Original Article

Association of inflammation with anaemia in patients with chronic kidney disease not requiring chronic dialysis

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Abstract

Background. Anaemia associated with chronic kidney disease (CKD) has substantial public health importance. However, the association of haemoglobin concentrations with inflammation in the setting of decreased kidney function is not well established.

Methods. We analysed cross-sectional data from 7389 outpatient adults, who were referred by general practitioners for routine blood testing between June 2006 and June 2007. Glomerular filtration rate (eGFR) was estimated by the abbreviated Modification of Diet in Renal Disease (MDRD) equation. Multivariable linear regression analysis was used to identify factors independently associated with haemoglobin concentrations across eGFR categories as the main outcome.

Results. Of the 7389 participants included in the analytic cohort 2221 (30.1%) participants had eGFR ≥90 mL/min/m², 4310 (58.3%) 60–89 mL/min/m² and 858 (11.6%) <60 mL/min/m². There were significant, graded, increases in high sensitivity C-reactive protein (hs-CRP) and haemoglobin concentrations across eGFR categories independent of age, gender, plasma glucose and lipids (P<0.0001 for trends). In the multivariable regression analysis, increased hs-CRP concentrations were independently associated with lower haemoglobin concentrations at different stages of eGFR (P<0.0001 for all). Other independent predictors of lower haemoglobin were older age, female gender and lower eGFR.

Conclusions. Our findings suggest that increased plasma hs-CRP concentrations are independently associated with anaemia in the setting of decreased kidney function in a large cohort of unselected adult outpatients.

Keywords: anaemia; chronic kidney disease; inflammation

Introduction

Anaemia is a common complication of chronic kidney disease (CKD) and has been shown to be a primary risk factor for adverse cardiovascular outcomes in those who have CKD [1]. Recently there has been considerable interest in the relationship between haemoglobin targets and major cardiovascular outcomes in patients with CKD stage 3 and 4 [2,3]. The publication of two large randomized control trials of recombinant human erythropoietin in CKD patients—Cardiovascular Risk Reduction by Early Anaemia Treatment with Epoetin beta (CREATE) [2] and Correction of Haemoglobin and Outcomes in Renal Insufficiency (CHOIR) [3]—have received intense attention and discussion by the nephrology community [4–6].

The primary cause of anaemia in CKD patients is mainly related to a deficiency in the synthesis of endogenous erythropoietin from the diseased kidney. However, the role of chronic inflammation in the development of anaemia and erythropoiesis stimulating agents (ESA) hyporesponsiveness is now gaining increasing attention as potential factors that might adversely affect patient outcomes [7,8]. Erythropoiesis may be inhibited by several pro-inflammatory cytokines such as interleukin-1, tumour necrosis factor-alpha (TNF-α) and interferon gamma (IFN-γ) [8]. Furthermore, high plasma concentrations of C-reactive protein (CRP) have shown to be associated with anaemia and ESA hyporesponsiveness in chronic haemodialysis patients [9–11]. Nevertheless, there is currently a paucity of data regarding whether anaemia is associated with a low-grade chronic inflammatory state even in subjects with CKD not requiring dialysis.

Knowledge of the relationship of chronic inflammation with anaemia at different levels of kidney function is essential for our understanding and management of adequate ESA treatments in CKD patients not requiring renal replacement therapy. We have therefore performed the following cross-sectional analysis using data from a Clinical Chemistry Laboratory, with the purpose of examining the relationship of inflammation with kidney function loss and, specifically, to examine the possible confounding effect that
this factor (i.e. inflammation) may exert on the observed association between anaemia and progressive reduction in kidney function.

Subjects and methods

We performed a retrospective analysis on the database of a Laboratory Information System of the Clinical Chemistry Laboratory at the Verona University Hospital to retrieve results of haemoglobin, serum creatinine, glucose, lipids and high sensitivity C-reactive protein (hs-CRP), which were performed on 7389 outpatient adults (aged 18 years or older) consecutively referred by general practitioners for routine blood testing between June 2006 and June 2007.

