Original Paper

High Levels of Soluble Tumor Necrosis Factor Receptors 1 and 2 and Their Association with Mortality in Patients Undergoing Hemodialysis

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Key Words

Tumor necrosis factor · Laplace regression · Mortality · Inflammation

Abstract

Objective: Circulating soluble tumor necrosis factor receptors 1 and 2 (sTNFR1 and sTNFR2) are associated with chronic kidney disease (CKD) progression in patients with CKD or diabetes, and with higher mortality. However, data in patients with end-stage renal disease are scarce. Therefore, we analyzed serum levels of sTNFR1 and sTNFR2 and investigated their association with inflammatory markers and mortality in dialysis patients. **Research Design and Methods:** This was a longitudinal cohort study of 207 prevalent patients (median age 66 years, 56% men) undergoing hemodialysis in Stockholm, Sweden. Demographics, clinical characteristics, including comorbidities and laboratory data, were obtained at baseline, together with prospective follow-up for mortality. **Results:** The median sTNFR1 and sTNFR2 levels were 17,680 ng/l [95% confidence interval (CI) 17,023–18,337] and 24,450 ng/l (95% CI 23,721–25,179), respectively. During a follow-up of 31 months (interquartile range, 21–38), 77 patients died. There was no association between the levels of sTNFRs and mortality in Cox regression models, and no consistent trend towards higher or lower mortality was seen in Laplace regression models. sTNFR1 and sTNFR2 levels were highly associated with other inflammatory markers including interleukin-6, pentraxin 3 and TNF- α . **Conclusions:** Prevalent hemodialysis patients have several-fold higher levels of sTNFRs compared to previous studies in CKD stage 4 patients. As no consistent association between TNFR and mortality was observed, clinical implications of measuring these receptors to predict outcome end-stage renal disease patients provide limited results. © 2015 S. Karger AG, Basel

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Introduction

End-stage renal disease (ESRD) is a serious condition where the survival often is limited to a few years, and where traditional cardiovascular risk factors have been shown to be of little prognostic importance [1]. Novel markers of prognosis in this high-risk population are thus warranted [2].

Evidence from studies in rats supports a causal role for soluble receptors for tumor necrosis factor (TNF)- α (sTNFRs: sTNFR1 and sTNFR2) in diabetic nephropathy [3]. A causal link between sTNFRs in diabetic nephropathy is further supported by studies in patients with diabetes showing that higher circulating sTNFRs are associated with increased risk of progression of micro- to macro-albuminuria [4], as well as of progression of chronic kidney disease (CKD) [5, 6], and development of ESRD [7].

Higher levels of sTNFR1 have been associated with mortality in the community-based setting [8], in rheumatoid arthritis [9], and diabetic kidney disease [10]. Yet, to the best of our knowledge, there are no studies investigating the levels of sTNFR1 and sTNFR2 in ESRD patients, and their association with mortality.

Based on the associations between sTNFRs, mortality and deterioration of kidney function [5, 7, 11], we hypothesized that these receptors affect causal processes that influence the survival of hemodialysis (HD) patients. Accordingly, we aimed to explore the levels of sTNFRs, and whether their concentration associates with mortality in a cohort of carefully phenotyped prevalent HD patients.

Methods

Patients

The current study was conducted at the Karolinska University Hospital in Stockholm (including four satellite dialysis units), Danderyds Hospital, and Uppsala Academic Hospital. It is an ancillary analysis from frozen samples in a cross-sectional cohort study with prospective follow-up that originally aimed at investigating the variability of inflammatory markers over time in prevalent HD patients. Recruitment occurred from October 2003 through March 2004. Data on demographics, comorbidities (i.e. diabetes mellitus and cardiovascular disease) were obtained from the patient records and blood samples were collected. More details about the study and its participants have been described in detail previously [12, 13]. Blood samples were collected before the dialysis session. The plasma was separated within 30 min, and samples were kept frozen at -70°C if not analyzed immediately. Sufficient plasma samples were available in 207 of the recruited patients who were included in the present analysis.

The Ethics Committee of Karolinska Institutet at Karolinska University Hospital Huddinge approved the study protocol, and informed consent was obtained from all patients.

