Nontraditional cardiovascular risk factors in end-stage renal disease

Studies on inflammatory markers and thyroid hormones

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Nontraditional cardiovascular risk factors in end-stage renal disease

Studies on inflammatory markers and thyroid hormones

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Voor mijn ouders

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Chapter 1

General Introduction

Chronic kidney disease and end-stage renal disease

In human physiology, the kidneys serve an important task as illustrated by their capacity to regulate volume status, plasma osmolality, blood purification, vitamin D metabolism, and hematopoiesis. Thus, when the kidneys fail, a cascade of pathological consequences is initiated. Kidney failure can occur in an acute setting, being then referred to as acute kidney injury, or in a more lingering, chronic form, which is known as chronic kidney disease.[1] Chronic kidney disease encompasses a broad spectrum of renal dysfunction syndromes in which 5 severity stages are recognized. On one side of the spectrum is stage 1, which corresponds to the presence of renal disease without any loss in renal function. On the other extreme end is stage 5 chronic kidney disease and end-stage renal disease (*ESRD*), in which the estimated glomerular filtration rate (eGFR) has fallen below 15 mL/min/1.73m^2.[1]

Throughout the different stages of chronic kidney disease, increasingly intensive treatment is necessitated to prevent decompensation of the various physiological systems. In end-stage renal disease, and despite optimal pharmacological treatment, the kidneys are no longer able to maintain homeostasis and renal replacement therapy is necessary. This can be done by either renal transplantation or dialysis, the latter option crudely coming in two forms: 1) hemodialysis and 2) peritoneal dialysis. In hemodialysis, blood is flushed through an extracorporeal device and purified by a semipermeable membrane. In peritoneal dialysis, the endogenous peritoneal membrane assists in the removal of uremic toxins and fluids. Both forms of dialysis generally yield an improvement in prognosis and reduction in symptoms.

Cardiovascular risk in end-stage renal disease

Despite dialysis treatment, mortality rates are approximately eight times higher in patients with end-stage renal disease than in age- and sex- matched individuals without renal disease.[2] Approximately 50 and 10 percent of all patients with end-stage renal disease survive 5 and 10 years after initiation of dialysis, respectively.[2] Although mortality hazard for both cardiovascular and non-cardiovascular causes are an eight fold higher than in age and sex matched individuals,[2] the majority of deaths in ESRD is attributable to cardiovascular causes.[3] Congruently, the incidence of coronary artery disease, left ventricular hypertrophy, and congestive heart failure is also substantially elevated in end-stage renal disease.[4,5] This increased cardiovascular risk cannot be explained by an increased occurrence of so called traditional risk factors (e.g. smoking, hypertension and obesity).[6]

During the last decade, we have come to realize that when renal function declines, a wave of so called nontraditional cardiovascular risk factors overshadows the importance of traditional ones.[7] So far, several non-traditional cardiovascular risk factors have been described including an increased sympathetic nerve activity, hemodynamic overload, endothelial dysfunction, oxidative stress, an increased grade of inflammation, and hormonal alterations, all of which havng profound effects on the cardiovascular system.[7] For many of these disturbances, however, uncertainty remains on whether they represent causal risk factors or merely epiphenomena of other processes. This thesis aimed at studying the states of sustained inflammation and low thyroid hormones as plausible candidates for cardiovascular risk factors in patients with end-stage renal disease.

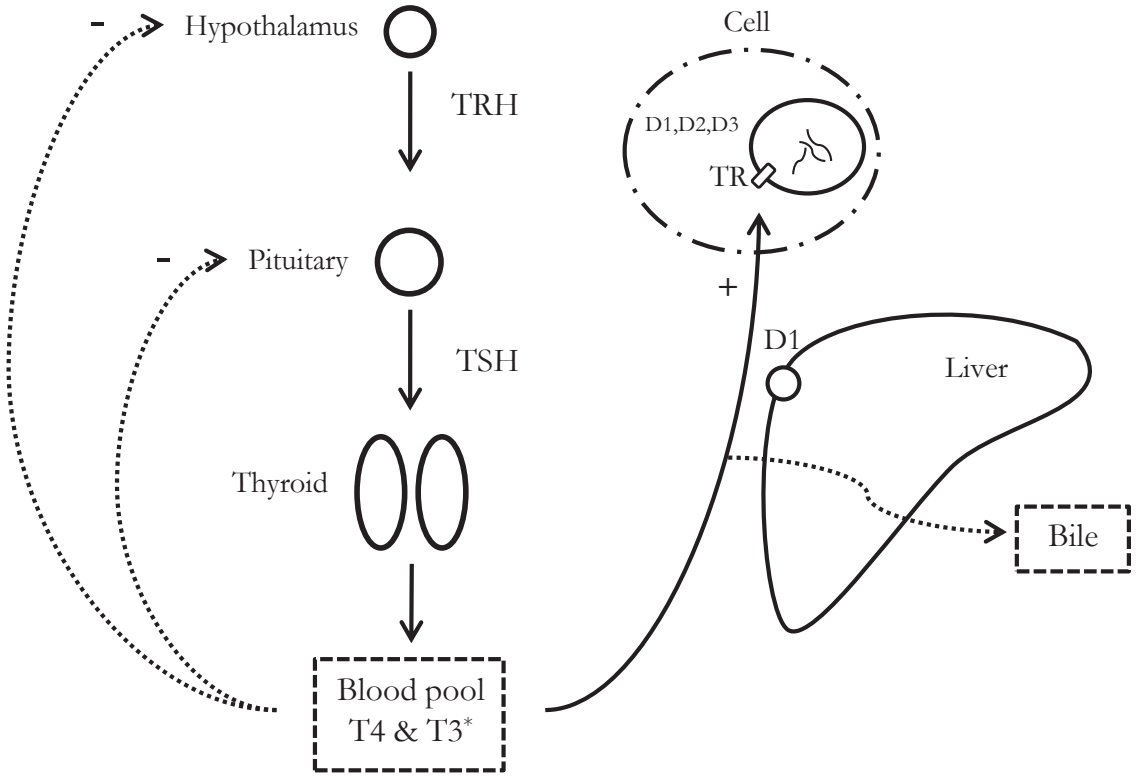

Figure 1. The hypothalamic-pituitaric-thyroidal (HPT)-axis. TRH: Thyroid Releasing Hormone, TSH: Thyroid stimulating hormone, T4: thyroxine, T3, triiodothyronine, TR: thyroid hormone receptor. D1: Deiodinase type 1 (D2: type 2, D3: type 3), TR: thyroid hormone receptor. * The largest part of the blood pool is bound to thyroxine binding globuline (TBG), transthyretin and albumin.

Inflammation and non-thyroidal illness in end-stage renal disease

1. An elevated inflammatory state in end-stage renal disease

In end-stage renal disease, an increased inflammatory state, as expressed by elevations in serum inflammatory markers, is encountered in the majority of patients.[8] As will be reviewed in **Chapter 2**, a variety of stimuli, such as infections, comorbidities, accumulation of toxins and fluid overload, contribute to its genesis. Partly dependent on the type of stimulus, the inflammatory cascade is initiated with the recruitment of polymorphonuclear cells and monocytes and activation of the acute phase response. Whereas some parts of this inflammatory cascade are different, depending on the type of stimulus, a rather non-specific and common feature is that of the acute phase response belonging to the innate immune system.[9]

The acute phase response is hallmarked by elevations in serum positive acute phase reactants (e.g. C-reactive protein (*CRP*)), and decreases in negative acute phase reactants (e.g. albumin and transferrin). The function of the acute phase response has not been fully elucidated but is believed to serve several purposes including; 1) the opsonization and entrapment of pathogens, 2) activation of complement pathways, 3) modulating immune response differentiation, and 4) assistance in tissue repair.[10] Apart from these immune modulatory functions, the acute phase response seems responsible for other provisionary adaptations, meant to prevail in the face of illness. These adaptations include adjustments in the central thermostat causing an increase in body temperature (fever), stimulating protein degradation, and the initiation of a hibernating modus by a central and peripheral downregulation of the Hypothyroidal-Pituitaric-Thyroidal (*HPT*)-axis.[11] Although functional to certain pathogens, this response seems dysfunctional in the face of chronic kidney disease and end-stage renal disease.

2. Nonthyroidal illness in end-stage renal disease

The regulation of serum thyroid hormone levels is a complex process in which multiple endocrine organs are involved. As illustrated in **Figure 1**, under direct influence of pituitaric Thyroid Stimulating Hormone (*TSH*), which is again produced in response to hypothalamic Thyroid Releasing Hormone (*TRH*), the thyroid gland synthesizes free Thyroxine (*fT4*) and, to a lesser extent, the biologically more active free triiodothyronine (*fT3*). After being released into the blood stream, a great portion of fT4 and fT3 binds to binding proteins such as Thyroxine-Binding Globulin (*TBG*), transthyretin (*TTR*), and albumin. The complex of protein bound thyroid hormones is measured as total levels (*TT3* and *TT4* for total triiodothyronine and total thyroxine, correspondingly) in the blood. At a tissue level, different subtypes of deiodinase enzymes regulate local fT3 levels by activating and inactivating fT4 and fT3, respectively. Serum fT4 and fT3 levels provide a direct feedback to hypothalamic TRH production, and pituitaric TSH production thereby yielding a tight control of serum levels.

Alterations in thyroid hormone levels are found in a large proportion of all patients with ESRD.[12-15] These alterations constitute part of a so called "Nonthyroidal illness syndrome". This syndrome is defined as the presence of thyroid hormone alterations in the absence of primary disease in the HPT-axis.[11] Throughout its spectrum, a wide variety of thyroid hormone derangements occur. The origin of these alterations is thought to be offset by successive changes at all levels of the HPT-axis. Factors responsible for these changes specifically associated to end-stage renal disease pertain to an increased inflammatory state, protein-energy wasting, comorbidities, accumulation of iodine and medication usage (as will be extensively reviewed in **Chapter 5**). Although nonthyroidal illness can be viewed upon as an adaptive state serving to survive in times of scarcity and disease, several adverse cardiovascular consequences could make it inappropriate in the setting of end-stage renal disease.

Fluctuations of serum inflammatory markers and thyroid hormones over time

Because the majority of triggers for the genesis of an inflammatory response and nonthyroidal illness fluctuate over time, also the presence and severity of these both risk factors show a large temporal oscillation. When interpreting the impact of the inflammatory response and nonthyroidail illnes on cardiovascular outcome, it seems essential to take into account this temporal variation for a number of reasons. Firstly, a dose response association between different variability patterns and outcome would strengthen the belief in causality. Secondly, it could provide insight in underlying triggers for both risk factors. Thirdly, it could assist in discovering pathways intermediating both risk factors and cardiovascular death which could in turn contribute to the identification of potential treatment targets. Finally, from a predictive point of view, knowledge of factors' temporal variability may improve identification of patients at highest risk and those who may benefit from treatment.

Main study questions

This thesis aimed at increasing our understanding on two plausible risk factors for cardiovascular disease in patients with end-stage renal disease. The following two main questions were addressed:

1. Is there an association between an increased inflammatory state and (cardiovascular) mortality in patients in end-stage renal disease and which mechanisms could contribute to such link?

2. Is there an association between thyroid hormone alterations and (cardiovascular) morbidity and mortality in patients with end-stage renal disease and which mechanisms could contribute to such link?

Patient populations

The studies included in this thesis were performed in cohorts from the Netherlands and Sweden. Below follows a short description of the patient populations included:

Dutch cohorts:

- For the *Netherlands Cooperative Study on the Adequacy of Dialysis* (NECOSAD) study, incident dialysis patients from 38 dialysis centers in the Netherlands were recruited and collected between 1997 and 2002. Out of the total cohort, serum C-reactive protein levels were assessed at 3 and 6 months after start of HD therapy in 472 patients. After inclusion, patients were followed over time during which events of death and censoring due to other reasons were recorded.

- The *Leiden 85 Plus Study* is a population-based cohort of 85-year old individuals. Between 1997 and 1999, all residents of Leiden, the Netherlands, celebrating their 85th birthday (belonging to the 1912-1914 birth cohort) were asked to participate. Out of the 705 individuals who were found eligible, 14 died before the recruitment phase, 92 refused participation and 37 participants refused blood sampling, leaving 562 participants to be included in the current study. During follow-up, participants were visited annually until reaching the age of 90 years or death.

Swedish cohorts:

- The *Mapping of Inflammatory Markers in Chronic Kidney Disease I* (MIMICK-I) cohort comprises prevalent patients with end-stage renal disease undergoing maintenance hemodialysis therapy at the Karolinska University Hospital and its satellite dialysis units throughout the city of Stockholm. From October 2003 through September 2004, 254 patients were invited to participate. Six declined and one subject was not included because of an active HIV infection. 247 patients were followed for 12 weeks during which clinical characteristics were gathered and blood was withdrawn on a weekly basis. After 12 weeks, 23 patients had insufficient data and were excluded. Eventually, a total of 224 patients were included for the current analyses.

- The *Mapping of Inflammatory Markers in Chronic Kidney Disease II* (MIMICK-II) cohort was designed to study inflammatory marker variability in patients on peritoneal dialysis (PD) and follows the same design as MIMICK-I. In this case, included patients were individuals undergoing maintenance PD in the city of Stockholm. Recruitment lasted from March 2008 to April 2011. All patients on maintenance PD therapy in the region of Stockholm (n =164) were invited to participate. Out of these 164 individuals, 80 were excluded because of unwillingness to participate (n=55), imminent transplantation (n=6), death (n=2), a switch to hemodialysis (n=8), or because of medical or mental disorders that precluded their entry into the study (n=9). Eventually, the cohort comprised 84 patients.

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Chapter 2

Monitoring of inflammation in patients on dialysis: forewarned is forearmed

Christiaan L. Meuwese, Peter Stenvinkel, Friedo W. Dekker & Juan J. Carrero.

Nature Reviews Nephrology. 7, 166-176 (2011).

Abstract

Current evidence about the effects of inflammation on the outcomes of patients with advanced chronic kidney disease (*CKD*) generally originates from single measurements of inflammatory biomarkers. Patients with CKD, however, are exposed to persistent low-grade inflammation and levels of serum inflammatory markers are subjected to a substantial variability over time, being influenced by multiple processes, such as transient infections, comorbidities, and the intermittent stimulus of dialysis. Understanding and evaluating inflammation in the context of its time-dependent oscillations in renal disease fluctuation is therefore important. Nevertheless, the relationship between longitudinal inflammatory variation and risk prediction has so far been addressed in only a few studies, not all of which have been sufficiently powered. Consequently, uncertainty exists about how to interpret the findings of these studies in the clinical setting. The purpose of this Review is to explore the reasons and implications of variability in levels of inflammatory biomarkers in patients with uremia, specifically focusing on C-reactive protein (*CRP*) measurements. We also discuss the value of repeated versus single measurements of inflammation in the clinical setting and provide solutions to reduce both sample size and intraindividual variability in hypothetical, randomized controlled trials aimed at reducing CRP levels in patients undergoing hemodialysis.

Keypoints:

- In patients with end-stage renal disease, inflammatory markers are subject to substantial variability over time, and are influenced by transient infections, comorbidities, and the intermittent stimulus of dialysis.

- Insufficient evidence exists about the implications of regular CRP screening in patients undergoing dialysis; multiple measures of CRP seem to offer predictive advantages with single determinations.

- Regular CRP screening could identify short-term variation in levels of inflammatory markers associated with mortality, which could facilitate risk stratification of patients with chronic kidney disease.

- Regular CRP screening for individual patients could enable extensive exploration of underlying causes of inflammation and the assignment of appropriate treatment.

- When designing a randomized controlled trial to lower CRP level in patients on hemodialysis, sample size and intrapatient variability can be reduced by estimating inflammation at each time point with averaged measurements for each individual.

Introduction

The high mortality risk of patients with chronic kidney disease (*CKD*)[1–3] has been partly attributed to the inflammatory state,[4,5] a condition encountered in the vast majority of patients with CKD, and especially in those with end-stage renal disease (*ESRD*). In patients with renal failure, the systemic concentrations of both pro-inflammatory and anti-inflammatory cytokines are several-fold higher than concentrations in healthy individuals,[6] as a result of both decreased renal clearance and increased production of cytokines. Indeed, several dialysis-related factors (such as membrane bioincompatibility, dialyzate backflow, and endotoxemia) and factors not related to dialysis (such as infections, comorbidities, intercurrent clinical events [including sepsis, exacerbation of pulmonary diseases, heart failure, gastrointestinal diseases] genetic factors, and diet) might contribute to increased cytokine production and, therefore, a persistent state of inflammation.[7–11]

The robust evidence available concerning single measurements of various inflammatory biomarkers as independent predictors of infectious complications and mortality in patients with CKD[12–15] has justified the use of such measurements for identifying patients at increased risk of inflammation. C-reactive protein (*CRP*) is the prototype marker currently used for inflammatory monitoring in clinical units.[16] In the uremic milieu, patients are exposed to a low-grade, persistent inflammation that is subject to a substantial intraindividual and interindividual variability.[7,17,18] The coexistence of CKD with background inflammation and fluctuation of cytokines emphasizes the importance of understanding and evaluating inflammation in the context of its time-dependent oscillations as the process of renal disease fluctuates. In this Review, we explore the reasons for, and the implications and clinical importance of inflammatory biomarker variability in the setting of uremia with a focus on CRP measurements given their extended use in the clinical setting. The advantages of regular monitoring of inflammation in patients undergoing dialysis as a means of risk stratification within patient groups is also discussed.

The dynamic inflammatory response

In response to tissue damage, bacterial particles, tissue necrosis, or other provoking stimuli, polymorphonuclear cells and monocytes are triggered and recruited to the affected area. These cells initiate the inflammatory cascade by producing a diverse range of proinflammatory and anti-inflammatory cytokines, which are involved in autocrine, paracrine, and endocrine communication.[19] Cytokines expand the inflammatory response to a systemic level via the endocrine pathway by initiating production of acute-phase proteins, including CRP, in the liver.[19] Systemic manifestation of this orchestrated system is soon apparent. Levels of proinflammatory mediators synthesized locally at the area of damage and/or infection, such as interleukin (*IL*)-6 or pentraxin 3 (*PTX-3*), are the first to increase systemically, typically 1.5–3.0 hours following tissue injury.[20–22] CRP levels, however, increase slightly later, about 6–8 hours following injury,[20,21] and peak after >24 hours from hospital admission in patients who have experienced myocardial infarction.[22,23]

Of all the acute-phase proteins and plasma markers of vascular inflammation, CRP has been studied the most extensively in patients with CKD. Depending on the severity of the inflammatory stimulus, CRP levels can increase by up to 500 times the normal level. CRP has a half-life of 19 hours,[24] this prolonged half-life makes it easy to detect CRP in the blood. Established methods for measuring CRP serum levels seem reliable, with relatively low coefficients of variation.[25] CRP is currently thought to be a 'bystander' marker of vascular inflammation, rather than a 'culprit' and risk factor for vascular disease.[11,26,27] Biological intraindividual and inter individual variability in CRP level exists, however, even among healthy individuals in whom CRP levels are low or undetectable.[28–30] This biological variability could be related to the reflective nature of CRP, and gradually increases in inflammatory and atherosclerotic conditions.[31] In early population studies, the 4-year reproducibility of CRP measurements was equivalent to those for cholesterol and blood pressure measurements.[30] Interindividual variability results from differences in both demographic factors and comorbidities between patients.[30,32–37] Intraindividual variability, on the other hand, is associated with the presence and influence of transient intercurrent events and the dynamic response of the immune system.[30] In 1,800 healthy Japanese patients who were tested twice during a 1-year period, increasing age was associated with larger intraindividual CRP variation in men than in women;[38] this difference could affect the risk assessment of CRP values, and, therefore, the sex of the patient needs to be taken into account when CRP is used to assess risk in age-related diseases.[39] Proinflammatory cytokines are also subject to a considerable day-to-day variation; intraindividual IL-6 variability was reported in healthy adults, with a mean age of 59 years, during six consecutive daily fasting measurements,[40] describing an index of individuality of 0.20 and a standard error of the mean (*SEM*) of 0.32 pg/ml. Such an SEM indicates that observed differences of ≤0.32 pg/ml in a trial should be understood as part of the normal daily fluctuation of IL-6 levels.

In patients undergoing dialysis, the intraindividual and interindividual variability of serum CRP levels might be even greater than the variability in the general population. However, this variability has been investigated considerably less in the dialysis population. The main cause of CRP variability in the setting of renal dialysis are intercurrent clinical events.[41–43] However, other factors could also contribute to variability, as intraindividual CRP variation in patients undergoing hemodialysis who are free from intercurrent clinical events is very high.[44] Additional interindividual variation might result from decreased renal function and the uremic environment, comorbidities, and protein-energy wasting.[18,45,46] Intraindividual variation, however, could be enhanced by membrane bioincompatibility, dialyzate backflow, endotoxemia and the intermittent nature of hemodialysis.[7,10,41,47–49] In addition, residual renal function can further contribute to variation in CRP levels among patients undergoing dialysis.[50]

Quantifying the magnitude of variability specifically attributable to dialysis-related and nondialysis-related factors is difficult in the clinical setting. However, as demonstrated by Kaysen et al.,[42] the intermittent activation of the acute-phase response often spans multiple dialysis sessions, thereby suggesting that the causative value of the dialysis session per se is of less importance in inducing and maintaining the inflammatory response. Finally, genetic determinants might influence the

intraindividual variability of the inflammatory response, as suggested for both CRP variance in relation to cardiovascular disease risk[51] and IL-6 response following vaccination in the general population.[52] In patients undergoing hemodialysis, Girndt et al. observed that a polymorphism in the gene encoding IL-10 associated with reduced production of IL-10 was associated with reduced intrapatient CRP variability assessed monthly over a 6-month period (**Figure 1**).[53]

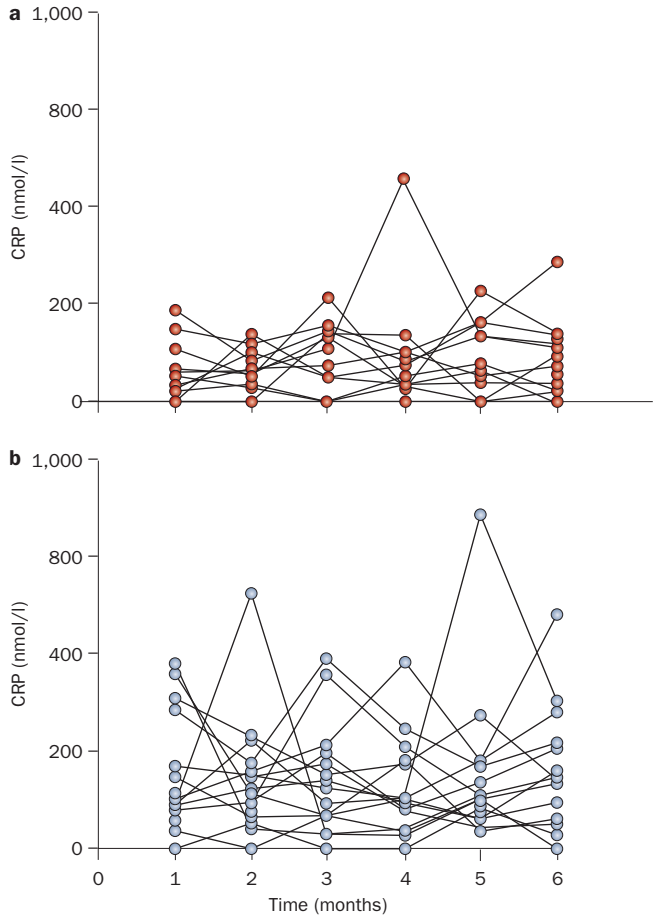

Figure 1. Course of CRP levels in three selected patients. Intraindividual CRP variability could, in part, be genetically determined. The figure depicts CRP values of 15 patients a) an IL-10 'high-producer' genotype or b) an IL-10 'low-producer' genotype. CRP measurements were taken during the first week of each month before dialysis, regardless of whether or not the patient had complications. The IL-10 'low-producer' genotype seemed to be associated with more frequent elevations in CRP than the 'high-producer' genotype. The mean CRP measurement in the 'low-producer' group was 173.3 nmol/l (18.2 mg/l) ± 15.2 nmol/l (1.6 mg/l), and in the 'high-producer' group the mean CRP measurement was 80 nmol/l (8.4 mg/l) ± 16.2 nmol/l (1.7 mg/l) (P = 0.002,Mann–Whitney test). To convert values to mg/l, divide by 9.524. Abbreviation: CRP, C-reactive protein. Permission obtained from Nature Publishing Group © Girndt. M. et al. Kidney Int. 62, 949–955 (2002).

Single measurement assessment

In the general population, strong links exist between elevated levels of systemic inflammatory markers during a single assessment and an increased risk of cardiovascular morbidity and mortality.[5,32] Such links are also observed for the majority of cytokines and acute-phase reactants in various subpopulations of patients with CKD, including individuals in the early stages of this disease,[5,54] patients with ESRD awaiting their first dialysis session,[55] individuals undergoing hemodialysis[12,14,56–63] and patients on peritoneal dialysis.[64–67] In addition, single CRP measurements have been positively associated with left ventricular hypertrophy (*LVH*),[67] myocardial infarction,[66] and hospitalization[68] in patients undergoing dialysis. Despite the fact that many comparative studies suggest that IL-6 might be the best outcome predictor in early and advanced CKD,[56,64,69,70] CRP measurement is still the prototypic marker of uremic inflammation, owing to the widespread availability of this method. Cytokines and acute-phase proteins are substantially elevated in patients with uremia.[71–73]

Although several, arguably, arbitrary CRP cut-off values have been suggested for patients with CKD.[12,54,67,74] At present, no consensual definition of 'uremic inflammation' exists in terms of levels of CRP and other inflammatory markers. Furthermore, safe target CRP values for patients with CKD are still undefined. Follow-up periods for studies in which single measurements of inflammatory biomarkers were used typically range from 2 years to 10 years.[5,12,14,54–67] From a clinical point of view, and misinterpreting traditional Cox models, it would be incorrect to assume that a single CRP measurement would predict the probability of death within 10 years. Indeed, conditional or time-stratified analyses show that CRP is an excellent predictor of risk in the short term (1 year of follow-up).[75] Over an extended period (2–3 years of follow-up) however, the association between CRP and mortality diminishes as other comorbidities and conditions take part in the patient's prognosis.[76] Using periodical CRP monitoring for short-term risk prediction only therefore seems pertinent.

Biological variance associated with single measurements of CRP could have been incorrectly misinterpreted as a negative aspect of using this biomarker as a clinical tool. Increased variation in CRP levels would, however, have imposed a reduction in point estimates towards the null hypothesis in all of the aforementioned studies that included a single baseline CRP measurement.[5,12,14,54–67] As such, the observed relative risk associated with inflammation might be underestimated compared with the true relative risk.[77] Platz et al. reassessed mortality risk ratios attributed to single CRP measurements by correcting for intraindividual and interindividual variances (calculated from three determinations 2 years apart) in 50 healthy men aged >55 years (mean age at baseline 64.9 years).[78] The investigators reported an intraclass correlation coefficient of 0.66 for CRP levels measured at three time points over a 4-year period, which we consider to indicate good consistency over time. This observation indicates the following: if the CRP level is measured once, and, assuming no other errors exist, if the observed relative risk for an elevated CRP level is 2.06, then the true relative risk equals 3.0. Thus, although a single CRP measurement probably underestimates the true risk, it could be considered a valid estimate. A single measurement, however, prevents clinicians from gaining important information on the variability of the inflammatory response and the underlying processes behind this variation. **Figure 2** demonstrates that a single measurement of CRP level is probably insufficient for medical decision-making in day-to-day clinical practice.

Longitudinal CRP changes

To the best of our knowledge (details on the literature search are specified in **Appendix 1**), as many as 10 small-to medium studies, all but one examining patients undergoing hemodialysis, have addressed the consequences of fluctuating levels of CRP, IL-6, or tumor necrosis factor (*TNF*) on mortality or cardiovascular disease (**Table 1**). with respect to study design and methods of analysis, these studies can be grouped into three different categories: 1) ' summary measures', 2) 'long-term fluctuation' and 3) 'intradialytic fluctuation'. In the first category, Snaedal et al. assessed whether summary measures of repeated measurements (average, median or upper/lower values

within a given time period) offer a predictive gain as compared with a single assessment.[18] In this report, CRP levels were measured every week over a 3-month period in 224 patients who were undergoing hemodialysis.[18] As shown in **Figure 3**, an elevation of 10 nmol/l (1 mg/l) in median or mean CRP levels contributes a statistically significant 1.2–1.3% increase to the all-cause mortality hazard ratio.[18] As an example, a patient with a mean CRP value of 143 nmol/l (15 mg/l) has a 15% increased risk of death during follow-up compared with a patient with a mean CRP value <48 nmol/l (<5 mg/l). As such, the results from this study indicate that multiple measurements might offer improved mortality prediction.

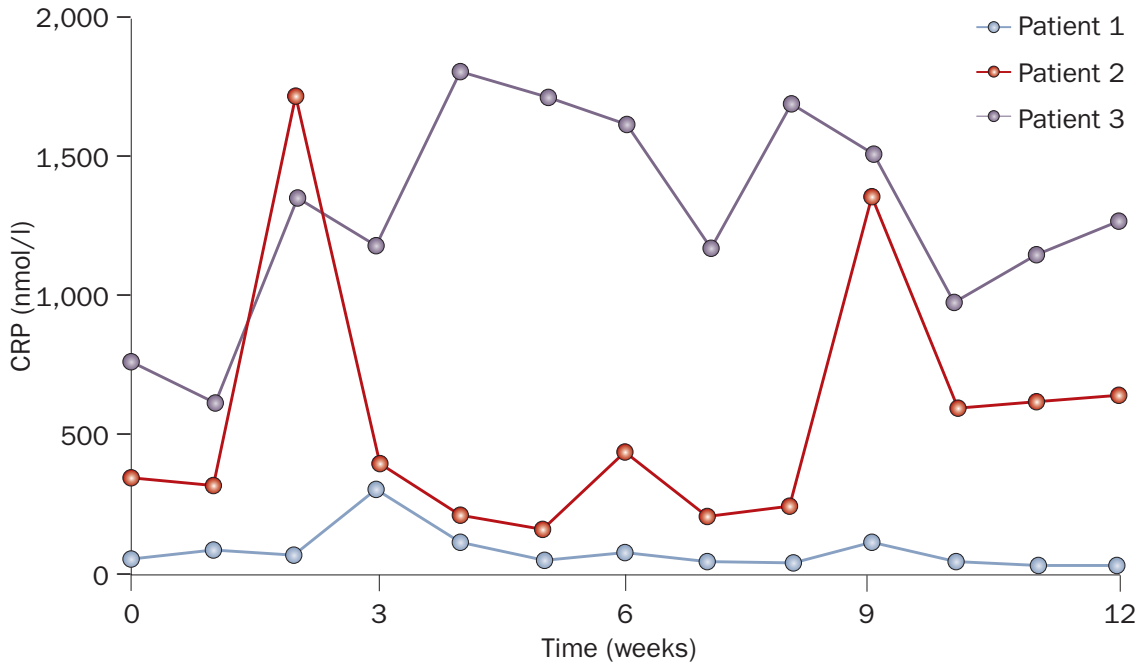

Figure 2. Weekly CRP variability in three selected hemodialysis patients at Karolinska University Hospital, Stockholm, Sweden. Three different hypothetical patients illustrate the usefulness of regular monitoring of the inflammatory response in patients undergoing dialysis. In patient 1, CRP levels remained low during the entire follow-up period apart from during a cold at week 3. In patient 2, CRP levels were, on average, within the 'smoldering' range (mostly remaining within the range 48–476 nmol/l (5–50 mg/l)). However, acute increases in weeks 2 and 9 denoted infections that were appropriately treated. In patient 3, CRP levels were high, indicating acute inflammatory processes that should have alerted the clinician about appropriate treatment and prognosis. Although a single measurement would have sufficed for patient 1, it would have provided misleading information in the other two cases. Regular monitoring in these cases could help to detect systemic CRP elevations warranting a work-up for the source of inflammation and appropriate palliative measures. To convert values to mg/l, divide by 9.524. Abbreviation: CRP, C-reactive protein.

A second category of studies assessed the temporal variation of inflammatory biomarkers during several consecutive measurements over a longer time period that spanned multiple dialysis sessions. In these reports, the analysis included three different categorization strategies: 1) persistently low CRP levels, 2) persistently high CRP levels, or 3) CRP elevations and/or decreases. Study periods varied from 3 months to 6 months.[74,76,79] These studies consistently showed that patients with persistently elevated CRP levels exhibited the worst prognosis (that is, all-cause mortality) as compared with the other groups, followed by patients that presented a rise or a fall in CRP during the observation period [74,76,79] (**Figure 4**). Such observations also seem to be valid for patients undergoing peritoneal dialysis [80] and are further reinforced by the results reported by Kim et al. who demonstrated that LVH was more prevalent among patients on hemodialysis who had persistently elevated CRP levels.[81] We could also demonstrate that this pattern of mortality linked to CRP variation is identical to that seen with variation in IL-6 and TNF.[79] An unexpected finding, however, was that the correlation between changes in these inflammatory markers was not very strong, leading to the hypothesis that various inflammatory pathways contribute in parallel to the pathogenesis of CKD.[79] In a more complex analysis, Rao et al. used a time-dependent Cox model to analyze, on a yearly basis, the influence of IL-6 levels on mortality in 206 stable patients undergoing hemodialysis.[82] In comparison with baseline values, hazard ratios were higher when IL-6 levels were incorporated as a time-dependent covariate.[82] These study results are similar to results from studies on the variability of parathyroid hormone[83] or hemoglobin,[84] which suggests that fluctuation in risk biomarkers in patients undergoing dialysis might affect both clinical decision-making and patient outcome.

A third category of studies examines the association between intradialytic changes in serum CRP levels and death and/or development of cardiac disease. The investigators of these studies base their hypotheses on the putative intermittent proinflammatory stimuli of the dialysis procedure. Although some studies reported elevated CRP levels following a single dialysis session,[85–87] the effect of hemoconcentration during the hemodialysis session was taken into consideration. The observation of Park et al., that a proinflammatory response to a single hemodialysis session was associated with LVH, could be a consequence of this lack of correction.[58] The only study that, despite postdialytic CRP correction, showed an association between intradialytic CRP elevations and mortality[7] is, in our opinion, difficult to interpret in light of the biological plausibility of a rise in CRP within 6–8 h following tissue injury.[88] Indeed, similar analyses looking at intradialytic CRP changes from our group, including two independent cohorts of European patients undergoing hemodialysis, could not replicate these findings.[89] Moreover, as evidenced by statistically nonsignificant Pearson correlation coefficients, the congruency between CRP changes amidst consecutive dialysis sessions was poor.[89]

Monitoring inflammatory markers

In determining how inflammatory markers should be measured, the key points to consider are the reasons why CRP needs to be measured and the likelihood that diagnostic and therapeutic strategies not currently being used might change on the basis of these test results. A substantial amount of evidence included in the 2003 scientific statement by the American Heart Association (AHA) and Centers for Disease Control and Prevention (CDC)[90] is still applicable to patients undergoing dialysis. However, despite 10 years of extensive research on the causes and effects of uremic inflammation,[91] no randomized trials with testing of inflammatory markers as the primary intervention have been performed, nor have cost-effectiveness analyses been completed to assess additional costs or cost savings through the use of such tests. Consequently, the following suggestions about the routine monitoring of inflammatory markers are not evidence-based and reflect the authors' opinion only. Although lacking a precise definition, according to the literature from western countries, CRP levels in patients with uremia are usually higher than the >29 nmol/l (3 mg/l) level that indicates a high mortality risk in the general population.[92,93] A pragmatic cut-off value for serum CRP concentrations, which are typically observed in western patients with ESRD, would be 48 nmol/l (5 mg/l).[94] Individuals with CRP values <48 nmol/l (<5 mg/l), however, might still be at increased mortality risk. On the basis of published data from pooled European cohorts, a cut-off point of 95 nmol/l (10 mg/l) has been proposed for uremia-related inflammation[95] and is often used in research studies for the prediction of mortality. However, this cut-off value has not been approved for use in clinical practice. Notably, a substantially increased risk of mortality was already associated with a CRP concentration of 29 nmol/l (3 mg/l) in a large cohort of Japanese patients undergoing dialysis (adjusted HR 1.64, P = 0.04).[96] However, because ethnic differences exist, and lower CRP levels are found in Asian dialysis patients,[37] these results might not apply to other populations.

