Subclinical hypothyroidism is linked to micro-inflammation and predicts death in continuous ambulatory peritoneal dialysis

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Abstract

Background. Low T3 is a frequent alteration in patients with ESRD. This derangement has been recently linked to inflammation in haemodialysis patients. Whether this association holds true in peritoneal dialysis patients has not been studied.

Methods. We investigated the relationship between low-grade inflammation [IL-6, C-reactive protein (CRP) and serum albumin levels] and free triiodothyronine (fT3) in a cohort of 41 CAPD patients (mean age, 66 years; M, 26; F, 15) without heart failure and inter-current illnesses.

Results. CAPD patients had lower fT3 levels (2.7 ± 0.8 pg/ml) than healthy subjects (3.7 ± 1.0 pg/ml, P < 0.001) of similar age. Free T3 levels were directly related to those of serum albumin (r = 0.52, P < 0.001) and inversely to IL-6 (r = -0.30, P = 0.05) and CRP (r = -0.54, P < 0.001). Age (r = -0.61, P < 0.001), haemoglobin levels (r = 0.32, P = 0.05) and diastolic blood pressure (r = 0.50, P = 0.001) were also related to fT3. In multiple regression models adjusting for all variables related to fT3, CRP and albumin were retained as independent correlates of fT3.

During the follow-up (2.8 ± 1.7 years) 27 patients died. Plasma fT3 levels were lower in patients who died (2.5 ± 0.8 pg/ml) compared with survivors (3.3 ± 0.5 pg/ml P = 0.001). In Cox analyses, fT3 was a significant predictor of mortality independent of the main traditional as well as non-traditional risk factors.

Conclusions. The relationship between fT3, CRP and serum albumin suggests that inflammation–malnutrition might be involved in the low T3 syndrome in CAPD patients. Thyroid dysfunction might be implicated in the pathogenic pathway which links micro-inflammation to survival in PD patients.

Keywords: CAPD; inflammation; low T3 syndrome; thyroid

Introduction

The notion that end-stage renal disease (ESRD) [1–4] affects thyroid function has solid ground and ESRD is now formally listed as an established cause of chronic non-thyroidal illness [5]. A variety of alterations in thyroid hormone levels and/or metabolism have been described in patients with ESRD and low plasma triiodothyronine (T3) has been consistently found to be the most common disturbance in thyroid function in this population [1–4].

Peritoneal dialysis (PD) patients frequently display low T3 levels [6–9] as an effect of impaired extra-thyroidal T4 to T3 conversion [10], or as a phenomenon secondary to peritoneal loss of thyroid binding globulin [11]. The recent observation that biomarkers of inflammation are consistently associated with low T3 levels in haemodialysis (HD) patients [12] is a stimulating new finding which may have implications also for PD patients. Indeed, a low-grade inflammatory state, signalled by high levels of IL-6 and C-reactive protein (CRP), is frequently observed in PD patients [13–18]. Because risk factors for inflammation in PD patients do not coincide with those in HD patients, we thought that it is important to confirm the inflammation-T3 link in PD patients and explore the association between low T3 and hard outcomes (death) in this population. In this prospective study, we have therefore investigated the steady-state relationship between biomarkers of inflammation and T3 in a cohort of CAPD patients and tested the prognostic value of T3 in the same cohort.
Patients and methods

Study population

Patients belonging to the CAPD cohort enrolled in the CREED study (Cardiovascular Risk Extended Evaluation in Dialysis Patients) [19] formed the basis of the present study. The criteria for enrolment were being on dialysis for more than 6 months, the absence of clinical evidence of heart failure [20] and no inter-current acute illness. Patients were free of peritonitis for at least 3 months at time of enrolment. This cohort represented about 80% of the whole CAPD population of the CREED study. Among 51 eligible patients, 41 patients (26 M and 15 F, mean age 66 ± 16 years) were not affected by thyroid diseases and were not taking drugs known to interfere with thyroid function (i.e. amiodarone, β-blockers, lithium).