Anthropometrics and Nutrition

Body mass index was defined as the body weight in kilograms divided by the square of patient height in meters. Self-reported appetite is a part of the subjective global assessment (SGA) questionnaire and it includes six different components: three subjective assessments that are performed by the patient and that concern the patient's history of weight loss, incidence of anorexia, and incidence of vomiting and three assessments that are performed by the evaluators and that are based on the subjective grading of muscle wasting, the presence of edema, and the loss of subcutaneous fat. On the basis of these assessments, each patient received a nutritional status score: (1) normal nutritional status, (2) mild malnutrition, (3) moderate malnutrition, and (4) severe malnutrition. For the purposes of the current study, protein-energy wasting (PEW) was defined as an SGA score >1 .

Laboratory Analysis

The soluble receptors sTNFR1 and sTNFR2 were analyzed using a commercially available ELISA kit (DY225 and DY726; R&D Systems, Minneapolis, Minn., USA). The assays had a total coefficient of variation (CV) of approximately 6%. High-sensitivity C-reactive protein (hsCRP) was measured by nephelometry.

Table 1. Baseline characteristics

Patients	209
Men	118 (56)
Age, years	66 (63–69)
Dialysis vintage, months	29 (24–33)
sTNFR1, ng/l	17,680 (17,023–18,337)
sTNFR2, ng/l	24,450 (23,721–25,179)
Body mass index	24 (23–26)
CRP, mg/l	6.7 (5.2–8.1)
IL-6, pg/ml	8.4 (7.5–9.3)
PTX3, ng/ml	10.2 (9.1–11.3)
TNF- α , pg/ml	14.1 (13.5–14.7)
Plasma albumin, g/l	35 (34–36)
Subjective global assessment >1	93 (45)
Diabetes prevalence	49 (23)

Values are n (%) or medians (Bonnet-Price 95% CI).

Interleukin-6 (IL-6) was quantified in serum by an immunometric assay on an Immulite Analyzer according to the instructions of the manufacturer (Siemens Medical Solutions Diagnostics, Los Angeles, Calif., USA). Pentraxin 3 (PTX3) was determined by an ELISA kit (Perseus Proteomics, Tokyo, Japan), as were the TNF- α levels (Immulite; DPC, Siemens, Los Angeles, Calif., USA). Additional biochemical analyses were performed using routine methods at the Department of Clinical Chemistry at Karolinska University Hospital Huddinge.

Follow-Up

Survival, censored at transplantation, was determined from the day of examination, with a median follow-up period of 31 months (interquartile range, IQR, 21–38).

Statistical Analysis

Bonnet-Price 95% confidence intervals (CI) were estimated for all continuous variables, and used to test if there were any significant differences in the levels of sTNFRs in individuals with high and low SGA (cutoff SGA >1). We tested the correlation between sTNFR1 and sTNFR2, as well as other inflammatory markers using Spearman's rank correlation. The statistical software package STATA 12.1 (Stata Corp, College Station, Tex., USA) was used.

Longitudinal Analyses

In our primary analyses, we modelled sTNFR1 and sTNFR2 as per SD increment. Cox regression was used to calculate hazard ratios (HR) with 95% CI, and Laplace regression was used to calculate the difference in time (in months) until a certain percentage of the ESRD patients died during follow-up [14]. We used 10, 25 and 50% of the patients as cutoffs, to identify survival at different time points during follow-up. Bootstrap methods were used to calculate the 95% CIs in Laplace regression with an amendment for Laplace regression in Stata. The following multivariable models were used: (1) Model A was adjusted for age, sex, dialysis vintage and presence of diabetes mellitus. All these are well-known factors that may influence the prognosis in ESRD patients. (2) Model B was, in addition to the factors in model A, also adjusted for CRP, albumin and PEW, to see if our estimates are independent of inflammation as well as albumin (often used as a marker of nutritional status in ESRD patients), and PEW diagnosis.

Results

Baseline characteristics are shown in table 1. The mean value ($\pm$ SD) of sTNFR1 and sTNFR2 was $17,588 \pm 4,643$ and $25,862 \pm 12,550$ ng/l, respectively. Figure 1a and b shows histograms of sTNFR1 and sTNFR2.