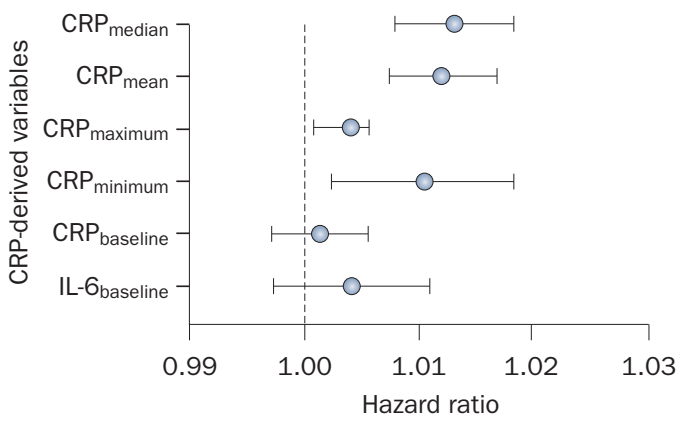

Figure 3. Prediction of all-cause mortality with single and averaged measurements in prevalent patients undergoing hemodialysis. Ratios indicate the increase in risk per 10 mg/L in CRP. Abbreviation: CRP, C-reactive protein. Permission obtained from Elsevier © Snaedal, S. et al. Am. J. Kidney Dis. 53, 1024–1033 (2009).

Table 1. Studies addressing the effect of multiple measurements of inflammatory marker levels on mortality or on a cardiovascular end point

Study	Number of patients, type of dialysis	Dialysis vintage (months)	Marker, baseline levels	Measurements[3]	Follow-up (months)	Outcome measurement	Conclusion
Summary measures							
Snaedal *et al.*[17]	224, HD	28 (14–57)**	hs-CRP	3 months, 12	29.0 (11)#	Mortality	Average CRP levels superior to baseline in predicting mortality
Long-term fluctuation							
Nascimento *et al.*[70]	180, HD	59 (36)#	CRP	6 months, 12	21	Mortality	Persistent high > solitary high > persistent low[3]
Den Elzen *et al.*[72]	635, HD and PD	3	CRP	3 months, 2	27.2 (0.0-78.5)##	Mortality	Persistent high > solitary high > persistent low[3]
Kim *et al.*[77]	52, HD	41.8 (33.7)#	hs-CRP	3 weeks, 2	Cross-sectional	LVH	Persistent high > persistent low[3]
Ates *et al.*[76]	98, CAPD	>3	CRP	20 months, 5	33.9#	Mortality	Persistent high > solitary high > persistent low[3]
Meuwese *et al.*[75]	201 HD [2] 472 HD	28 (15–57)** 3	(hs-)CRP, IL-6 and TNF	3 months, 2	38.4 (17.4-45.1)** 27.2 (11.9-47.9)**	Mortality	Persistent high > increase > decrease > persistent low
Rao *et al.*[78]	198, HD[1]	44.4 (52.8)#	IL-6	4 years, 1–5	31.2 (20.2)#	Mortality	Time dependent IL-6 levels > baseline values[3]
Intradialytic fluctuation							
Korevaar *et al.*[6]	115, HD	9 (5)#	CRP	1 HD session, 2	22.5 (17.4)#	Mortality	Intradialytic CRP increase predicts mortality[3]
Park *et al.*[54]	118, HD	23 (2–225)§§	CRP	1 HD session, 2	Cross-sectional	LVH	Intradialytic CRP increase associates with LVH[3]
Meuwese *et al.*[85]	190, HD‡ 94, HD	29 (15–57)** 6 (6–12)**	CRP hs-CRP	1 HD session, 2	41.3 (22.2–48.5) 18.4 (9.3–41.6)	Mortality	Intradialytic CRP variation does not predict mortality[3]

Hemodialysis (HD), peritoneal dialysis (PD), continuous ambulatory peritoneal dialysis (CAPD). [1]A sample from the Hemodialysis (HEMO) study. During the 5 years of follow-up, 10 out of 198 patients could fulfill all 5 measurements. The dialysis vintage was retrieved from Cheung et al.[116] [2] In this study, two cohorts were included in which separate analyses were performed. [3] Period during which measurements took place and number of measurements. Total include baseline measurements. [3]Highest mortality in groups in order. Characteristics of LVH in most prevalent in groups are displayed in the following order:#Mean (standard deviation), **Median (IQR), ##Mean (range), §§Median (range). When summaries were given for separate groups in a study, a pooled estimate for the total population was calculated. Abbreviations: CAPD, continuous ambulatory peritoneal dialysis; CRP, C-reactive protein; hs-CRP, high sensitivity CRP (nmol/l), HD, hemodialysis; interleukin-6 (pg/ml), LVH, left ventricular hypertrophy; PD, peritoneal dialysis; TNF, tumor necrosis factor (pg/ml).

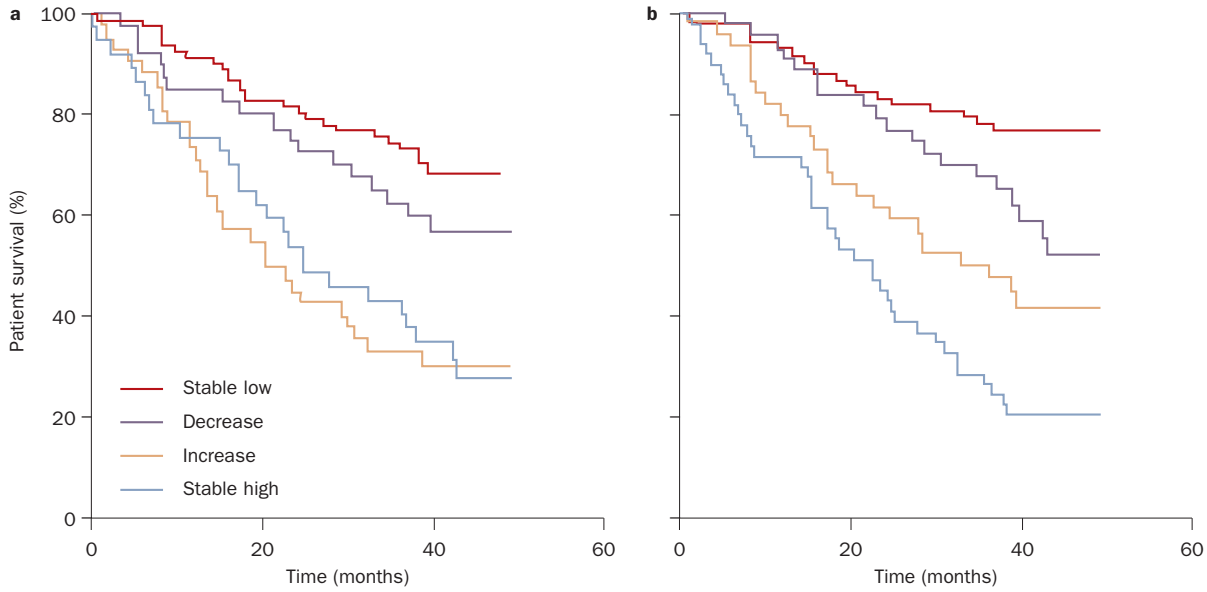

Figure 4. Kaplan–Meier survival curves according to trimestral variation patterns. a) CRP and b) IL-6 variations. Variation groups were created according to the tertile distribution at each time point. Survival curves in four different variability patterns are highlighted: a 'decrease' group, which contained individuals having a decrease in CRP levels from the upper tertile to the middle or lower tertile or from the middle tertile to the lower tertile; an 'increase' group which contained patients in whom CRP levels increased from the lower tertile to the middle or upper tertile, or from the middle tertile to the upper tertile; a 'stable high' group containing patients in whom levels remained in the highest tertiles; and a 'stable low' group containing patients who had both values in the lower or middle tertile. Abbreviation: CRP, C-reactive protein.

Why should we assess CRP?

In dialysis units, monthly CRP estimation could help to monitor the presence of contaminated water or dialysis fluid, audit vascular access status, and ensure optimization of dialysis protocols and dialysis. From a preventive point of view, however, CRP screening should not be used as an alternative to screening for major risk factors in determining patient risk, but should complement clinical judgment. Additional reasons for performing CRP measurements could be to motivate individuals with persistently elevated CRP levels to improve their lifestyles (by smoking cessation, dietary modification, exercise, and/or weight loss) or to comply with drug therapies.

To date, no solid evidence exists demonstrating the advantage of regular CRP monitoring in dialysis units. However, in an analysis from the ecological Dialysis outcomes and Practice Patterns Study,[16] Kawaguchi et al. reported that cardiovascular mortality was lower in renal facilities that measured CRP levels in ≥50% of patients. These data suggest that regular CRP monitoring could aid physicians' judgment and decision making, positively affecting overall patient survival. However, the design of ecological studies calls for caution in their interpretation owing to their susceptibility to the ecological fallacy. In this case, it is possible that units with a higher occurrence of CRP testing exhibited systematic differences in the use of materials affecting vascular access, dialysis filters, dialysis regimes or medicines. These factors could confound the observed inverse association between increased monitoring of CRP and cardiovascular mortality.

When should we assess CRP?

At the patient level, regular CRP screening could lead to further investigations into the underlying causes of inflammation and the assignment of appropriate treatment. During short-term monitoring, the most clinically interesting patients are those presenting a 'smoldering', chronically elevated CRP level in the range of 48–476 nmol/l (5–50 mg/l) (**Box 1**). Possible causes of these smoldering elevations include graft-related or catheter-related infections, peripheral arterial disease, silent coronary ischemia, ulcers, inflammatory bowel disease, malignancies, periodontitis, or hepatitis. According to the AHA/CDC recommendations, a second CRP measurement taken 2 weeks after the first might be useful in identifying transient processes while reducing biological variation in usual clinical practice.[90] Patients with elevated CRP levels within this smoldering range should undergo an extensive clinical work-up, whether or not they exert clinical symptoms. This scenario, in our view, is the most important and justified use for CRP screening at present. What has not been established, however, is how a clinical work-up should be performed in patients undergoing hemodialysis who have elevated CRP levels but no clinical signs of inflammation. In patients with rapidly rising CRP levels or levels consistently >476 nmol/l (>50 mg/l), the clinician should undertake all measures to detect overt infection, as well as other conditions associated with elevated CRP levels, such

Box 1. Possible causes of inflammation according to CRP ranges in patients undergoing dialysis*

CRP 47.6–476 nmol/L (smoldering or chronically raised)

- Failed kidney transplant in situ
- Biofilm (grafts, catheters, hemodialysis machine)
- Silent (encapsulated) infection of AV or arterial grafts
- Chronic obstructive uropathies
- Calciphylaxis
- Cholesterol emboli
- Peripheral arterial disease
- Silent cardiac ischaemia (myocardial ischaemia, stroke)
- Congestive heart failure
- Ischaemic ulcers, neuropathic and venous ulcers
- Chronic obstructive pulmonary disease
- Inflammatory bowel disease
- Periodontal inflammation
- Arthritis
- Hepatitis
- Major surgery

CRP >476 nmol/l (acute infection)

- Underlying renal diagnosis (infected cysts in autosomal dominant polycystic kidney disease)
- Vasculitis relapse, sinusitis, otitis
- Discitis, osteomyelitis, endocarditis
- Urinary tract infection/urosepsis, biliary sepsis
- Septicaemia, any cause (foreign material)
- Malignancy, de-novo and recurrent

* Normal CRP <47.6 nmol/l. Abbreviation: CRP, C-reactive protein. Adapted with permission from Oxford University Press © Wanner, C. et al. Nephrol. Dial. Transplant. 22 (Suppl 3), iii7–iii12 (2007).

as malignancy or relapse of vasculitis.[94] These diagnoses clearly show dynamic changes in the individual CRP distribution curve over time.[94] The studies addressing longitudinal changes in inflammatory status discussed above could assist clinicians in their interpretation of the outcomes of monitoring the inflammatory status. Clearly, persistent CRP elevations or increasing trends in CRP levels indicate patients at high risk of dying, and efforts should be made to address the causes of such elevations.

CRP measurements for mortality prediction

The evidence for a correlation between elevated CRP levels and an increased mortality risk among patients undergoing dialysis (even with multivariate adjustment for traditional risk factors) is abundant.[76,97] However, whether CRP measurements add prognostic value beyond traditional risk factor algorithms (such as the Framingham risk factor score) is not clear. Mallamaci et al. studied the predictive value of a composite of CRP and brain natriuretic peptide (BNP) levels in 246 patients undergoing dialysis.[98] The investigators observed that, by adding these two biomarkers to a basic score that was estimated using factors such as age, sex, smoking status, presence of diabetes mellitus and cardiovascular disease, and level of albumin, the explained variance increased by 9.9% for all-cause mortality and by 10.5% for cardiovascular mortality.[98] To our knowledge, no study has assessed the predictive gain that CRP measurements alone offer in addition to risk stratification by traditional risk factors in patients undergoing dialysis.

The study by Danesh et al. addressed the question of whether CRP level adds prognostic gain to traditional risk factors in patients with cardiovascular disease.[32] The investigators compared 2,459 patients who had a nonfatal myocardial infarction, or died as a result of coronary heart disease, with 3,989 healthy controls, all of whom were followed for 12 years. Danesh et al. reported that CRP measurements provided limited predictive value for mortality over and above established risk factors such as hypertension, cholesterol, and smoking. This study was acknowledged by the AHA/CDC as being indicative that insufficient evidence existed to support the use of CRP as a clinical tool in the prediction of cardiovascular events.[90] Modern epidemiological approaches have, however, helped us to gain further insight into the potential of such a clinical tool through the discrimination of deceased patients and the reclassification of risk by introducing novel biomarkers into a model based on traditional risk factors.[99,100] The study by Blankenberg et al., published in 2010, added CRP, n-terminal pro-BNP, and troponin I to a conventional risk model in three independent cohorts.[101] Adding any single biomarker separately to the established risk model did not improve risk estimation. By contrast, incorporation of all three biomarkers effectively reclassified the true mortality risk in 11% of the patients.[101] Such a risk model could have important implications in common risk algorithms for the general population; however, whether and how such a model would help patients undergoing dialysis who are already at high risk of mortality is unclear.

Managing increased CRP levels

No clinical trials targeting a decrease in inflammation as a means of improving outcome in patients undergoing dialysis have been performed. Therefore, current recommendations for treating inflammation in patients with CKD are patient-specific and mainly include identifying and treating the cause of the inflammatory response. The first step in dealing with increased CRP levels in this patient group is the treatment of intercurrent events and comorbidities that might cause inflammation. The next step would be to evaluate and, if possible, handle potential dialysis-related causes of inflammation. Various nonpharmacological anti-inflammatory treatment strategies, such as physical training and nutritional interventions,[91] could be considered at this stage. Another consideration is that inflammation does not occur alone and clearly impinges upon many other metabolic manifestations of uremia, perhaps acting as a catalyst and magnifying the risk of other concurrent risk factors.[102] For instance, higher doses of erythropoietin-stimulating agents are needed to maintain target hemoglobin levels in patients experiencing inflammation.[103,104] Thus, the best treatment for patients undergoing hemodialysis who have elevated biomarkers of inflammation would probably be multifaceted. Interventional studies reported since 2008 have suggested that a variety of drugs, such as cholecalciferol,[105] sevelamer,[106] angiotensin- convertingenzyme inhibitors,[107] pentoxifylline[108] and statins[109] have anti-inflammatory effects. Finally, on the basis of the observation that chemokine receptor type 5 (*CCR5*) polymorphisms influence the outcome of patients undergoing dialysis who are experiencing inflammation,[110] blockade of CCR5 could provide a novel therapeutic approach in some individuals.[111] Randomized, placebo controlled trials that specifically target inflammation in patients with CKD are eagerly awaited.

Anticytokine therapies are gaining importance in treating diseases with an elevated inflammatory component, such as rheumatoid arthritis.[112] However, some concerns have been raised about the use of such therapies, as they are usually mediated through the blockade of cellular and/or molecular functions that presumably have an important role in host defense. For instance, because TNF, IL-1 and IL-6 are key factors in both the innate and adaptive host defense system, increased rates of infections with the use of these therapies have been reported.[113] Additionally, since TNF plays an important role in granuloma formation and the defense against intracellular pathogens, reactivation of tuberculosis has been observed with TNF inhibitors in patients with diseases other than CKD.[113] In patients undergoing dialysis, the safety of TNF blockers (specifically etanercept) has been assessed in two small, but important, reports. Don et al. initially designed a 3-month intervention trial with subcutaneous etanercept (25 mg twice weekly) in six patients undergoing hemodialysis who had normal albumin (>42.0 g/l) or CRP levels (<48 nmol/l [<5 mg/l]).[114] The investigators reported no adverse effects during the treatment phase or subsequent 6-month follow-up. The pharmacokinetics of etanercept in patients undergoing hemodialysis were similar to those in individuals with normal renal function and, therefore, administration of etanercept to patients on hemodialysis was deemed feasible without dose adjustment. Following on from this research, in 2010 the same group reported the results of an intervention trial where 10 patients

undergoing hemodialysis were randomly assigned to receive etanercept or placebo over a 44-week period.[115] Risk of infection was a major exclusion criterion, and patients were allowed to participate if both hypoalbuminemia (>38.0 g/l) and inflammation (CRP >48 nmol/l [>5 mg/l]) were present. Unfortunately, <6% of the screened patients met the inclusion criteria and the study was therefore not sufficiently powered to detect potential changes in the primary outcome (an increase in albumin and prealbumin levels).[115] However, an important message from this report is that, again, administration of etanercept for more than 7 months seems to be safe and is not associated with the occurrence of adverse events.

Sample size in a hypothetical trials

At present, we await adequately powered, randomized controlled trials targeting inflammation as a treatment strategy in patients undergoing hemodialysis. However, on the basis of reported variability of the inflammatory response in patients with CKD, one would expect such studies to include, a priori, a large sample size. Intraindividual variability can be the result of measurement error and/or variability in the biomarker of interest (in this case CRP) or of patient-specific factors. In both cases, a practical solution to overcome the effects of such variability involves averaging consecutive measurements in each individual patient. To reduce variability due to measurement error, one solution would be to use the average of duplicate CRP measurements at each time point. However, given the reliable existing methodology for measuring CRP levels, variability caused by measurement error is not a major issue.[25] Intrapatient variability caused by patient-specific factors should be taken into account when a relatively longer period (for instance, weeks) is considered between successive measurements.

In practical terms, the primary outcome of a hypothetical study design can be defined as the average of two or more consecutive CRP levels measured some time apart. The following example illustrates that, by using the average of two or more consecutive CRP measurements taken 1 week apart, the minimum number of individuals needed in a hypothetical randomized controlled trial examining the CRP-lowering ability of a drug can be reduced. In this example, we use a cohort of 167 prevalent patients undergoing hemodialysis from the Stockholm region,[18] in whom CRP levels were measured weekly for 12 consecutive weeks. All CRP measurements were logarithmically transformed, because of non-normalcy in their distribution. Changes in the CRP levels, along with their standard deviations, were calculated over a 6-week period (during which a theoretical intervention could have taken place) on the basis of three different scenarios. In the first scenario, preintervention and postintervention values of CRP were both based on single measurements. In the second scenario, preintervention and postintervention values of CRP were both based on the average of two weekly measurements (two consecutive measurements 1 week apart). In the third scenario, preintervention and postintervention values of CRP were both based on the average of three weekly measurements (three consecutive measurements one week apart).

Table 2. Theoretical reduction in the sample size per arm of a hypothetical randomized controlled trial based on the use of average weekly CRP measurements to define end points[1]

	Δ CRP[2]	SD[3]	Reduction (%)[4]
End points based on single measurements	- 0.005	0.523	100
End points based on the average of two weekly measurements	0.016	0.442	71
End points based on the average of three weekly measurements	0.017	0.416	63

[1]The table illustrates a simulation of a hypothetical randomized controlled trial in which an increasing number of measurements causes a decrease in the standard deviation. This, in turn, effectively reduces the minimal number of subjects needed per arm. [2]Δ CRP; Changes in serum C-reactive protein levels over a 6-week period. Because of non-normality, CRP levels at both time points were logarithmically transformed. [3]SD, Standard deviation of the change in log-serum CRP levels. [4]Reduction in the minimum numbers of patients needed for a hypothetical trial. The squared ratios were multiplied by a hundred to obtain percentages. The scenario based on single measurements served as the reference category (100%).

From the logarithmic changes in CRP levels and their standard deviations, the reduction in the number of patients needed was calculated. This reduction equals the squared ratio of the standard deviation based on a single preobservation and postobservation, and on the standard deviation based on multiple measurements. As shown in **Table 2**, when the number of measurements is increased, the standard deviation of the change decreases gradually. Consequently, the minimum numbers of patients needed also decreases. This solution to high numbers of participants required would offer certain complexity in the accomplishment of the trial, but would provide a considerable reduction in the minimum numbers of individuals needed.

Conclusions

Inflammation is a prominent characteristic of patients undergoing dialysis. Although CRP is considered to be the prototypic inflammatory biomarker, its levels fluctuate substantially over time. Regular monitoring of CRP levels could provide us with important information on the existence of the processes behind this variability. Identifying and treating underlying proinflammatory processes is currently the only demonstrated way to tackle inflammation in this patient population. A number of studies in the dialysis setting pinpoint CRP levels as a powerful predictor of outcomes. Whether measurement of CRP levels adds predictive power beyond traditional risk factors is currently unclear. The available evidence suggests that regular monitoring of the inflammatory status could be an informative clinical tool for assessing disease progression and predicting outcomes. Finally, intraindividual CRP variability could be used to our advantage to reduce the number of patients necessary to adequately power future randomized controlled trials.

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Chapter 3

Trimestral variation patterns of C-reactive protein, interleukin-6 and tumor necrosis factor-α are similarly associated with survival in hemodialysis 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 hemodialysis patients is unknown. We therefore studied survival in relation to trimestral variation of CRP, interleukin-6 (*IL-6*) and tumor necrosis factor-α (*TNF-a*).

Methods: In 201 prevalent hemodialysis patients from the Mapping of Inflammatory Markers in Chronic Kidney Disease (MIMICK) 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 calculated by the Cox proportional hazard model and Pearson's test was used to correlate changes. CRP analyses were replicated in 472 incident hemodialysis patients from the Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD).

Results: 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 3.38 [2.31-4.94]; adjusted 2.33 [1.58-3.45]).

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

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 there 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 impact of intra-individual variability of the inflammatory response on mortality. 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 tumor necrosis factor-α (*TNF-a*) and survival in two well-characterized cohorts of hemodialysis (*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 months observational period, 3 patients died, 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 centers 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 centers and while being informed, all patients consented. In both cohorts, comorbidity was classified according to Davies et al.,[12] on a 7 point

scale which was later on simplified into 3 risk categories (low, medium and high comorbidity risk). Nutritional status was quantified by means of the subjective global assessment which, for reasons of simplicity, was trans-calculated to a 3 point scale.[13] Body mass index (*BMI*) was calculated as the body weight (kg) divided by the squared height (m^2).

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 analyzed immediately. CRP in the NECOSAD was analyzed 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 Analyzer (Immulite, DPC, Siemens, California) similarly to plasma IL-6 and TNF- α levels (Immulite DPC, Siemens, California). Serum albumin levels were measured with bromecresol purple in the MIMICK cohort, whereas immunonephelometry was used in the NECOSAD.

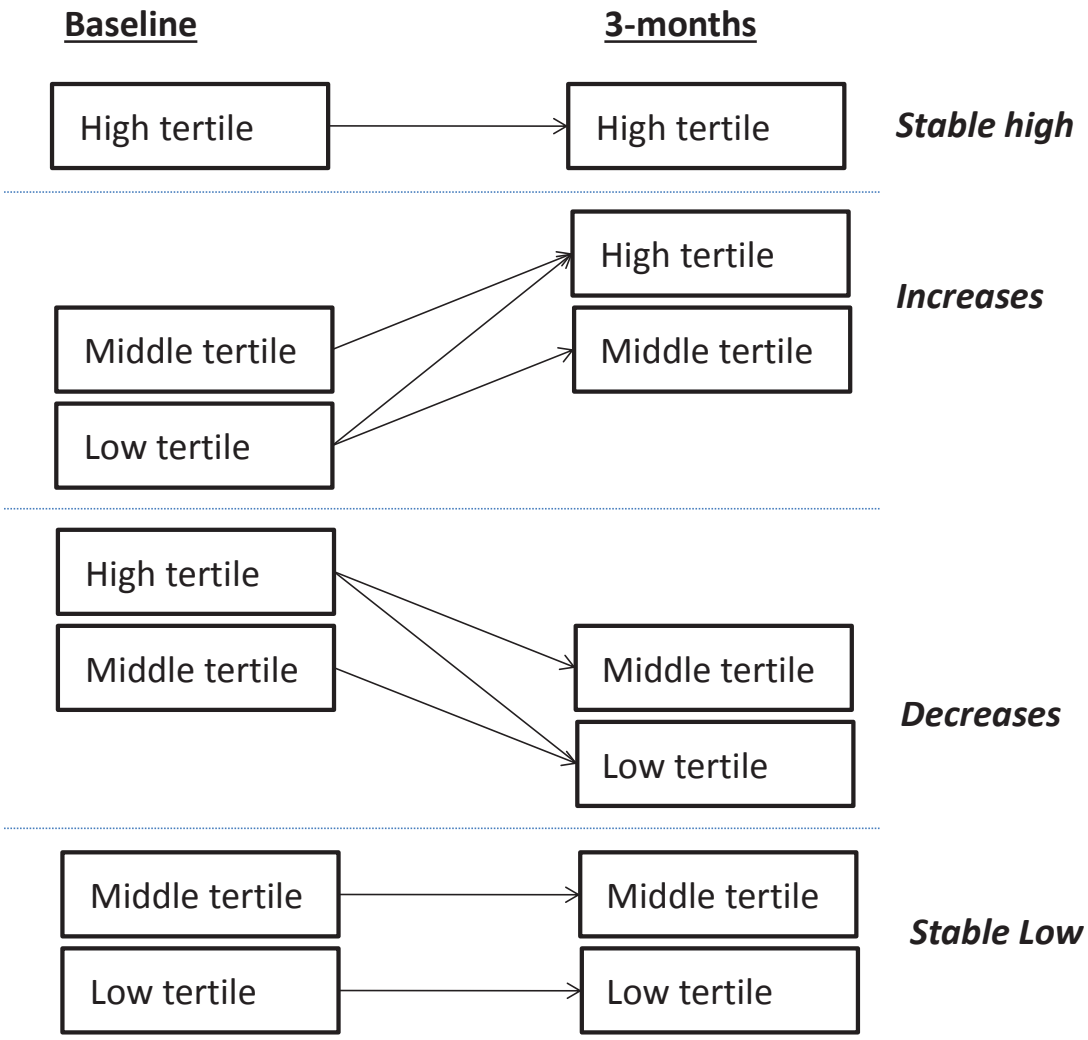
Statistical Methods

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 of the studied biomarker at both time points (**Figure 1**). From the nine possible combinations, four groups were created by clustering patients with changes in the same direction: a) Individuals who showed a change to lower tertiles were classified as the "decrease" group; b) Individuals showing an increase to upper tertiles were assigned to the "increase" group; c) Individuals with both values within the highest tertile of distribution were labeled as "stable high" group; d) Individuals with both values within the lower or the middle tertile were labeled as "stable low" group. The same procedure was applied for each inflammatory mediator and for each cohort separately. Additionally, an extra categorization was performed for CRP values using the internationally accepted CRP thresholds (the CDC/AHA) of >3 mg/L and >10 mg/L.[14]

To test differences between the four groups, Kruskal Wallis, one-way ANOVA, and Chi square tests were applied for normally, non-normally distributed and categorical data, respectively. In the analysis of survival, Kaplan Meier curves were created and Cox proportional hazards model were used to calculate hazard ratios (*HR*). Since age, sex, comorbidities and nutritional status are known to interact with inflammation and influence mortality, they were included as confounders in multivariate Cox models. To take into account the potential confounding effect of baseline 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 groupings. Also, in both cohorts, hazard ratios were recalculated using one cut-off value for CRP (10 mg/L) on both time points to define the different groupings.

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 hazard ratios, a 95% confidence intervals (95%CI) not including 1 and for all other tests, a p-value smaller than 0.05, were considered to be statistically significant. Kaplan Meier figures were created using Prism 5.02 (Graphpad, 1992).

Figure 1. Classification of trimestral variation patterns for each inflammatory marker



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.

Table 1. Baseline characteristics of patients included in the study and after stratification according to CRP tertile variation categories in MIMICK subjects[1]

	All patients	Stable low[1]	Decrease[1]	Increase[1]	Stable high[1]	p-value
	n=201	n=82	n=40	n=42	n=37	
Baseline characteristics						
Men, %[4]	56.7	52.4	62.5	59.5	56.8	0.731
Age, years[5]	62.9 (14.2)	60.6 (15.4)	62.9 (14.3)	63.0 (15.0)	66.6 (11.5)	0.220
BMI, kg/m^2[,5]	24.5 (5.2)	24.2 (4.8)	24.4 (4.9)	24.4 (5.0)	25.2 (7.1)	0.842
Dialysis vintage, months[6]	28 (15 to 57)	29 (17 to 54)	29 (16 to 55)	19 (8 to 63)	34 (11 to 62)	0.287
Time dialysis per week, hours[6]	12.0 (12.0 to 13.5)	12.0 (12.0 to 13.5)	12.0 (12.0-13.5)	12.0 (12.0 to 13.5)	12.0 (10.9 to 12.50)	0.397
Low/medium/high Davies score, %[4]	20.4/56.2/23.4	31.7/48.8/19.5	15.0/57.5/27.5	7.1/69.0/23.8	16.2/56.8/27.0	0.048
Protein-energy wasting , %[3,4]	46.0	36.2	47.5	50.0	61.1	0.083
CRP at baseline, mg/L[6]	6.4 (2.6 to 18.5)	2.9 (1.2 to 6.0)	18.0 (6.5 to 31.3)	4.9 (2.5 to 8.1)	29.0 (22.0 to 47.5)	-
ΔCRP, mg/L[6]	-0.3 (-4.3 to 4.5)	-0.2 (-1.0 to 0.7)	-12.2 (-25.2 to -4.2)	9.9 (6.8 to 25.9)	-3.0 (-13.5 to 13.5)	-
IL-6 at baseline, pg/mL[6]	8.6 (5.1 to 15.0)	5.6 (3.5 to 8.9)	10.3 (7.3 to 15.8)	8.6 (5.1 to 12.7)	18.9 (11.0 to 26.9)	<0.0001
ΔIL-6, pg/mL[6]	0.2 (-2.2 to 2.8)	0.2 (-1.4 to 2.0)	-2.1 (-7.0 to 0.4)	4.90 (-0.1 to 12.0)	0.6 (-4.9 to 7.4)	<0.0001
TNF-α at baseline, pg/mL[6]	13.9 (11.1 to 16.8)	12.6 (10.6 to 15.3)	14.4 (11.3 to 16.9)	13.5 (10.9 to 15.9)	15.9 (13.8 to 18.3)	0.005
ΔTNF-α, pg/mL[6]	-0.2 (-1.9 to 1.7)	0.4 (-1.4 to 2.5)	-0.6 (-3.0 to 0.6)	-0.2 (-1.8 to 2.8)	-1.1 (-2.4 to1.3)	0.020
Characteristics during follow-up						
N. of deaths[4]	97	25	17	29	26	<0.0001
Time till death, months[6]	17.3 (8.4 to 28.1)	18.0 (10.6 to 30.8)	21.5 (8.3 to 31.7)	13.5 (8.3 to 23.0)	18.3 (6.6 to 28.9)	0.059

1. CRP variation groups were constructed according to changes in tertiles of the distribution at baseline and 3 months measurement (see methods).
2. Protein-energy wasting was defined as a SGA>1 (see methods).
3. ΔCRP was calculated by subtracting the level at zero months from the level at three months.
4. Differences between groups in categorical data were tested by means of a Chi square test.
5. Data are expressed as mean (SD). Differences between groups were tested by means of a one-way ANOVA test.
6. Data are expressed as median (IQR). Differences between groups were tested by means of a Kruskal-Wallis test.

Table 2. Baseline characteristics of patients included in the study and after stratification according to CRP tertile variation categories in NECOSAD subjects[1]

	All patients	Stable low[1]	Decrease[1]	Increase[1]	Stable high[1]	p-value
	n=472	n=179	n=115	n=95	n=83	
Baseline characteristics						
Men, %[4]	57.2	54.2	57.4	56.8	63.9	0.538
Age, years[5]	62.2 (13.8)	58.3 (14.8)	64.3 (13.3)	62.5 (13.2)	67.5 (10.2)	<0.0001
BMI, kg/m[2,5]	24.6 (4.2)	24.7 (3.8)	23.9 (3.9)	24.4 (3.5)	25.8 (6.0)	0.018
Time dialysis per week, hours[6]	9.0 (8.0 to 12.0)	9.0 (8.0 to 12.0)	9.0 (8.0 to 12.0)	9.0 (8.0 to 12.0)	9.0 (8.0 to 12.0)	0.552
Low/medium/high Davies score, %[4]	39.2/50.8/10.0	45.8/45.3/8.9	37.4/55.6/7.0	45.3/47.4/7.4	20.5/60.2/19.3	0.001
Protein-energy wasting , %[2,4]	29.9	24.5	37.0	23.2	46.8	0.002
CRP at baseline, mg/L[6]	6.0 (3.0 to 16.0)	3.0 (3.0 to 6.0)	15.0 (7.0 to 23.0)	3.0 (3.0 to 7.0)	24.0 (17.0 to 48.0)	-
ΔCRP, mg/L [3,6]	0.0 (-4.0 to 4.0)	0.0 (0.0 to 1.0)	-9.0 (-19.0 to -3.0)	9.0 (5.0 to 17.0)	1.0 (-14.0 to 18.0)	-
Characteristics during follow-up						
N. of deaths[4]	206	57	56	36	57	<0.0001
Time to death, months[6]	20.1 (8.6 to 32.7)	28.0 (15.5 to 39.8)	19.3 (5.3 to 32.3)	20.4 (7.7 to 39.4)	12.8 (5.4 to 23.1)	<0.0001

1. CRP variation groups were constructed according to changes in tertiles of the distribution at baseline and 3 months measurement (see methods).
2. Protein-energy wasting was defined as a SGA>1 (see methods).
3. ΔCRP was calculated by subtracting the level at zero months from the level at three months.
4. Differences between groups in categorical data were tested by means of a Chi square test.
5. Data are expressed as mean (SD). Differences between groups were tested by means of a one-way ANOVA test.
6. Data are expressed as median (IQR). Differences between groups were tested by means of a Kruskal-Wallis test.

Table 3. Crude and adjusted mortality risk for each variation category according to tertile categorization

	Groups, [1]	Crude, HR (95% CI)		Adjusted, HR (95% CI)[2]	
MIMICK cohort					
CRP	Stable low	1.00		1.00	
	Decrease	1.43	(0.77 to 2.65)	1.38	(0.73 to 2.60)
	Increase	3.27	(1.91 to 5.60)	3.13	(1.79 to 5.45)
	Stable high	2.98	(1.71 to 5.20)	2.79	(1.58 to 4.94)
IL-6	Stable low	1.00		1.00	
	Decrease	1.94	(1.02 to 3.68)	1.76	(0.92 to 3.38)
	Increase	3.38	(1.86 to 6.14)	3.62	(1.96 to 6.69)
	Stable high	5.73	(3.30 to 9.97)	3.80	(2.10 to 6.87)
TNF-α	Stable low	1.00		1.00	
	Decrease	1.27	(0.70 to 2.29)	1.43	(0.79 to 2.60)
	Increase	1.89	(1.09 to 3.25)	1.81	(1.04 to 3.14)
	Stable high	2.46	(1.49 to 4.07)	1.59	(0.92 to 2.74)
NECOSAD cohort					
CRP	Stable low	1.00		1.00	
	Decrease	1.68	(1.15 to 2.46)	1.45	(0.98 to 2.16)
	Increase	1.27	(0.83 to 1.94)	1.39	(0.91 to 2.15)
	Stable high	3.38	(2.31 to 4.94)	2.33	(1.58 to 3.45)

1. The stable-low group (see methods) served as the reference category.
2. After adjustment for age, sex, Davies Comorbidity score, protein-energy wasting (SGA>1), and log (vintage on dialysis).

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, characteristics of follow-up are presented. In the MIMICK cohort, no difference was observed among the groups except for the severity of comorbidities 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 significantly different between all groups. In the NECOSAD cohort however, significant differences existed with regard 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 1a-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 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 with 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 inflammatory markers (deltas, as continuous variables) over the 3 months 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 crude (3.38 [2.31-4.94]) and adjusted (2.33 [1.58-3.45]) Cox analysis (**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.

Table 4. Correlation matrix of changes in inflammatory markers

	ΔCRP	ΔIL-6
ΔIL-6	0.468**	-
ΔTNF-α	0.103	0.190*

Correlations were calculated by means of Pearson correlation tests; *p=0.05; ** p<0.001.