Laboratory methods

Venous blood from outpatients was routinely collected in the morning on fasting subjects. If a participant had more than one blood test ordered over the year, only the first result was included in the analysis. Haemoglobin concentration was determined by a fully automated haematological analyser ADVIA 120TM (Bayer Diagnostics, Newbury, Berkshire, UK). Serum creatinine, glucose, total cholesterol and triglyceride concentrations were assayed by standard enzymatic procedures on Roche/Hitachi Modular System (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's specifications and employing proprietary reagents. High sensitivity hs-CRP concentrations were measured by a nephelometric assay on a Behring Nephelometer II (BN II, Dade Behring GmbH, Marburg, Germany). A 3.0 mg/L threshold for hs-CRP was selected, since it indicates a higher relative cardiovascular risk when added to traditional risk factors [12]. Kidney function was calculated by using the formula developed and validated in the Modification of Diet in Renal Disease (MDRD) study. The MDRD formula was as follows: estimated glomerular filtration rate (eGFR) = 175.0 × (serum creatinine\(^{-1.154}\)) × (age\(^{-0.203}\)) × 1.212 (if black) × 0.742 (if female) [13].

Statistical analysis

Data are expressed as mean ± SD or frequencies. Excluding those \((n = 3)\) who had eGFR values ≥200 mL/min/1.73 m² left 7389 participants who were included in the final analysis. For statistical purposes, participants were categorised according to four clinical categories of eGFR values (i.e. <30, 30–59, 60–89 and ≥90 mL/min/1.73 m²). Skewed variables (triglycerides and hs-CRP) were logarithmically transformed to improve normality prior to the analysis and then back-transformed to their natural units for presentation in the text. Statistical analyses included the one-way analysis of variance (with Fisher-PLSD tests for multiple comparisons between groups), the chi-squared test with Yate’s correction for continuity (for categorical measures) and the analysis of covariance. Multivariable linear regression analyses were also performed to identify factors independently associated with haemoglobin concentrations for each category of eGFR (Table 2). In these analyses, we combined subjects with eGFR <30 mL/min/1.73 m² and those with eGFR 30–59 mL/min/1.73 m² in a single one eGFR category, given the low number of those \((n = 50)\) with eGFR <30 mL/min/1.73 m². Haemoglobin (entered as continuous measure) was considered as the dependent variable, whereas age, gender, fasting plasma glucose, total cholesterol, triglyceride, hs-CRP concentrations and baseline eGFR were included as covariates in these multivariable regression models. \(P < 0.05\) were considered statistically significant.

Results

Cumulative results for serum creatinine and hs-CRP tests were retrieved for 7389 outpatient adults (M/F = 2642/4747) with a broad spectrum of age [mean age (±SD): 57 ± 17 years; range: 18–95]. In the whole population, the mean (±SD) concentrations of haemoglobin, eGFR and hs-CRP were 14.0 ± 1.5 g/dL (range: 4.4–19 g/dL), 81.1 ± 19 mL/min/1.73 m² (12–186 mL/min/1.73 m²) and 5.6 ± 14 mg/L (0.7–424 mg/L), respectively. The prevalences of women with haemoglobin <12 g/dL or <11 g/dL or <10 g/dL were 9.9%, 3.7% and 1.1%, respectively. Frequencies of men with haemoglobin <12 g/dL or <11 g/dL or <10 g/dL were 6.3%, 2.6% and 1.0%, respectively. There were significant differences \((P < 0.0001)\) by the chi-squared test) between men and women among those with haemoglobin <12 g/dL or <11 g/dL but not <10 g/dL. Moreover, the frequency of individuals with hs-CRP ≥3 mg/L was 37.9% \((n = 2800)\).

The clinical and biochemical characteristics of participants, stratified by eGFR category, are shown in Table 1. Overall, 858 (11.6%) subjects had eGFR <60 mL/min/1.73 m², 47 of whom had eGFR between 15 and 29 mL/min/1.73 m² and 3 subjects had eGFR <15 mL/min/1.73 m²; most participants (58.3%) had an eGFR of 60–89 mL/min/1.73 m².

As shown in Table 1, lower levels of eGFR were significantly associated with older age, female gender, elevated fasting plasma glucose, cholesterol and triglyceride concentrations. Notably, plasma hs-CRP concentrations increased steadily with decreasing eGFR levels. Similarly, the prevalence of participants with hs-CRP ≥3 mg/L was remarkably greater among those with lower eGFR levels. In contrast, haemoglobin concentrations decreased progressively with decreasing eGFR, and the prevalence of anaemia (by any criteria) increased across eGFR categories. Interestingly, the graded increases in hs-CRP and haemoglobin concentrations across eGFR categories remained significant \((P < 0.0001)\) for both by the analysis of covariance) after adjustment for age, gender, plasma glucose and lipids (not shown). The above-mentioned significant trends did not change when participants \((n = 50)\) with eGFR <30 mL/min/1.73 m² were excluded from the analysis or combined with those with eGFR in the range 30–59 mL/min/1.73 m².