Patients were treated with four 2 l (2.5 l in three patients) exchanges per day, using a standard dialysate containing glucose (Na 132, lactate 35 mmol/l, Ca 1.75 mmol/l). In seven patients, one bag was an icodextrin (7.5 g/dl) solution. Dialysis prescription aimed at obtaining a total Kt/V of at least 1.8/week. The median duration of CAPD treatment was 38 months (interquartile range 12.5–65 months), and 56% of the patients had been on this treatment modality for more than 2 years. The median residual diuresis was 136 ml (interquartile range: 0–370 ml) and the median residual glomerular filtration rate [Creatinine Cl(Urea Cl)/2] 0.14 ml/min (interquartile range 0–0.9 ml/min). The cause of chronic renal disease was nephroangiosclerosis in 14, greater renal disease in 8, unknown in 8, polycystic kidneys in 5, tubulo-interstitial nephritis in 3, and nephropathy associated with Laurence Moon Biedl syndrome in 1 patient. Two patients had diabetic nephropathy but diabetes as a comorbidity was present in five additional patients.

Control group

Thyroid hormone levels were also determined in a control group composed of 31 healthy individuals recruited from the clinical and laboratory staff and from a series of healthy senior members of an association supporting our institution. The control group was well matched to the patients as for sex and age (15 M, 16 F, average age 61 years).

Blood pressure measurements

Blood pressure values were obtained by averaging home blood pressure measurements (10–20 measurements/month).

Laboratory measurements

Free plasma triiodothyronine (fT3) and thyroxine (fT4) were measured by commercially available RIA kits (Byk-Sangtek Diagnostica, Dietzenbach, Germany) and thyrotropin (TSH) by a sensitive IRMA (Byk-Sangtek Diagnostica, Dietzenbach, Germany). The intra-assay CV of these hormones ranged from 2.8% to 4.7%; and the inter-assay CV from 6.5% to 7.1%. The upper limit of TSH of this assay is 3 mIU/l. CRP was measured by using a commercially available kit (immunoturbidimetric method, lower limit of detection ≤3.5 mg/l) (Behring, Scoppito, L’Aquila, Italy). Serum levels of IL-6 were measured by ELISA with the use of Quantikine High Sensitivity kits (intra-assay CV: 2.6%; inter-assay CV: 4.5%) (R&D Systems Inc, Minneapolis, USA).

Serum albumin concentration was measured by the bromocresol green method.

Follow-up

After the initial assessment patients were followed up for an average time of 2.8 ± 1.7 years. Each death occurring during the follow-up was reviewed and assigned an underlying cause by a panel of five physicians. As a part of the review process, all available medical information about each death was collected. This information always included study and hospitalization records. In the case of an out-of-hospital death, family members were interviewed by telephone to better ascertain the circumstances surrounding death.

Statistical analysis

Data are presented as mean ± SD, median and inter-quartile range or as percent frequency and comparison between groups were made by t-test, Mann–Whitney test or chi-squared test, as appropriate.

To test the independent link between inflammation and fT3, we divided patients into tertiles on the basis of the plasma concentration of IL-6. Tested covariates included thyroid hormones as well as a series of traditional risk factors (age, male gender, previous CV events, smoking, diabetes, arterial pressure, heart rate, antihypertensive treatment, cholesterol and triglycerides), risk factors peculiar to dialysis patients (haemoglobin, calcium and phosphate) and markers of inflammation–malnutrition (albumin and CRP). The independent association between plasma fT3 and IL-6, CRP and albumin was analysed further by simple and multiple linear regression analyses adjusting for other factors which were associated to fT3 on univariate analysis.

The prognostic power of low fT3 for death was analysed by Kaplan–Meyer survival analysis and by the Cox’s proportional hazards method. Due to the small number of deaths, the independent risk of plasma fT3 for all cause mortality was analysed with a parsimonious approach based on bivariate Cox models. In these analyses, we tested fT3 as a fixed covariate and relevant risk factors for death (considered one by one) as a second covariate.

All calculations were made using a standard statistical package (SPSS for Windows).