Discussion

The present study is the first to concurrently analyze the implications of CRP, IL-6 and TNF-α trimestral variation on outcome in HD patients. Our results show a similar survival pattern for all three biomarkers. Both an increase as well as a persistent elevation in all these biomarkers was 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 amongst 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 amongst HD patients with persistently elevated CRP levels.[16] A novel finding in our study is that increasing levels of CRP over the 3 months period in prevalent patients are also associated with a higher mortality risk in both uni- and multi-variate analysis, agreeing with a small report in patients on continuous peritoneal dialysis.[8]

Table 5. Crude and adjusted mortality risk for each variation category according to the established CRP thresholds of 3 and 10 mg/L

	Groups[1]	Crude	Adjusted
		HR (95%CI)	HR (95%CI)[2]
MIMICK cohort			
CRP	Stable low	1.00	1.00
	Decrease	1.30 (0.67 to 2.52)	1.33 (0.67 to 2.62)
	Increase	3.25 (1.84 to 5.73)	2.77 (1.55 to 4.93)
	Stable high	2.92 (1.69 to 5.07)	2.36 (1.34 to 4.16)
NECOSAD cohort			
CRP	Stable low	1.00	1.00
	Decrease	1.39 (0.92 to 2.11)	1.16 (0.76 to 1.79)
	Increase	1.35 (0.89 to 2.05)	1.28 (0.84 to 1.96)
	Stable high	2.62 (1.81 to 3.81)	1.86 (1.27 to 2.72)

1. The stable-low group (see methods) served as the reference category.
2. Adjusted for age, sex, Davies score, protein-energy wasting (SGA>1), and log (vintage on dialysis).

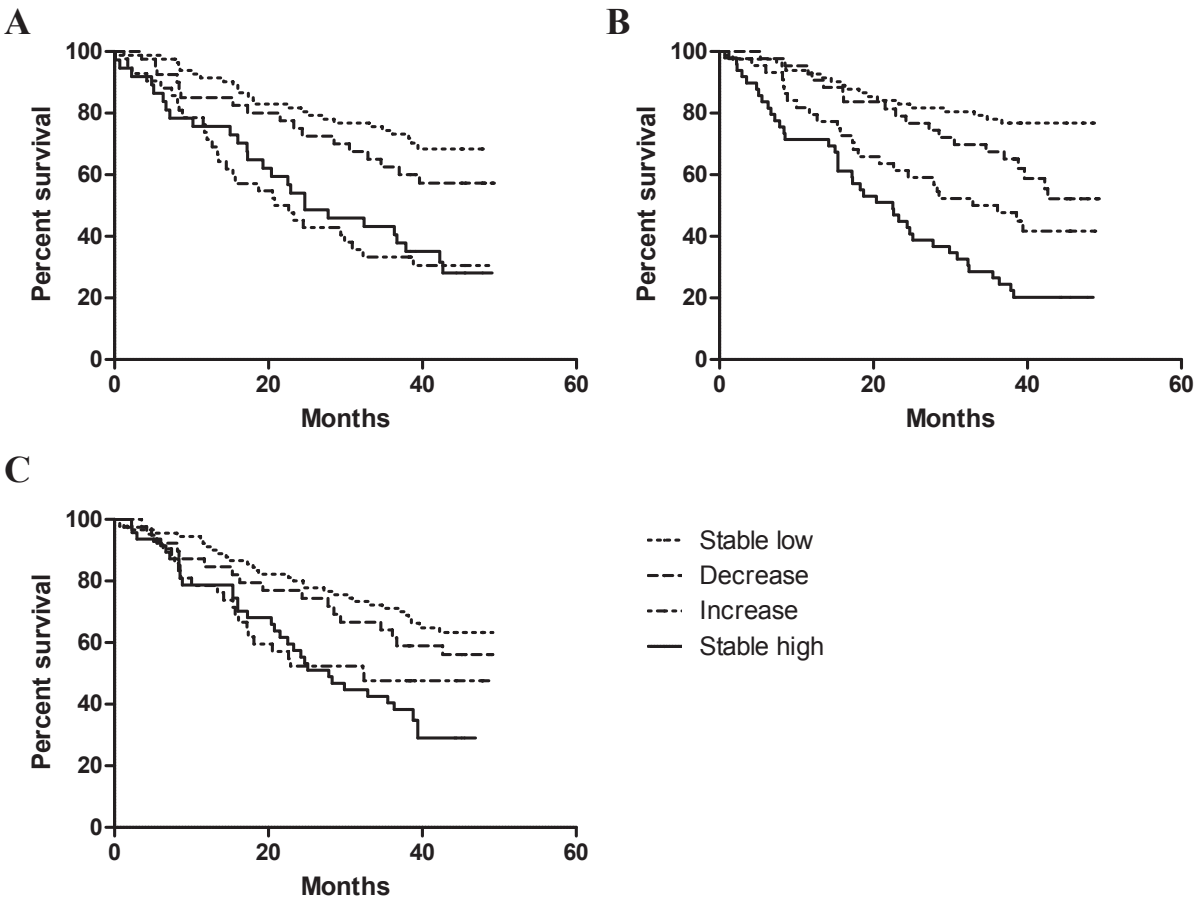


Figure 2. Kaplan-Meier survival curves in MIMICK patients according to CRP (Panel A), IL-6 (Panel B) and TNF-α (Panel C) variation groups.

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 must also be noted that the association between inflammatory marker variability and mortality was the strongest for IL-6. A finding which is in line with previous observations with single measurements.[3,18] 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 three month variation patterns of these inflammatory markers.

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. Whilst 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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Chapter 4

Variations in C-reactive protein during a single hemodialysis session do not associate with mortality

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Abstract

Background: An increase in C-reactive protein (*CRP*) levels during a single hemodialysis (*HD*) session has been associated with mortality. These associations, however, are difficult to understand from the current understanding of CRP metabolism.

Methods: In 190 Swedish HD patients from the Mapping of Inflammatory Markers in Chronic Kidney Disease (MIMICK) cohort, CRP was measured before and after a HD session. During follow up, events of death and censoring were recorded and hazard ratios were calculated and analyzed as a function of CRP variation. Results were replicated in 94 Dutch HD patients from the Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD). In this cohort, also correlation and kappa statistics were calculated to assess concordance in CRP changes amidst multiple dialysis sessions from the same individuals.

Results: In both cohorts, mean CRP values did not increase during a single HD session. In the MIMICK, median (IQR) dialysis vintage was 29.0 (14.8-57.0) months. In both crude (HR [95% CI]: 1.008 [0.971 to 1.047]) and multivariate Cox models (0.996 [0.949 to 1.046]), no association was observed with mortality. In the NECOSAD, individuals endured 6.0 (6.0 to 12.0) months on dialysis. No association was found with mortality neither in a crude (0.961 [0.908 to 1.018]) nor in an adjusted analyses (0.978 [0.923 to 1.037]). Finally, the concordance between changes in different sessions was poor.

Conclusions: CRP changes during a single HD session do not associate with mortality, thereby adding to the biological uncertainty concerning the ability of CRP to rise in such short period.

Introduction

Patients with chronic kidney disease (*CKD*), and especially those with end stage renal disease (ESRD), are at considerable increased risk of premature death.[1] Since the surplus in CKD mortality is strongly associated with a state of persistent inflammation and chronic activation of the acute phase response, the identification of factors involved in the pathogenesis of the inflammatory response is of considerable therapeutic interest.[2-5]

In addition to CKD related factors, such as decreasing renal function, comorbidities, infections or the uremic environment per se,[6] the hemodialysis (*HD*) procedure has been suggested to play a pivotal role in the development of inflammation. Indeed, several studies have shown that intradialytic activation is associated with increased fractional synthesis rates of albumin and fibrinogen as well as of pro-inflammatory cytokines, leading to a state of increased muscle-protein catabolism.[7-10] While the long-term consequences of intradialytic activation (that is, increased inflammation) on the muscle may, indeed increase the mortality risk,[11,12] it is uncertain whether these inflammatory fluctuations are as such valid prognosticators of the patient's outcome. A previous study based on the NECOSAD cohort reported that an increase in C-reactive protein (*CRP*) during a single HD session was positively related to subsequent mortality.[13] However, while CRP is predominantly produced in the liver as induced by Interleukin-6 (*IL-6*) and typically raises after 6-8 hours from a major tissue damage,[14] this somewhat surprising finding is difficult to understand from the current biological perspective. Since external validation of observational findings in unrelated populations is a fundamental step in the assessment of purported risk factors, the predictive power for death of intradialytic CRP changes has still to be confirmed in other cohorts of dialysis patients. This confirmation is of particular relevance because CRP levels appear highly variable across different studies in ESRD patient.[15-17] With this background in mind, we used available data from two historical cohorts of dialysis patients to investigate and further clarify the association between changes of plasma CRP levels during a single HD session and subsequent outcome.

Subjects 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.[18] This cohort includes adult patients (n=224) on maintenance HD therapy recruited during the period of October 2003 to September 2004 in six dialysis units in the Stockholm-Uppsala (Sweden) region. Out of these individuals, 190 patients had CRP and body weight measurements performed both before and after the index session. A comparison between the included individuals and the overall cohort revealed no differences in general characteristics (data not shown). The study Ethics Committee of Karolinska Institutet, Stockholm, Sweden approved the study protocol and informed consent was obtained from each

patient. From inclusion on forward, events of death were recorded, with no loss to follow up.
The second cohort is a selection from the Netherlands Cooperative Study on the Adequacy of Dialysis (*NECOSAD*) study, described in more detail elsewhere.[19] This is a prospective follow up study in which all new ESRD patients from 38 dialysis centers in the Netherlands were asked to participate between 1997 and 2002. Patients were re-assessed every six months. During follow up, events of death and censoring, due to renal transplantation, leaving the study or the end of follow up, were prospectively recorded. For the current analysis, individuals were selected conditionally on having completed at least a second HD session six months after an initial observation with an identical protocol. Ninety-six patients met these criteria and again, no major differences were observed with regards to general baseline characteristics when compared with the overall cohort (data not shown). Of these ninety-six individuals, an overlap of eleven patients existed with the sample reported on by Korevaar et al..[13] The Medical Ethics Committees of all participating Dutch centers approved the protocol and informed consent was obtained from every individual.
For the MIMICK cohort, plasma CRP levels were measured before and after a single dialysis session (index session). The same measurements were recorded in the NECOSAD individuals, which additionally collected data on one or two additional consecutive dialysis sessions (six months apart as per protocol). Co-morbidity was classified according to Davies et al.,[20] on a seven point scale which was later on simplified into three risk categories (low, medium and high co-morbidity risk). Nutritional status was measured by means of the subjective global assessment which was trans-calculated to a three-point scale.[21] Weight was recorded before and after each dialysis and rounded up to the nearest decimal. Body Mass Index (BMI) was calculated as the body weight (kg) divided by the squared height (m).

Biochemical Methods

In both cohorts, venous blood was obtained immediately before and after the dialysis session. In the MIMICK cohort, samples were stored at -80°C for a period ranging between 2 to 14 months before high-sensitivity CRP measurements were performed. High-sensitivity CRP concentration was measured by commercial immunometric assays (LKCRP, DPC, Siemens, California) on an Immulite Analyzer (Immulite, DPC, Siemens, California), which had a detection limit 0.1 mg/L. Coefficient of variation for a 20-fold repetitive measurement of a single sample is reported of 7.5%. In the NECOSAD cohort, samples were stored at -80 °C degrees for a period ranging from 4 to 8 years before high-sensitivity CRP measurements were performed. We recently reported that this different storage time did not affect the measurements.[22] Measurements were done by means of particle-enhanced immunonephelometry using a standard CardioPhase high-sensitivity CRP for BNII (Dade Behring Holding GmbH, Liederbach, Germany) which had a detection limit 0.1 mg/L. Coefficient of variation for a 20-fold repetitive measurement of a single sample is reported of 7%.[22] While in the MIMICK cohort serum albumin levels were measured with bromecresol purple, immunonephelometry was used in the NECOSAD.

Statistical Methods

For both the MIMICK as well as the NECOSAD cohort, a change in serum CRP levels over a HD session was calculated as the levels after- minus before- dialysis in which an increase is reflected by a positive value. Body weight change was calculated as the weight before- minus the weight after- dialysis. In addition, relative body weight change (Relative Δ body weight) was calculated by dividing the change during dialysis by the weight before dialysis and multiplying this value by 100 to obtain percentages. To take into account potential volume shifts during the index session (hemoconcentration), CRP levels after dialysis were adjusted by means of the formula proposed by Bergström & Welhe[23] based on bodyweight change. As a sensitivity analysis in the NECOSAD, correction for hemoconcentration was also done using the albumin ratio during HD (described in more detail by Korevaar et al.[13]). Because this approach did not yield any differences in findings, results are not presented. To assess whether the mean change of serum CRP levels (both unadjusted and adjusted for hemoconcentration) deviated from zero (no change), one sample t-test statistics were calculated.

In each cohort, individuals demonstrating an increase in serum CRP levels during the HD session (>0 mg/L) were grouped as "increase" group, while individuals showing either no-change in CRP or a decrease (≤0 mg/L) were classified as "no-increase" group. In the comparison of baseline characteristics between both groups in each cohort, data is presented as means plus standard deviations (SD) and medians plus interquartile ranges (*IQR*), depending on the variable distribution and parametric and non-parametric tests were applied as appropriate. In the analyses of survival in MIMICK subjects, the Cox proportional hazards model was used to calculate hazard ratios for a change in CRP over the index session. Since age, gender, comorbidity, nutritional status, and dialysis vintage are known to influence CRP levels and mortality, they were included as confounders in a multivariate Cox model. Moreover, to take into account the potential confounding effect of CRP levels before dialysis, the logarithmic transformed value was included in the crude as well as in the multivariate analysis, together with the relative body weight change. The proportional hazards assumption was tested by calculating the correlation between the Schoenfeld residuals of each covariate and the survival rank for each patient. For all variables, this test was non-significant, indicating no violation of the proportional hazards assumption.

To assess the concordance in changes of CRP between subsequent hemodialysis sessions in NECOSAD individuals, Spearman correlation coefficients and Cohens Kappas were calculated. Identical statistical methods were used for the survival analyses in NECOSAD patients. Since individuals in the NECOSAD sample were selected on having fulfilled measurements during at least two dialysis sessions, follow up started from the second session onwards. For the same reason, characteristics at the time of the second session were included as confounders in the multivariate Cox model. For all the statistical tests, SPSS version 17.0 (SPSS Inc., Chicago. IL. USA) was used. Furthermore, a 95% confidence interval (*95%CI*) not including 1 for all hazard ratios and a p-value < 0.05 for all other tests was considered to be statistically significant. Kaplan Meier curves were created using Prism 5.02 (Graphpad, 1992).

Table 1. Mean change of serum CRP levels during a single hemodialysis session, both crude and after correction for hemoconcentration

	Uncorrected	Corrected by albumin [1]	Corrected by body weight [2]
MIMICK, n=190			
Δ CRP, mg/L #	1.99 (5.23) *	-	-0.76 (4.49)
Relative Δbody weight, % #	-3.5 (1.9) *		
NECOSAD, n=94			
Δ CRP 1st HD session, mg/L#	1.03 (8.75)	-0.79 (7.78)	-1.83 (7.73) *
Relative Δbody weight 1st HD, % #	-2.5 (1.6) *		
Δ CRP 2nd HD session, mg/L #	1.17 (6.60)	0.41 (5.38)	-0.90 (5.57)
Relative Δbody weight 2nd HD, % #	-2.7 (1.6) *		

1. Corrected by means of the albumin ratio. In 9/94 individuals, albumin was not measured.
2. Corrected by means of the formula suggested by Bergstrom and Welhe[21] based on body weight change.

\# Means plus SD. Deviations from zero were tested with a one sample t-test, with a * denoting $p<0.05$.

Results

In a crude analysis, mean CRP levels were significantly increased after the HD session in the MIMICK cohort (**Table 1**), but not in the two NECOSAD recorded sessions. Correction of CRP levels after the HD session for hemoconcentration made these differences disappear.

In the MIMICK cohort, mean age (SD) was 62.6 (14.0) years and median (IQR) vintage on dialysis 29.0 (14.8-57.0) months. Furthermore, of all individuals, 56.3% were men and 26.3% had diabetes mellitus. As shown in **Table 2**, no substantial differences were observed between patients who increased or did not increase in their CRP levels during a HD session with regards to age, sex, dialysis vintage, time on dialysis per week, relative body weight change, co-morbidities and type of dialyzer used. However, BMI and body weights before- and after- dialysis were higher in the CRP increase group.

During a median follow up of 41.3 (IQR: 22.2 to 48.5) months, 87 out of 190 patients died, of which 58/125 (46.4%) and 29/65 (44.6%) in the no-increase and increase groups, respectively. As shown in the Kaplan Meier survival curve (**Figure 1**), no difference in survival was noted between the two groups. When a change of CRP during the index session was analyzed as a continuous variable in a univariate Cox proportional hazards model, no association was found with mortality neither in the crude analyses, (HR [95%CI]: 1.008 [0.971 to 1.047]), nor after adjustment for CRP levels before dialysis, age, sex, malnutrition (SGA score), Davies co-morbidity score, relative body weight change and dialysis vintage (0.996 [0.949 to 1.046]). In the NECOSAD cohort, individuals were on average 65.2 (13.7) years of age and endured a median (IQR) time of 6.0 (6.0 to 12.0) months on dialysis (**Table 3**). When the sample was restricted to changes during the first session, no substantial differences with respect to age, gender, co-morbidity, type of filter, BMI and relative body weight change were observed between the increase group and no-increase group. However, the increase in serum albumin levels was substantially higher in the increase group.

Table 2. Baseline characteristics of MIMICK patients according to the CRP change during a single HD session

	No increase group (ΔCRP ≤0 mg/L) n = 125	Increase group (ΔCRP >0 mg/L) n = 65	P value
Age, years #	62.3 (14.0)	63.2 (14.0)	0.6
Men, % ¶	58.4	52.3	0.4
BMI, kg/m2 [1,#]	24.0 (5.4)	25.6 (5.0)	0.05
CRP before, mg/L *	6.3 (1.5 to 21.0)	7.6 (2.1 to 24.0)	0.7
CRP change, mg/L [2,#]	-2.1 (4.2)	1.9 (3.8)	-
Body weight before, kg #	72.5 (18.0)	77.4 (17.1)	0.07
Body weight change, kg [3,#]	-2.6 (1.3)	-2.7 (2.0)	0.8
Relative body weight change, % [4,#]	-3.6 (1.7)	-3.4 (2.3)	0.5
Dialysis vintage, months *	28.0 (12.5 to 57.0)	30.0 (15.0 to 57.0)	0.6
Time on dialysis per week, hours *	12.0 (12.0 to 13.5)	12.0 (12.0 to 13.5)	0.4
Type of dialyzer, Synth/cell. based, % ¶	95.2/4.8	93.8/6.2	0.7
Part with Diabetes Mellitus, % ¶	24.0	30.8	0.3
Comorbidity, low/middle/high, [5,¶]	24.8/52.8/22.4	15.4/52.3/32.3	0.2
Malnourished, % [1,6,¶]	47.2	53.1	0.9

1. In a few subjects, not all characteristics were measured.
2. CRP changes were calculated as CRP after- minus before the index session, in which serum CRP concentrations after the index session were adjusted for volume shifts during dialysis by use of the formula suggested by Bergstrom and Welhe. [23].
3. Weight change was calculated as weight before dialysis minus weight after dialysis.
4. Relative body weight change was calculated as body weight change divided by body weight before dialysis times hundred.
5. Comorbidity was defined according to Davies (scale 1 to 3).
6. Malnourishment was defined as a SGA score >2 on a scale of 1 to 3.

¶ Categorical variables were compared using a Chi square test.

* Data are expressed as median (IQR) and differences were tested by means of a Mann Whitney U test.

\# Data are expressed as mean (SD) and difference were tested by means of an independent sample t-test.

In the NECOSAD cohort, when comparing changes amidst different sessions, a low concordance and agreement existed between the changes in the index session and the second session (n=94), as expressed by non-significant kappa and correlation statistics with a value close to zero (**Table 4**). Out of the 94 patients, having fulfilled measurements during the first and second measurement, 47 attended a third HD session in which CRP variation was assessed. Again, concordance and agreement between both sessions were very low (**Table 5**). During the median follow up of 18.4 (IQR: 9.3-41.6) months, 52 patients died. However, no differences were observed between the patients that showed or did not show an increase in their CRP value during the HD session (**Figure 2**). No association was observed between ΔCRP as a continuous variable with mortality in a crude Cox model (0.961 [0.908 to 1.018]) nor when adjusted for the abovementioned confounders (0.978 [0.923 to 1.037]).

Table 3. Baseline characteristics of NECOSAD patients according to the CRP change during a single HD session

	No increase group (ΔCRP ≤0 mg/L) n = 61	Increase group (ΔCRP >0 mg/L) n = 33	P value
Age, years#	64.4 (14.3)	66.6 (12.5)	0.4
Men, %¶	49.2	63.6	0.2
BMI, kg/m2 [1,#]	25.0 (4.8)	26.1 (3.3)	0.2
CRP before, mg/L*	11.4 (3.0 to 21.3)	8.6 (3.1 to 21.5)	0.5
CRP change, mg/L [2,#]	-3.6 (8.9)	1.5 (2.3)	-
Albumin before, g/L #	38.7 (3.9)	39.0 (4.0)	0.7
Albumin change, g/L [3,#]	2.4 (4.3)	5.1 (4.7)	0.006
Body weight before, kg #	74.5 (13.5)	76.4 (12.8)	0.5
Body weight change, kg [4,#]	-1.8 (1.2)	-2.1 (1.0)	0.2
Relative body weight change, % [5,#]	-2.4 (1.7)	-2.6 (1.3)	0.6
Dialysis vintage, months*	6.0 (6.0 to 12.0)	6.0 (6.0 to 12.0)	0.7
Time on dialysis per week, hours*	10.5 (8.0 to 12.0)	12.0 (9.0 to 12.0)	0.2
Type of dialyzer, Synth/cell. based, %¶	86.0/14.0	92.7/6.3	0.5
Part with Diabetes Mellitus, %¶	9.8	6.1	0.1
Comorbidity, low/middle/high, % [6,¶]	39.3/52.5/8.2	36.4/57.6/6.1	0.9
Malnourished, % [1,7,¶]	32.7	27.3	0.7

1. In a few subjects, not all characteristics were measured.
2. CRP changes were calculated as CRP after- minus before- the index session, in which serum CRP concentrations after the index session were adjusted for volume shifts during dialysis by use of the formula suggested by Bergström and Welhe. [23]
3. Albumin changes were calculated as Albumin after- minus before- the index session.
4. Weight change was calculated as weight before dialysis minus weight after dialysis.
5. Relative body weight change was calculated as body weight change divided by body weight before dialysis times hundred.
6. Comorbidity was defined according to Davies (scale 1 to 3).
7. Malnourishment was defined as a SGA score ≥2 on a scale of 1 to 3.

¶ Categorical variables were compared using a Chi square test.

* Data are expressed as median (IQR) and differences were tested by means of a Mann Whitney U test.

\# Data are expressed as mean (SD) and difference were tested by means of an independent t-test.

In a sensitivity analysis in the MIMICK cohort, only individuals with a vintage on dialysis under 15 months were included (n=44). Moreover, in additional analyses, cut-off values were chosen differently, testing the median CRP change, the 75th percentiles, or even including a third stable group (-0.5 to 0.5mg/L change) to the increase (>0.5 mg/L) and decrease (<-0.5 mg/L change) Group. All these analyses did not yield any difference in findings. Because the previous study[13] was performed with a non high-sensitivity CRP assay, we further excluded in the survival analyses of both cohorts all patients with a CRP concentration before, and/or after dialysis under 3 mg/L. Finally, we also re-ran the survival analyses without correction for hemoconcentration. Also in these analyses, findings did not change.

Table 4. Percentage of individuals with concordant/discordant CRP changes during the first and second HD sessions in the NECOSAD sample

n=94	**Change during 2nd session**	
Change during 1st session	Increase	Decrease
Increase, n (%)	15 (16.0)	18 (19.1)
Decrease, n (%)	23 (24.5)	38 (40.4)

$\varkappa$= 0.075, p= 0.5, Pearson correlation: -0.01 (p=0.9)

Table 5. Percentage of individuals with concordant/discordant CRP changes during the second and third HD sessions in the NECOSAD sample

n=47	**Change during 3rd session**	
Change during 2nd session	Increase	Decrease
Increase, n(%)	5 (10.6)	11 (23.4)
Decrease, n(%)	10 (21.3)	21 (44.7)

$\varkappa$ = -0.01, p=0.9, Pearson correlation: 0.07 (p=0.6)

Discussion

The current study demonstrated in two independent European cohorts that on average, CRP levels do not significantly change during a single HD session. In addition, we demonstrated a lack of association between these changes and mortality. Furthermore, it was observed that the concordance and correlation in CRP changes amidst different sessions was very poor.

In both cohorts, corrected CRP levels did not increase during dialysis. These findings are in disagreement with previous works in which serum CRP levels were found to be elevated after a HD session.[8,24-26] In all these studies, however, CRP levels after dialysis were not adjusted for hemoconcentration, leading possibly to a misinterpretation of the results. Indeed, the significant crude CRP increase observed in the HD session of MIMICK patients was explained by the effect of hemoconcentration, as volume extraction (indicated by relative body weight change) was higher in this patient material. In a cross sectional study, Park et al.[26] found characteristics of left ventricle hypertrophy (LVH) to be more prevalent amongst individuals who showed an increase in CRP levels during dialysis (responders). However, as basal levels were substantially higher in responders and no correction for the effect of hemoconcentration on CRP measurements took place, it could be argued that the observed rise in serum CRP levels is, at least partly, secondary to the effect of fluid removal during dialysis. Moreover, ultrafiltration was slightly higher in the response group, thereby aggravating this effect. In this perspective, the association between an increase in CRP during dialysis and LVH could be considered as an invalid consequence of the known association between high CRP levels and LVH.[27]

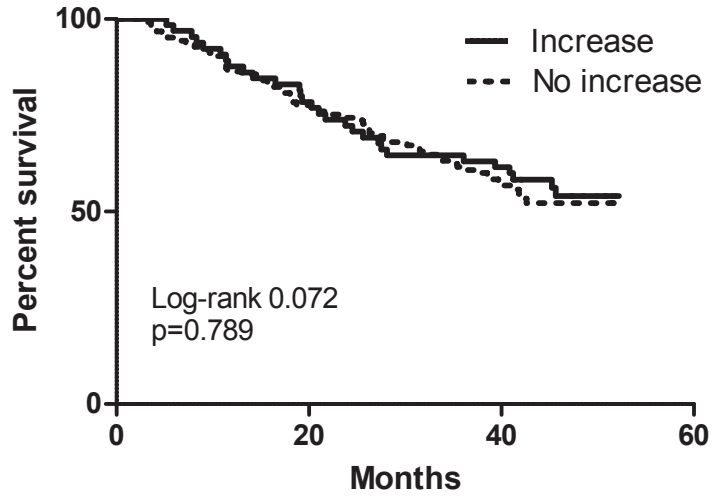

Figure 1. MIMICK sample: Survival plots by change of serum CRP during dialysis

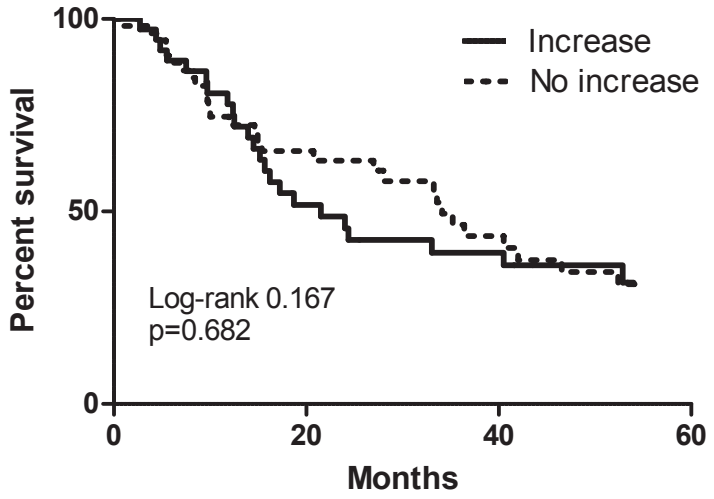

Figure. 2. NECOSAD sample: Survival plots by change of serum CRP during dialysis

Most importantly, the current study contradicts our previous finding in the NECOSAD cohort in which an increase of 1 mg/L in serum CRP levels during dialysis was associated with a statistically significant 8 percent higher mortality risk during follow up.[13] There are some important similarities and differences between these two studies, which are of importance when analyzing the strengths and limitations of the current analysis. Firstly, whereas Korevaar et al.[13] studied HD patients with a median vintage of 6.0 months on dialysis, the MIMICK cohort is composed of HD patients with a median vintage of 29.0 months on dialysis. Thus, it could be argued that a survival bias masked a potential association between a change in serum CRP levels and mortality in the MIMICK population. More specifically, when considering a pro-inflammatory response to extracorporeal circulation to be a subject-specific characteristic, individuals with a more pronounced inflammatory response to dialysis would have died before reaching 29 months on HD therapy. This reasoning is further supported by the fact that on average, age was lower and dialysis vintage approximately three fold higher in the MIMICK cohort as compared with the NECOSAD cohort. However, the theory of a possible survival bias is contradicted by the absence of an association between changes of CRP during dialysis and mortality in the current NECOSAD sample and in the sensitivity analysis of MIMICK patients, restricted to those being less than 15 months on dialysis.

Secondly, it could be argued that while CRP levels in our previous observation[13] were measured using an assay with a detection limit of 3 mg/L, our current analysis utilized high-sensitivity CRP assays. Nonetheless, sensitivity analyses excluding CRP values <3 mg/L in the present analyses also showed no differences in outcome prognostication in both cohorts. Additional limitations of our study include the fact that the protocol was not specifically designed to evaluate changes during the HD session, and several factors that potentially could influence CRP generation or degradation during the dialysis session, such as fluid status, were not taken into account. Finally, it would have been desirable to measure CRP repeatedly after the HD session over a longer time window to study whether CRP rose afterwards.

When putting these results in the context of the current understanding of CRP biology, more uncertainties add to the previously observed association between an intradialytic CRP change and mortality. When the dynamics of the acute phase response was studied after the aggressive stimulus of cutting the sternal bone during open heart surgery, CRP levels rose after approximately 7 hours.[14,28] Because a dialysis session typically lasts 4-5 hours, an increase of CRP within this period is biologically less plausible. On the other hand, while extrahepatic CRP production has been reported in adipocytes[29] and endothelial cells[30] in experimental conditions, we cannot exclude the possibility that specific phenotypes, for example obese individuals, or certain genetic profiles may translate, under the exposure to the uremic milieu,[31] in a faster CRP production from alternative tissues in response to the intermittent HD stimulus. However, this hypothesis has to date no substantiation and it is unknown if, and to what extent other tissues may contribute to systemic CRP levels in the context of uremia. In the interpretation of the current results, we have assumed the established statement that the vast majority of circulating CRP comes from hepatic production.[32] Also, as Kaysen et al.[15] pointed out, the acute phase response generally spans multiple dialysis sessions, thereby suggesting the value of intradialytic causes to be of less importance. Moreover, intradialytic changes in inflammatory markers seem to be very complex and influenced by many factors, such as adhesion of molecules to the dialyzer membrane, shifts between extravascular and intravascular compartments, and activation of the inflammatory response by extracorporeal circulation.[33,34]

As apparent by the lack of consistency amidst the different HD sessions in our study, CRP is probably not a valid marker to monitor the intradialytic inflammatory response. Focus should instead go out to more adequate markers. For example, in a study by Boehme et al.,[35] blood leaving the dialyzer produced more Pentraxin 3 (*PTX3*) than blood withdrawn before the dialysis session. In another study by Friedrich et al.[36] intracellular RNA levels in leucocytes encoding TNF-alpha, IL-1 beta and IL-8 increased significantly during dialysis. Since the intracellular compartment does not seem to be affected by volume extraction during dialysis, no differences are encountered when adjusting values after dialysis for hemoconcentration in this specific study.

Based on findings in the current study and the difficulty to explain an increase in CRP levels within the course of a single HD session from a biological perspective, we conclude that CRP changes during dialysis do not associate with mortality. However, whether patients exhibiting a more pronounced inflammatory response to extracorporeal circulation, have an increased mortality risk, is still of substantial interest. Therefore, future research should focus on understanding the consequences of activating other inflammatory markers or interleukins during HD.

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Chapter 5

Nonthyroidal illness and the cardiorenal syndrome

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Nature Reviews Nephrology. 9, 599-609 (2013).

Abstract

The cardiorenal syndrome represents a final common pathway for renal and congestive heart failure and heralds a poor prognosis. Factors that link the failing heart and the failing kidneys, the so called cardiorenal connectors, are therefore of clinical and therapeutic interest. Alterations in the levels and function of thyroid hormones that fit the spectrum of nonthyroidal illnesses could be considered to be cardiorenal connectors as both renal failure and heart failure progress with the development of nonthyroidal illness. In addition, circumstantial evidence suggests that nonthyroidal illness can induce deterioration in the function of the heart and the kidneys via multiple pathways. As a consequence, these reciprocal associations could result in a vicious cycle of deterioration that likely contributes to increased mortality. In this Review, we describe the evidence for a pathophysiological role of nonthyroidal illness in the cardiorenal syndrome. We also discuss the available data from studies that have investigated the efficacy of thyroid hormone replacement therapy in patients with renal failure and the rationale for interventional trials to examine the effects of normalization of the thyroid hormone profile in patients with renal failure and congestive heart failure.

Key points

- Cardiac disease and renal disease are frequently accompanied by nonthyroidal illness (that is, alterations in thyroid hormones in the absence of hypothalamic–pituitary–thyroidal disease).

- General factors (such as inflammation and nutritional deficiencies) and pathophysiological factors that are specific to cardiac and renal disease contribute to the development of nonthyroidal illness. The presence of nonthyroidal illness is associated with an increased risk of cardiovascular death in patients with cardiac and/or renal disease.

- Nonthyroidal illness can result in deterioration of cardiovascular and renal function via several pathways.

- The genesis of nonthyroidal illness in cardiac and renal failure as well as its deleterious effects on both organ systems suggest that nonthyroidal illness acts as a cardiorenal connector in the cardiorenal syndrome.

Introduction

Cardiac and renal function are closely connected in both physiological and pathological states.[1] Heart failure can lead to renal failure and vice versa, a condition commonly known as cardiorenal syndrome.[1] As the presence of this syndrome is associated with increased mortality,[2] factors that explain the connection between the failing heart and failing kidneys are of clinical interest.[3] Several of these so-called cardiorenal connectors have been identified and include inflammation, oxidative stress, anaemia and increased sympathetic nerve activity.[3] These factors, however, do not seem to fully explain the increased mortality of patients with cardiorenal syndrome.

Many patients with congestive heart failure (CHF)[4] and/or chronic kidney disease (CKD)[5] present with alterations in the levels of thyroid hormones in the absence of an apparent underlying dysfunction in the hypothalamus–pituitary–thyroid (HPT) axis. This condition, known as nonthyroidal illness, was traditionally considered to be the result of a physiological adaptation to slow metabolism and save energy in the presence of disease.[6] In patients with CHF[7,8] or CKD,[9–11] nonthyroidal illness is associated with an additional 2–3- fold increase in mortality. Direct effects of a low thyroid hormone state on both cardiovascular and renal function may explain this risk association.

Both CHF and CKD drive the development of non- thyroidal illness, which in turn could result in a deterioration in both renal and cardiac function (**Figure 1**). In this Review, we discuss the hypothesis that nonthyroidal illness is a cardiorenal connector in the genesis and maintenance of the cardiorenal syndrome, and describe available data from interventional studies that have investigated the potential beneficial effects of thyroid hormone supplementation in CHF and CKD.

Nonthyroidal illness

Thyroid hormone abnormalities that result from nonthyroidal illness are encountered in a variety of diseases, including severe infections, liver disease, severe malnutrition and psychiatric morbidities.[12] In mild-to-moderate disease states, levels of free and total triiodothyronine decrease, reverse triiodothyronine accumulates in the serum and levels of free thyroxine might increase slightly (**Figure 2**).[12,13] Severe forms of nonthyroidal illness (such as those observed in the majority of patients in intensive care units), are typified by a reduction in the levels of thyroid-stimulating hormone (*TSH*; also known as thyrotropin) and free and total thyroxine.[14] These alterations seem to be induced by functional changes that occur at all levels of the HPT axis, including an impaired central feed- back mechanism,[12] a reduction in the binding capacity of thyroxine binding globulin,[14] alterations in iodothyronine deiodinase activity and in the transport of thyroid hormones across cell membranes,[15] and differential expression and activity of the nuclear thyroid hormone receptor subtypes α and β.[16,17] Importantly, nonthyroidal illness occurs in patients who did not have overt dysfunction of the HPT axis prior to disease.