In multivariable linear regression analyses (Table 2), there was an inverse and independent association between hs-CRP and haemoglobin concentrations in each category of eGFR and in the whole cohort, which implies that an
Inflammation and anaemia in CKD

Table 1. Clinical and biochemical characteristics of the whole cohort of individuals (n = 7389) grouped according to estimated glomerular filtration rate (eGFR) categories

<table>
<thead>
<tr>
<th>Estimated eGFR categories (mL/min/1.73 m²)</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td></td>
</tr>
<tr>
<td>30–59</td>
<td></td>
</tr>
<tr>
<td>60–89</td>
<td></td>
</tr>
<tr>
<td>≥90</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>Gender (female, N)</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td></td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td></td>
</tr>
<tr>
<td>haemoglobin ≤10 g/dL (%)</td>
<td></td>
</tr>
<tr>
<td>Haemoglobin ≥12 g/dL (%)</td>
<td></td>
</tr>
<tr>
<td>Haemoglobin ≥11 g/dL (%)</td>
<td></td>
</tr>
<tr>
<td>Haemoglobin ≥12 g/dL (%)</td>
<td></td>
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</tbody>
</table>

Data are expressed as means ± SD or frequencies. P-values for trend by the one-way analysis of variance (for continuous variables) or the chi-squared test (for categorical measures). hs-CRP: high sensitivity C-reactive protein.

*P < 0.05 or less by Fisher-PLSD tests for multiple comparisons between groups (i.e. each GFR category versus the next lower GFR category for hs-CRP and haemoglobin only).

Table 2. Independent predictors of haemoglobin concentrations (included as a continuous measure) in each category of estimated glomerular filtration rate (eGFR in mL/min/1.73 m²) and in the whole cohort of participants

<table>
<thead>
<tr>
<th>Independent predictors</th>
<th>&lt;60</th>
<th>60–89</th>
<th>≥90</th>
<th>Whole cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>858</td>
<td>4310</td>
<td>2221</td>
<td>7389</td>
</tr>
<tr>
<td>Age</td>
<td>−0.16</td>
<td>−0.08</td>
<td>−0.05</td>
<td>−0.10</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>−0.17</td>
<td>−0.43</td>
<td>−0.54</td>
<td>−0.42</td>
</tr>
<tr>
<td>Baseline eGFR</td>
<td>0.34</td>
<td>0.06</td>
<td>0.09</td>
<td>0.18</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>−0.14</td>
<td>−0.19</td>
<td>−0.21</td>
<td>−0.19</td>
</tr>
</tbody>
</table>

Data are presented as standardized beta coefficients by multivariable linear regression analyses. P < 0.0001 for all variables presented in the table. Note that fasting plasma glucose, total cholesterol and triglyceride concentrations were also entered in the four different regression models but did not independently correlate with haemoglobin concentrations. hs-CRP: high sensitivity C-reactive protein.

Discussion

Anaemia is commonly seen in individuals with CKD and its etiology is complex and multifactorial [14]. Our results show that while anaemia is closely associated with a reduction in eGFR levels, much of this association appears to be the result of confounding by associated factors, especially the presence of chronic inflammation as reflected by increased plasma hs-CRP concentrations. Our findings support and extend to a large cohort of unselected outpatients with a wide range of age and kidney functions the observations from haemodialysis studies [9–11] regarding a strong relationship between anaemia and chronic inflammation, and suggest that chronic inflammation is associated with lower haemoglobin concentrations even in the early stages of CKD.

In observational studies of chronic dialysis patients, the requirement of high doses of ESA is related to the degree of anaemia [9–11]. In addition, participants with high ESA dose requirements also have higher levels of plasma inflammatory markers such as CRP and interleukin-6 [9]. Furthermore, these inflammatory markers have been shown to predict lower haemoglobin concentrations in patients on dialysis and to be responsible for ~10% of the observed variability in the ESA dose requirements [15]. Although increasing attention is being paid to understanding and treating anaemia among CKD patients not yet requiring renal replacement therapy [6] and explaining the relationship between progressive kidney disease and inflammatory biomarkers [16], to our knowledge,
the effect of underlying inflammation on haemoglobin concentrations in patients with early stages of CKD has not been extensively evaluated.