Study power

On the basis of published analyses in HD patients [12], we hypothesized that correlation coefficients defining the association between inflammation markers and T3 were about 0.40. Assuming that correlations of similar strength also exist in PD patients, we calculated that with 41 patients our study had an 80% power to detect as statistically significant (P-value <0.05, two tailed) prospectively tested associations between the same variables in PD patients.
Table 1. Main demographic, clinical and biochemical data of dialysis patients (n = 41)

<table>
<thead>
<tr>
<th></th>
<th>I tertile</th>
<th>II tertile</th>
<th>III tertile</th>
<th>P for trend</th>
<th>fT3 vs r and (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;5.9 pg/ml</td>
<td>6.0–10.9 pg/ml,</td>
<td>&gt;10.9 pg/ml,</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>n = 14</td>
<td>n = 13</td>
<td>n = 14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>57 ± 19</td>
<td>69 ± 14</td>
<td>72 ± 11</td>
<td>0.015</td>
<td>−0.61 (0.001)</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>12 (86%)</td>
<td>7 (54%)</td>
<td>7 (50%)</td>
<td>0.05</td>
<td>0.12 (0.45)</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>9 (64%)</td>
<td>7 (54%)</td>
<td>7 (50%)</td>
<td>0.45</td>
<td>−0.03 (0.86)</td>
</tr>
<tr>
<td>Diabetics, n (%)</td>
<td>2 (14%)</td>
<td>2 (15%)</td>
<td>3 (21%)</td>
<td>0.62</td>
<td>−0.16 (0.32)</td>
</tr>
<tr>
<td>Anti-hypertensive therapy, n (%)</td>
<td>10 (71%)</td>
<td>8 (61%)</td>
<td>10 (71%)</td>
<td>1.0</td>
<td>0.14 (0.37)</td>
</tr>
<tr>
<td>With previous CV events, n (%)</td>
<td>5 (36%)</td>
<td>5 (38%)</td>
<td>6 (43%)</td>
<td>0.70</td>
<td>−0.14 (0.39)</td>
</tr>
<tr>
<td>On treatment with EPO, n (%)</td>
<td>6 (43%)</td>
<td>4 (31%)</td>
<td>8 (57%)</td>
<td>0.45</td>
<td>−0.04 (0.81)</td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>141 ± 18</td>
<td>139 ± 18</td>
<td>140 ± 20</td>
<td>0.87</td>
<td>0.14 (0.39)</td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>84 ± 8</td>
<td>79 ± 13</td>
<td>76 ± 10</td>
<td>0.06</td>
<td>0.50 (0.001)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>78 ± 12</td>
<td>79 ± 11</td>
<td>81 ± 10</td>
<td>0.65</td>
<td>0.03 (0.88)</td>
</tr>
<tr>
<td>fT3 (pg/ml)</td>
<td>3.11 ± 0.70</td>
<td>2.87 ± 0.83</td>
<td>2.32 ± 0.72</td>
<td><strong>0.008</strong></td>
<td>−</td>
</tr>
<tr>
<td>fT4 (ng/100ml)</td>
<td>1.24 ± 0.24</td>
<td>1.19 ± 0.19</td>
<td>1.24 ± 0.15</td>
<td>1.00</td>
<td>−0.26 (0.10)</td>
</tr>
<tr>
<td>TSH (mIU/l)</td>
<td>1.81 ± 1.75</td>
<td>1.41 ± 1.25</td>
<td>2.49 ± 1.91</td>
<td>0.28</td>
<td>0.07 (0.68)</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>198 ± 51</td>
<td>207 ± 38</td>
<td>218 ± 45</td>
<td>0.51</td>
<td>0.03 (0.85)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>185 ± 101</td>
<td>179 ± 126</td>
<td>187 ± 93</td>
<td>0.88</td>
<td>0.20 (0.21)</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>10.6 ± 2.1</td>
<td>11.6 ± 2.1</td>
<td>9.7 ± 1.7</td>
<td>0.21</td>
<td>0.32 (0.05)</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>8.8 ± 1.0</td>
<td>9.2 ± 1.8</td>
<td>8.6 ± 0.8</td>
<td>0.63</td>
<td>0.003 (0.98)</td>
</tr>
<tr>
<td>Phosphate (mg/dl)</td>
<td>5.9 ± 1.4</td>
<td>5.6 ± 0.9</td>
<td>4.9 ± 1.3</td>
<td>0.07</td>
<td>0.26 (0.09)</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.5 ± 0.3</td>
<td>3.3 ± 0.3</td>
<td>3.3 ± 0.3</td>
<td><strong>0.05</strong></td>
<td>0.52 (0.001)</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>4.6 (3.4–8.0)</td>
<td>4.8 (3.4–16.1)</td>
<td>16.7 (7.9–44.7)</td>
<td><strong>0.001</strong></td>
<td>−0.54 (0.001)</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>4.05 (2.7–5.6)</td>
<td>7.8 (7.2–8.95)</td>
<td>20.1 (11.8–26.8)</td>
<td><strong>0.001</strong></td>
<td>−0.30 (0.05)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD, median and inter-quartile range or as percent frequency, as appropriate. Patients are divided into three groups on the basis of IL-6 tertiles, and P for trend tests the differences among the tertiles; r is the Pearson coefficient of correlation between fT3 and the variables. Significant differences between tertiles, and significant correlations are indicated in bold.