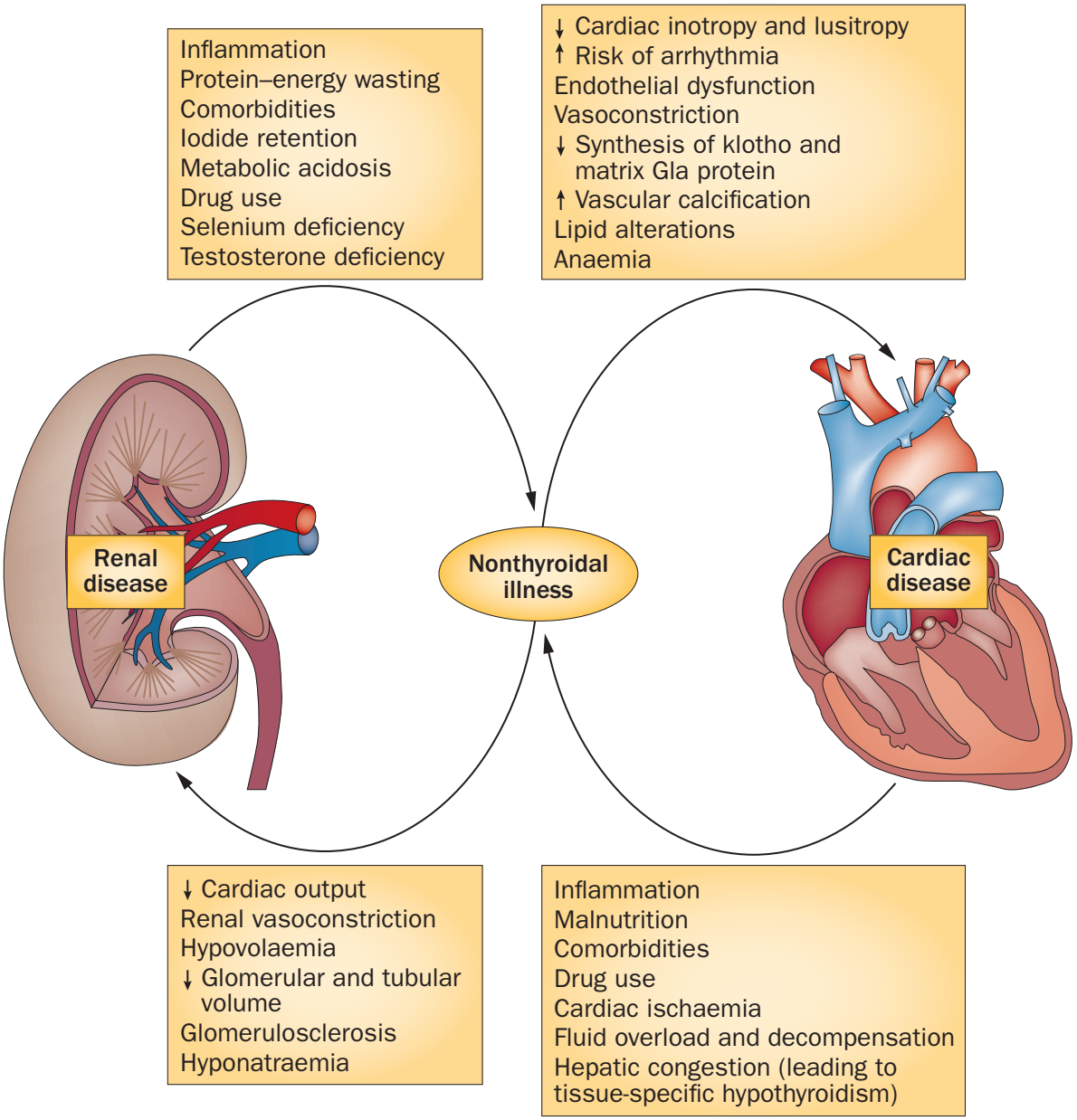

Figure 1. Nonthyroidal illness as a cardiorenal connector. Various mechanisms could drive the genesis of nonthyroidal illness in patients with chronic kidney disease and/or congestive heart failure and explain the potential negative consequences of nonthyroidal illness on cardiac and renal function.

Below, we describe the genesis and prevalence of nonthyroidal illness in CKD and in CHF. Some important considerations must, however, be taken into account. Firstly, nonthyroidal illness reflects a heterogeneous spectrum of abnormalities in thyroid hormone levels. As a consequence, various studies might differ in the thyroid hormones and cut-off values used to define nonthyroidal illness. The definition of nonthyroidal illness is further complicated by the existence of considerable inter-assay variability in measurements of the levels of free triiodothyronine and free thyroxine, particularly if the older radioimmunoassay method is used.[18] Secondly, as many of the available studies excluded the sickest patients (for example those with unstable CHF) the prevalence of nonthyroidal illness might be underestimated.

Thirdly, in nearly all of the available studies of nonthyroidal illness in cardiac and renal disease, serum levels of thyroid hormones were assumed to reflect the tissue levels of these hormones. The expression and activity of the various iodothyronine deiodinase subtypes, co-activator complexes, and nuclear thyroid hormone receptor isoforms, however, seems to differ between tissues and organs.[15] To date, this issue has been understudied in both cardiac and renal disease.

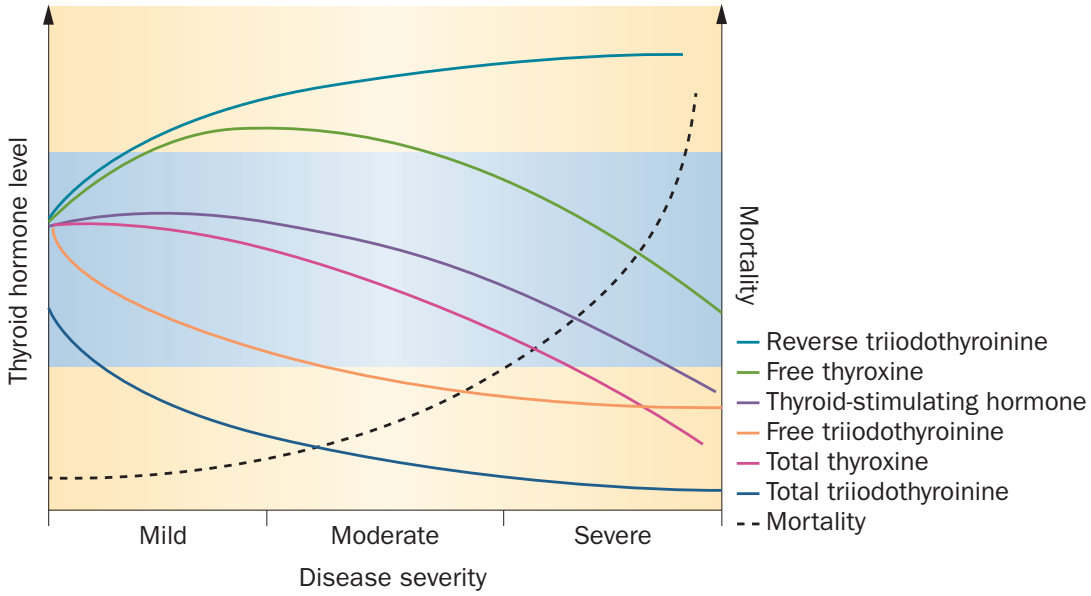

Figure 2. Thyroid hormone levels in nonthyroidal illness. Worsening of disease severity is associated with increasing abnormalities in thyroid hormone levels. The blue band indicates the normal range of thyroid hormone levels. Permission obtained from Society of Endocrinology © Warner, M.H. et al. J. Endocrinol. 205, 1–13 (2010).

Genesis of nonthyroidal illness

In renal disease

The prevalence of nonthyroidal illness gradually increases as renal function declines and recent studies have reported that clinically low serum concentrations of free and/or total triiodothyronine are present in more than 75% of individuals with end-stage renal disease (ESRD).[11,19,20] Low serum levels of free and total thyroxine have been reported in 10–50% of patients with ESRD,[21–24] and low levels of TSH have been reported in up to 10% of patients with ESRD.[21] The bioactivities of thyrotropin-releasing hormone (TRH; also known as prothyroliberin) and TSH may also be reduced in these patients.[20,25] In contrast to classical nonthyroidal illness, however, total serum levels of reverse triiodothyronine are not increased in patients with ESRD,[20] possibly as a result of the increased transfer of reverse triiodothyronine to extravascular tissues.[20] In acute kidney injury (AKI), a reduction in the levels of total and/or free triiodothyronine in combination with low levels of total and/ or free thyroxine and an increase in reverse triiodothyronine concentrations has been reported in up to 80% of patients.[26,27] As TSH levels infrequently exhibit a compensatory increase,[20] low levels of free thyroxine also seem to be part of the nonthyroidal illness spectrum rather than constituting a state of true hypothyroidism.[24]

Both general factors and renal-specific factors may explain the high prevalence of nonthyroidal illness in patients with ESRD.[19,28] A persistent chronic state of inflammation is observed in the majority of patients with ESRD[29] and is strongly associated with the presence of nonthyroidal illness in patients on dialysis.[30,31] In rats, infusion of interleukin-1 resulted in alterations in thyroid hormone levels that were similar to those seen in nonthyroidal illness in humans.[32] Nutritional disorders that are associated with the development of nonthyroidal illness[12] are common in patients with ESRD,[33] and hypoalbuminaemia (a proxy for nutritional status) was inversely correlated with the presence of nonthyroidal illness in these patients.[10,31] In CKD, nutritional disorders often coexist with a systemic break down of various muscle proteins and perturbations in the levels of appetite controlling hormones (that is, protein–energy wasting).[34] These abnormalities are also considered to be risk factors for nonthyroidal illness.[12]

Together, inflammation and protein–energy wasting may affect thyroid hormone physiology at all levels of the HPT axis, by mechanisms including decreasing the activity of type 1 iodothyronine deiodinase (which converts thyroxine into active triiodothyronine) and the thyroid-hormone-binding capacities of various proteins.[12,21] Finally, anaemia, which is common in patients with ESRD is associated with a decrease in triiodothyronine levels.[35] To some extent, the development of nonthyroidal illness also seems to be a direct consequence of factors related to the loss of renal function. For example, iodide retention owing to renal dysfunction causes thyroidal iodide oversaturation, which inhibits thyroid hormone oxidation and release, eventually resulting in functional hypothyroidism (a mechanism known as the Wolff–Chaikoff effect).[36] Low levels of free triiodothyronine have also been reported in patients with metabolic acidosis.[37] A further contribution to the development of nonthyroidal illness is made by comorbid diseases that are associated with CKD (such as, diabetes mellitus, gout and intermittent infections) and the use of certain drugs commonly used to treat patients with CKD, such as corticosteroids, amiodarone, propranolol and lithium.[38,39] In addition, phosphate binders such as calcium carbonate may reduce the intestinal absorption of L-thyroxine (a synthetic form of thyroxine).[40] In patients with pre-existing thyroidal disease receiving thyroxine therapy, this phenomenon may affect thyroxine dosages.

Researchers have suggested that loss of thyroid hormone binding proteins during dialysis might account for deficiencies in total levels of thyroid hormones in patients receiving dialysis. However, levels of total thyroxine and thyroxine-binding globulin correlate poorly in patients on dialysis, suggesting that the mechanism of thyroid hormone deficiencies in these patients is more complex than initially assumed.[23] Finally, because iodothyronine deiodinase activity is selenium dependent, abnormalities in peripheral conversion that result in low levels of total and free triiodothyronine have been partly attributed to selenium deficiency, which is common in patients with ESRD.[41] Testosterone deficiency is also a frequent finding in these patients[42] and is associated with a decrease in the activity of hepatic type I iodothyronine deiodinase in rats.[43]

In cardiac disease

CHF attributable to ischaemic and nonischaemic causes is often accompanied by low levels of free and/ or total triiodothyronine and elevated levels of reverse triiodothyronine.[8] These derangements are reported to be prevalent in ≤58% of patients with CHF7,[44–48] and are observed even during the early stages of the disease.[49] A strong dose-relationship exists between a reduction in serum levels of free and total triiodothyronine and the severity of symptoms in patients with decompensated[47,50] and compensated CHF.[51] In the settings of acute myocardial infarction[52,53] and cardiac arrest,[52,54] serum levels of free triiodothyronine and free thyroxine sometimes decrease below detection limits. In CHF, a reduction in the serum levels of total and free triiodothyronine parallels worsening of disease[48,55] and is induced by general and disease-specific factors. Firstly, inflammation is induced by both atherosclerosis[56] and CHF[57] and is associated with the presence of nonthyroidal illness in CHF.[7] Malnutrition and anaemia also contribute to the development of nonthyroidal illness by blunting pituitary responses to TRH and reducing the peripheral conversion of free thyroxine into active free triiodothyronine.[58,59] Certain drugs that are routinely prescribed by cardiologists also drive this process.[47] For example, the potent type III anti-arrhythmia drug, amiodarone, affects the thyroid via its high iodine content, alteration of triiodothyronine receptor binding affinity and iodothyronine deiodinase activity and its metabolites, which act as weak antagonists of free triiodothyronine.[60] Propranolol treatment was shown to selectively lower total triiodothyronine levels without significantly altering total thyroxine concentrations in hyperthyroid and hypothyroid patients, likely reflecting an effect of this drug on iodothyronine deiodinase activity.[39] Lastly, high dosages of furosemide (≤500 mg) have been associated with a reduction in the binding capacity of thyroxine binding globulin.[61]

Risk factors for nonthyroidal illness that are specifically linked to cardiac disease include those that are induced by CHF and ischaemia. In rodent cardiomyocytes, the activity of type 3 iodothyronine deiodinase (the major thyroid hormone inactivating enzyme) was selectively upregulated after induction of cardiac ischaemia[62] and right ventricular pressure overload.[63] This upregulation was hypothesized to result in a systemic lowering of triiodothyronine levels.[1] A similar decrease in serum triiodothyronine levels after streptococcal infection was, however, observed in mice with knock-out of deiodinase type 3 and in wild type controls, suggesting that deiodinase type 3 may not have an important role in the regulation of systemic triiodothyronine levels.[64]

Interestingly, type 3 iodothyronine deiodinase knock-out mice subjected to adrenergic overdrive developed restrictive cardiomyopathy, whereas wild type mice developed left ventricular dilatation.[65] As the liver is one of the major sites of conversion of free thyroxine to free triiodothyronine,[66] hepatic congestion in patients with right sided CHF might contribute to further inhibition of iodothyronine deiodinase activity and to the lowering of systemic triiodothyronine levels.[45,46]

Nonthyroidal illness and organ dysfunction

Cardiac dysfunction in renal disease

Cardiovascular mortality is more than eight times higher in patients with ESRD than in the general population,[67] and is further increased in patients with cardiorenal syndrome.[2] In observational studies, this increase in cardiovascular death in patients with ESRD has been partly attributed to the presence of nonthyroidal illness.[9–11]

The increased mortality that is associated with nonthyroidal illness can be explained by several biological mechanisms. At a cardiac level, low levels of free triiodothyronine are associated with an increase in left ventricular mass and a reduction in left ventricular function in patients on dialysis.[31] This observation is consistent with the finding of reduced left ventricular systolic dysfunction and impaired diastolic performance in Langendorff-prepared (that is, in vitro perfused) hearts of mice with starvation-induced nonthyroidal illness compared with those from well-fed, euthyroid animals.[68] In these animals, both phases of cardiac function improved after triiodothyronine substitution.[68] As cardiomyocytes have hardly any deiodinase activity[69] and are, therefore, largely dependent on free triiodothyronine in serum, the cardiac consequences of nonthyroidal illness might be equivalent to those of overt hypothyroidism.[70,71] During overt hypothyroidism, structural alterations occur in the heart that are associated with a reduction in its inotropic and lusitropic properties.[72] These alterations are the result of direct effects of thyroid hormones on the transcription of genes encoding myosin-α (also known as myosin-6), sarcoplasmic/endoplasmic reticulum Ca2+ ATPase (SERCA),[72–75] and β-1 adrenergic receptor proteins,[76,77] via stimulation of thyroid hormone response elements in the promoter regions of these genes (**Figure 3**). Thyroid hormones also negatively regulate the synthesis of myosin-β (also known as myosin-7) subtypes of adenylyl cyclase,[78] and phospholamban, an inhibitor of SERCA activity.[72] A low thyroid hormone state will, therefore, result in a cardiac phenotype encompassing upregulation of myosin-β, phospholamban and adenylyl cyclase, and a reduction in the activity of myosin-α, the β-1 adrenergic receptor and SERCA.

As is apparent from thyrotoxic atrial fibrillation (that is, atrial fibrillation associated with hyperthyroidism), thyroid hormone physiology also affects cardiac electrophysiological properties. In a study of patients with CHF, nonthyroidal illness was associated with an increased frequency of non-sustained ventricular tachycardias.[46] A poorer haemodynamic situation (that is, lower cardiac index and greater congestion) and higher levels of noradrenaline could, however, migh have accounted for this difference. Nonetheless, in patients on haemodialysis, the presence of nonthyroidal illness is associated with an increase in the occurrence of ventricular late potentials (as assessed on signal-averaged electrocardiograms) and in the risk of ventricular tachyarrhythmias.[79] In patients with hypothyroidism, cases of QT interval prolongation and torsades de pointes ventricular tachycardias have also been reported.[80,81] These events might be the result of a decrease in the activity of voltage-gated potassium channels and in the expression of Na+/K+ ATPase and the Na+/Ca2+ exchanger in response to low levels of thyroid hormones.[72,81]

At the vascular level, increased arterial calcification in patients with ESRD[82] could be partially explained by the presence of nonthyroidal illness. This hypothesis is suggested by studies in which levels of free triiodothyronine in patients with ESRD were inversely associated with coronary artery calcification (CAC) scores,[83,84] intima-media thickness[84] and measures of systemic arterial stiffness.[83–85] Several potential mechanisms might underlie an association between nonthyroidal illness and arterial calcification in patients with ESRD. Firstly, in ESRD a low thyroid hormone state is associated with endothelial dysfunction,[86,87] which is generally considered to be a prodromal step in atherosclerosis.[56] Secondly, a low thyroid hormone state exerts direct effects on vascular smooth muscle cells, promoting vasoconstriction and thereby increasing peripheral vascular resistance.[88] Thirdly, ex-vivo studies have indicated that low levels of free triiodothyronine induce downregulation of the synthesis of Klotho[89] and matrix Gla protein,[90] which are important inhibitors of the calcification process. Fourthly, low levels of free triiodothyronine were associated with high blood lipid levels that normalized after treatment with thyroid-hormone analogues.[91]

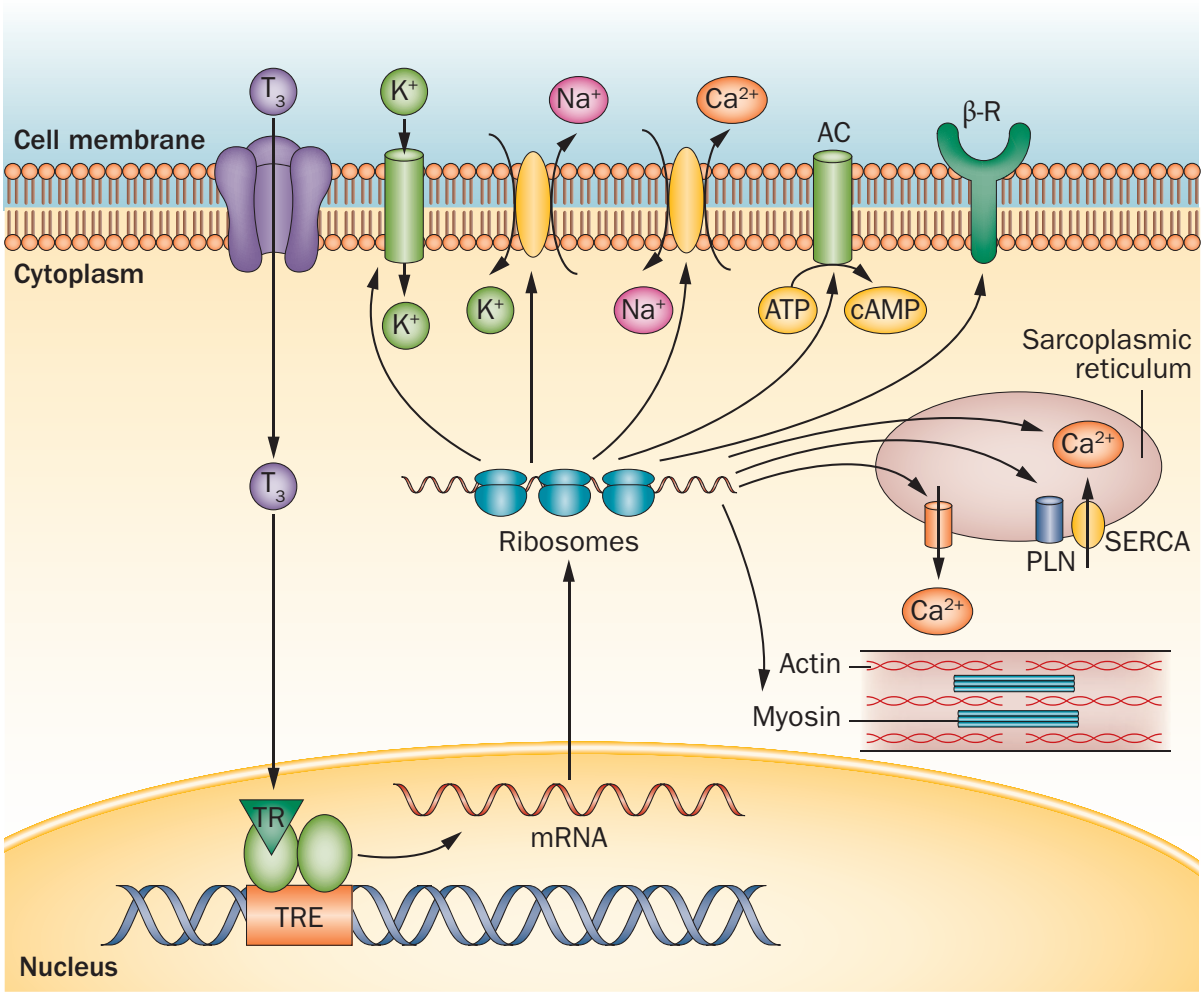

Figure 3. Direct effects of T3 on the cardiomyocyte. Binding of T3 to thyroid hormone receptors in the nucleus of the cardiomyocyte activates thyroid hormone response elements leading to the transcription of genes encoding myosin-α, SERCA and β-R, increased expression of voltage-gated K+ channels, Na+/K+ ATP-ase and the Na+/Ca2+ exchanger, and downregulation of myosin-β, AC and PLN. Abbreviations: AC, adenylyl cyclase; β-R, adrenergic β-1 receptor; PLN, phospholamban; SERCA, sarcoplasmic/ endoplasmic reticulum Ca2+ ATPase; T , triiodothyronine; TR, thyroid hormone receptor; TRE, thyroid hormone response element.

Renal dysfunction in cardiac disease

Renal dysfunction is common among patients with CHF and is associated with increased mortality in this population.[2] Various researchers have attributed this increased mortality to the presence of nonthyroidal illness.[48,92] Circumstantial evidence for this association comes from studies in patients with overt hypothyroidism. These patients often show reduced renal function that normalizes in response to restoration of thyroid hormone levels.[93,94] Recently, thyroid hormone replacement therapy in patients with subclinical hypothyroidism was shown to attenuate the slope of renal function loss.[95,96] Although thyroid hormone physiology directly affects the production of serum creatinine and levels of cystatin C,[97] the association between thyroid and renal function may not solely reflect a direct effect of thyroid hormone levels on renal function. Systemic myopathy, which is common in patients with hypothyroidism, could cause an increase in serum creatinine levels and, therefore, result in a reduction in estimated glomerular filtration rate (*eGFR*) that does not reflect worsening of renal function. However, hypothyroidism has also been associated with reduced renal function in studies that used gold-standard assessments of renal function (such as clearance of radio-iodinated hippuran, inulin or ^{51}Cr-edetic acid),[98–100] and micropuncture studies have shown a reduction in single nephron filtration rate in rodents with experimentally-induced hypothyroidism.[101]

The putative effects of nonthyroidal illness on kidney function can be subdivided into effects on the prerenal and renal compartments. In the pre-renal compartment, hypothyroidism may cause a decrease in renal plasma flow[98,102] that can be explained by several mechanisms. Firstly, cardiac output decreases in low triiodothyronine states induced by ESRD,[31] thereby reducing renal perfusion. Secondly, the renal vasculature and the systemic circulation show a diminished sensitivity to adrenergic and vasodilatory stimulation, leading to a further decrease in renal blood flow.[103,104] Thirdly, systemic levels of vasodilators, such as insulin-like growth factor-1, are reduced, shifting the balance to a relative vasoconstriction in the renal vessels.[105] Lastly, hypothyroidism has been associated with a decrease in circulating plasma volume.[98]

At the renal level, a low thyroid hormone state results in a reduction in the weight of the kidney cortex and medulla that seems to be reversed after restoration of euthyroidism.[106] There is a lack of studies of direct effects of a low thyroid hormone state on the glomerulus itself. A small, uncontrolled pathological study, reported basement membrane thickening, glomerulosclerosis and alterations in the mesangial matrix in renal specimens obtained from deceased patients with hypothyroidism.[107] Observations of a reduction in intravascular volume and impairment of urine concentration in patients with hypothyroidism have been attributed to hypothyroidism-induced tubular dysfunction.[108] Namely, a decline in eGFR in a hypothyroid state causes a decrease in sodium and water delivery to the diluting segments of the kidney, which in turn reduces reabsorption of sodium and water.[108] Reabsorption of salts in the setting of hypothyroidism seems to be further hampered by downregulation of the Na+/K+/CL2–exchanger and of the activity of Na+/K+ ATPase.[109,110] Another frequent phenomenon in hypothyroidism concerns hyponatraemia, which has been associated with inappropriate secretion of anti-diuretic hormone (*SIADH*).[110]

Increased tubular toxicity due to a hypothyroid-myopathy-induced increase in the levels of serum myoglobulin and creatinine phosphokinase could also affect renal function.[111]

Therapeutic options

Nonthyroidal illness in renal disease

In several studies, renal transplantation resulted in an increase in the serum levels of free, total and reverse triiodothyronine,[112,113] indicating a role of kidney dysfunction per se in these derangements. However, thyroid hormone concentrations initially decreased in the first few days after transplantation, likely reflecting postoperative triggers for nonthyroidal illness (that is, delayed graft function and introduction of immunosuppressive steroidal therapy).[112] Nevertheless, a study in 20 patients with ESRD and hypothyroidism reported an average reduction of 55% in the required dose of l-thyroxine 6 months after renal transplantation.[114]

Targeting disease-specific risk factors for the development of nonthyroidal illness also improves thyroid hormone derangements. Restriction of dietary iodide,[36] adoption of a low protein and phosphorus diet,[115] erythropoietin therapy,[35,116] correction of metabolic acidosis[37,117] and normalization of selenium levels[41] all resulted in increased levels of triiodothyronine and/or normalization of other thyroid hormone profiles in patients with renal disease and nonthyroidal illness.

Thyroid hormone supplementation accelerated renal recovery in rodents with renal insufficiency,[118–120] but evidence for a beneficial effect of such therapy in humans with kidney disease is limited. Several studies have investigated the effects of thyroid hormone supplementation in these patients (**Tables 1 and 2**; details on our literature search are specified in **Appendix 2**). A placebo-controlled randomized clinical trial of twice-daily thyroxine (150 μg delivered intravenously) versus placebo in 59 patients with AKI and nonthyroidal illness was terminated prematurely because of increased mortality in the treatment group.[121] It is plausible that because of reduced iodothyronine deiodinase activity in these patients,[14] the relatively high dosages of thyroxine that were administered could have suppressed TSH production without providing a higher bioavailability of free triiodothyronine.[122] Indeed, levels of free triiodothyronine did not significantly increase in the study participants during or after thyroxine supplementation.[121] Similar observations were reported in rats with nonthyroidal illness owing to starvation.[123] In addition, a combination of L-triiodothyronine and L-thyroxine (but not L-thyroxine alone) normalized tissue levels of free triiodothyronine in all organs of thyroidectomized rats.[124] On the basis of these findings, we speculate that l-triiodothyronine supplementation (with or without L-thyroxine) would be the preferred therapeutic approach in the context of CKD-related nonthyroidal illness. Interestingly, in a study of paediatric patients with AKI (n=8), diuresis commenced within 46 hours of initiation of L-thyroxine treatment in all participants.[125] Possible inferences from this study are, however, limited due to the lack of a control arm.

Table 1. Interventional studies of thyroid hormone substitution in patients with AKI

Study (design)	Participants, (age, years)*	Exclusion criteria	Intervention (no. of patients)	Setting, (follow-up)	Outcome parameters	Findings
Acker *et al.* 2000[121] (double-blinded, placebo-controlled RCT)	Patients with AKI (56.1 ± 2.7)	Azotaemia due to prerenal, postrenal or obstetric causes, acute glomerulonephritis, interstitial nephritis, hepatorenal syndrome or NYHA class IV heart failure	L-T4 300 µg IV twice daily for 48 h (28) Placebo (31)	ICU (14 days)	Mortality, renal recovery	Mortality was significantly increased in the L-T4 group (43%) versus the placebo group (13%; *P*= 0.001) No statistically significant difference in recovery of renal function or requirement for dialysis between the groups Sustained reduction in TSH levels in the L-T4 group.
Straub *et al.* 1976[125] (case series)	Patients with AKI not on dialysis (8.8 ± 2.2)	None	Oral L-T4 5-6 µg/kg per day, 1–2 daily doses (8)	Hospital ward (3–5 days)	Start of diuresis Serum urea and creatinine levels	In all patients diuresis occurred <6 h after initiation of L-T4 therapy.
Adamovich *et al.* 1992[126] (non-RCT)	Newborn infants with perinatal asphyxia‡ (3.7 h, 1–9 h§)	None	L-T4 100 µg IV, in two 50 µg doses in 24 h (15) Conventional treatment (13)	NICU (1 day)	Hypoxic stress, recovery of glomerular and tubular function, respiratory recovery	Compared with conventional treatment, L-T4 therapy resulted in a more rapid reduction in measures of hypoxic stress and faster recovery of glomerular and (to a lesser extent) tubular function No difference in respiratory recovery between the groups

*Mean ± SD or mean, range. ‡5 min Apgar score ≤7. §Mean gestational age 37.2 weeks, range 34–42 weeks.
Abbreviations: AKI, acute kidney injury; ICU, intensive care unit; IV, intravenous; -T4, Levo-thyroxine; NICU, neonatal intensive care unit; RCT, randomized controlled trial; TSH, thyroid-stimulating hormone.

Another uncontrolled, non-randomized clinical trial in 15 newborn infants with perinatal asphyxia reported a beneficial effect of L-triiodothyronine on renal recovery but not on respiratory recovery.[126] In two classic case series of patients with CKD, near physiologic doses of l-triiodothyronine resulted in a negative nitrogen balance, a finding that was considered to suggest that thyroid hormone supplementation should not be used in these patients.[127,128] However, another case series and a single-blinded randomized controlled trial reported favourable effects of thyroid hormone substitution on lipid profiles in patients with ESRD.[91,129] Finally, a randomized clinical trial of thyroid hormone replacement therapy in 38 patients with clinical or biochemical signs of delayed graft function within 24 h after renal transplantation found no significant differences in renal recovery or 1-year graft survival in the placebo and treatment groups.[130]

Table 2. Interventional studies of the effects of thyroid hormone substitution in patients with CKD

Study (design)	Participants, (age, years)*	Exclusion criteria	Intervention (no. of patients)	Sampling/ setting	Outcome	Findings
Lim *et al.* 1985[127] (cross-over trial)	Patients with CKD on RRT‡with T_3 levels less than 1 SD of the population mean (42.8 ± 15.9)	TSH levels >10 μU/mL, previous thyroid disease, clinically unstable, angina pectoris or CHF	Oral L-T_3 50 μg daily in four doses for 2 weeks then oral sodium ipodate 1 g once daily for 2 weeks (7 + 4 healthy controls)	At baseline and during the treatment periods	TH profile, nitrogen balance Basal oxygen consumption	L-T_3 treatment decreased levels of T_4 and TSH and increased levels of T_3; sodium ipodate had the opposite effect L-T_3 treatment increased nitrogen excretion in patients with CKD but not in controls; sodium ipodate therapy had little effect on nitrogen balance L-T_3 increased basal oxygen consumption in controls but not in patients with CKD.
Lim *et al.* 1989[128] (cross-over trial)	Stable patients with CKD and adequate nutritional status on RRT§ (38.7 ± 5.2)	Diabetes mellitus, CHF or angina pectoris	IV L-T_3 0.8 μg/kg per day in three or four equal doses for 7 days (11 + 7 healthy controls)	At baseline, during the treatment period and after 2 week wash-out	TH profile Nitrogen balance Protein metabolic state (leucine turnover kinetics)	L-T_3 treatment decreased levels of T_4 and TSH and increased levels of T_3. L-T_3 treatment resulted in a significant decrease in nitrogen balance in patients with CKD but only a small decrease in controls. L-T_3 treatment induced a significant increase in leucine nitrogen flux.
Carter *et al.* 1977[129] (case series)	Stable patients with CKD >12 m on HD (40.6 ± 10.6)	Diabetes mellitus, CHF or angina pectoris	Oral L-T_3 every 8 h, increasing dose during a 12 week period‖ (5)	At baseline and during the treatment periods	TH profile, Serum lipids. Symptomatic changes	During the treatment period total T_4 levels decreased but levels of TSH and free T_3 did not change. Total cholesterol and triglyceride levels decreased during the treatment phase. No change in symptoms during or after L-T_3 treatment.
Bommer *et al.* 1997[91] (single-blinded RCT)	Patients with CKD on HD with LP(a) levels >250 mg/l (58.8 ± 11.0)	Relapse of vasculitis, decompensating diabetes, septicaemia, tuberculosis, occlusive bowel disease, noncompliance, transfer to another centre	4 weeks placebo then oral d-T_4 for 16 weeks, starting dose 2 mg daily (15) 8 weeks placebo then oral d-T_4 for 12 weeks, starting dose 2 mg daily¶ (11) Placebo (13)	At baseline, and during the treatment and washout periods	Serum lipid levels	Significant reductions in levels of Lp(a) and total, LDL and VLDL cholesterol during d-T_4 therapy with corresponding increases during washout. No change in levels of Lp(a) or total, LDL and VLDL cholesterol in the placebo group.
Acker *et al.* 2002[130] (double-blinded, placebo controlled RCT)	Kidney transplant recipients aged >18 y with primary delayed graft function (46.2 ± 13.3)	AKI (due to acute allograft rejection, tacrolimus nephrotoxicity or delayed graft function), high cardiac risk, coronary ischaemia, myocardial infarction or cardiac dysrhythmia	L-T_3 0.2 mg/kg bolus followed by 0.2 mg/kg infused over 6 h within 24 h after kidney transplantation (20) Placebo (18)	ICU or internal ward (1 year follow-up)	Renal recovery, time from transplantation to recovery, hospitalization, 1 year patient and graft survival	No significant difference in outcomes between the treatment and placebo groups.

*Mean ± SD. ‡HD (n = 6) or CAPD (n = 1). §HD (n = 9) or PD (n = 2). ‖Dosages were increased at 3 weekly intervals, doses of 15 μg, 30 μg, 60 μg, and 90 μg per day. ¶Dose increased by 2 mg every 2 weeks to a maximum dose of 6 mg per day. Abbreviations: AKI, acute kidney injury; CAPD, continuous ambulatory peritoneal dialysis; CHF, congestive heart failure; CKD, chronic kidney disease; d-T_4, d-thyroxine; HD, haemodialysis; ICU, intensive care unit; IV, intravenous; LP(a), lipoprotein(a); L-T_3, Levo-triiodothyronine; L-T_4, Levo-thyroxine; PD, peritoneal dialysis; RCT, randomized controlled trial; RRT, renal replacement therapy; SD, standard deviation; T_3, triiodothyronine; TH, thyroid hormone, TSH, thyroid-stimulating hormone.

To date, no studies in patients with ESRD have examined the effects of targeting nonthyroidal illness on cardiovascular endpoints. Below we discuss how this field is evolving more rapidly in cardiac diseases.

