>
<th>ΔIL-6</th>
<th>ΔTNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔIL-6</td>
<td>0.468a</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>ΔTNF-α</td>
<td>0.103b</td>
<td>0.190b</td>
<td></td>
</tr>
</tbody>
</table>

Correlations were calculated by means of Pearson correlation tests. 

aP < 0.001. 
bP = 0.05.
significantly different between all groups. In the NECOSAD cohort, however, significant differences existed with respect to age, BMI, comorbidities and malnutrition across the four CRP variation groups: patients with elevated CRP levels on both time points were older, more often malnourished, had a higher BMI and more comorbidities.

In the MIMICK cohort, survival was assessed for each inflammatory biomarker after a median (IQR) follow-up of 38.4 (17.4–45.1) months, during which 97 (48.3%) individuals died. As shown in Figure 2A–C, a similar mortality pattern was observed for fluctuation categories of CRP, IL-6 and TNF-α. Crude and adjusted HRs are presented in Table 3. As compared with patients from the stable low group (reference), individuals who increased or had a persistently elevated concentration of inflammatory biomarkers experienced an increased mortality risk, both crude and adjusted. The magnitude of the HRs for these two categories was from a clinical perspective—similar. For the case of IL-6, a decrease during the observational period was also associated to an increased mortality risk in the crude analysis [HR 1.94 (95% CI 1.02–3.68)]. Although adjustments made significance disappear, the magnitude of the HR was still substantial [1.76 (0.92–3.38)].

Table 4 illustrates a correlation matrix among the changes in each inflammatory marker (deltas, as continuous variables) over the 3-month period. The correlation between ΔCRP and ΔIL-6 was substantial. On the other hand, correlations between ΔIL-6 and ΔTNF-α and between ΔCRP and ΔTNF-α were relatively weak.

In the NECOSAD cohort, during a median (IQR) follow-up of 27.2 (11.9–47.9) months, 206 (43.6%) individuals died. In crude [3.38 (2.31–4.94)] and adjusted [2.33 (1.58–3.45)] Cox analyses (Table 3), patients within the stable high group exhibited the highest mortality hazard. Patients who showed decreases in CRP concentrations also presented an increased mortality risk in crude analysis [1.68 (1.15–2.46)], being barely lost after adjustment for confounders [1.45 (0.98–2.16)] and reaching a similar magnitude as the increase group.

As sensitivity analyses, we used in both cohorts different cut-off values based on quartiles; however, results did not change (data not shown). Finally, Table 5 repeats the analysis using the established CRP thresholds of 3 and 10 mg/L. Results confirm the same trend, namely that stable high levels associated with the highest mortality risk, followed by the increase group in the MIMICK cohort.

**Discussion**

The present study is the first to concurrently analyse the implications of CRP, IL-6 and TNF-α trimestral variations on outcome in HD patients. Our results show a similar survival pattern for all three biomarkers. Both an increase and a persistent elevation in all these biomarkers were linked to a poor prognosis during the follow-up period. The concordance between the changes of these biomarkers was low, especially between ΔCRP and ΔIL-6 with ΔTNF-α.

Our finding of an increased mortality risk among individuals with persistently elevated serum CRP levels is in agreement with previous studies in HD patients, which substantially differed regarding lengths and frequency of CRP measurement [6,7,15]. Also, our observations accord with findings from a cross-sectional study in which measures of cardiac hypertrophy were more prevalent among HD patients with persistently elevated CRP levels [16]. A novel finding in our study is that increasing levels of CRP over the 3-month period in ‘prevalent’ patients are also associated with a higher mortality risk in both uni- and multivariate analyses, agreeing with a small report in patients on continuous peritoneal dialysis [8]. Results were partially replicated in a second cohort of ‘incident’ patients, in whom both increases and decreases of CRP were similar in magnitude in adjusted analyses. The inclusion of incident or prevalent patients in each of these cohorts, together with different baseline characteristics, may have influenced at this level.

Our analysis also longitudinally assesses the implications of IL-6 or TNF-α variation on HD outcome. We found a similar relation between mortality and variation patterns for all three biomarkers, thereby complementing the reported associations between single measurements of these markers and mortality [3,17]. In this context, it expands cross-sectional observations [3,18] showing that IL-6 is the best predictor of outcome in HD patients. While the cross-sectional correlations between single measurements of TNF-α, CRP and IL-6 are consistently reported to be strong [19,20] and a congruent pattern of altered cytokine profiling is observed cross-sectionally in HD patients [21], we anticipated a better correlation between the 3-month variation patterns of these inflammatory markers. Because the observed survival patterns are similar, this observation leads us to hypothesize that different pathways may trigger each of these biomarkers to rise and fall. While age, sex, comorbidities and clinical events (fever, antibiotics use, vomiting) were the main predictors of the 12-week variability of CRP in HD patients [22], to our knowledge, no studies have addressed the factors associated with the longitudinal variability of TNF-α or IL-6 in HD patients. Because CRP is a crude marker of systemic inflammation and both IL-6 and TNF-α are, instead, more related to local and tissue-specific inflammatory processes [23,24],
these findings may be expected. Also, differences in local activity and tissue specificity may influence risk profiles.

Several differences should be acknowledged as limitations: firstly, the cohorts share different incident (NECOSAD) and prevalent (MIMICK) designs. Secondly, while in the MIMICK CRP was measured with a high-sensitivity assay, a non-high-sensitivity assay with a detection limit of 3 mg/L was used in the NECOSAD. Nonetheless, sensitivity analyses using other cut-offs did not alter our findings, and we could recently demonstrate an excellent agreement between high-sensitivity and non-high-sensitivity CRP measurements on mortality prediction in this same patient material [25]. A final drawback is that this study was performed in patients treated with HD, who may be different from patients treated with other dialysis modalities. This would limit the generalizability of our findings to other populations.

Altogether, our study indicates that persistent elevation and increases of CRP, IL-6 and TNF-α over a short period of time associate with a worse outcome. Since the correlation between these changes was not strong, it is likely that different inflammatory pathways are in parallel influencing the CKD patient’s risk. While our findings may be of help to physicians when interpreting the evolution of the inflammatory response in HD patients, the question whether reduction of inflammation or stabilization of its variability could translate into improved survival remains, however, unanswered.

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Conflict of interest statement. None declared.

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