**Results**

Table 1 shows the main baseline characteristics of the patients divided into three groups on the basis of IL-6 tertiles. At time of enrolment 16 patients (39%) had a history of at least one previous CV event (myocardial infarction = 6, stroke = 3, angina = 4, TIA = 4, arrhythmia = 3, lower limb ischaemia with claudication = 5).

Patients on CAPD had lower fT3 levels (2.7 ± 0.8 pg/ml) than healthy subjects (3.7 ± 1.0 pg/ml, P < 0.001). Figure 1 shows the scatter plot of fT3 values. No difference in fT3 levels was observed between patients on icodextrin (3.0 ± 0.8 pg/ml) and those not using icodextrin (2.7 ± 0.8 pg/ml, P = 0.5). Free T4 did not differ between CAPD patients (1.22 ± 0.19 ng/100 ml) and healthy controls (1.25 ± 0.22 ng/100 ml). Seven patients (i.e. 17%) had TSH levels between 3.5 and 7.7 mIU/l, above the upper limit (cutoff: 3 mIU/l) of the normal range, but well below the values observed in overt hypothyroidism.

When patients were divided into three tertiles on the basis of IL-6 plasma concentration it emerged that those in the third tertile had lower fT3 levels, were older, had lower serum albumin and higher CRP concentrations (Table 1). These categorical associations were fully confirmed by linear regression analysis. Serum fT3 levels were indeed directly associated with serum albumin (r = 0.52, P = 0.001) and inversely to IL-6 (r = −0.30, P = 0.05) CRP (r = −0.54, P < 0.001) and age (r = −0.61, P < 0.001) (Table 1 last column, Figure 2). We also observed a direct association of fT3 with diastolic blood pressure (r = 0.50, P = 0.001) and haemoglobin levels (r = 0.32, P = 0.05) (Table 1 last column). As expected, IL-6, CRP and albumin were significantly interrelated (Table 2). The IL-6–CRP association being the strongest (P = 0.001) and the CRP–albumin the weakest (P = 0.09) among these relationships (Table 2).

In multiple regression analyses with fT3 set as the outcome variable and albumin, CRP, IL-6, age, diastolic blood pressure and haemoglobin as predictor variables, CRP and albumin were retained as independent correlates of fT3 (Table 3).
Patients were followed up for 2.8±1.7 years. During this period, 27 of them died (Table 4). In a crude analysis, fT3 levels were lower in patients who died (2.5±0.8 pg/ml) as compared with survivors (3.3±0.5 pg/ml, \( P = 0.001 \)), while free T4 (1.21±0.18 vs 1.25±0.22 ng/100 ml, \( P = 0.61 \)) and TSH levels (median 1.30 mIU/l interquartile range (0.75–2.20) vs 2.05 (0.37–3.92), \( P = 0.47 \)) did not differ.

In a Kaplan–Meyer analysis, the overall risk of death was progressively higher from the third to the first tertile of fT3 levels (Figure 3). In parsimonious Cox analyses adjusting for other risk factors, fT3 was confirmed as a significant predictor of mortality (Table 5).