Nonthyroidal illness in cardiac disease

In patients with mild-to-moderate heart failure and nonthyroidal illness, serum levels of total triiodothyronine normalize rapidly after heart transplantation.[46] Increases in triiodothyronine levels have also been observed in patients with CHF undergoing interventions that enhance haemodynamics, such as physical exercise programmes,[45] cardiac synchronization therapy,[131] and dobutamine infusion.[132] At least eight small studies have investigated the haemodynamic effects of thyroid hormone substitution using L-triiodothyronine,[133–135] L-thyroxine,[136–138] and the synthetic thyroid hormone analogue 3,5-iiodothyropropionic acid (DITPA)[139,140] in these patients. In a phase II randomized controlled trial that evaluated the safety of DITPA versus placebo in patients with New York Heart Association (NYHA) class I–III CHF, cardiac index improved significantly in the treatment versus the placebo group.[139] The trial was terminated prematurely, however, because of adverse effects and a trend towards increased mortality in the treatment group.[139] A different bioactivity of DITPA as compared with L-thyroxine, particularly in the high dosages administered, is thought to have accounted for these effects. In contrast to L-triiodothyronine and L-thyroxine, DIPTA is also poorly tolerated by mice.[141]

The key therapeutic approach for the successful management of nonthyroidal illness might be to restore thyroid hormone deficiencies and maintain levels of TSH, free thyroxine and free triiodothyronine within the normal ranges. Physiological doses of L-triiodothyronine and L-thyroxine seem to be well tolerated and are not associated with adverse events.[8] Although a substantial heterogeneity exists among the available data, supplementation with L-triiodothyronine and L-thyroxine was generally considered to be safe and resulted in improved haemodynamic status, characterized by increased exercise capacity, pressure development rate, and cardiac-output index, together with a reduction in peripheral vascular resistance.[8] Finally, in a placebo-controlled randomized trial that included 20 patients with CHF and nonthyroidal illness,[3] days of l-triiodothyronine therapy (20 $\mu g/m^2$) maintained levels of free triiodothyronine in the normal range and resulted in a substantial improvement in neuroendocrine profiles and ventricular performance.[134]

In 1972, the only placebo-controlled randomized controlled trial of thyroid hormone supplementation (D-thyroxine, 6 mg daily) in patients with acute myocardial infarction conducted to date was terminated because of a higher occurrence of arrhythmias and mortality in the treatment group than in the placebo group.[142] Although the inactive D-thyroxine used in this study was likely contaminated with active L-thyroxine, resulting in a dose that was the equivalent of many times the level of endogenous thyroxine produced daily by the thyroid, this negative finding led to its use being terminated.[143]

Finally, a recent systematic review concluded that compared with placebo, triiodothyronine was associated with a statistically significant increase in cardiac index without an increase in the risk of atrial fibrillation or death soon after cardiac surgery.[144] In many of the included studies, however, sample sizes were small and the duration of follow-up was short so that firm conclusions cannot be drawn from these data.

Future studies

Additional studies are needed to improve our understanding of the nature and consequences of nonthyroidal illness in patients with cardiorenal syndrome. For example, in patients with nonthyroidal illness, thyroid hormone levels may differ substantially between the serum and tissues[15] but tissue levels of these hormones have scarcely been investigated in the setting of cardiorenal syndrome. Moreover, beneficial effects of thyroid hormone supplementation for the treatment of nonthyroidal illness have been shown in animal models of ESRD[119,120] and CHF[68] but additional studies in models of cardiorenal syndrome are required. Thyroid hormone replacement therapy in this setting is controversial (**Box 1**). Arguments in favour pertain to potential improvements in organ function and decreases in the rate of atherosclerosis progression. Furthermore, the available data suggest that supplementation of thyroxine and triiodothyronine in physiological dosages is well tolerated. As discussed above, treatment of disease-specific risk factors for the development of nonthyroidal illness also resulted in normalization of thyroid hormone derangements without reported adverse events.

Box 1. TH supplementation in nonthyroidal illness

Arguments in favour

- ↑ Cardiac inotropy and lusitropy.[8,144]
- ↓ Peripheral vascular resistance.[88]
- Improves the neuroendocrine profile.[134]
- ↓ Rate of atherosclerosis.[152]
- ↑ Renal function.[93,95]
- Physiological doses of l-thyroxine and l-triiodothyronine seem to be well tolerated in patients with renal failure[124,125,127] and in patients with congestive heart failure.[8]
- Animal studies have shown beneficial effects.[68,119,120]
- Indirect evidence exists of restoration of thyroid disorders without reported adverse effects through restriction of dietary iodide,[36] use of a low protein and phosphorus diet,[115] EPO therapy,[35,116] correction of metabolic acidosis[37,117] or normalization of selenium levels.[41]

Arguments against

- ↑ Mortality in studies that used high dosages of DITPA,[139] d-thyroxine,[142] or l-thyroxine.[121]
- ↑ Cardiac oxygen consumption.[70]
- ↑ Occurrence of atrial arrhythmias.[146]
- ↑ Protein catabolism.[127,128]

Abbreviations: DITPA, 3,5-diiodothyropropionic acid; EPO, erythropoietin; TH, thyroid hormone

The main argument against thyroid hormone substitution originates from the two studies that showed an association between such therapy and increased mortality in patients with AKI and in patients with acute myocardial infarction.[121,142] These studies, however, utilized thyroid hormone analogues or thyroxine in supra-physiological dosages. A more sensitive physiological approach would involve the restoration of thyroid hormone levels to within the normal range. In addition, the possibility exists that thyroxine supplementation could increase myocardial contractility and heart rate,[70] resulting in an increase in myocardial oxygen consumption that could theoretically result in cardiac ischaemia, particularly in the presence of pre-existing coronary artery disease. Some studies have, however, suggested that thyroid hormone therapy might improve myocardial efficiency, endothelial function and coronary flow reserve.[70,145] In regards to the fear of a possible increase in risk of atrial fibrillation,[146] thyroid hormone substitution in physiological dosages did not increase this risk in patients with nonthyroidal illness after cardiac surgery.[144]

In two small studies that included patients with ESRD, protein catabolism increased in response to triiodothyronine supplementation.[127,128] This finding may, however, simply reflect the correction of a hypothyroid state and could possibly be compensated by adequate protein intake. Last but not least, some similarities can be drawn between nonthyroidal illness and other cardiorenal connectors, such as inflammation, sympathetic stress and anaemia, which could be interpreted as an adaptive response of the human body to illness. In the case of anaemia, although haemoglobin level is strongly associated with patient outcomes, and mechanisms that could explain the deleterious effect of anaemia on patient outcomes have been documented, large intervention studies aimed at normalizing haemoglobin levels in patients with heart failure and in CKD have not shown a benefit of the therapy.[147]

If randomized controlled trials exploring the hypothesis that thyroid hormone supplementation at physiological levels could improve outcomes in patients with cardiorenal syndrome are to be initiated, triiodothyronine supplementation may be the preferred intervention. The development of thyroid hormone analogues with specificity for the various nuclear subtypes of THR has evolved rapidly and these agents are also a potential therapeutic option.[148] Other therapies targeted at thyroid hormone metabolism are also in development. In a small cross-over trial that included 14 patients with protracted critical illness, a combination of thyroid releasing hormone with growth hormone-releasing peptide (*GHRP*)-2 increased the serum levels of TSH, total triiodothyronine and total thyroxine.[149] Notably, these changes were accompanied by a reduction in markers of catabolism (that is, urea production and urinary excretion of collagen cross-links) and an increase in markers of anabolism (that is, levels of serum osteocalcin and leptin).[149]

If interventions in the HPT-axis prove to be of benefit to patients with nonthyroidal illness attributable to renal failure and/or congestive heart failure, increased awareness among physicians, more extensive screening of patients and a more universal definition of nonthyroidal illness will be required.[122,150] According to current screening recommendations, most patients with nonthyroidal illness (that is, those with selectively low levels of total and/or free triiodothyronine) should be considered to be euthyroid.[151] Conversely, patients with severe disease and low levels of free thyroxine are not likely to suffer from primary hypothyroidism but rather from a more severe form of nonthyroidal illness.[150] However, in patients with nonthyroidal disease, low triiodothyronine levels typically precede low levels of total and free thyroxine. If interventions in the HTP-axis do prove to be of benefit to patients with cardiorenal syndrome, we suggest that levels of TSH, free thyroxine and total and/or free triiodothyronine should be included in a screening tool for nonthyroidal illness.

Conclusions

The available evidence suggests that nonthyroidal illness develops in patients with either CHF or CKD and has adverse effects on the heart and on the kidneys. This finding leads us to describe nonthyroidal illness as a cardiorenal connector that is involved in the vicious cycle of the cardiorenal syndrome. Existing studies of the effects of thyroid hormone supplementation in patients with nonthyroidal illness are limited by their design, small sample sizes and/or short durations of follow-up. As thyroid hormone replacement therapy in physiological dosages is generally well-tolerated, a need exists for large scale trials to test these interventions in patients with cardiac disease and/or renal failure.

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Chapter 6

Baseline levels and trimestral variation of triiodothyronine and thyroxine and their association with mortality in maintenance hemodialysis patients

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Abstract

Background: Conflicting evidence exists with regards to the association of thyroid hormone levels and mortality risk in patients with end-stage renal disease (*ESRD*). This study assesses the association between basal and trimestral variation of thyroid stimulating hormone (*TSH*), triiodothyronine (*T3*) and thyroxine (*T4*) and cause-specific mortality in dialysis patients.

Methods: In 210 prevalent hemodialysis patients, serum T3, T4, TSH, and interleukin-6 (*IL-6*)were measured 3 months apart. Cardiovascular and non-cardiovascular deaths were registered during follow-up. Based on fluctuations along tertiles of distribution, four trimestral patterns were defined for each thyroid hormone: persistently low, decrease, increase, and persistently high. The association of baseline levels and trimestral variation with mortality was investigated with Kaplan–Meier curves and Cox proportional hazard models.

Results: During follow-up, 103 deaths occurred. TSH levels did not associate with mortality. Patients with relatively low basal T3 concentrations had higher hazards of dying than patients with high levels. Longitudinally, patients with persistently low levels of T3 during the 3-month period had higher mortality hazards than those having persistently high levels. These associations were mainly attributable to cardiovascular-related mortality. The association between T4 and mortality was not altered after adjustment for T3 levels.

Conclusions: Hemodialysis patients with reduced T3 or T4 levels bear an increased mortality risk, especially due to cardiovascular causes. This was true when considering both baseline measurements and trimestral variation patterns. Our longitudinal design adds observational evidence supporting the hypothesis that the link may underlie a causal effect.

Introduction

A reduction in kidney function encompasses a number of alterations in thyroid hormone metabolism that renders a particular "Nonthyroidal illness" syndrome[1] in patients with chronic kidney disease (*CKD*), where low levels of both Total (*T3*) and free triiodothyroinine (*fT3*) are hallmark findings. Recent reports suggest that as many as 70% of patients with end-stage renal disease (*ESRD*) present with low T3 levels,[2-5] and as many as 20-25% have subclinical hypothyroidism.[6,7] The underlying pathophysiology of these derangements is likely multifactorial, involving iodine retention, altered serum protein binding capacity, systemic inflammation and peripheral deiodinase activity.[8,9]
These abnormalities have traditionally been considered as a physiological mechanism to save energy in response to uremic wasting.[10] However, growing evidence suggests T3 to participate in the pathophysiology of endothelial dysfunction,[11] atherosclerosis[12] and cardiac abnormalities[13] of CKD patients. Some studies, with somewhat conflicting results, have also implicated thyroid alterations in the mortality risk of CKD patients.[14-16] Zoccali et al.[14] were the first to report a positive association between low fT3 levels and all-cause mortality in stable hemodialysis (*HD*) patients, with similar findings in peritoneal dialysis (*PD*) patients.[17] We could further confirm and expand these observations in a cohort of euthyroid incident HD patients, in which the variance of all-cause and cardiovascular (*CV*) mortality was better explained by T3 than by fT3 levels.[16] In contrast, a study in 87 HD patients could not confirm these findings,[15] and a more recent retrospective report found that the association between fT3 and all-cause mortality was confounded by the nutritional status of the patients.[5] Whereas existing evidence on the association between thyroid hormones and mortality is limited to studies considering single measurements of hormone levels, it is presently unknown whether thyroid hormone fluctuation over time impacts on mortality hazards. In apparently healthy individuals, thyroid hormone concentrations show a narrow intra-individual variation,[18] although certain circadian and seasonal (mainly due to seasonal differences in iodine intake) fluctuations have been observed.[19] In diseased individuals, however, thyroid hormone concentrations seem subjected to a higher fluctuation as influenced among others by wasting syndromes, persistent inflammation and cardiac comorbidities.[19] No studies, to the best of our knowledge, have assessed variation of thyroid hormones in CKD patients and their association with mortality outcome. Against this background, the objective of the present study was to evaluate the association between both basal levels and longitudinal (3-month) variation of T3, T4 and thyroid stimulating hormone (*TSH*), and all-cause and cause-specific mortality. We did so in a well-defined cohort of maintenance HD patients.

Methods and patients

The Medical Ethics Committee at Karolinska University Hospital and Uppsala University approved the study protocol, and all patients consented to participate. This is a post-hoc analysis from the observational Mapping of Inflammatory Markers in Chronic Kidney Disease (*MIMICK*) cohort,

consisting of prevalent HD patients in the Stockholm region. The protocol has been described elsewhere in more detail.[20,21] The inclusion period ranged from October 2003 till September 2004. Laboratory measurements and patient characteristics were gathered at baseline and after 3 months. From inclusion onward, patients were followed for the occurrence of fatal events. Out of 224 patients originally included in this cohort, TSH, T3 and T4 were not measured in 6 individuals due to limited plasma volume. The patients were on angiotensin-converting enzyme inhibitors and/or angiotensin II receptor antagonists (n=72), betablockers (n=109), calcium-channel blockers (n=57), diuretics (n=104), statins (n=72), antiplatelet drugs (n=65), erythropoesis-stimulating agents (n=211), and iron substitution (n=144). Prescribed medication possibly influencing thyroid hormone levels was extracted from medical records: 16 patients were receiving levothyroxine and one of these 16 patients additionally used amiodarone. No patients were receiving lithium medication or propranolol. Comorbidity was classified according to Davies et al.[22], on a seven point scale which was simplified into a three risk category scale (low, medium and high comorbidity risk). Nutritional status was evaluated by means of the subjective global assessment (*SGA*).

Causes of death were retrieved from death certificates and classified as cardiovascular (*CV*) or non-CV. CV mortality was defined as death due to myocardial ischemia or infarction, cardiac arrest or unknown sudden death, acute as well as chronic heart failure, cerebrovascular accidents, cerebral hemorrhage, and ruptured aortic aneurysm. Non-CV death was defined as that not attributable to a cardiovascular origin. Individuals with unknown causes of death (n=13) were grouped within the non-CV group.

Biochemical Methods

Venous blood samples were drawn with the patient in a supine position and stored at -70 °C if not analyzed immediately. Concentrations of high-sensitivity C-reactive protein (*CRP*) and serum albumin (bromcresol purple) were determined using routine methods at the Department of Laboratory Medicine, Karolinska University Hospital, Huddinge, Sweden. Serum IL-6 concentrations were quantified in an automated immulite analyzer (Siemens Healthcare Diagnostics, Los Angeles, CA, USA). Thyroid hormones levels were also assessed on an Immulite system, using commercially available immunometric assays for T3 [analytical sensitivity (AS), 0.54 nmol/L; total coefficient of variation (CVs), 13.2% and 5.4% at the levels of 0.95 and 6.02 nmol/L)], T4 [AS, 5 nmol/L; total CVs, 8.4% and 6.3% at the levels of 49 and 167 nmol/L], and TSH [AS, 0.004 mIU/L; total CVs, 12.5%, and 4.6% at the levels of 0.016 and 1.3 mIU L]. Results are expressed as the average of two measurements.

Statistical Methods

Because existing evidence suggests that up to 70% of ESRD and dialysis patients present with low T3 levels,[3,5] in our study, we chose to define low T3 or T4 categories as those below the 66th percentile of distribution (higher tertile as the reference group). For the analysis of trimestral thyroid hormone variation (two time points: at study inclusion and after 3 months), we classified patients

according to their shift along tertile distribution at each time point, a strategy described in more detail elsewhere[23] and illustrated in **Figure 1**. From the nine possible combinations, four groups were created by clustering patients with changes in the same direction: (i) individuals who showed a change to lower tertiles were classified as the 'decrease' group, (ii) individuals showing an increase to upper tertiles were assigned to the 'increase' group, (iii) individuals with both values within the highest tertile of distribution were labeled as a 'persistently high' group and (iv) individuals with both values within the lower or the middle tertile were labelled as a 'persistently low' group. Differences between groups were tested by means of parametric (independent sample t-test, one-way ANOVA), non-parametric (Mann-Whitney U and Kruskal-Wallis test), and Chi-square tests as appropriate. Correlation was assessed by means of Pearson or Spearman correlation coefficients, as appropriate. Intra-patient variability of two measurements is depicted as a coefficient of intra-patient variation for each thyroid hormone. Survival during follow-up was analyzed by the Kaplan Meier method and hazard ratios (HR) were calculated with Cox-proportional hazard models with different degrees of adjustment for potential confounders. Age, sex, comorbidity, protein-energy wasting (PEW), serum albumin levels, smoking history, levothyroxine prescription, dialysis vintage, and IL-6 were considered as possible confounders in the associations. In these models, T3, IL-6, and vintage on dialysis were logarithmically transformed because of a non-normal distribution. In order to test whether the effect of T4 levels at baseline and variation groupings on mortality was due to the effect of T3 (in the causal pathway), we performed an additional adjustment for Log T3 or delta T3, respectively.

As a sensitivity analysis, Cox analyses were repeated in biochemically euthyroid patients only, being defined as presenting with normal TSH (reference range 0.1-4.5 mIU/L) and T4 (reference range 57.9-169.9 nmol/L). Finally, Cox analyses were adjusted by CRP instead of IL-6 as a surrogate of inflammation. HRs with 95% confidence intervals (95% CI) not including one were considered to be statistically significant. For all other tests, differences with p-values below 0.05 were considered to be statistically significant. SPSS version 17.0 (SPSS Inc., Chicago, USA) was used to analyse the study material.

Figure 1. Classification of trimestral variation patterns for each thyroid hormone based on shifts between tertiles of distribution

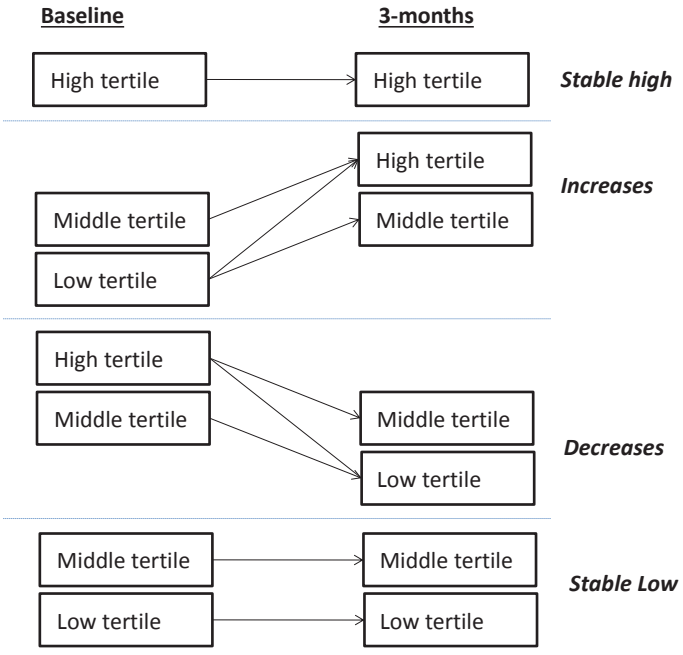


Table 1. Baseline characteristics according to T3 and T4 dichotomization in 218 prevalent hemodialysis patients [1]

	Low T3 n = 146	High T3 n = 72	p-value	Low T4 n = 147	High T4 n = 71	p-value
Age, years [3]	64.0 (13.8)	61.0 (15.1)	0.16	62.2 (14.5)	64.6 (13.8)	0.26
Men, % [4]	53	60	0.33	63	39	0.001
Dialysis vintage [5]	26 (12 – 50)	32.0 (17 – 63)	0.07	26 (12 – 55)	31 (17 – 61)	0.15
Comorbidity, % [2,4]	14/58/28	32/51/17	0.004	16/57/27	27/55/18	0.12
Smoking history, % [4]	17	20	0.60	15	25	0.07
SGA>1, % [4]	49	44	0.57	37	67	<0.0001
Albumin, g/L [3]	34.3 (4.8)	35.3 (4.1)	0.21	35.1 (4.2)	33.7 (5.2)	0.03
IL-6, pg/mL [5]	9.2 (5.5 – 15.7)	7.0 (4.3 – 12.1)	0.03	8.4 (4.8 – 14.6)	8.5 (5.1 – 15.4)	0.34
TSH, mIU/L [5]	1.40 (0.82 – 2.66)	1.40 (0.84 – 2.24)	0.43	1.41 (0.93 – 2.48)	1.30 (0.62 – 2.64)	0.30
T4, nmol/L [3]	64.40 (48.90 – 74.60)	83.70 (68.53 – 97.80)	<0.0001	60.5 (48.9 – 69.5)	94.0 (86.2 – 104.2)	-
T3, nmol/L [5]	0.73 (0.60 – 0.82)	1.11 (1.03 – 1.28)	-	0.76 (0.60 – 0.90)	1.00 (0.82 – 1.20)	<0.0001

1. Low T3 and Low T4 were defined as < 66th percentile (lower and middle tertile).
2. Davies score (see methods) showing the percentage of patients with low/medium/high comorbidity risk.
3. Means (SD). Differences were tested by independent t-test.
4. Percentages. Differences were tested by Chi square test.
5. Medians (IQR). Differences were tested by Mann-Whitney U tests.

Results

Basal thyroid hormone levels and its association with mortality

In all 218 individuals, median (IQR) levels of TSH, and T3 were 1.35 (0.84 – 2.52) uIU/mL, and 0.82 (0.67 – 1.03) nmol/L, respectively. Basal T4 levels were on average (SD) 70.44 (24.65) nmol/L and positively related to T3 levels (rho: 0.529, P<0.001). Thyroid hormone disorders on the basis of circulating hormones were common: 146 patients were classified as having a Low T3 syndrome, 11 patients were classified as having overt hypothyroidism, and additional 4 patients as having subclinical hypothyroidism. General and thyroid specific characteristics of studied patients are presented in **Table 1**, according to low levels of T3 (≤66th percentile; ≤0.94 nmol/L) and T4 (≤ 66th percentile; ≤77.2 nmol/L). Patients with low T3 presented with a higher prevalence of comorbidities, increased IL-6 levels and lower T4 concentrations. Patients with low T4 were more often men, malnourished and presenting with lower T3 levels. In univariate analyses, IL-6 positively associated with TSH (Spearman's rank test rho=0.16, P=0.01) and negatively with T3 (rho= -0.17, P=0.009). CRP levels positively associated with T4 (rho=0.19, P=0.003).

Table 2. Mortality hazard ratios (HR) and 95% confidence intervals (95% CI) according to basal T3 and T4 in 218 prevalent hemodialysis patients

	Crude, HR (95%CI)	Model 1, HR (95%CI)[1]	Model 2 HR (95%CI)[2]	Model 3 HR (95%CI)[3]
	Low T3, < 66th percentile (≤0.94 nmol/L)			
All-cause mortality	1.8 (1.1 – 2.8)	1.5 (0.9 – 2.4)	1.6 (1.0 – 2.6)	-
CV-mortality	2.8 (1.2 – 6.4)	2.5 (1.1 – 5.7)	2.6 (1.1 – 6.0)	-
	Low T4, < 66th percentile (≤77.2 nmol/L)			
All-cause mortality	1.2 (0.8 – 1.9)	1.7 (1.1 – 2.8)	2.1 (1.3 – 3.5)	2.1 (1.1 – 4.0)
CV-mortality	1.8 (0.9 – 3.8)	2.6 (1.2 – 5.9)	3.1 (1.3 – 7.0)	2.6 (1.0 – 6.8)

1. Model 1: Adjusted for age, sex, Davies Comorbidity score, protein-energy wasting (SGA>1), smoking, log-(vintage on dialysis) and serum albumin levels.
2. Model 2: Additionally adjusted for log-IL-6
3. Model 3: Variables included in Model 2 plus baseline log-T3 levels.

* The group with thyroid hormone levels in the highest tertile served as the reference group.

During a median (inter quartile range; IQR) follow-up period of 38.2 (17.6 – 45.1) months, 103 deaths occurred (40 CV and 63 non-CV) of which 77 occurred in the Low T3 group and 26 in the High T3 group. As shown in **Table 2**, the crude and adjusted hazards of dying (by all causes) were 1.8 and 1.6 times higher, respectively, in patients with low T3 levels than in those with high T3 levels. This was mainly due to CV deaths, where HRs were considerably higher in magnitude (crude HR [95%CI]: 2.8 [1.2 – 6.4]) and remained so after adjustment for confounders (adjusted HR: 2.7 [1.2 – 6.3]). A similar analysis was performed for T4. A statistically significant association between low T4 levels and an increased all-cause and CV mortality was observed after adjustment for confounders (adjusted HR: 3.0 [1.3 – 6.8]). Further adjustment for log-T3 levels did not materially alter the strength of these associations, although a 16% reduction in magnitude was

observed for CV mortality. Neither low T3, nor low T4 levels were significantly associated with non-CV mortality. TSH levels did not associate with any outcome measure (data not shown).

Table 3. General characteristics according to T3 trimestral variation in 210 hemodialysis patients[1]

	Persistently low n = 77	Decrease n = 41	Increase n = 44	Persistently High n = 48	p-value
Age, years [3]	65.7 (12.1)	63.2 (15.6)	62.6 (15.3)	58.4 (14.8)	0.06
Men, % [4]	52	61	61	54	0.68
Dialysis Vintage [5]	24 (11–48)	26 (13–49)	34 (16–63)	44 (20–71)	0.05
Comorbidities, % [2,4]	14/55/31	24/59/17	16/59/25	31/50/19	0.21
Smoking history, % [4]	18	18	9	24	0.30
SGA>1, % [4]	49	44	46	48	0.96
Albumin, g/L [3]	35.0 (4.5)	35.1 (4.6)	33.7 (4.1)	35.7 (4.2)	0.16
IL-6 pg/mL [5]	9.3 (5.8–16.6)	7.8 (4.2–14.8)	9.1 (5.5–11.4)	6.2 (4.3–10.1)	0.05
TSH, mIU/L[5]	1.51 (0.85–2.75)	1.66 (1.15–2.64)	1.20 (0.73–2.38)	1.12 (0.80–2.18)	0.20
T4, nmol/L[3]	59.38 (21.45)	78.18 (21.14)	66.48 (25.23)	85.46 (21.66)	<0.0001
T3, nmol/L [4]	0.67 (0.60–0.82)	0.96 (0.80–1.06)	0.72 (0.60–0.84)	1.19 (1.03–1.30)	-

1. Groups defined according to basal and 3-month variation in tertile distribution (See Methods).
2. Davies score (see methods) showing the percentage of patients with low/medium/high comorbidity risk.
3. Means (SD). Differences were tested by one-way ANOVA.
4. Percentages. Differences were tested by Chi square test.
5. Medians (IQR). Differences were tested by Kruskal Wallis tests.

Trimestral thyroid hormone variation and its association with mortality

Thyroid hormones were assessed again after three months in 210 patients. Median (IQR) coefficients of intra-patient variation were for T3: 7.8 (2.6 – 12.5)%, T4: 7.1 (4.0 – 12.0)%, and TSH: 14.0 (5.4 – 24.8)%. In **Table 3**, general and thyroid specific characteristics are depicted across four different T3 variation groups (see Methods), not observing major differences among these groups. The same was true for T4 variation groups (data not shown). In univariate correlation analyses, delta TSH associated with delta IL-6 (rho= 0.17, P=0.01), while delta T3 associated with delta serum albumin (rho=0.22, P=0.01). **Figure 2** shows the Kaplan Meier curves for T3 and T4 trimestral variation, both being clearly associated with patient outcome. **Table 4** shows crude and adjusted Cox models. Patients with persistently low T3 levels presented the highest hazard for all-cause mortality (HR 2.7 [1.5 –5.0)]) as compared with subjects having persistently high levels. This was particularly true for the association with CV mortality (HR 4.0 [1.3 – 11.7]), while no association between T3 variation and non-CV mortality was observed (data not shown). Adjustment for confounders did neither alter the magnitude of the association nor the statistical significance. Of note, elevated HRs, albeit statistically non-significant, were observed for patients showing increases or decreases of T3 levels during the 3-month follow-up versus those having persistently high concentrations.

Patients with persistently low T4 levels showed elevated hazards for all-cause mortality as compared with patients having persistently high T4 levels, an association that reached statistical significance after adjustment for confounders. Higher hazards were observed for prediction of CV-mortality (crude HR 2.6 [1.0 – 7.0]). In both cases, further adjustment for trimestral T3 variation (as a continuous variable) did not significantly affect the results. Of note, a decrease in T4 was also associated with elevated hazards for CV-mortality. No association was observed between T4 variation patterns and non-CV mortality. Trimestral TSH variation patterns were not associated with outcome. In a sensitivity analysis, all baseline and longitudinal Cox analyses were repeated in biochemically euthyroid patients only (n=210 at baseline, n=202 with available data at both time points). Additionally, Cox adjustment was done with CRP instead of IL-6. In both cases, results were not different (data not shown).

Table 4. Mortality hazard ratios (HR) and 95% confidence intervals (95% CI) according to T3 (Panel A) and T4 (Panel B) trimestral variation in 210 prevalent hemodialysis patients [1]

Panel A	Crude, HR (95%CI)	Model 1, HR (95%CI)[2]	Model 2, HR (95%CI)[2]	
All-cause mortality				
T3 Increase	1.4 (0.7 – 2.8)	1.1 (0.6 – 2.3)	1.3 (0.6 – 2.6)	-
T3 Decrease	1.5 (0.7 – 3.0)	1.3 (0.6 – 2.7)	1.4 (0.7 – 2.9)	-
Persistently low T3	2.7 (1.5 –5.0)	2.2 (1.2 – 4.1)	2.4 (1.3 – 4.5)	-
CV-mortality				
T3 Increase	2.4 (0.7 – 7.8)	2.1 (0.7 – 7.3)	2.3 (0.7 – 7.6)	-
T3 Decrease	1.8 (0.5 – 6.5)	1.8 (0.5 – 6.5)	1.8 (0.5 – 6.7)	-
Persistently low T3	4.0 (1.3 – 11.7)	3.5 (1.2– 10.5)	3.6 (1.2 – 10.9)	-
Panel B	Crude, HR (95%CI)	Model 1, HR (95%CI)[2]	Model 2, HR (95%CI)[3]	Model 3, HR (95%CI)[4]
All-cause mortality				
T4 Increase	0.9 (0.4 – 1.7)	1.5 (0.7 – 3.3)	1.9 (0.9 – 3.4)	2.0 (0.9 – 4.4)
T4 Decrease	1.8 (0.9 – 3.4)	2.0 (1.0 – 3.9)	1.7 (0.9 – 3.6)	1.6 (0.8 – 3.1)
Persistently low T4	1.5 (0.8 – 2.7)	1.9 (1.0 – 3.6)	2.0 (1.1 – 3.8)	2.1 (1.1 – 4.0)
CV-mortality				
T4 Increase	0.9 (0.3 – 3.5)	1.8 (0.5 – 7.1)	1.9 (0.5 – 7.5)	2.2 (0.5 – 8.8)
T4 Decrease	2.9 (1.0 – 8.4)	3.2 (1.0 – 10.0)	3.0 (0.9 – 9.3)	2.6 (0.8 – 8.0)
Persistently low T4	2.6 (1.0 – 7.2)	3.6 (1.2 – 10.6)	3.7 (1.3 – 10.8)	3.8 (1.3 – 11.2)

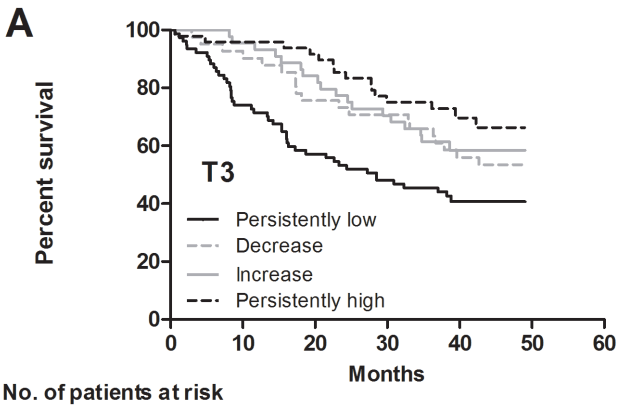
1. The group with persistently elevated levels served as the reference category in each analysis.
2. Model 1: Adjusted for age, sex, Davies Comorbidity score, protein-energy wasting (SGA>1), smoking history, log (vintage on dialysis), and serum albumin levels.
3. Model 2: Additionally adjusted for log-IL-6 levels.
4. Model 3: Variables included in Model 2 plus T3 changes (as a continuous variable).

Discussion

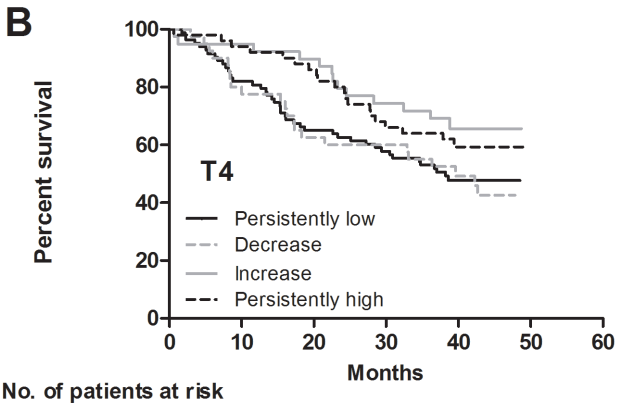
In this study, we show that both basal levels and trimestral variation of T3 and T4 are associated with increased mortality, particularly due to cardiovascular causes. Adjusting in the causal pathway suggests that the mechanisms associating low T4 levels with an elevated mortality rate may be, at least in part, independent of T3 levels.

Our observation linking low basal T3 levels with increased all-cause mortality is in agreement with some,[5;14;16;17] but not all preceding literature.[15] While earlier studies[5;14;17] only studied the association between T3 and fT3 and all-cause mortality, our study also shows that mortality prediction is mainly attributable to CV causes of death. The lack of mortality prediction observed by Fernandez-Reyes et al.[15] could relate to the selection of patients having survived at least 12 months, the low number of events encountered and the strict inclusion criteria which excluded, among others, patients with abnormal fT3 and fT4 levels. In this sense, our analysis shows that patients with both baseline and longitudinal low T3 levels have a relatively shorter time to death, possibly suggesting a stronger impact of thyroid hormones on short-term outcome. A recent study from Ozen et al.[5] showed that adjustment for CRP and s-albumin abrogated the association between fT3 and all-cause mortality, and the authors concluded that poor nutritional status confounded the investigated association. The acute-phase reactant nature of s-albumin

Figure 1. Kaplan–Meier curves for T3 and T4 trimestral variation. Kaplan–Meier survival curves for all-cause mortality according to T3 (A) and T4 (B) trimestral variation in 210 prevalent hemodialysis patients.



No. of patients at risk					
Persist. high	48	47	46	37	22
Increase	44	43	38	32	17
Decrease	41	38	32	30	23
Persist. low	77	58	45	39	21



No. of patients at risk					
Persist. high	50	48	44	34	23
Increase	39	38	36	30	17
Decrease	40	32	26	25	16
Persist. low	83	69	55	49	28

and its strong associations with the inflammatory status however, make these results difficult to contextualize.[24;25] In our own patient population, adjustment for inflammation (IL-6) and malnutrition (SGA) did not affect this association. Furthermore, Zoccali et al.[14] reported in their study that, since adjustment for fT3 abrogated the association between inflammation and patient outcome, fT3 may be an intermediate pathological of systemic inflammation. In our data, as well as in the study conducted by Ozen et al.[5], adjustment for inflammation did not affect the link between T3 and mortality. Also, associations between trimestral thyroid hormone changes and variation of inflammatory markers were somewhat weak and inconsistent in our study. One limitation that makes our studies not fully comparable is, that while Zoccali et al.[14] and Ozen et al.[5] measured free fractions, we report on total T4 and T3 levels only. Although adjustment for s-albumin (one of the main transporters of T3 in plasma) did not modify the observed associations, we should acknowledge that fT3 is not tantamount of T3. Several studies have, nevertheless, suggested anti-atherosclerotic effects of fT3 on the vascular bed via its effect on mitochondrial oxidative systems, induction of vasodilatatory molecules, inhibition of angiotensin II receptor expression and down-stream signal transduction, mechanisms all of which do not necessarily involve the inflammatory cascade.[26-28]

In support of the association between T3 and the prediction of cardiovascular death in our study, a previous report[11] in patients with moderate to severe kidney disease reported that T3 levels inversely associated with flow mediated vasodilatation, and that this observation was dependent on multivariate adjustment for asymmetric dimethylarginine levels. At a cardiac level, low T3 levels in CKD patients are associated with reduced left ventricular function, increased left ventricular mass[13] and elevated intima-media thickness[12]. Our study also adds novel evidence on the suggested impact of trimestral T3 variation on all-cause and CV mortality in ESRD. Patients with persistently low T3 levels exhibited the highest hazards of dying irrespective of other concomitant risk factors. Interestingly, also an increased variation in T3 during the observation period (e.g. both increases and decreases in concentration) resulted in elevated hazards albeit statistically not significant, leading us to hypothesize that also an increased T3 fluctuation may link to an adverse outcome. This finding accords with a previous study in critically ill patients,[29] in which a fast decline in T3 and T4 (without a concomitant rise in TSH levels) was observed prior to death. Our longitudinal analysis, by including two observations 3 months apart in the same individual, represents a step forward from previous evidence by virtue of observing the subject specific temporal order of events. Therefore, it is interesting to pinpoint that fluctuation of thyroid hormones over time exists in ESRD patients, and that this fluctuation links to a worse outcome. What factors drive this, and whether uremic thyroid fluctuation is higher than in other diseases remains, for now, unknown.