Fig. 1. **a** Histogram of sTNFR1. **b** Histogram of sTNFR2.

Table 2. Multivariable Cox regression models showing the association between sTNFR1 and sTNFR2 with mortality

Biomarker	Model	HR	95% CI	p value
sTNFR1	A	1.18	0.92–1.53	0.18
	B	1.07	0.82–1.40	0.61
sTNFR2	A	1.08	0.96–1.22	0.21
	B	1.04	0.90–1.19	0.61

Model A was adjusted for age, sex, number of days on dialysis before baseline and presence of diabetes mellitus. Model B was, in addition to the factors in model A, also adjusted for CRP, albumin and SGA.

The Spearman rank correlation (ρ) between sTNFR1 and sTNFR2 was 0.73 ($p < 0.001$). The median levels of both receptors were slightly higher in patients with PEW based on their Bonnet-Price 95% CIs ($p < 0.05$). No significant differences in the levels of sTNFR1 ($p = 0.82$) or sTNFR2 (0.14) were seen in patients with and without diabetes (data not shown).

Both sTNFR1 and sTNFR2 were associated with inflammatory markers, i.e. CRP, IL-6, PTX3 and TNF- α at baseline. Spearman's rank correlations: sTNFR1 and CRP ($\rho = 0.34$, $p < 0.001$), sTNFR2 and CRP ($\rho = 0.31$, $p < 0.001$), sTNFR1 and IL-6 ($\rho = 0.25$, $p < 0.001$), sTNFR2 and IL-6 ($\rho = 0.25$, $p < 0.001$), sTNFR1 and PTX3 ($\rho = 0.27$, $p < 0.001$), sTNFR2 and PTX3 ($\rho = 0.29$, $p < 0.001$), sTNFR1 and TNF- α ($\rho = 0.19$, $p = 0.005$), sTNFR2 and TNF- α ($\rho = 0.43$, $p < 0.001$) (data not shown).

Table 2 shows the association between SD increments in the serum levels of sTNFR1 and sTNFR2, and mortality in Cox regression models. There were no significant associations. Table 3 presents the association between SD increments in the serum levels of sTNFR1 and sTNFR2, respectively, and survival time in the Laplace regression models. In agreement with the Cox regression models, neither SD increments in sTNFR1 nor sTNFR2 were significantly associated with any difference in survival time until the first 10 and 25% of the HD patients had died. There was a significant difference ($p = 0.04$) in the median survival for every SD increment in sTNFR1 in model A, which was attenuated and no longer significant in model B. No difference in median survival was seen for sTNFR2.

Table 3. Multivariable Laplace regression models showing the difference in survival of the ESRD patients per SD increment in sTNFR1 or sTNFR2

Percentile	Model	sTNFR1			sTNFR2		
		months	95% CI	p value	months	95% CI	p value
10%	A	−0.49	−5.52, 4.53	0.85	−4.26	−17.00, 8.49	0.51
	B	2.11	−9.01, 13.24	0.71	−0.25	−28.79, 28.30	0.99
25%	A	−2.22	−5.33, 0.89	0.16	−3.86	−15.12, 7.41	0.50
	B	0.76	−5.55, 7.07	0.81	0.48	−0.73, 1.69	0.44
50%	A	−3.70	−7.18, −0.22	0.04	−0.48	−1.18, 0.22	0.18
	B	−0.66	−4.17, 2.86	0.71	−0.21	−1.10, 0.69	0.65

Model A was adjusted for age, sex, number of days on dialysis before baseline and presence of diabetes mellitus. Model B was, in addition to the factors in model A, also adjusted for CRP, albumin and SGA.

Discussion

High levels of sTNFR1 and sTNFR2 were found in a cohort of prevalent HD patients, yet no consistent association with mortality was seen in the Cox and Laplace regression models making the clinical implications of high levels of sTNFR1 and sTNFR2 unlikely to play a major role in the extremely complicated uremic milieu with multiple risk factors [2].