In the CHOIR study [3], the largest study to date in CKD patients not receiving dialysis, higher haemoglobin concentrations were found to be associated with an increased risk (hazard ratio 1.34; \( P = 0.03 \)) of developing future cardiovascular events (death, myocardial infarction, hospitalization for congestive heart failure and stroke). The mechanisms that underlie this excess in cardiovascular events noted in the patients randomized to a higher haemoglobin concentration are unclear. Whether this increased risk relates to the higher haemoglobin per se or to the means by which this was achieved—i.e. use of higher doses of erythropoietin—remains controversial. Moreover, a recent meta-analysis further confirmed an increased risk of all-cause mortality (hazard ratio 1.17; \( P = 0.031 \)), arteriovenous access thrombosis (hazard ratio 1.34; \( P = 0.0001 \)) and poorly controlled blood pressure (hazard ratio 1.27; \( P = 0.004 \)) in patients with CKD randomized to higher haemoglobin targets [17].

Recombinant human erythropoietin not only increases blood viscosity as a result of increased erythrocyte mass but may also increase thrombotic risk via increased inflammation and anti-fibrinolytic activity, which can occur irrespective of haemoglobin concentration [18]. Overall, these observations arise the possibility that participants included in the CHOIR study could have had higher levels of plasma inflammatory markers. Of note, plasma inflammatory markers have not been measured in the CHOIR study, although the age-adjusted prevalence of hs-CRP \( \geq 2.2 \text{ mg/dL} \) has been previously reported to be present in 44% and 69% of subjects with eGFR of 59–30 mL/min/1.73 m² and 29–15 mL/min/1.73 m², respectively [19].

The reasons for the elevation in plasma inflammatory markers with the progressive decline in eGFR are poorly known. However, inflammation-induced anaemia and resistance to erythropoietin are common features in patients with advanced CKD. Elevated levels of inflammatory cytokines, enhanced oxidative stress and alterations in iron metabolism—conditions associated with inflammatory states—may be implicated in the development of anaemia [20]. For example, interferon-\( \gamma \) and TNF-\( \alpha \) by diminishing colony formation of burst-forming unit-erythroid cells (BFU-es) and colony-forming unit-erythroid cells (CFU-Es) can exacerbate anaemia [21–23]. The marked inhibitory effects of these inflammatory cytokines on erythroid progenitor cells may be mainly related to the ability of these cytokines to decrease endothelial nitric oxide production, which is known to stimulate the proliferation of erythroid progenitor cells [24]. Hence, inflammation is an established risk factor for end-stage renal disease (ESRD)-associated anaemia, where it is usually attributed to blood-dialyzer interactions, impurities within the dialysate or other coexisting comorbid diseases [25,26]. In the present cohort, we show that the presence of increased plasma concentrations of hs-CRP in association with mild-to-moderate reductions in eGFR occurs in a substantial proportion of subjects (i.e. \( > 55\% \) of participants with eGFR \( 60 \text{ mL/min/1.73m}^2 \)) had an hs-CRP concentration \( \geq 3 \text{ mg/L} \), and long before the need for renal replacement therapy.

The current study has some potential limitations that should be noted. First, given the cross-sectional design of the study, we cannot be certain that plasma hs-CRP concentrations affect anaemia rather than vice versa. Second, no information was available on medications use and co-existing medical conditions. Third, the definition of kidney function was based on eGFR rather than on more precise measurement of kidney function, such as iothalamate clearance. Although the MDRD equation has been shown to be more accurate than the Cockcroft–Gault equation for persons with GFR \(< 90 \text{ mL/min/1.73 m}^2 \), questions remain about the generalisability of the MDRD study equation. Finally, whether these observations can also be extended to non-Caucasian ethnic groups remains to be determined. However, we believe most of these potential limitations should be consistently attenuated by the very large sample size of the study—which is possibly representative of the adult population living in Verona (given that our Clinical Chemistry Laboratory is the largest one of Verona and it serves \( \sim 90 \text{ 000 outpatient adults per year} \)—and by the finding of strong, graded, associations between hs-CRP, haemoglobin concentrations and kidney function tests even within the reference intervals.

In summary, this study shows that elevated hs-CRP concentrations are independently associated with lower haemoglobin concentrations in a large cohort of unselected outpatient adults. Furthermore, the relationship between hs-CRP concentrations and anaemia appears to be evident at early stages of CKD. Future prospective studies are needed to confirm whether elevated levels of acute-phase reactants are associated with anaemia in subjects with CKD not requiring dialysis.

Conflict of interest statement. None declared.

References


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