Another novelty in our analysis is the association between baseline and trimestral variation of T4 levels and (cardiovascular) mortality. Although this is the first study to report so in a dialysis population, findings are in line with previous evidence in non-renal patients with non-thyroidal illness.[29;30] An interesting aspect in our analysis is that the strength of the association between low T4 levels and mortality was not fully affected by further adjustment for T3 levels. This may suggest

that the effect of T4 on outcome is, at least in part, not dependent on its metabolite and that both may participate in the increased CV risk. According to recent literature however, adjusting in the causal pathway may not be so straightforward as initially thought of,[31] and caution is needed in the interpretation of this finding which warrants confirmation in further studies. Nevertheless, our previous report in incident dialysis patients also observed, by means of receiving operator characteristics (ROC) analysis a significant, albeit weak association between T4 levels and all-cause mortality.[16] The recent study by Takamura et al.[32] in euthyroid patients demonstrated that carotid intima-media thickness was inversely and independently associated with T4 and fT4, suggesting an increased cardiovascular risk in subjects with low T4 even within the normal reference range. Differential effects of T4 and T3 on immune cells have also been reported: T4 stimulated while T3 inhibited peripheral lymphocyte proliferation.[33]

In the interpretation of our results, some additional limitations must be addressed: Causes of death were extracted by death records and not confirmed by autopsies, which could result in some degree of misclassification. As unknown causes of death were denoted as non-CV, this could translate, in any case, into an underestimation of the observed effect towards the null hypothesis, and the true hazards may possibly be bigger. The inclusion of prevalent dialysis patients may infer into a survival bias, although preceding literature contemplates also prevalent patients in their designs. Finally, ours is an observational study and confounding by unmeasured factors cannot be ruled out.

To conclude, patients with reduced T3 and/or T4 levels bear an increased mortality risk, especially due to cardiovascular causes. This was true when considering both baseline measurements and trimestral variation patterns. Our longitudinal design adds important observational evidence -although non-decisive- that the link may underlie a causal effect.

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Chapter 7

Non-thyroidal illness: a risk factor for coronary calcification and arterial stiffness in patients undergoing peritoneal dialysis?

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Abstract

Objectives: Low triiodothyronine levels, as part of the non-thyroidal illness syndrome, are common in dialysis patients and have repeatedly been shown to be associated with increased (cardiovascular) mortality rates. We hypothesized that increased vascular calcification may intermediate this relationship.

Methods: A total of 84 patients from the Stockholm region receiving maintenance peritoneal dialysis were included in the study. Serum concentrations of free triiodothyronine (*fT3*), thyroxine and thyroid stimulating hormone were measured. Coronary artery calcium (*CAC*) scores were assessed by cardiac computed tomography scans. Surrogates of arterial stiffness included aortic diastolic and systolic blood pressures, pulse pressure, augmentation pressure and Buckberg's subendocardial viability ratio measured by pulse waveform analyses. Patients were subsequently followed and events of death and censoring were recorded. Thyroid hormone concentrations were associated with CAC scores, measures of arterial stiffness and all-cause mortality. The associations between CAC scores and arterial stiffness surrogates and mortality were also determined to evaluate a possible causal pathway.

Results: Both CAC scores and arterial stiffness surrogates were substantially higher in individuals with low fT3 levels. These associations persisted in multivariate logistic and linear regression analyses. During a median (interquartile range) follow-up of 32 (22–42) months, 24 patients died. Both fT3 levels below the median value [HR crude 4.1, 95% confidence interval (CI) 1.4 to 12.6] and CAC scores above the median value (HR crude 5.8, 95% CI 1.7 to 20.1) were strongly associated with mortality.

Conclusions: In patients undergoing peritoneal dialysis, fT3 levels were strongly associated with arterial stiffness, coronary artery calcification and mortality. We speculate that the association between non-thyroidal illness and mortality may be partly mediated by acceleration of vascular calcification.

Introduction

Coronary artery disease (*CAD*)[1] and myocardial ischaemia[2] frequently occur in patients with end-stage renal disease (*ESRD*), and are directly associated with increased risk of mortality.[3,4] CAD in ESRD is characterized by media thickening (Mönckeberg media sclerosis) and heavily calcified plaques.[5] Furthermore, cardiac valve calcification[6] and systemic atherosclerosis[7] are also common in patients with ESRD. Therefore it appears that uraemic risk factors, such as hyperphosphataemia, inflammation and depletion of vascular calcification inhibitors [fetuin-A and matrix gla protein (*MGP*)] may promote the calcification of the vasculature on a systemic level.

Thyroid hormone alterations due to chronic disease are frequently observed in ESRD and are characterized by low serum concentrations of triiodothyroinine (T3) with normal thyroid-stimulating hormone (TSH) levels. This pattern is known as non-thyroidal illness,[8] and its prevalence increases progressively with reduced renal function; it is found in approximately 70% of individuals with ESRD.[8,9] A low T3 level in patients with chronic kidney disease (*CKD*) has long been considered a benign compensatory response to malnutrition and wasting. However, recently, these abnormalities have been associated with a two- to three-fold elevated mortality rate in patients undergoing haemodialysis (*HD*)[10-12] or peritoneal dialysis (*PD*).[13] This excess mortality risk seemed to be most specific for cardiovascular causes.[11]

Whether a low T3 concentration in ESRD is causally implicated in the high cardiovascular disease risk of CKD is still unknown, but an accelerated rate of atherosclerosis represents a potential mechanism. In support of this, free T3 (*fT3*) levels were negatively associated with carotid artery intima–media thickness, arterial stiffness and pulse wave velocity in 137 non-diabetic HD patients.[9] In patients undergoing PD, an inverse association was described between fT3 levels and arterial stiffness.[14] In addition, non-thyroidal illness has been implicated in uraemic endothelial dysfunction,[15,16] and dyslipidaemia.[17] This is further supported by the demonstration of overt and subclinical hypothyroidism as true cardiovascular disease risk factors in the community.[18,19]

Thus, the aim of this study was to test the hypothesis that vascular calcification mediates the effect of non-thyroidal illness on mortality. To assess vascular calcification, we performed non-contrast cardiac computed tomography (*CT*) and pulse wave-form analyses in a cohort of prevalent patients undergoing maintenance PD.

Methods and materials

Study population

We conducted a longitudinal follow-up study in a cohort of prevalent PD patients from the Stockholm region. This cohort was originally designed to investigate inflammatory marker variability over time. Subjects were recruited between March 2008 and April 2011. All patients who were receiving PD therapy in the Stockholm region were invited to participate (n=164).

Of these, 55 patients did not provide informed consent, six underwent transplantation, two died, eight switched to haemodialysis and nine were excluded because of medical conditions (including mental disorders) that precluded their entry into the study. Measurements were performed weekly during a period of 3 months in the remaining 84 patients. The causes of ESRD in these patients were diabetes mellitus (n=10), renovascular disease (n=10), glomerulonephritis (n=5), immunoglobulin A nephropathy (n=6) and other (n=20), or unknown (n=33). From inclusion onwards, events of death and censoring due to the end of follow-up or renal transplantation were recorded. Comorbidity was scored based on the 7-point scale of Davies et al.[20] simplified into a three-category comorbidity risk scale (low, medium and high). Nutritional status was evaluated using subjective global assessment (SGA) according to the method of Detsky et al..[21] Among all patients, 60 (71%) used beta-blockers and 27 (32%) calcium antagonists. In addition, 47 subjects (56%) used angiotensin-converting enzyme inhibitors or angiotensin antagonists and 65 (77%) and 19 (23%) were receiving alfacalcidiol and calcitriol, respectively. The study protocol was approved by the Ethics Committee of Karolinska Institutet at Huddinge University Hospital (Stockholm, Sweden) and informed consent was obtained from all patients.

Arterial stiffness

In 74 patients, central aortic pressure waveforms were recorded by applanation tonometry at the radial site using a SphygmoCor device (AtCor Medical, Sidney, Australia). The methodology utilizes a validated transfer function that creates aortic pressure wave shapes on the basis of 20 sequential peripheral arterial waveforms.[22,23] In this way, aortic systolic and diastolic pressures were derived. Aortic pulse pressure was calculated by subtracting the central diastolic from the systolic blood pressure (*BP*). The difference between the first and second systolic peaks of the aortic waveform defined the aortic augmentation pressure. Patients with a negative aortic augmentation pressure were considered to have a value of 0 mmHg in the analysis. Buckberg's subendocardial viability ratio (*SEVR*) was calculated by dividing the diastolic by the systolic pressure time index. An experienced nurse performed the pulse waveform measurements in a temperature-controlled room, with patients in a supine position after resting for 5 min to acclimatize.

Cardiac CT protocol and coronary artery calcium score measurements

In 66 patients, cardiac CT scans were performed using an electrocardiogram (*ECG*)-gated technique on a 64-channel detector scanner [LightSpeed VCT, General Electric (GE) Healthcare, Milwaukee, WI, USA] in cine mode. A standard non-contrast protocol was used with the following parameters: tube voltage 100 kV, tube current 200 mA, rotation time 350 ms, slice thickness 2.5 mm and displayed field of view 25 cm.

The data were transferred to a dedicated workstation (Advantage Workstation 4.4, GE Healthcare) for further processing and analysis using coronary artery calcium (*CAC*)-scoring software (Smartscore 4.0, GE Healthcare). A standard threshold of 130 Hounsfield units for identifying calcified plaque was adopted. CAC scores were expressed in Agatston units (*AU*) as previously described in

detail.[24] Total CAC score was calculated as the sum of the CAC scores in the left main artery, the left anterior descending artery, the left circumflex artery and the right coronary artery. CAC scores were expressed in percentiles with respect to the distribution for an age- and sex-matched reference population.[25]

Biochemical measurements

Venous blood was collected after an overnight fast; all blood samples were centrifuged immediately and stored at −70 °C until required for analysis. Serum high-sensitivity C-reactive protein (*CRP*), calcium, phosphate, creatinine, urea and albumin levels (bromocresol purple) were determined using routine methods at the Department of Laboratory Medicine, Karolinska University Hospital. Serum levels of IL-6 were quantified using an Immulite system (Siemens Healthcare Diagnostics, Los Angeles, CA, USA). Plasma analyses of thyroid hormones were performed using a Roche Modular E/Cobas E analyser and commercially available electrochemiluminescence immunoassays for fT3 [analytical sensitivity (AS), 0.4 pmol/L; total coefficients of variation (CVs), 6.27% and 3.36% at 3.16 and 10.03 pmol/L, respectively], fT4 (AS, 0.3 pmol/L; total CVs, 2.05% and 3.03% at 12.01 and 34.70 pmol/L, respectively) and TSH (AS, 0.014 mU/L; total CVs, 2.16% and 2.42% at 0.995 and 5.530 mU/L, respectively). Results are expressed as the average of two measurements. Residual glomerular filtration rate (rGFR) was calculated as previously reported and expressed in mL/min/1.73m^2.[26]

Statistical analysis

Patient characteristics are presented across two fT3 strata (based on the median value) as means (SD or SEM), medians [interquartile range (*IQR*)] or percentages, and compared appropriately.

Linear regression analyses were performed to study associations between thyroid hormones and measures of arterial stiffness. Due to non-normality, CAC scores were categorized and a linear trend of thyroid hormones across these categories was investigated using a polynomial linear term in one-way ANOVA. Additionally, logistic regression models were applied to quantify the effect of thyroid hormone levels on CAC scores, dichotomized at the median value (920 AU). Univariate and multivariate regression models were used for analyses. In the multivariate models, age, sex, dialysis vintage and Davies scores were considered as confounders. For this purpose, dialysis vintage was logarithmically transformed due to non-normality. For the models including thyroid hormones as exposure, SGA, which showed the strongest association with non-thyroidal illness and mortality, was added. Because thyroid hormone status could also influence cytokine production,[27] and we were restricted in the number of covariates due to our small sample size, inflammation was not included in the primary analyses. In a sensitivity analysis, however, we examined the results after adjustment for log(IL-6). Assumptions underlying linear and logistic regression analyses were tested and not found to be violated.

Survival was analysed using Kaplan–Meier methodology. Only in the plot, mortality follow-up was restricted to the point at which 20% of patients were still at risk (44 months).[28] Cox proportional hazards models were fitted on the whole follow-up period and used to calculate hazard ratios (*HR*). To anticipate the possibility of monotone likelihood due to small numbers of events, Cox models were re-run after applying a shrinkage factor according to a modified version of the Firth method.[29] The same confounders described above were included in multivariate Cox models.
Primary analyses were performed using SPSS 20 (IBM Inc., New York, NY, USA). SAS version 9.3 (SAS Campus Drive, Cary, NC, USA) was used for Cox models with inclusion of a shrinkage factor. In linear regression analyses, beta values with 95% confidence intervals (CIs) not including 0 and odds ratios with 95% CIs not including 1 were considered statistically significant. For all other tests, a P-value lower than 0.05 was considered to indicate statistical significance. Figures were created with Prism 5.02 (Graphpad, 1992).

Results

The study population consisted of 84 PD patients; 68% were men, 24% had diabetes and 19% were smokers. The mean (SD) age of participants was 63.7 (14.1) years. Subjects with serum fT3 levels below the median value (3.95 pmol/L) had a higher prevalence of diabetes and protein–energy wasting (**Table 1**), whereas those with relatively low fT3 levels tended to have lower haemoglobin and albumin levels, lower rGFR but higher inflammatory biomarker levels (IL-6 and CRP). Mean serum calcium and- phosphate levels and median dialysis vintage did not differ significantly between the fT3 groups.
Measures of arterial stiffness, including aortic systolic BP, pulse pressure and augmentation pressure were significantly higher in the group with low fT3 values (**Table 2**). Results from linear regression analyses (**Figure 1** and **Table 3**) showed statistically significant univariate associations between fT3 and aortic systolic BP, pulse pressure, augmentation pressure and SEVR. After adjustment for confounders, beta values remained statistically significant for aortic systolic BP, pulse pressure and augmentation pressure. A trend remained for the association between fT3 and SEVR, which was not different when heart rate and systolic BP were excluded from the analyses. Log(TSH) was strongly associated with aortic pulse pressure (β 17.7, 95% CI 5.2 to 30.2), aortic augmentation pressure (β 7.1, 95% CI 1.2 to 13.0) and SEVR (β -20.1, 95% CI -38.5 to -1.7). Adjustment for log(IL-6) levels did not significantly alter the results.
The majority of the patients had CAC scores above 1000 AU. Calcification affected all three coronary vessels in almost all patients. Compared to age- and sex-specific distributions among the general population 25, 73% of study subjects had CAC scores above the 90th percentile. As presented in **Table 2**, median total CAC scores were significantly higher in subjects with low versus high fT3 levels [1527, interquartile range (IQR) 594–2507 vs. 438, IQR 56–1824; P = 0.01]; this relationship was also true for the different coronary arteries separately. Conversely, fT3 levels showed a gradual decline across CAC categories (P for trend = 0.001). In logistic regression analyses (**Table 3**), the

Table 1. Baseline characteristics of the 84 prevalent PD patients according to median values of free triiodo thyronine (fT3)

	Low fT3 group ≤3.95 pmol/L n = 42		High fT3 group >3.95 pmol/L n = 42		p-value
General characteristics					
Men, %[1]	60		76		0.10
Age, years[2]	65	(13)	62	(15)	0.33
Patients with medium/high Davies score, %[1]	55/21		60/7		0.15
Diabetes mellitus, %[1]	31		17		0.08
Protein–energy wasting, %[1,4]	50		30		0.09
Dialysis vintage, months[c]	15	(7 to 32)	11	(6 to 21)	0.44
CAPD, %[1,5]	78.6		76.2		0.794
Laboratory measurements					
Creatinine, mmol/L[2]	738	(168)	710	(181)	0.46
Residual eGFR, mL/min/1.73m[3,3]	2.3	(1.0 to 3.2)	3.2	(2.1 to 5.8)	0.008
Albumin, g/L[2]	29.5	(4.6)	33.2	(3.9)	<0.0001
Haemoglobin, mmol/L[2]	116	(12)	121	(10)	0.04
IL-6, pg/L[3]	8.2	(4.9 to 17.2)	5.1	(2.8 to 7.6)	0.001
C-reactive protein, mg/L[3]	5.2	(1.7 to 19.9)	3.3	(0.9 to 7.1)	0.05
TSH, mIU/L[3]	2.1	(1.3 to 3.2)	2.2	(1.4 to 3.1)	0.72
fT3 , pmol/L[2]	3.4	(0.3)	4.5	(0.4)	-
fT4, pmol/L[2]	12.8	(2.5)	13.8	(3.5)	0.12
Calcium, mmol/L[2]	2.3	(0.2)	2.3	(0.2)	0.31
Phosphate, mmol/L[2]	1.7	(0.6)	1.7	(0.4)	0.54

1. Differences between groups for categorical data were tested by chi-squared test.
2. Data are expressed as mean (SD); differences between groups were tested using an independent sample t-test.
3. Data are expressed as median (interquartile range); differences between groups were tested by the Mann–Whitney U test.
4. Protein–energy wasting defined as subjective global assessment score >1.
5. The remaining patients underwent automated peritoneal dialysis.

TSH, thyroid-stimulating hormone; fT4, free thyroxine; CAPD, continuous ambulatory peritoneal dialysis; eGFR, estimated glomerular filtration rate.

risk of having a CAC score >920 AU was higher in those with low versus high fT3 levels [odds ratio (OR) 3.4, 95% CI 1.2 to 9.4], an association that persisted after adjustment for confounders. After adjustment for log(IL-6), point estimates remained approximately the same while the confidence interval broadened substantially (OR 3.4, 95% CI 0.7 to 15.7). During a median (IQR) follow-up of 32 (22–42) months, a total of 24 (29%) patients died. Of these deaths, 17 (41%) and seven (17%) occurred in the low and high fT3 groups, respectively (Fig. 2). In crude analyses, individuals in the low fT3 group had a higher mortality risk (HR 4.1, 95% CI 1.4 to 12.6) than those with high fT3 levels (**Table 4**). The point estimate was reduced after adjustment for confounders and even further after adjustment for CAC categories. Regarding to the association between CAC scores and mortality, 15 (46%) subjects died in the group with CAC scores >920 AU as compared

to three (9%) patients with CAC scores ≤920 AU. The high CAC group had higher risks of death in both crude (HR5.8, 95% CI 1.7 to 20.1) and adjusted (HR 5.6, 95% CI 1.4 to 22.9) Cox models. After applying a shrinkage factor to the Cox models, the associations between low fT3 and mortality (crude HR 2.6, 95% CI 1.2 to 6.6; adjusted HR 1.9, 95% CI 0.8 to 5.1) and between high CAC scores and mortality (crude HR 5.2, 95% CI 1.8 to 19.8; adjusted HR 4.5, 95% CI 1.3 to 20.0) remained. Measures of arterial stiffness were not associated with mortality (data not shown).
Thyroid hormone levels did not differ between those who used or did not use beta-blockers (data not shown). Patients undergoing continuous ambulatory PD were not significantly different from those undergoing automated PD with respect to demographic characteristics, thyroid hormone levels, total CAC scores and mortality (data not shown). Finally, demographic characteristics, thyroid status and survival were not significantly different in patients who did and did not undergo cardiac CT scanning (data not shown).

Table 2. Coronary artery calcium (CAC) scores and measures of arterial stiffness according to median free triiodothyronine (fT3) values

	Low fT3 group ≤3.95 pmol/L		High fT3 group >3.95 pmol/L		p-value
Pulse waveform analyses	*n* = 38		*n* = 38		
Aortic systolic BP, mmHg[1]	134	(4)	120	(3)	0.005
Aortic diastolic BP, mmHg[1]	82	(2)	81	(2)	0.57
Aortic PP, mmHg[1]	52	(3)	40	(2)	0.006
Aortic AP, mmHg[1]	15	(1)	10	(1)	0.15
SEVR, %[1]	132	(5)	138	(4)	0.001
Coronary CT scans	*n* = 33		*n* = 33		
Total CAC score[2]	1527	(594–2507)	438	(56–1824)	0.01
LM CAC score[2]	70	(0–166)	22	(0–99)	0.41
LAD CAC score[2]	655	(203–1109)	194	(46–594)	0.002
LCx CAC score[2]	197	(74–617)	45	(0–269)	0.02
RCA CAC score[2]	339	(86–900)	33	(0–734)	0.04

1. Data are expressed as mean (SEM); differences between groups were tested with one-way ANOVA.
2. Data are expressed as median (IQR); differences between groups were tested using the Kruskal–Wallis test.

BP, blood pressure; PP, pulse pressure; AP, augmentation pressure; SEVR, subendocardial viability ratio; CT, computed tomography; CAC, coronary artery calcium; LM, left main stem; LAD, left anterior descending; LCx left circumflex; RCA, right coronary artery.

Figure 1. Association between free triiodothyronine (T3) and aortic systolic blood pressure (BP) (A), pulse pressure (PP) (B), augmentation pressure (AP) (C) and subendocardial viability ratio (SEVR) (D)

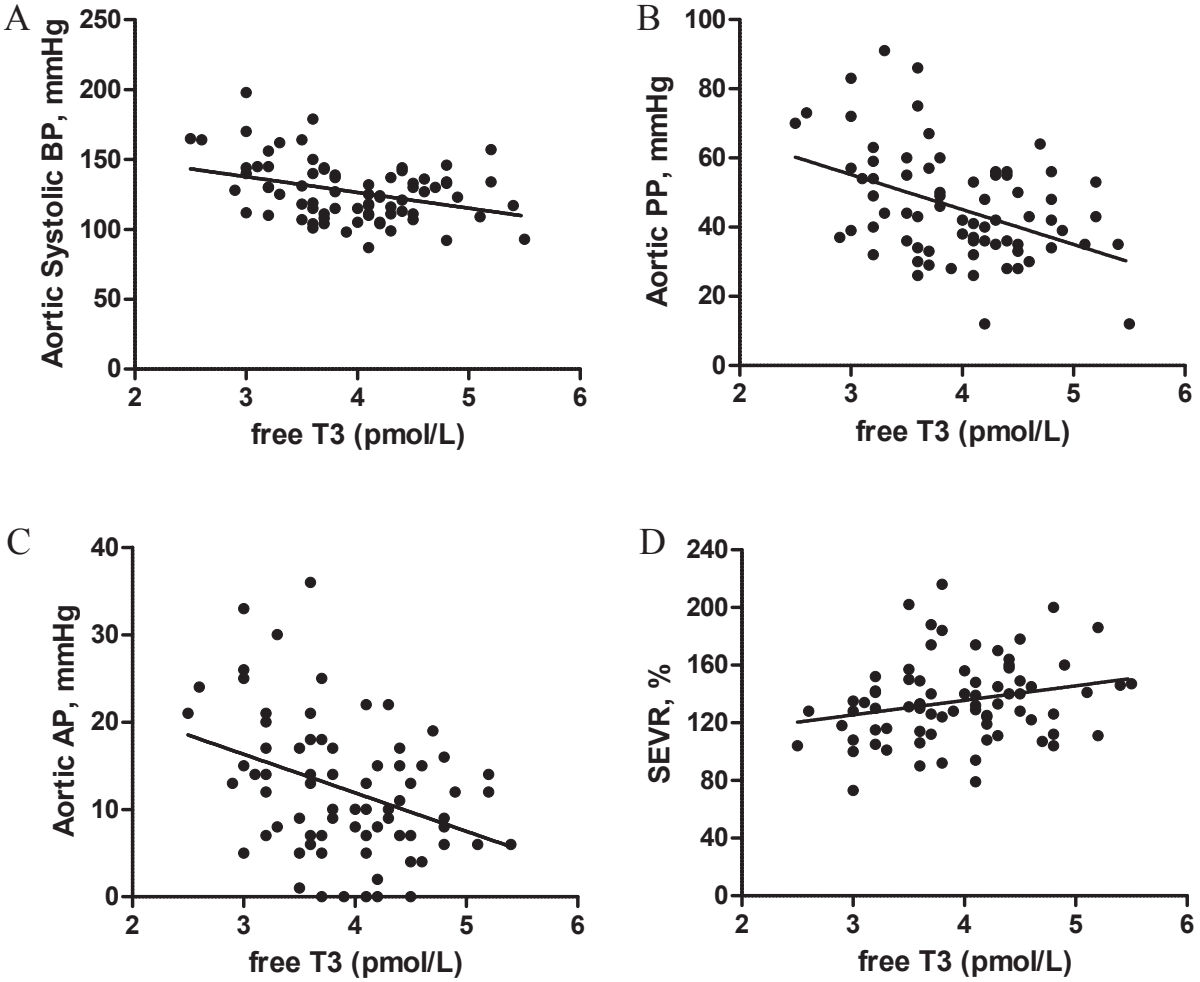

Table 3. Regression analyses of associations between serum free triiodothyronine (fT3) levels and coronary artery calcium (CAC) scores and measures of arterial stiffness

			Crude		Adjusted [2]	
Logistic regression	Low fT3 - CAC score[1]	OR (95%CI)	3.4	(1.2 to 9.4)	4.5	(1.1 to 19.1)
Linear regression	fT3- Aortic systolic BP	β (95% CI)	-11.3	(-18.2 to -4.3)	-11.8	(-19.1 to -4.4)
	fT3- Aortic diastolic BP	β (95% CI)	-1.2	(-5.5 to 3.2)	-2.3	(-6.8 to 2.2)
	fT3- Aortic PP	β (95% CI)	-10.1	(-15.1 to -5.1)	-9.4	(-14.6 to -4.3)
	fT3- Aortic AP	β (95% CI)	-4.4	(-7.0 to -1.8)	-3.1	(-5.7 to -0.5)
	fT3- SEVR	β (95% CI)	10.1	(0.6 to 19.6)	7.5	(-0.7 to 15.8)

1. FT3 levels and CAC scores were dichotomized according to the median values (3.95 pmol L1 and 920 Agatston units,respectively); for fT3, the highest group served as the reference.
2. Adjusted for age, gender, Davies comorbidity score, log (dialysis vintage) and SGA. The association between fT3 and SEVR was additionally adjusted for heart rate and systolic blood pressure.

BP, blood pressure; SEVR, subendocardial viability ratio; PP, pulse pressure; AP, augmentation pressure; SGA, subjective global assessment; OR, odds ratio; CI, confidence interval.

Discussion

In the present study, we found that serum fT3 levels were associated with measures of coronary calcification and arterial stiffness. Both fT3 and CAC, but not arterial stiffness, were associated with mortality. As 73% of our patients had CAC scores above the 90th percentile of age- and sex-specific distributions from the general population,[25] our data confirm previous findings that this patient population is subject to accelerated vascular calcification. CAC scores above 400 AU in the general population are considered to increase the short-term risk of cardiovascular events and death.[30] Compared to other PD[31,32] and HD[33] populations, the CAC scores in our cohort were considerably higher. This may be due to the fact that CAC scores increase with age[34,35], and our group of prevalent patients were on average 10 years older than in the previous studies.[31–33]

Figure 2. Kaplan–Meier survival curves according to free triiodothyronine (fT3) (A) and coronary artery calcium (CAC) score (B) categories

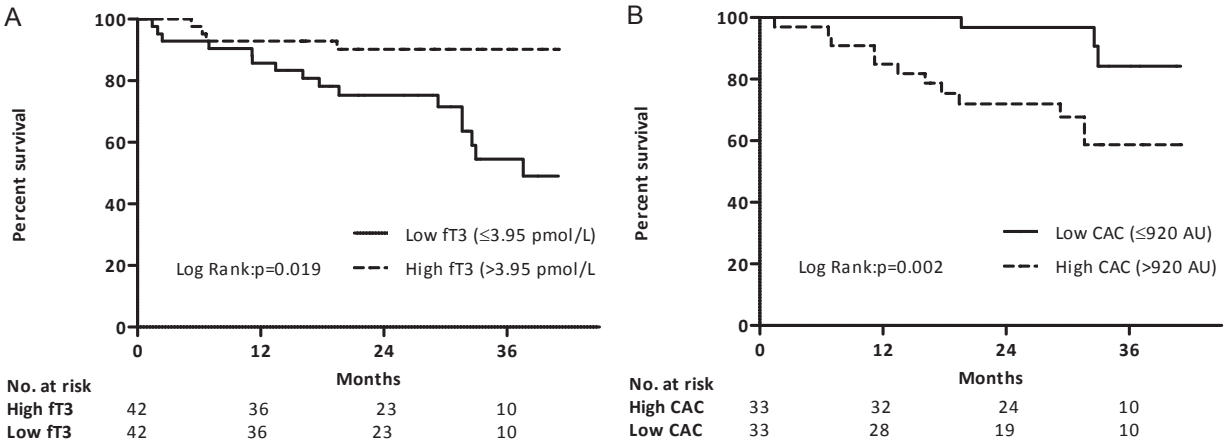

Table 4. Cox regression analyses of the association between free triiodothyronine (fT3) and coronary artery calcium (CAC) scores and mortality

Exposure[1]	Crude HR (95% CI)	Adjusted[2] HR (95% CI)	Adjusted[3] HR (95% CI)
Low fT3	4.1 (1.4–12.6)	3.1 (0.9–10.1)	2.4 (0.7–8.6)
High CAC	5.8 (1.7–20.1)	5.6 (1.4–22.9)	-

1. Exposures were dichotomized according to the median value. The complementary group served as the reference group.
2. Adjusted for age, sex, log(dialysis vintage) and Davies comorbidity score. The association between fT3 and mortality was additionally adjusted for SGA (see Methods).
3. Additionally adjusted for CAC score categories.

SGA, subjective global assessment; HR, hazard ratio; CI, confidence interval.

The chief finding of the present study is an inverse association between serum fT3 levels and CAC scores. Notably, this association remained in multivariate models including SGA and IL-6 levels, suggesting an association independent of the protein–energy wasting syndrome. Our results partially agree with the observation in HD patients of an inverse association between fT3 and CAC scores; however, this correlation did not remain after multivariate adjustment.[9] The approach of covariate selection may, however, have resulted in adjustment within the causal pathway in the previous study and could explain this divergence in results. Our finding of an inverse association between fT3 and measures of arterial stiffness agrees with the results of other studies in PD[14] and HD patients[9] also supporting a role of adverse effects of non-thyroidal illness on the systemic vasculature. Findings of studies in the general population suggest that overt and subclinical hypothyroidism are both true cardiovascular disease risk factors.[18,19] Four possible mechanisms might explain how a low T3 milieu could augment atherosclerosis and vascular calcification. First, a low T3 state in ESRD has been linked to dyslipidaemia, which is reversible after thyroid hormone replacement.[17] Secondly, non-thyroidal illness is associated with endothelial dysfunction,[15,16] and promotes vasoconstriction by a direct effect on vascular smooth muscle cells.[36] Thirdly, in agreement with previous studies in non-renal patient,[37] our data show that non-thyroidal illness is associated with inflammation, which may be indirectly related to low T3 levels, vascular calcification and cardiovascular death via low fetuin-A levels.[38] Finally, ex vivo studies have demonstrated that the expression of Klotho[39] and MGP[40] are T3 dependent. The finding of Mizuno et al.[39] that T3 significantly increased the expression levels of the membrane form of the klotho gene is of interest as premature ageing and vascular calcification are prominent features of the uraemic phenotype.[41] Moreover, the observation by Sato et al.[40] that physiological concentrations of T3 facilitate MGP gene expression in smooth muscle indicates that thyroid hormone replacement may be a future option to treat vascular calcification. Indeed, 45 years ago it was already known that cretinism was associated with vascular calcification, especially in patients who did not receive sufficient thyroid hormone replacement.[42] The fact that thyroid hormones regulate skeletal development and synthesis and secretion of vitamin K-dependent proteins[43] may support an indirect link between non-thyroidal illness and vascular calcification.

The positive association between fT3 levels and mortality in our study is in agreement with an earlier report that mortality hazards were 3.2 times higher per 1 pg/L decrease in serum fT3 levels in 41 patients undergoing continuous ambulatory PD.[13] Further, our results are consistent with findings in HD patients.[10-12] We previously showed that this association was predominantly accounted for by cardiovascular-specific deaths.[11] In agreement with previous data,[3,4] our current analyses also illustrate that higher CAC scores were very strongly associated with consequent death. Although adjustment in a causal pathway may not be easy to interpret,[44] the effect estimate of the association between fT3 and mortality was reduced considerably after adjustment for CAC categories, suggesting a mediating role for coronary calcification. No association between measures of arterial stiffness and mortality was apparent. It is conceivable however that patients with high as well as those with low aortic BP, augmentation pressure and pulse pressure are at increased risk of death.

That is, low aortic augmentation and pulse pressures could reflect poor systolic left ventricular function. As pulse waveform analysis is less influenced by this phenomenon, Blacher et al.[45] were indeed able to show an association between this measure of arterial stiffness and subsequent mortality.

Some limitations should be acknowledged when interpreting our findings. Although our sample size and the number of events during follow-up were limited, associations remained strong and independent of confounders considered. Nevertheless, larger studies are indicated to verify our findings and to test whether non-thyroidal illness is linked to low levels of circulating inhibitors of vascular calcification, such as fetuin-A and MGP. Furthermore, the lack of CT data in 18 out of 84 patients could have resulted in a selection bias. However this is unlikely as demographic characteristics, thyroid status and survival were not different between responders and non-responders. It should also be acknowledged that, as we only assessed calcification in coronary arteries, we do not know whether non-thyroidal illness is also associated with increased calcification at other arterial sites. However, by inclusion of mortality follow-up data and the description of a biologically plausible causal chain, we do provide important evidence for this possibility. This may support the design of studies aimed at testing whether restoration of the low T3 syndrome may reduce the high risk of atherosclerotic complications in the CKD population.

In conclusion, serum fT3 levels were inversely associated with CAC scores and measures of arterial stiffness in prevalent PD patients. Both fT3 and CAC scores were also associated with mortality. Specific mechanistic and intervention studies are warranted to clarify the nature of the intriguing link between non-thyroidal illness and vascular calcification.

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Chapter 8

Thyroid status and renal function in older persons in the general population

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Abstract

Background: Prevalence estimates of thyroid dysfunction and chronic kidney disease both increase with age. The aim of this study was to investigate the cross-sectional association between low thyroid function and renal function in subjects aged 85 years and to assess whether a low thyroid function at age 85 years is associated with an accelerated decline in renal function during follow up.

Methods: We included 558 participants from the Leiden 85-plus Study. At baseline (age 85), thyroid stimulating hormone (*TSH*), free thyroxine (*fT4*) and free triiodothyroinine (*fT3*) levels were measured. Thyroid function groups were created using clinical cut-off values of TSH and fT4. Serum creatinine concentrations were determined at baseline and annually during a 5 year follow-up period. Estimated glomerular filtration rates (*eGFR*) were calculated by means of the MDRD equation.

Results: At baseline, subjects with higher levels of TSH and lower levels of fT4 and fT3 had lower renal function. Participants with hypothyroidism (mean 53.7 (2.0) mL/min/1.73m^2) and subclinical hypothyroidism (55.7 (2.1) mL/min/1.73m^2) had lower mean eGFR (*SE*) than participants with normal thyroid function (59.5 (0.7) mL/min/1.73m^2); the highest eGFR was observed in participants with hyperthyroidism (eGFR (61.5 (3.1) mL/min/1.73m^2) (p-for trend=0.004). There was no association between thyroid hormone levels at baseline and the change in renal function during follow-up.

Conclusions: Although low thyroid function was associated with lower renal function at age 85 years, an association between a low thyroid function and change in renal function over time was absent. Our findings question the causal relevance of the thyroid status for the deterioration of renal function in the oldest old.

Introduction

Renal function declines with increasing age.[1] Up to 47 percent of individuals aged 70 years and over are estimated to suffer from some stage of Chronic Kidney Disease (*CKD*).[2] Throughout all age strata, CKD is associated with an increased risk of adverse cardiovascular outcomes, such as myocardial infarctions, heart failure and death.[3,4] In the general population, CKD is commonly caused by diabetes mellitus, and hypertension,[5] but treatment of these risk factors does not fully prevent the decline in renal function with advancing age. Therefore, identification of other risk factors for a decline in renal function, that are potentially amenable for treatment, is needed.

Alike CKD, overt and subclinical hypothyroidism are common disease entities in the general population, especially in older persons.[6,7] Up till 14 % of individuals aged ≥80 years are reported to have elevated serum thyroid stimulating hormone (*TSH*) levels.[6] In the general population, overt hypothyroidism and subclinical hypothyroidism are both associated with an increased cardiovascular risk,[8,9] which could be attributed to various cardiovascular effects of thyroid hormones.[9] A low thyroid hormone state has been associated with adverse blood lipid alterations,[10] endothelial dysfunction,[11] and accelerated atherosclerosis.[12]

Several small observational studies indicated a decline in renal function in patients with overt hypothyroidism as estimated by serum creatinine measurements[13-15] and labeled edetic acid.[16] These alterations attenuated or even reversed after thyroid hormone supplementation.[13-15] Two very recent reports additionally showed a negative effect of subclinical hypothyroidism on renal function over time in patients with pre-existing CKD.[17,18] Potential pathophysiological mechanisms connecting a low thyroid function to a decrease in renal function could pertain to a decrease in cardiac output, direct vasoactive effects, a reduction in the size of glomeruli, and promotion of arteriosclerosis as induced by low serum thyroid hormone concentrations.[19]

Although prevalence estimates of a low thyroid function and CKD both increase with age, it is unknown whether subclinical and overt hypothyroidism are also associated with a deterioration of renal function over time specifically in older persons. Therefore, the aim of this study was to investigate the association between low thyroid function and renal function specifically in an elderly population. For this purpose, we investigated the cross-sectional association between thyroid status and renal function in subjects 85 years and assessed whether a low thyroid hormone state at age 85 years associated with an accelerated decline in renal function over time. This is of special interest as negative effects of commonly appreciated risk factors in the general population,[20,21] including the impact of a low thyroid hormone state on mortality,[7,22-24] proved absent in the oldest old.