Higher levels of sTNFRs have been shown to be associated with a risk for early renal decline in a study of patients with type 1 diabetes, normal renal function, and no proteinuria [7] as well as development of ESRD in patients with type 2 diabetes without proteinuria [6], suggesting that these markers may contribute to the pathogenesis of renal decline. Higher levels of sTNFR1 were associated with earlier mortality in patients with diabetes and an estimated glomerular filtration rate <60 ml/min or microalbuminuria [10]. High levels of sTNFRs have also been associated with mortality in patients with rheumatoid arthritis [9] and in a multiethnic community-based setting [8]. As no consistent associations with mortality were observed in the present study, this indicates that sTNFRs seem to be less promising as biomarkers of early mortality in patients with established ESRD. As far as we know, the association between sTNFRs and mortality has not been studied previously in dialysis patients.

In the present study, the lack of an association between higher levels of sTNFRs and mortality may be explained by the following potential mechanisms. Higher levels of sTNFRs have been observed among patients with diabetic nephropathy with insulin resistance, higher insulin doses and with a larger waist-hip ratio, supporting the notion of a metabolic dysfunction with high sTNFR levels [15]. Yet, high levels of sTNFRs could also be associated with wasting, which has been shown to induce TNF- α activity [16]. This is supported by our finding of slightly higher levels of sTNFRs in patients with PEW. However, the difference in the present study was not large enough to justify sTNFR measurements as a means to estimate SGA or mortality risk.

Another explanation for the findings towards the null, despite high levels of sTNFRs, could be that the kidney disease process in HD patients had been going on for too long for the observed levels of sTNFRs to be of importance for the outcome. It is also possible that the levels of the receptors were too high to show risk variability.

Finally, as the sTNFRs are eliminated mainly by the kidneys, it is possible that the increased circulating concentrations of the sTNFRs in the present study merely are a consequence of the low or absent glomerular filtration in HD patients, rather than being a marker for causal mechanisms in ESRD leading to mortality.

As far as we know, the present study is the first to investigate sTNFRs as risk markers in HD patients. The levels of sTNFR1 and sTNFR2 were 3–4 times higher than what has previously been reported in CKD stage 4 patients (i.e. a mean value of 4–6,000 ng/l [17], performed with the same methods in the same laboratory). However, in the present study, the Spearman rank correlation between sTNFR1 and sTNFR2 was in accordance with what has been reported in patients with a CKD stage ≥ 3 (0.78) [7], and higher than what has been shown in the community-based setting (0.59) [17]. The high correlation between sTNFR1 and sTNFR2, which is in agreement with previous studies, could be viewed as a validation and support that the high levels of sTNFRs in dialysis patients are a part of the pathophysiological processes taking place in these patients. The high correlation between the sTNFRs and other inflammatory markers in the present study also portray that sTNFRs are a part of the underpinning inflammatory disease process in HD patients.

Progression of CKD in diabetic nephropathy has been suggested to be explained by hyperglycemia affecting the levels of oxidative stress [18, 19]. Oxidative stress increased the TNF- α activity [18], in particular TNFR2 [20], which was also salient in the present study. Yet, there was no significant difference in the median levels of sTNFR1 or sTNFR2 between patients with and without diabetes, indicating that other pathways may explain the high levels in our cohort.

Clinical Implications

While sTNFRs may be useful as markers of kidney function decline and progression of CKD as shown in previous studies [4–7, 17], our findings indicate that their prognostic use in prevalent HD patients seems limited. Yet, the high activity of the sTNFRs and the high correlations with inflammatory markers in these patients calls for additional studies exploring the TNF pathway and its association with morbidities in further detail in these patients [21, 22]. A small study with etanercept in HD patients showed small effects on inflammation biomarkers [23].

Strengths and Limitations

The strengths of our investigation include the prospective longitudinal study design, the detailed characterization of study participants, and the relatively large sample of patients with no loss to follow-up. Limitations include the observational design, including prevalent patients with varying disease duration and low statistical power in some of our analyses, especially when considering the first 10% who died in the Laplace regression models.

Conclusions

Patients undergoing HD have several-fold higher levels of sTNFRs than individuals with normal or even severely reduced kidney function, suggesting that they have a high inflammatory activity. The prognostic utility of sTNFRs in prevalent ESRD patients seems limited. Further studies are needed to shed light on this issue.

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Disclosure Statement

The study was investigator-initiated and -driven. The authors have no conflicts of interest to report.

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