**Discussion**

Our observations confirm that low-T3 levels are commonly found in ESRD patients on PD and show that this alteration is linked to low-grade inflammation and death in this population.

Like HD patients, CAPD patients frequently display alterations in thyroid function of various severity, ranging from the low-T3 syndrome to subclinical and frank hypothyroidism [6–9]. Previous studies have explored risk factors for disturbed thyroid function
peculiar to PD, like loss of thyroid binding globulin with PD fluids [11] or thyroid hormone synthesis suppression induced by iodine used as disinfectant [21]. Inflammation is now emerging as one of the most important causes of deranged thyroid function associated with chronic or acute non-thyroidal illness [22–26]. In particular, experimental as well as clinical studies indicate that the inflammatory cytokine network plays a central role in the genesis of the low T3 syndrome. IL-6 decreases the mRNA of liver type 5'-deiodinase (D1) [22] as well as of thyroid type 5'-D1 [23] and these and other mechanisms are implicated in the low T3 induced by bacterial endotoxins [24]. The critical role of IL-6 in this syndrome is nicely epitomized by the observation that the suppressive effect of inflammation on T3 is markedly attenuated in IL-6 knock-out mice [24]. Consistent with this experimental evidence are clinical surveys showing strong inverse associations between IL-6 and thyroid hormone levels in patients with chronic or acute non-thyroidal disorders [25,26]. In a recent survey [12], we showed that inflammatory markers are strongly associated with low T3 in HD. Such an association may be causal in nature because in CKD patients studied sequentially before/after inter-current infective episodes, T3 plasma concentration mirrors plasma IL-6 and CRP levels [12]. Whether low T3 is associated with inflammation in PD patients has not been investigated to date. The question is of relevance mainly because it was emphasized that risk factors for inflammation in PD patients do not coincide with those observed in HD patients.

The present survey is the first to show that inflammation is linked to the low-T3 syndrome also in CAPD patients. Indeed, we observed inverse relationships between fT3 levels, CRP and IL-6, as well as a direct relationship with serum albumin levels, which is a negative acute phase reactant and a nutrition marker as well. It should be noted that in the multivariable analysis the link between CRP and Albumin with fT3 was apparently stronger than that of IL-6 with the same outcome variable. This finding is difficult to interpret on biological grounds because the three inflammatory biomarkers were interrelated.

While our study was powerful enough to explore the association between inflammatory markers and T3, it was underpowered to study the independent association between this factor and hard outcomes with a full multivariate Cox analysis including all established predictors of mortality in CAPD. Nonetheless, our analysis based on parsimonious (bivariate) statistical models shows that low T3 is not only a strong univariate predictor of mortality but also that it is independently associated with this outcome, thus suggesting that low T3 in CAPD patients may not be an innocent finding.

The clinical implications of the low-T3 syndrome in ESRD are still unknown mainly because most patients are clinically euthyroid and do not require thyroid
hormone replacement therapy. It was suggested that low-T3 syndrome may be seen as a protective adaptation against protein wasting [10]. However, the benignity of low-T3 syndrome has been questioned, and recently an association between fT3 levels and background cardio-vascular complications has been found in a cross-sectional study of HD patients [12].

While the link between inflammation and T3 was quite consistent, it should be clearly recognized that the observational nature of our study does not allow conclusions as to the nature (causal or non-causal) of this association in PD patients. This study establishes for the first time an association between inflammatory biomarkers and fT3 in PD patients and generates the hypothesis that thyroid dysfunction is implicated in the pathogenic pathway which links micro-inflammation to survival in CAPD patients, an issue which remains to be confirmed in larger prospective cohort studies and in properly designed intervention studies. As to the possibility of an intervention study, small studies show that thyroid hormone administration improves heart function in patients with subclinical hypothyroidism [27,28]. Interventions could also be aimed at correction of acidosis, a manoeuvre which has been found to improve the low-T3 syndrome in HD patients [29] and/or at correcting the inflammatory state.

Conflict of interest statement. None declared.

References


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