Methods and material:

Study population

The Leiden 85-plus Study is a population-based prospective follow-up study of 85-year-old inhabitants of Leiden, the Netherlands. The study protocol has been described in detail previously.[21] In short, between 1997 and 1999, all residents of Leiden, the Netherlands, celebrating their 85th birthday (belonging to the 1912-1914 birth cohort) were contacted and asked to participate. Out of the 705 individuals who were found eligible, 14 died before the recruitment phase and 92 refused participation leaving a total of 599 individuals to be enrolled in the study (response rate 87%).[21] Within one month after their 85th birthday, participants were visited at home. During these visits, participants underwent face-to-face interviews, performance tests were done and a venous blood sample was drawn. 37 participants refused blood sampling. In the present analyses, 558 participants were included with complete information on thyroid hormone status and renal function. Participants were visited annually until reaching the age of 90 years or death. At age 88, 376 individuals underwent a second blood withdrawal. The medical ethical committee of the Leiden University Medical Centre approved the study protocol, and informed consent was obtained from all participants.

Laboratory measurements

Blood was withdrawn in a supine position and analyzed immediately. Plasma levels of TSH and free thyroxine (*fT4*) were measured in a fully automatic fashion using an Elecsys 2010 system (Hitachi, Tokyo, Japan). For TSH, the coefficients of variation (*CV*) ranged between 5% and 11%. For fT4, CVs varied between 5% and 8%. An electrochemiluminescence technique was applied (Boehringer, Mannheim, Germany). Plasma levels of free triiodothyronine (*fT3*) were determined by a microparticle enzyme immunoassay (Abbott Diagnostics, Abbott Park, Ill) for which CVs were between 3% and 8%.

The following thyroid hormone groups were created based on serum TSH and fT4 levels as widely accepted;[25] 1) Euthyroidism; TSH levels between 0.5 and 4.5 mIU/L. 2) overt hypothyroidism; TSH >4.5 mIU/L and fT4 <13 pmol/L, 3) subclinical hypothyroidism; TSH >4.5 mIU/L and fT4 between 13 and 23 pmol/L, 4) overt hyperthyroidism; TSH <0.5 mIU/L and fT4 >23 pmol/L and 5) subclinical hyperthyroidism; TSH levels <0.5 mIU/L and fT4 concentrations between 13 and 23 pmol/L. Since only two subjects had overt hyperthyroidism, groups 4 and 5 were merged into one category named "hyperthyroidism". Three patients, two of whom with low TSH and low T4 levels and one with high TSH and high T4 levels, fell out of our classification. When new thyroid dysfunction was discovered (n=39) subjects were referred to their general practitioner for further work-up.[22]

Serum creatinine concentrations were measured according to the Jaffé method (Hitachi 747; Hitachi, Tokyo, Japan). For the primary analyses, glomerular filtrations rates were estimated using the four variable version of the Modification of Diet in Renal Disease Study (*MDRD*) formula[26] which has been validated in older adults.[27] Subjects were divided into three eGFR groups (<30, 30-60, >60 mL/min/1.73m^2). For the purpose of sensitivity analyses, creatinine clearances were calculated using the Chronic Kidney Disease Epidemiology Collaboration (*CKD-EPI*)[28] and Cockroft-Gault formulas.[29] Plasma C-reactive protein (*CRP*) levels were assessed by utilization of a Hitachi 747 automated analyzer at the day the sample was drawn.

Information on the presence of disease was obtained from general practitioners and nursing home physicians. The presence of cardiovascular disease was defined as a history of a cerebrovascular accident or transient ischemic attack, angina pectoris, myocardial infarction, peripheral vascular disease (including a history of arterial grafting, endarterectomy, and/or angioplasty), an electrocardiogram suggesting myocardial ischemia or past infarction, and a history of heart failure.[30] Diabetes mellitus (*DM*) was considered present when diagnosed by a primary care physician, when routine non-fasting glucose levels exceeded 11.0 mmol/L, or when an individual was being prescribed anti-diabetic medication. Past and/or currently active malignancies were grouped into one category. Also, a simple physical examination was performed which included an assessment of weight, height, and blood pressure. With an intervening period of two weeks, blood pressure was measured twice by using a mercury sphygmomanometer.[31] For every measurement, patients had rested for at least 5 minutes and performed no vigorous exercise in the preceding 30 minutes. Information on the use of thyroid medication (antithyroid medication and/or thyroxine supplementation) was obtained from pharmacy records.

Statistical analyses

Baseline characteristics were presented as means with standard deviation (*SD*), medians plus interquartile ranges (*IQR*) or numbers with percentages (%) across thyroid hormone groups. Differences between thyroid hormone groups were tested by means of one-way ANOVA, Kruskal Wallis and Chi square tests, as appropriate.

Mean (standard error of the mean (*SE*) or 95% confidence interval (*95%CI*)) eGFR values within tertiles of thyroid hormone distributions or thyroid hormone groups at baseline were calculated using univariate and multivariate linear regression models. Univariate models (*Model 1*) comprised tertiles of the specific thyroid hormone distribution or the different thyroid hormone groups as independent variables. In multivariate models, sex, DM, smoking, the presence of cardiovascular disease (composite score ≥1), malignancies, and amiodarone usage were added as possible confounders (*Model 2*).

To examine the effects of the different thyroid hormone groups and thyroid hormone concentrations at baseline on change of renal function over time, linear mixed models were fitted. A model with fixed intercept and slope and unstructured covariance matrix was adopted because of its best fit as judged upon by the maximum likelihood estimate method and Akaikes information criterium. Mean annual changes per group were estimated by means of multivariate models including the baseline variables sex, DM, smoking, cardiovascular disease, malignancies, and amiodarone treatment as possible confounders. The effect of thyroid hormone status on the change in renal function over time was evaluated by implementation of an interaction term between thyroid hormone state and time.

As an alternative approach, regression lines were fitted on the repeated measurements for each individual separately. Then these betas were pooled within the different thyroid hormone strata (tertiles for each hormone) and groups (as earlier specified) and compared by means of a one-way ANOVA test. In addition, we compared the percentage of individuals developing stage 4 or 5 CKD (<30 mL/min/1.73m^2) during follow-up between the different thyroid function groups.

As sensitivity analyses, models were rerun 1) in three different baseline strata of renal function, 2) only in the 535 individuals not on drugs interfering with thyroid hormone measurements (thyroxine and/or antithyroid drugs), 3) specifically in those who lived to celebrate their 90th birthday (n=299), and in order to exclude those with possible non-thyroidal illness 4) only in subjects having CRP levels < 5 mg/L (n=317). Also, creatinine clearances as estimated with the Cockcroft Gault and CKD-EPI formulas were used as outcome variables. Further, multivariable models were further adjusted for systolic blood pressure, C-reactive protein levels, BMI, and total cholesterol levels. Finally, we investigated the association between different thyroid hormone change patterns over a 3 year period (85-88 years of age) and progression of renal function in the years thereafter. For this purpose, we categorized patients into four categories: 1) Those having elevated TSH levels (>4.5 mIU/L) at age 85 and 88 (Persistent hypothyroid group, n=31), 2) those having TSH levels between 0.5 and 4.5 mIU/L at both time points (Persistent euthyroid group, n=276), 3) those persistently having levels < 0.5 mIU/L (Persistent hyperthyroid group, n=12) and 4) patients changing categories (Change group, n=53).

In linear regression analyses and linear mixed models, betas with 95 percent confidence intervals (95%CI) not including 0 were considered statistically significant. For all other tests, a p-value smaller than 0.05 indicated statistical significance. All analyses were performed using SPSS 20 (IBM Inc., New York, USA). Figures were created using Prism 5.02 (Graphpad, 1992).

Table 1. Baseline characteristics of the study population according to thyroid status

	O hypothyroidism n=40	SC hypothyroidism n=35	Euthyroidism n=451	O and SC hyperthyroidism n=29	p-value
General					
Men, n, %[1]	9 (22.5)	7 (20.0)	161 (35.7)	10 (34.5)	0.104
BMI, kg/m^2	28.5 (4.8)	28.2 (4.1)	26.7 (4.5)	25.1 (4.3)	0.040
Smoker, n, %[1]	5 (12.5)	5 (14.3)	74 (16.4)	4 (13.8)	0.888
Malignancy, n, %[1]	6 (15.0)	11 (31.4)	76 (16.9)	5 (17.2)	0.178
Institutionalized, n, %[1]	13 (32.5)	2 (5.7)	81 (18.0)	5 (17.2)	0.027
Cardiovascular profile					
Previous CVD, n, % [1,2]	21 (52.5)	23 (65.7)	205 (45.5)	16 (55.2)	0.090
DM, n, %[1]	7 (17.5)	7 (20.0)	65 (14.4)	2 (6.9)	0.483
CRP, mg/L[3]	4.0 (2.0-8.0)	8.0 (3.0-11.0)	3.0 (1.0-7.0)	5.0 (1.0-10.0)	0.007
Thyroid profile					
TSH, mIU/L[3]	6.45 (5.57-8.27)	5.57 (5.05-6.66)	1.67 (1.18-2.34)	0.19 (0.01-0.37)	-
fT4, pmol/L	10.16 (1.24)	15.10 (1.41)	14.58 (2.31)	17.70 (3.92)	-
fT3, pmol/L	3.20 (0.59)	3.32 (0.64)	3.39 (0.52)	3.65 (0.72)	-
Medication usage					
Thyroid hormone, n(%)[1]	2 (5.0)	6 (17.1)	6 (1.3)	2 (6.9)	<0.001
Antithyroid med, n, %[1]	1 (2.5)	2 (5.7)	0 (0.0)	1 (3.4)	<0.001

1. Categorical data are presented as numbers plus percentages and differences between groups were tested by means of a X^2-square test.
2. CV disease comprised a composite score of a history of a myocardial infarction, angina pectoris, heart failure, peripheral arterial disease and/or cerebrovascular accident/transient ischemic attack. A score ≥ 1 indicated presence of CV disease.
3. Nonnormally distributed data are presented as Medians plus interquartile ranges, differences were tested by means of a Kruskal-Wallis test.

O: Overt. SC: Subclinical, CV: cardiovascular, DM: Diabetes Mellitus, CRP: C-reactive protein, TSH: thyroid stimulating hormone, fT4: free thyroxine, fT3: free triiodothyronine.

Results

Of the total study population, 33.6 percent was male and 18.3 percent was institutionalized in a care home or a nursing home. 82 (14.8%) participants had diabetes mellitus, 52.2% (n=291) suffered from a history of cardiovascular disease, and 98 (17.6%) participants had a past or current malignancy. The mean (SD) eGFR, as calculated with the MDRD formula, was 59.0 (14.4) mL/min/17.3m^2 (CKD-EPI: 49.0 (13.4), Cockroft-Gault: 45.4 (11.5) mL/min). In **Table 1**, baseline characteristics are compared between the different thyroid hormone groups. As compared to euthyroid subjects, those with overt and subclinical hypothyroidism were more frequently women and had more comorbidities. Also, they had higher BMI and CRP levels and were prescribed more frequently thyroxine as well as anti-thyroid medication.

Table 2. Baseline levels of renal function within tertiles of distributions of TSH and thyroid hormones

	Tertiles of distribution of thyroid hormones			
	Lower	Middle	Higher	p-for trend[1]
	TSH, mIU/L			
	0.01 – 1.38	1.39 – 2.35	2.35 – 33.0	
n	186	188	184	
Crude eGFR, mean (SE)[2]	60.1 (1.1)	60.3 (1.0)	56.6 (1.1)	0.021
Adjusted eGFR, mean (SE)[3]	61.5 (2.8)	61.9 (2.8)	58.2 (2.7)	0.037
	fT3, pmol/L			
	0.77 – 3.19	3.20 – 3.63	3.63 – 6.60	
n	201	199	144	
Crude eGFR, mean (SE)[2]	57.8 (1.0)	58.8 (1.0)	61.1 (1.2)	<0.0001
Adjusted eGFR, mean (SE)[3]	59.3 (2.8)	59.5 (2.7)	62.1 (2.8)	0.074
	fT4, pmol/L			
	8.4 – 13.4	13.5 – 15.3	15.3 – 30.9	
n	189	190	174	
Crude eGFR, mean (SE)[2]	57.4 (1.0)	59.4 (1.0)	60.0 (1.1)	0.083
Adjusted eGFR, mean (SE)[3]	57.4 (2.8)	60.2 (2.8)	61.5 (2.7)	0.005

1. The p-for trend was calculated by means of regression analysis.
2. Crude means and SDs were calculated by means of regression analyses.
3. Adjusted means and SDs were calculated by means of multivariate regression analyses including sex, diabetes mellitus, smoking, cardiovascular disease, malignancies, and amiodarone usage as possible confounders.

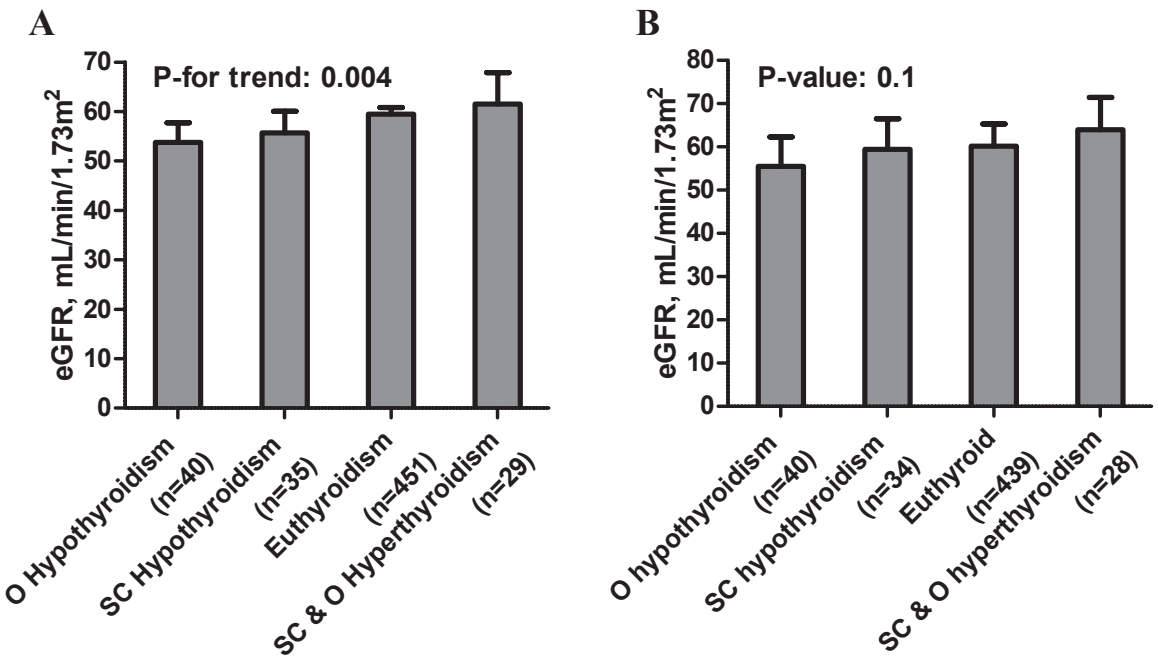
* In these analyses, a maximum of 14 individuals did not provide data.

The median (IQR) TSH level was 1.82 (1.16-2.90) mIU/L. 451 (81.2%) participants were cassified in the euthyroid group, and 40 (7.2%) and 35 (6.3%) of participants were classified as having hypothyroidism and subclinical hypothyroidism, respectively. 4.9 percent (n=27) had subclinical hyperthyroidism and 0.4 percent (n=2) suffered from overt hyperthyroidism (5.3 percent in total). Median creatinine (IQR) levels were 92 (81-107) μmol/L translating into a mean (SD) eGFR (MDRD) of 59.0 (14.4) mL/min/1.73m^2. 305 participants had an eGFR <60 mL/min/1.73m^2 (CKD ≥3) of whom 7 and 2 values fitting CKD stage 4 (15-30 mL/min/1.73m^2) and 5 (<15 mL/min/1.73m^2), respectively.

As apparent from **Figure 1A**, 85-year old participants with overt hypothyroidism (53.7 (2.0) mL/min/1.73m^2) and participants with subclinical hypothyroidism (55.7 (2.1) mL/min/1.73m^2) had a lower mean baseline eGFR (SE) than participants with normal thyroid function (59.5 (0.7) mL/min/1.73m^2). The highest eGFR was observed in participants with hyperthyroidism (61.5 (3.1) mL/min/1.73m^2). After adjustment for confounders (**Figure 1B**), a trend remained. In **Table 2**, mean (95% CI) eGFR values are presented across tertiles of distribution of the different thyroid hormones. The eGFR was lower in participants with higher TSH levels (p=0.021). eGFR values were lower within the lower fT3 tertiles (<0.0001) and, although not statistically significant, also lower in lower fT4 tertiles (p=0.083). After adjustment for possible confounding variables, associations were statistically significant for TSH (p=0.0037) and for fT4 (p=0.005).

Throughout a median follow-up of 5 years, during which 259 individuals died, the eGFR declined on average (SE) with -0.25 (SE 0.13, p=0.052) mL/min/1.73m^2 per year. **Figure 2** shows the estimated adjusted mean (95%CI) annual changes in eGFR across thyroid function groups as obtained from linear mixed models. No significant differences were observed in the change in eGFR between thyroid function groups. In a second approach in which individual specific betas (slopes) were pooled within the different thyroid hormone groups (**Appendix 3**), similar results were found (p=0.149). No association between baseline thyroid hormone concentrations as continuous variables and the change in eGFR over time was present. Also, the percentage of individuals developing new CKD stage 4 or 5 did not differ between the thyroid function groups (p=0.755, data not shown).

Figure 1. Mean crude (A) and adjusted (B) eGFR (mL/min/1.73 m^2) across different thyroid hormone groups at baseline



Mean (95% CI) eGFR at baseline within the different thyroid hormone groups. The value for P for trend was calculated by means of polynomial trend analysis in a one-way ANOVA test. B, Mean adjusted eGFR (mL/min/1.73 m^2) across different thyroid hormone groups at baseline. Mean (95% CI) eGFR at baseline within the different thyroid hormone groups was adjusted for sex, DM, smoking, the presence of cardiovascular disease (composite score 1), malignancies, and amiodarone usage. The value for P trend was calculated by means of linear regression analysis. O, overt; SC, subclinical.

In sensitivity analyses, subgroup analyses in three different baseline strata of renal function, solely in survivors reaching age 90 (n=299), and in those with CRP levels below 5 mg/L yielded no different results. Results did not materially change with respect to the effects of basal thyroid hormone status on change in eGFR over time when renal function was estimated with CKD-EPI and Cockcroft Gault formulas (results not presented). Further adjustment for systolic blood pressure, C-reactive protein levels, BMI, and total cholesterol levels in multivariable models neither changed findings. In the 535 individuals not on drugs interfering with thyroid hormone measurements, results were not different as in total the population (**Appendix 4**). Finally, we did not observe an association between different thyroid hormone groups as defined upon two thyroid hormone measurements in time (85 and 88 years of age, see methods) and renal function at 88 years of age and change in renal function from that point on forward (results shown in **Figure 1 and 2** of **Appendix 5**).

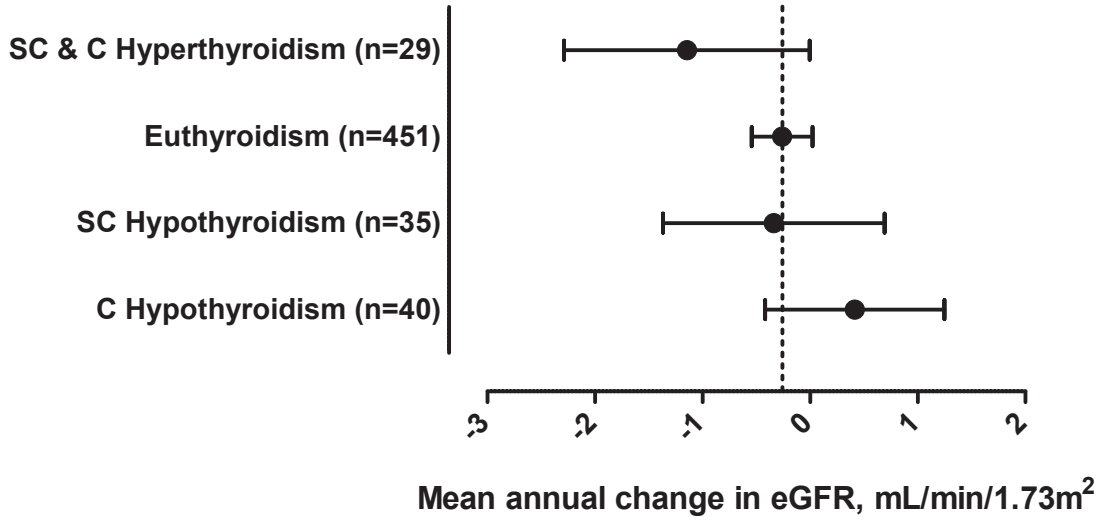


Figure 2. Mean annual change in eGFR (milliliters per minute per 1.73 m^2) across different thyroid function groups. Mean (95% CI) annual change in eGFR per group was calculated by means of multivariate linear mixed models including sex, DM, smoking, cardiovascular disease, malignancies, and amiodarone usage as possible confounders. Negative values indicate a decline, whereas a positive value indicates an improvement in renal function over time. The mean annual changes in eGFR within the different thyroid function groups did not differ significantly from the euthyroid group (reference, dotted line). O, overt; SC, subclinical.

Discussion

In this community based sample of the oldest old, positive cross-sectional associations between thyroid function and renal function were observed. Over time, thyroid function was not associated with change of renal function.

In our cross sectional analyses, a low thyroid status associated with lower eGFR values at baseline in univariate as well as multivariate models when compared to participants with euthyroidism and overt- and subclinical- hyperthyroidism. These findings align with cross-sectional observations from other large scaled community-based surveys.[32,33] To our knowledge, this is the first study to investigate the longitudinal association between thyroid hormone status and the change in renal function over time in the oldest old. We observed no longitudinal association as such. Although statistically non-significant, one may interpret the findings in **Figure 2** as a slight trend in which overt hypothyroidism conveys a protective and hyperthyroidism a harmful effect on the change in renal function over time. However, this explanation seems unlikely. Not only had those with overt hypothyroidism a lower, and those with overt/subclinical hyperthyroidism a higher eGFR at baseline, it is biologically less plausible that hypothyroidism is protective against a decline in renal function. We therefore interpret this trend through the concept of "regression to the mean". In addition, analyses including the thyroid hormone measurements at 88 years of age further support the absence of an association between thyroid hormone status and (change of) renal function. Thus, our observations contrast with findings in younger individuals having overt hypothyroidism[13,14] and subclinical hypothyroidism[17,18] who experienced a faster decline in renal function which in turn seemed to be attenuated or reversed by thyroid hormone replacement therapy.[13,18]

It is of interest to speculate why the association between thyroid status and change of renal function over time is absent in the oldest old. As a result of selection due to survival, the oldest old may be least susceptible to the detrimental effects of common risk factors including a low thyroid status. Consequently, other pathophysiological mechanisms may be at play in this age category. This reasoning finds support in earlier studies in the oldest old indicating a reversal or disappearance of negative effects of traditional risk factors like hypertension and hypercholesterolemia[20,34] but also overt and subclinical hypothyroidism.[22,23,35] The association between subclinical hypothyroidism and risk for cardiovascular events seems to diminish specifically in the elderly[22,23,25,35] It has been suggested that a low thyroid function in the elderly represents a physiological downregulation of the HPT-axis, possibly benefitting life expectancy.[36] A possible explanation lies in a slower metabolic rate which related to an increased survival in several species.[37] In a recent study in families of nonagenarian siblings, a lower family mortality score was found to be associated with lower thyroid function in the offspring, leading the authors to speculate that low thyroid function may be an inheritable trait.[38] This would imply that a low thyroid function could already be of protective effect in specific subgroup of younger individuals. Throughout all of these explanations however, our findings question the causal relation between low thyroid function and decline in renal function in the oldest old, and as a result, question the benefits of thyroid hormone replacement in old age.

Notably, the positive cross-sectional association in our study between thyroid function and renal function could also be explained through the concept of reverse causality. Severe CKD commonly induces a hypothyroid state which exists in the absence of primary HPT-axis dysfunction.[39] Presence of this low thyroid state in states of disease, commonly referred to as non-thyroidal illness or low-T3 syndrome, associates with substantially increased mortality rates.[40,41] Consistently, a previous analysis in the Leiden 85 plus Study showed that low fT3 levels were associated with an increased mortality risk.[22] This finding did however not withstand multivariate adjustment and was contradicted by another study in which this association appeared absent.[23]

As TSH levels in non-thyroidal illness typically descend or remain within range, the finding of an increased prevalence of elevated TSH levels in the oldest old 6 does not fit this hypothesis. When we excluded those with CRP levels below 5 mg/L, cross-sectional associations between thyroid function and renal function remained present, pleading against non-thyroidal illness as an explanation for our results.

A strength of the present study is its population-based design with inclusion of the oldest old. As there were no exclusion criteria, the Leiden 85-plus Study is a representation of the very oldest in the general population. For the interpretation of our results, some general limitations have to be discussed. First, as thyroid status could possibly influence plasma creatinine levels via muscle metabolism and volume status, the eGFR may not be a good approximation of renal function in this association.[42] Nevertheless, overt hypothyroidism was also linked to a reduced eGFR as measured by labeled edetic acid.[16] In addition, sensitivity analyses using CKD-EPI and Cockcroft Gault formulas yielded similar results. Secondly, as subjects in whom new thyroid dysfunction was discovered were referred to their general practitioner, the possible initiation of treatment could have masked a possible effect. As our results did not change when analyses were repeated solely in those not on thyroid hormone therapy, this effect is unlikely of great importance. Lastly, bias due to competing events (death), and selection on basis of survival at age 85 could theoretically both have masked a true association.

In conclusion, in older persons in the general population, overt and subclinical hypothyroidism are associated with lower renal function at baseline but not with an additional decline in renal function over time. Ultimately, our findings suggest an absence of a causal relation between low thyroid function and decline in renal function in the oldest old. Further studies are warranted to disentangle the association between thyroid status and renal function throughout different age groups and whether thyroid hormone replacement therapy impacts positively on renal function in those with low thyroid function.

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Chapter 9

Summary & General Discussion

I. Summary of findings

Introduction

Patients with end-stage renal disease have a very poor prognosis.[1] Although mortality due to both cardiovascular causes and noncardiovscular causes are both an eight fold higher than in age and sex matched controls without end-stage renal disease, cardiovascular diseases remain the biggest cause of death.[2] The excess in cardiovascular morbidity and mortality cannot be explained by an increased prevalence of traditional risk factors (smoking, hypertension, dyslipidemia and obesity etc.). Rather, so called "non-traditional risk factors" may be more important in the context of reduced renal function and take over. In this thesis, two non-traditional risk factors plausibly involved in the increased cardiovascular risk of end-stage renal disease were studied: 1) An increased inflammatory state, and 2) thyroid hormone alterations fitting the spectrum of nonthyroidal illnesses. Because both risk factors, as a function of other triggers and disease severity, fluctuate over time, associations were studied in context of risk factors' temporal oscillations.

1. Inflammation

In **Chapter 2**, the reasons and implications of elevated serum inflammatory markers in patients undergoing dialysis are reviewed. In the discussion, specific focus was placed on C-reactive protein (*CRP*). Also, an overview of studies on this issue is presented. Further, we discuss the value of repeated versus single measurements of inflammatory markers in the clinical setting and provide solutions to reduce both sample size and intraindividual variability in hypothetical, randomized controlled trials aimed at reducing CRP levels in patients undergoing hemodialysis. In **Chapter 3**, the impact of trimestral variability patterns of CRP, interleukin-6 (*IL-6*) and tumor necrosis factor-α (*TNF-a*) on mortality was studied in 218 maintenance hemodialysis patients. Individuals were categorized on basis trimestral variability patterns identifying a stable high, stable low, increase and decrease group. As compared with those having persistently low levels of inflammatory markers during the three months observational period (reference), patients with variable levels over time and persistently elevated levels had increasingly higher mortality hazards, respectively. Adjustment for confounding factors did not alter findings much leading us to speculate on a possible dose response relationship in which a longer and more severe presence of the risk factor translates into increased mortality hazards. **Chapter 4** investigates the impact of a pro-inflammatory response versus a lower inflammatory activation on the stimulus of a single hemodialysis session on mortality. In 190 Swedish, and 94 Dutch hemodialysis patients, no difference in survival was noted between both groups. In addition, a low agreement between CRP changes in a first hemodialysis and second hemodialysis session was found in the Dutch cohort. Therefore, we conclude on the absence of an association between a change in serum CRP levels during dialysis and mortality rates in two independent cohorts. This reasoning is supported by a low biological likelihood of CRP to rise in such short timeframe.

2. Nonthyroidal illness

In **Chapter 5**, we introduce the concept of nonthyroidal illness and describe its genesis in renal and cardiac diseases. Both disease unspecific factors (like inflammation, protein-energy wasting, and comorbidities) and renal disease specific factors (iodide retention, metabolic acidosis, selenium deficiency, and certain medicaments) may be involved in its development. The potential impact of nonthyroidal illness on cardiac and renal functioning is further discussed. Because of its high prevalence in both settings, and its deleterious effects on both organ systems, we hypothesize on a possible role of nonthyroidal illness in the induction and maintenance of the cardio-renal syndrome. Finally, we provide results from a literature search focusing on the supplementation of thyroid hormone in the context of end-stage renal disease induced nonthyroidal illness. **Chapter 6** reports a study on the association between trimestral variation patterns of total triiodothyroinine and thyroxine over a three months period and mortality. In 218 patients on maintenance hemodialysis, we found that those having persistently low triiodothyronine levels had the highest cardiovascular mortality rates, followed by those having fluctuating levels over time, and those having persistently high (normal) levels. For trimestral variation patterns of serum thyroxine levels, a comparable association was seen. Interestingly, an association remained after adjustment for serum triiodothyronine levels, suggesting an independent effect of thyroxine on cardiovascular mortality. On basis of these findings, we conclude that in patients with end-stage renal disease, trimestral variation patterns of thyroid hormone concentrations are strongly associated with mortality.

In **Chapter 7**, a study in 84 patients on maintenance peritoneal dialysis is presented investigating coronary artery calcification scores and arterial stiffness as possible mediators in the association between nonthyroidal illness and mortality. Despite the low numbers of patients and events, a low triiodothyronine state associated strongly with mortality, arterial stiffness and coronary artery calcification (CAC) scores. A relatively high CAC score was strongly associated with higher mortality rates during follow-up. When the association between a low triiodothyronine state and mortality was adjusted for CAC scores, the magnitude of the effect attenuated. These findings support the hypothesis that vascular calcification could be involved as an intermediate in the association between nonthyroidal illness and mortality. **Chapter 8** describes a study in 558 elderly, home-dwelling individuals in which we investigated the association between thyroid status and progression of renal dysfunction over a 5-year observational period. Individuals were included during the year they turned 85 years of age and at inclusion, baseline characteristics were collected and thyroid hormone function was measured. During subsequent follow-up, serum creatinine concentrations were measured annually and events of death were recorded. At age 85, a low thyroid function was associated with lower renal function. During follow-up however, an association between a low thyroid function and change in renal function over time was absent. Our findings question the causal relevance of the thyroid status for the deterioration of renal function in the oldest old.

II. Methodological considerations

In our studies, several methodological difficulties were encountered and must be brought upfront. Whereas most of these limitations have already been addressed in the discussions of the different chapters, this sections aims at providing more general considerations. The discussion will be assisted by means of "Directed Acyclic Graphs" (*DAGs*) which give graphical insight into the associations between different variables (vertices). The term "acyclic" refers to the fact that there are no loops in the graph, so a specific factor cannot cause itself.

Selection bias

A common problem in scientific studies is selection bias, which concerns a systematic error that arises when the selection of a population occurs on the basis of both the exposure and the outcome.[3] Consequently, the association between exposure and outcome is different in participants versus the cohort which would have participated if all individuals would have been included. In our studies, two important sources of selection bias must be discussed;

1. Collider-stratification bias: As illustrated **Figure 1**, collider-stratification bias results when conditioning occurs on a collider (*C*), by definition being a consequence of both the exposure (*E*) and other risk factors (*U*) 4. C often concerns an inclusion criterion. By conditioning on C, E is artificially associated with the outcome (*O*) via U. U can specify confounding factors or factors that exist independently of the exposure (illustrated by the dotted line). Adjustment for U, if known, would abolish collider-stratification bias and leave the true association between E and O.

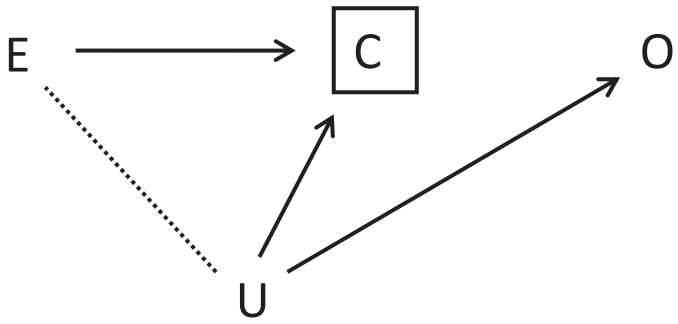

Figure 1. A Directed Acyclic Graph (DAG) illustrating collider-stratification bias. E: exposure, C: collider, U: other (un)known factors, O: outcome of interest.

2. Informative censoring: This type of bias arises when individuals can no longer develop the outcome of interest due to another reason which is associated with the exposure and outcome. Among the causes of this latter form of bias are loss to follow-up and competing risks. When competing risks occur, conventional Kaplan-Meier methodology can yield misleading results.[5]

1. Collider-stratification bias

In our studies ESRD (**Chapter 3,4,6** and **7**), individuals were eligible only when they were treated with dialysis and were thus required to have survived up to this point in time (C). Before this, inflammation and a low thyroid function (E) likely caused death in a number of patients. Accordingly, those with a higher grade of both risk factors but who survived to reach the eligibility criteria are of an unusual kind, typically having less other risk factors (U). By selectively including these patients, an artificial association can arise between inflammation/low thyroid hormone state (E) and mortality (O) via other (un)known factors (U). As a result, the association between a high inflammatory state/low thyroid hormone state and mortality artificially changes. This seems however unlikely because an increased mortality risk in those with higher grades of inflammation[6;7] and a low thyroid hormone status was also found in the general population.[8] Therefore no paradox need be explained. Also, adjustment for known factors (U) did not result in differential findings.

In **Chapter 8**, collider-stratification bias could have masked the presence of an association between thyroid hormone status (E) and the progression of renal dysfunction over time (O) in elderly subjects. While a low thyroid status is a risk factor for mortality (C), it is possible that those with a low thyroid function who have lived to celebrate their 85th birthday, have less other risk factors (U). This could have resulted in an artificial association between E and O, via U. Indeed, associations between a low thyroid function and faster progression of CKD were apparent in younger individuals (**Chapter 8**)[9;10] but proved absent in the elderly (this thesis). Two arguments could plead against collider-stratification bias explaining an absence of findings: 1) Adjustment for conventional risk factors (e.g. comorbidities, BMI, inflammation and blood pressure) did not alter findings suggesting a true absence of an association. 2) It is not unlikely that physiology is different at old age as compared with younger age.

In the evaluation of collider-stratification bias, it must be noted that the situation is likely more complex than assumed in **Figure 1**. The presence of a high inflammatory/low thyroid hormone state is, in contrast to other exposures (e.g. genes or medication usage), subject to a certain temporal variability over time (this thesis). As many patients gain and lose the exposure over time, the exposed group is not a fixed population. This phenomenon would require another vertex in the DAG indicating exposure status at time point 2 (E2). The same would hold for all the other factors implicating the differentiation of U into U1 and U2. On top of this, various factors can be thought of which influence E, U and O in turn, further complicating the DAG. Ultimately, DAGs in end-stage renal disease seem extremely complex due to the systemic nature of disease and alterations in almost all physiological systems, and thus are likely all incomplete.

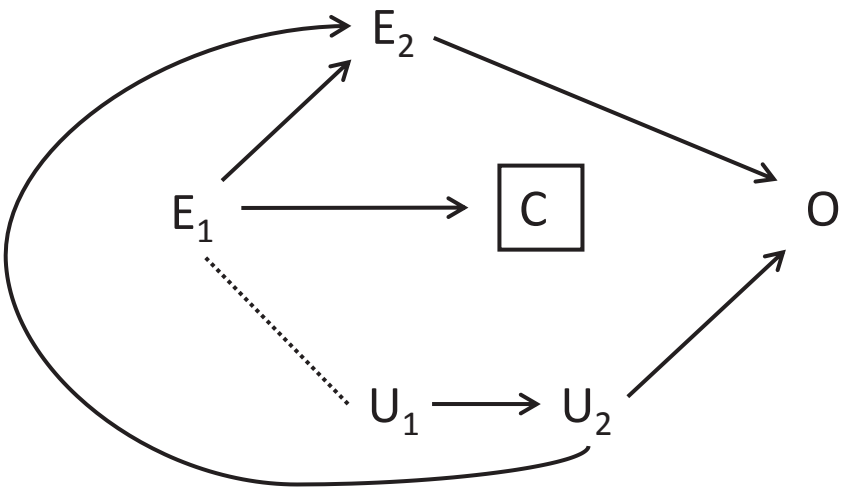

Figure 2. A directed acyclic graph (DAG) illustrating the complexity of collider stratification bias in end-stage renal disease. E1: exposure at timepoint 1, E2: exposure at timepoint 2, C: collider, U1: other (un)known factors at timepoint 1, U2: other (un)known factors at timepoint 2, O: outcome of interest.

2. Informative censoring

During the study period, loss to follow-up was low in all cohorts. This may be explained by two reasons; 1) mortality was the endpoint of interest in most of our studies being a characteristic which can be obtained through various sources. 2) dialysis patients depend on medical care and generally have a good liaison with their physicians whereby loss of patients is limited. In the Leiden 85 plus study, house visits contributed to a reduction in loss to follow-up.

As another cause of informative censoring, competing risks are important to discuss.[11] In dialysis, the occurrence of renal transplantation after which individuals are censored is a common competing risk. As we primarily used prevalent dialysis cohorts in which transplantation rate is lower, this cause of competing risks is of less relevance. Otherwise, when patients would die because of non-cardiovascular causes, they are not anymore at risk to die from a cardiovascular cause, yielding informative right censoring with the potential of bias. In a sensitivity analysis in Chapter 6, we calculated cumulative mortality probabilities.[11] Because our conclusions did not change and the conventional approach is more widely accepted, the latter form was adopted in the presentation of results. No Fine and Gray Cox regression models were applied.

Confounding

Observational studies are hindered by confounding, that is, when another factor, being associated with the exposure and outcome while not lying in the causal pathway, affects the association of interest.[12] The selection of confounders for specific associations in this thesis occurred on basis of biological knowledge and DAGs. Confounding was then corrected for by means of stratification or adjustment in multivariable regression models. Because of low numbers of events in the MIMICK II cohort (n=24), in the association between low fT3 levels and mortality (**Chapter 7**), we also tried

an approach by correcting for propensity scores rather than all confounders separately. The small patient population (n=84) however limited the construction of proper prediction models leading us to prefer the conventional way of adjustment for the final presentation of results. Findings were however not substantially different between both approaches. Although associations were quite extensively adjusted, residual confounding from unknown factors cannot be excluded.

Mediation analyses

In causal inference, mediation analyses are meant to clarify to what extent the association between exposure and outcome is mediated by a certain factor. A standard approach for mediation analyses is to regress the exposure on the outcome with and without the mediator included in the model. In mediation analyses, two limitations can be encountered. 1) The model is not robust when in fact effect measure modification exists. 2) Possible outcome-mediator confounding can arise when the mediator behaves as a collider (C). Adjustment for C would then result in a false association between the exposure and outcome via other factors.[13] Consequently, correction for these other factors (U) could remove this type of confounding.

In **Chapter 6**, we adjusted the association between T4 and mortality for serum T3 levels. Interestingly, an independent effect remained suggesting that T4 also acts via direct pathways on mortality. In **Chapter 7**, we observed an attenuation in the association between T3 levels and mortality after correction for coronary artery calcification scores, supporting the hypothesis of an intermediate role for coronary calcification. In both analyses, we tested the presence of interaction between exposure and mediator in multivariable Cox models which appeared to be absent. Also here, we considered the possibility of mediation-outcome confounding. A limitation in both mediation analyses was the fact that exposure and mediator were both measured at the same moment. Ideally, one would have measured the development of coronary artery calcification or occurrence of cardiac events during follow-up.

III. Inflammation and non-thyroidal illness as cardiovascular risk factors

Causal associations or merely epi-phenomena?

Several contextual arguments, as originally proposed by Hill,[14] strengthen the belief in a causal role for both an increased inflammatory state and nonthyroidal illness in cardiovascular risk augmentation. Firstly, a biological gradient is seen in several of the associations tested in this thesis, worse levels of the exposure (over time) coexisting with higher occurrences of the outcome. Secondly, as adjustment for confounders did not reduce effect estimates greatly, it can be speculated that adjustment for other, unknown, confounders will unlikely result in a disappearance of the found associations. Third, the concept of temporality was satisfied, meaning the exposure was there before the outcome occurred. Finally, associations were consistent within different cohorts from different countries.

Findings from the current thesis confirm and expand prior reports in other patient populations, animals and ex-vivo experiments (reviewed in **Chapters 2** and **5**). Many of these studies were, however, observational in nature and possibly hindered by confounding. In this respect, studies applying a Mendelian Randomization approach are of special interest. By utilizing the random distribution of genes in a certain population this approach can, under certain assumptions, provide estimates free of confounding for an association of interest.[15] As such, a significantly lower risk for developing coronary heart disease events and mortality was observed in patients with the functional interleukin-6 receptor (IL-6R) rs7529229 polymorphism versus those having the wild type allele, supporting a causal role for IL-6 signaling.[16;17] Interestingly, CRP polymorphisms were not associated with mortality,[17;18] implying that CRP is a marker of inflammation rather than a causative factor. Causal pathways linking a higher grade of inflammation to an increased cardiovascular risk pertain to the promotion of plaque formation, destabilization and eventually rupture.[19]

Also for nonthyroidal illness, several pathways have been suggested that connect this risk factor to an increased cardiovascular risk in end-stage renal disease (extensively reviewed in **Chapter 5**). To our knowledge, no studies have applied the Mendelian randomization concept to the association between thyroid hormone alterations and cardiovascular risk in end-stage renal disease.

Fluctuations of serum inflammatory markers and thyroid hormones over time

When evaluating the consequences of a high inflammatory state and nonthyroidal illness on cardiovascular risk, it is important to bear in mind their variable presence over time (this thesis). This may be valuable from both an etiological and predictive perspective. From an etiological perspective, the observation of a dose response association between both exposures and outcome strengthens the belief in causality (**Chapters 3** and **6**). Further, they could help in the identification of intermediate pathways. It is interesting to note that such intermediates are likely to show temporal variability as well. For example, the severity of atherosclerotic plaques likely varies over time, certain plaques regressing while others showing progression.[20] Finally, variability patterns provide information concerning the underlying stimulus (**Chapter 2**).

From a predictive point of view, a second measurement of a blood marker could add information by reducing measurement error and intra-patient variability (**Chapter 2**). It could thereby help in the discrimination between individuals with a true nonthyroidal illness syndrome and those with a random aberrant value in illness. Thereby it could also hold therapeutic consequences. For instance, thyroid hormone supplementation would theoretically only be of value in chronic- but not in a temporary state of deficiency. Also, multiple measurements add prognostic value (this thesis). Finally, a second measurement adds information, improving risk stratification in prediction models.

Clinical and scientific implications

Inflammation

As reviewed in **Chapter 2**, the monitoring of inflammatory levels could serve for retrieving the cause of inflammation in a single patient which could again guide therapeutic interventions to treat its underlying cause. Efforts to identify anti-inflammatory therapies of benefit for patients with end-stage renal disease have unfortunately been few and somewhat unsuccessful.[21] Although several substances were shown to result in a reduction of inflammatory marker levels, no clear benefit on primary endpoints has so far been reported. Recently, bardoxolone methyl generated much expectation on basis of its anti-oxidant effects via the NR2f pathway. Yet, a large scaled placebo randomized controlled trial investigating bardoxolone methyl in patients with end-stage renal disease and type 2 diabetes mellitus was terminated prematurely because of an increased cardiovascular event rate in the treatment arm.[22] A general fear for immune suppressive strategies concerns that of potential infectious side effect. As such, no specific therapy is currently registered for anti-inflammatory effects in patients with end-stage renal disease.

Further observational and interventional studies are needed to gain more insight in specific inflammatory pathways and potential interventions. It would be interesting to investigate whether the cardiovascular impact of the inflammatory response to different types of stimuli would be different. Also, a more thorough understanding in the genesis of the increased inflammatory levels is necessary.

Nonthyroidal illness

As is the case for an increased grade of inflammation, also studies on correction of thyroid hormone alterations in nonthyroidal illness due to end-stage renal disease have been largely negative. It must however be stated that the majority of studies were insufficient in sample size, design and had other major shortcomings (further exemplified in **Chapter 5**). In addition to that, our current knowledge on nonthyroidal illness seems insufficient to identify subjects who could benefit from thyroid hormone supplementation. Presumably, the aspect of temporal variability plays an important role in this. All in all, currently no evidence exists for thyroid hormone supplementation for this indication.

For the association between nonthyroidal illness and a possible increased cardiovascular risk, studies utilizing the Mendelian randomization approach could be of aid to further address the question of causality. Also, a universal definition should be introduced on nonthyroidal illness, possibly with different grades of severeness. Without any doubt, the temporal variability of thyroid hormone derangements in chronic end-stage renal disease must be implemented in such definition. The make-up of a solid definition and should assist in the identification of individuals in possible need

of thyroid hormone supplementation. As a next step, interventional studies should be designed with an adequate sample size and follow-up duration. In the design, special attention should also be given to the type of compound used. As deiodinase defects are a cornerstone aberration in nonthyroidal illness, the supplementation of thyroxine seems not legitimized. In the field of cardiology, new trials have been initiated in patients with heart failure and myocardial infarctions. This could form an incentive for studies in the field of nephrology.

IV. Conclusions

1. Inflammatory marker levels are elevated in the majority of patients with end-stage renal disease and associate strongly with (cardiovascular) mortality. Also variability patterns of inflammatory markers over a three months observational period strongly associate with (cardiovascular) mortality while changes of CRP during a single dialysis session do not.

2. Alterations in thyroid hormone levels fitting the spectrum of nonthyroidal illness are observed in the majority of patients with end-stage renal disease. The persistent and variable presence of these derangements strongly associate with (cardiovascular) mortality. This association may be intermediated by a higher coronary artery calcification and arterial stiffness.

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Chapter 10

Dutch Summary

Introductie

In het menselijk lichaam zijn de nieren betrokken bij een aantal belangrijke processen. Hiertoe behoren de vocht- en zoutbalans, het uitscheiden van afvalstoffen en de aanmaak van rode bloedcellen. Wanneer de nieren niet optimaal functioneren is behandeling met medicijnen noodzakelijk om deze processen alsnog goed te laten verlopen. In eindstadium nierfalen, wanneer de nieren bijna niet meer werken, is medicamenteuze therapie niet afdoende en moet de functie van de nieren worden vervangen. Dit kan op twee manieren: middels een niertransplantatie of dialyse. Transplantatie is echter alleen voor een selecte groep patiënten mogelijk. Bij dialyse kan het bloed op twee manieren kunstmatig gereinigd worden van afvalstoffen: 1) hemodialyse en 2) peritoneale dialyse (ook wel buikspoelingen genoemd). Bij hemodialyse wordt bloed vanuit de patiënt door slangen naar een dialysemachine geleid alwaar het wordt gereinigd. Vervolgens wordt 'schoon' bloed het lichaam van de patiënt weer ingevoerd. Bij peritoneale dialyse wordt gebruikgemaakt van het eigen buikvlies om afvalstoffen en overtollig vocht af te laten lopen. De patiënt laat hiertoe schoon vocht de buik inlopen om enkele uren later de buik te laten leeglopen. De keuze voor één van beide vormen van dialyse wordt door vele factoren bepaald waaronder de leeftijd, het aantal en de aard van andere ziekten van de patiënt, het sociale vangnet en de voorkeuren van de patiënt zelf.
Ondanks dialysebehandeling (zowel hemodialyse als peritoneale dialyse) hebben patiënten met eind stadium nierfalen tot op heden een slechte prognose. Vijf jaar na aanvang van dialysebehandeling is ongeveer 50 procent van alle patiënten overleden. Na tien jaar betreft dit zelfs 90 procent. Deze oversterfte bij dialyse patiënten wordt verklaard door het vaker voorkomen van hart- en vaatziekten (hartinfarcten, hartfalen, hartritmestoornissen, enzovoort), infecties, kanker en ondervoeding.
Het feit dat sterfte door hart- en vaatziekten ongeveer achtmaal vaker voorkomt in de dialysepopulatie kan tot op heden niet verklaard worden door risicofactoren die bij patiënten zonder nierziekten sterfte door hart- en vaatziekten veroorzaken (zoals roken, een te hoog cholesterolgehalte, hoge bloeddruk en obesitas). Deze risicofactoren worden beschouwd als de 'traditionele risicofactoren' voor hart- en vaatziekten. Gedurende de laatste jaren is gebleken dat in de dialysepopulatie 'niet-traditionele risicofactoren' belangrijk zijn voor het ontstaan van hart- en vaatziekten. Deze risicofactoren, waaronder inflammatie, zijn het gevolg van de nierziekte zelf en verminderen de functie van het hart en de bloedvaten. Onderzoek naar deze niet-traditionele risicofactoren is van belang voor het ontwikkelen van toekomstige therapieën voor dialysepatiënten.

In dit proefschrift werden twee niet-traditionele risicofactoren bestudeerd die mogelijk betrokken zijn bij het verhoogde sterfterisico door hart- en vaatziekten bij patiënten met eindstadium nierfalen: een verhoogde staat van ontsteking (inflammatie) en een verlaging van de concentratie schildklierhormoon veroorzaakt door nonthyroidal illness.

1. Een verhoogde inflammatoire staat

In de meerderheid van de dialysepatiënten bestaat een chronisch verhoogde inflammatoire staat. Hieraan lijken vele factoren bij te dragen waaronder een verminderde eliminatie van ontstekingsfactoren door de nieren, het vaker voorkomen van infecties, en prikkeling van het afweersysteem door dialyse. Deze gegeneraliseerde staat van inflammatie zou door verscheidene effecten op de vaatwand bij kunnen dragen aan het vaker voorkomen van hart- en vaat-ziekten en de daardoor veroorzaakte sterfte. De exacte invloed en werkingsmechanisme zijn echter niet compleet duidelijk.

2. Nonthyroidal illness

Bij ongeveer 70 procent van alle dialysepatiënten lijken schildklierhormoonconcentraties in het bloed te zijn verlaagd. Omdat deze afwijkingen niet veroorzaakt worden door ziekte in de schildklier zelf, wordt dit beeld ook wel "nonthyroidal illness" genoemd waarbij 'thyroid' schildklier betekent en 'illness' ziekte. Het nierfalen zelf lijkt via allerlei mechanismen de oorzaak van deze verlaging van de schildklierhormoonconcentraties. Lage spiegels van schildklierhormonen veroorzaken een lager metabolisme. Vroeger werd gedacht dat de lage schildklierhormoonconcentraties bij dialysepatiënten een aanpassing van het lichaam betekende waarin het lichaam ten tijde van ziekte langer kon volstaan met minder energie. Er bestaan echter aanwijzingen dat deze veranderingen nadelige consequenties zouden kunnen hebben op andere systemen. Schildklierhormonen zijn namelijk van invloed op de functie van hart en bloedvaten waarbij te lage schildklierhormoonwaarden het ontstaan van hart- en vaatziekten zouden kunnen bevorderen.

In de voorgaande studies bestudeerden we twee mogelijke niet-traditionele risicofactoren voor sterfte door hart en vaatziekten bij patiënten met eindstadium nierfalen. Omdat zowel een verhoogde staat van inflammatie als ontregelde schildklierhormoonconcentraties niet altijd aanwezig zijn, verrichten we meerdere metingen per patiënt in de tijd. Het proefschrift is opgedeeld in twee stukken: 1) dat deel dat de implicaties van inflammatie onderzocht en 2) het gedeelte dat studies naar de effecten van nonthyroidal illness omvat.

1. Inflammatie

In **hoofdstuk 2** beschrijven we in een review de oorzaak van verhoogde concentraties ontstekings waarden in het bloed van dialysepatiënten. Hiertoe behoren onder andere ziekten die dialysepatiënten vaak naast hun nierfalen hebben (bijvoorbeeld diabetes mellitus en hart- en vaatziekten), het vaker optreden van infecties en de prikkeling van het afweersysteem door dialyse. Vervolgens geven we de resultaten weer van een literatuurstudie naar onderzoeken die de associatie tussen verhoogde ontstekingswaarden en sterfte gedurende follow-up nagingen. Omdat de ernst van de inflammatoire staat wisselt over tijd wijden we ook een gedeelte aan de bespreking van studies die de waarde van herhaalde versus afzonderlijke metingen van ontsteking onderzochten. Aan het einde van dit hoofdstuk geven we een advies over hoe het aantal patiënten dat nodig is voor een studie theoretisch verminderd kan worden door meerdere metingen per patiënt te verrichten.

In **hoofdstuk 3** onderzochten we de invloed van veranderingen van inflammatiemarkers over drie maanden tijd op sterfte bij patiënten met eindstadium nierfalen. Tot de inflammatiemarkers die we onderzochten, behoorden C-reactief proteïne (*CRP*), interleukine-6 (*IL-6*) en tumornecrosefactor-α (*TNF-a*). Patiënten werden verdeeld in vier groepen op basis van de veranderingen in inflammatie markerwaarden die bij hen werden gemeten. De vier groepen werden als volgt gedefinieerd: 1) patiënten met stabiel lage concentraties, 2) degenen die een stijging lieten zien in serumwaarden, 3) degenen die een daling toonden, en 4) degenen met stabiel hoge waarden (de hoogste graad van inflammatie). Gedurende verdere follow-up in de tijd scoorden we welke patiënten van de initiële populatie overleden of de studie verlieten om andere redenen. We vonden dat, in vergelijking met patiënten die aanhoudend lage CRP-, IL-6- en TNF-α-waarden hadden gedurende drie maanden observatie, patiënten met wisselende waarden in de tijd (stijging of daling), en stabiel verhoogde waarden een sterk verhoogd sterfterisico hadden. De oorzaak van deze verhoogde risico's zou een langere blootstelling aan de risicofactor kunnen zijn geweest.
In **hoofdstuk 4** stelden we de vraag of patiënten die een hogere inflammatoire activatie zouden ontwikkelen op de stimulus van een hemodialysesessie een hoger sterfterisico zouden hebben dan patiënten die weinig inflammatie zouden ontwikkelen na een dialysesessie. Hiertoe maten we in 190 Zweedse en 94 Nederlandse hemodialysepatiënten bloedwaarden van CRP vóór en na een hemodialysesessie. De veranderingen in concentraties corrigeerden we voor de hoeveelheden vocht die tijdens dialyse aan de bloedbaan werden onttrokken omdat deze een (valse) stijging in CRP-concentraties zouden kunnen veroorzaken die niet toe te bedelen is aan een inflammatoire activatie. Tijdens de follow-up telden we telkens degenen die overleden en degenen die in leven bleven. In onze studie bevonden we geen verschil in overleving tussen patiënten in wie serum CRP concentraties stegen en degenen in wie de waarden ongeveer gelijk bleven of daalden. Ook vonden we bij dezelfde patiënten die gedurende twee hemodialysesessies metingen ondergingen geen overeenkomst in CRP-veranderingen. Op basis van deze gegevens concludeerden we dat veranderingen in bloed-CRP-waarden tijdens een dialysesessie (als maat voor de inflammatoire activatie) niet geassocieerd zijn met sterfte. Deze redenering werd ondersteund door een bevinding in de literatuur dat CRP-waarden vaak meer tijd nodig hebben om te stijgen dan de duur van een hemodialysesessie (mediane duur vier uur).

2. Nonthyroidal illness

In **hoofdstuk 5** introduceren we het concept 'nonthyroidal illness' en beschrijven we het ontstaan daarvan bij patiënten met eindstadium nierfalen. Hierbij treffen we een vergelijking met de ontstaanswijze van nonthyroidal illness bij patiënten met hartziekten. Op basis van een uitgebreide literatuur studie lijken zowel nierfalen-gerelateerde factoren (jodiumretentie, verzuring van het bloed, seleniumtekorten en gebruik van bepaalde geneesmiddelen) als niet-nierfalen-gerelateerde factoren (ontsteking, vermagering) een rol te spelen bij het ontstaan van nonthyroidal illness. Aansluitend bespreken we mogelijk nadelige gevolgen van nonthyroidal illness op hart- en vaatfunctie. Het is

aannemelijk dat lage schildklierhormoonwaarden, veroorzaakt door nonthyroidal illness, een verhoogd risico geven op sterfte door het bevorderen van vaatverkalking en verdikking van de hartspier. Tot slot tonen we een literatuuroverzicht van studies die de effecten van schildklierhormoon toediening op hart-en vaat-functie en sterfte bij patiënten met nonthyroidal illness, veroorzaakt door eindstadium nierfalen, onderzochten. Er wordt gespeculeerd dat een compensatie van het schildklierhormoontekort een gunstig effect zou kunnen hebben op deze eindpunten. Onze literatuurstudie toont dat er weinig grote studies bestaan over het effect van schildklierhormoonbehandeling met deze indicatie bij patiënten met eindstadium nierfalen. We komen tot het advies om verdere studies hiernaar te verrichten.

Hoofdstuk 6 rapporteert een studie waarin de associatie tussen veranderingen van schildklierhormoonconcentraties over drie maanden tijd en sterfte (door hart- en vaatziekten) werd onderzocht. Bij 224 hemodialysepatiënten werden T3-, T4- en TSH-concentraties bij inclusie en na drie maanden follow-up gemeten. Groepen werden gecreëerd op basis van veranderingspatronen zoals besproken in de samenvatting van hoofdstuk 3. Hier vertegenwoordigde echter de groep met stabiel lage concentraties de slechtste categorie. Gedurende verdere follow-up in de tijd scoorden we welke patiënten overleden of de studie verlieten. Overeenkomstig onze hypothese bevonden we dat bij patiënten met verlaagde schildklierhormoonwaarden (zowel lage T3- als T4-concentraties die passen bij de hoogste graad van nonthyroidal illness) de meeste sterfte optrad, gevolgd door de patiëntencategorie met wisselende waarden over de tijd en patiënten met aanhoudend hoge (bij benadering normale) waarden.

In **hoofdstuk 7** tonen we een studie naar de effecten van lage schildklierhormoonconcentraties op kransslagaderverkalking, vaatstijfheid en sterfte bij 84 patiënten die peritoneale dialyse ondergingen. Bij inclusie in de studie ondergingen de patiënten metingen van vaatstijfheid en CT-scans van het hart om de kransslagaderverkalking te scoren. Gedurende follow-up werd bijgehouden welke patiënten overleden of de studie verlieten. De opzet van de studie was gekozen met de gedachte dat het effect van lage schildklierhormoonconcentraties op sterfte geïntermedieerd zou kunnen worden door een verhoogde kransslagaderverkalking en vaatstijfheid. De resultaten tonen dat lage T3-waarden sterk geassocieerd waren met een verhoogd sterfterisico en een verhoogde kransslagaderverkalking en vaatstijfheid. Ook een verhoogde kransslagaderverkalking was, zoals tevens bevonden in andere studies, sterk geassocieerd met een verhoogd sterfterisico. Op basis van deze resultaten speculeren we dat een verhoogd sterfterisico bij patiënten met lage schildkierhormoonwaarden (met name T3) veroorzaakt wordt door verhoogde kransslagaderverkalking en vaatstijfheid.

In **hoofdstuk 8** presenteren we een onderzoek in een populatie van 85-jarigen zonder primaire nierziekten waarin we de effecten van lage schildklierhormoonwaarden op veranderingen in nierfunctie over tijd onderzochten. Tussen 1997 en 1999 werden alle inwoners van Leiden die hun 85e verjaardag vierden benaderd voor deelname aan de zogenaamde Leiden 85 plus studie. Bij de 558 deelnemers werden bij inclusie in de studie schildklierhormoonconcentraties in het bloed gemeten. In de vijf jaar follow-up daaropvolgend werd bij de participanten die nog in leven waren elk jaar de

nierfunctie gemeten (ook door middel van een bloedafname). Uit onze analyses bleek dat schildklierhormoonstatus op 85 jaar niet geassocieerd was met het nierfunctiebeloop in de jaren daarna. In de analyse van de gegevens vormt de hoge sterfte echter een probleem waarbij een mogelijke bias de interpretatie van de data bemoeilijkt. Verdere studies naar de effecten van schild klierfunctie op het beloop van nierfunctie zullen moeten uitwijzen of schildklierhormoonstatus een risicofactor is voor achteruitgang in nierfunctie over de tijd bij ouderen.

Conclusies

1. Bij een meerderheid van alle patiënten met eindstadium nierfalen worden verhoogde waarden van inflammatiemarkers (CRP, IL-6 en TNF-α) gevonden waarvan de aanwezigheid geassocieerd is met een sterk verhoogde sterfte. Ook veranderpatronen van inflammatiemarkers over drie maanden tijd zijn geassocieerd met sterfte gedurende follow-up. Hierin hadden patiënten met stabiel verhoogde waarden het hoogste sterfterisico, gevolgd door degenen met wisselende waarden (stijging en daling) en patiënten met stabiel lage waarden.

2. Bij een meerderheid van alle patiënten met eindstadium nierfalen worden verlaagde schildklier waarden gevonden welke veroorzaakt worden door het nierfalen zelf en passen binnen het spectrum van nonthyroidal illness. De aanwezigheid van verlaagde schildklierhormoonconcentraties bij patiënten met eindstadium nierfalen is geassocieerd met verhoogde kransslagaderverkalking, vaatstijfheid en risico op sterfte door hart en vaatziekten.

Chapter 11

Appendices

Appendix 1.

The following Pubmed searching queries were used to identify relevant publications on the topic of discussion. When relevant publications were retrieved, articles citing that publication afterwards were also tracked at ISI web of Knowledge.

("Kidney Failure, Chronic"[Mesh] OR "Chronic kidney failure"[All Fields] OR "End stage kidney disease"[All Fields] OR "End-stage kidney disease"[All Fields] OR "End stage renal disease"[All Fields] OR "End-stage renal disease"[All Fields] OR "End stage kidney failure"[All Fields] OR "End-stage kidney failure"[All Fields] OR "End stage renal failure"[All Fields] OR "End-stage renal failure"[All Fields] OR "chronic renal failure"[All Fields] OR "ESRD"[All Fields] OR "Renal Dialysis"[Mesh] OR "Renal Dialysis"[All Fields] OR "hemodialysis"[All Fields] OR "haemodialysis"[All Fields] OR "Extracorporeal dialysis"[All Fields] OR "peritoneal dialysis"[All Fields] OR "CAPD"[All Fields] OR "Hemodiafiltration"[All Fields] OR "Uremia"[Mesh] OR "uraemia"[All Fields] OR "uremia"[All Fields])

AND

("C-Reactive Protein"[Mesh] OR "c reactive protein"[All Fields] OR "c-reactive protein"[All Fields] OR "C-reactive protein (192-201) "[Substance Name] OR "CRP"[All Fields] OR "C-reactive protein (164-173) "[Substance Name] OR "Interleukin-6"[Mesh] OR "interleukin-6, 2-nitrophenylsulfenyl-Trp(36,160)- "[Substance Name] OR "Interleukin-6"[All Fields] OR "Interleukin 6"[All Fields] OR "IL 6"[All Fields] OR "IL-6"[All Fields] OR "Tumor Necrosis Factor-alpha"[Mesh] OR "Tumor Necrosis Factor alpha"[All Fields] OR "Tumor Necrosis Factor-alpha"[All Fields] OR "TNFalpha"[All Fields] OR "TNF alpha"[All Fields] OR "Cachectin"[All Fields])

AND

("Mortality"[Mesh] OR "Mortality"[All Fields] OR "Death"[All Fields] OR "Survival"[Mesh] OR "Survival"[All Fields] OR "Treatment Outcome"[Mesh] OR "outcome"[All Fields] OR "Cardiovascular Diseases"[Mesh] OR "Cardiovascular Diseases"[All Fields] OR "Cardiovascular Disease"[All Fields] OR "hypertrophy"[All Fields] OR "cardiomyopathy"[All Fields] OR "atherosclerosis"[All Fields] OR "arteriosclerosis"[All Fields])

AND

("Time Factors"[Mesh] OR "time factors"[All Fields] OR "Variation"[All Fields] OR "Variation"[All Fields] OR "Repeated"[All Fields] OR "Repeatedly"[All Fields] OR "Serial"[All Fields] OR "Multiple"[All Fields] OR "Consecutive"[All Fields] OR "Consecutively"[All Fields] OR "values"[All Fields] OR "Measurements"[All Fields] OR "monthly"[All Fields] OR "Daily"[All Fields] OR "Weekly"[All Fields] OR "Yearly"[All Fields] OR "Monitoring"[All Fields] OR "persistent"[All Fields] OR "Persistent"[All Fields] OR "Time-course"[All Fields] OR "Time course"[All Fields] OR "in Time"[All Fields] OR "twice"[All Fields] OR "thrice"[All Fields] OR "more"[All Fields] OR "regular"[All Fields] OR "regularly"[All Fields] OR "Interval"[All Fields] OR "Succeeding"[All Fields] OR "Successive"[All Fields] OR "Fluctuating"[All Fields] OR "fluctuation" OR "fluctuations"[All Fields] OR "course"[All Fields] OR "longitudinal"[All Fields])

Appendix 2.

Pubmed (from 1940 till August 2012) was searched with terms as enlisted below. Scripts were modified to fit an inquiry for Embase (from 1974) and Web-of Science (from 1945).

("Kidney Failure, Chronic"[Mesh] OR "Chronic kidney failure"[All Fields] OR "End stage kidney disease"[All Fields] OR "End-stage kidney disease"[All Fields] OR "Endstage kidney disease"[All Fields] OR "End stage renal disease"[All Fields] OR "End-stage renal disease"[All Fields] OR "Endstage renal disease"[All Fields] OR "End stage kidney failure"[All Fields] OR "End-stage kidney failure"[All Fields] OR "Endstage kidney failure"[All Fields] OR "End stage renal failure"[All Fields] OR "End-stage renal failure"[All Fields] OR "Endstage renal failure"[All Fields] OR "chronic renal failure"[All Fields] OR "ESRD"[All Fields] OR "Renal Dialysis"[Mesh] OR "Renal Dialysis"[All Fields] OR "hemodialysis"[All Fields] OR "haemodialysis"[All Fields] OR "Extracorporeal dialysis"[All Fields] OR "peritoneal dialysis"[All Fields] OR "CAPD"[All Fields] OR "Hemodiafiltration"[All Fields] OR "Uremia"[Mesh] OR "uraemia"[All Fields] OR "uremia"[All Fields] OR "Kidney Tubular Necrosis, Acute"[Mesh] OR "Acute tubular necrosis"[All Fields] OR "Acute renal insufficiency"[All Fields] OR "Acute tubular necrosis"[All Fields] OR "Acute tubulus necrosis"[All Fields] OR "Acute Kidney Injury"[Mesh] OR "Renal Insufficiency"[Mesh] OR "Renal insufficiency"[All Fields] OR "Nephritis, Interstitial"[Mesh] OR "Interstitial Nephritis"[All Fields])

AND

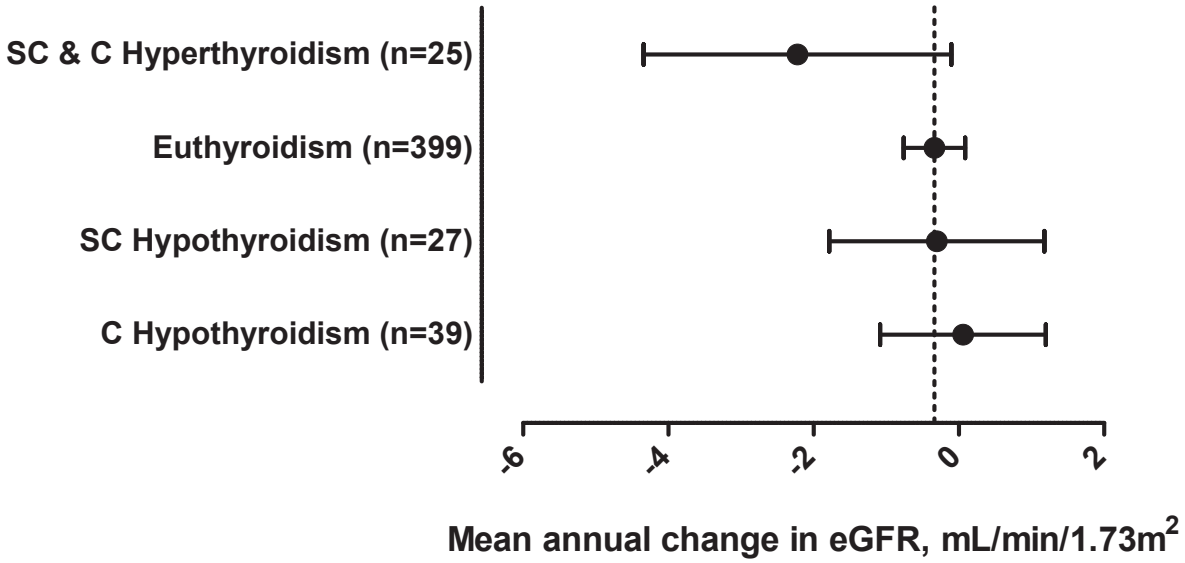
("Euthyroid Sick Syndromes"[Mesh] OR "Euthyroid Sick Syndromes"[All Fields] OR "Euthyroid Sick Syndrome"[All Fields] OR "Non-thyroidal Illness"[All Fields] OR "Nonthyroidal Illness"[All Fields] OR "Non thyroidal Illness"[All Fields] OR "Low-T3 Syndrome"[All Fields] OR "Low T3 Syndrome"[All Fields] OR "Low-T3 Syndromes"[All Fields] OR "Low T3 Syndromes"[All Fields] OR "Low-Triiodothyronine Syndrome"[All Fields] OR "Thyroid Hormones"[Mesh] OR "Thyroid hormone"[All Fields] OR "Thyroid hormones"[All Fields] OR "Thyroxine"[Mesh] OR "Thyroxine"[All Fields] OR "T4"[All Fields] OR "Tetraiodothyronine"[All Fields] OR "T3"[All Fields] OR "rT3"[All Fields] OR "Triiodothyronine"[Mesh] OR "Triiodothyronine"[All Fields] OR "Triiodothyronine, Reverse"[Mesh] OR "Thyrotropin"[Mesh] OR "TSH"[All Fields] OR "Thyroid Stimulating hormone"[All Fields] OR "Thyroid Stimulating hormones"[All Fields] OR "Thyroid-Stimulating hormones"[All Fields] OR "Thyroid-stimulating hormone"[All Fields] OR "Thyreotropin"[all fields] OR "Thyrotrophin"[all fields] OR "Thyrotropin-Releasing Hormone"[Mesh] OR "Thyrotropin-Releasing hormone"[All Fields] OR "Thyrotropin Releasing hormone"[All Fields] OR "Thyrotropin-Releasing hormones"[All Fields] OR "Thyrotropin Releasing hormones"[All Fields] OR "Thyroxine-Binding Proteins"[Mesh] OR "TBG"[All Fields] OR "Thyroxin-Binding Proteins"[All Fields] OR "Thyroxin-Binding Protein "[All Fields] OR "Thyroxin Binding Proteins"[All Fields] OR "Thyroxin Binding Protein"[All Fields])

AND

("Thyroxine"[Mesh] OR "Levo-thyroxine"[All Fields] OR "Levo thyroxine"[All Fields] OR "L-thyroxine"[All Fields] OR "L thyroxine"[All Fields] OR "Thyroxine"[All Fields] OR "T4"[All Fields] OR "Levothyroxine "[All Fields] OR "Levoxine"[All Fields] OR "Synthrox"[All Fields] OR "Thyrax"[All Fields] OR "Thyrox"[All Fields] OR "Levo-Thyrox"[All Fields] OR "Levo thyrox"[All Fields] OR "L Thyrox"[All Fields] OR "L-Thyrox"[All Fields] OR "Levo-T"[All Fields] OR "3,5-diiodothyropropionic acid"[All Fields] OR "DITPA"[All Fields] OR "3,5 DITPA"[All Fields] OR "Triiodothyronine"[Mesh] OR "Levo-triiodothyronine"[All Fields] OR "Levo-T3"[All Fields] OR "L-triiodothyronine"[All Fields] OR "L-T3"[All Fields] OR "L triiodothyronine"[All Fields] OR "L T3"[All Fields] OR "T3 Thyroid Hormone"[All Fields] OR "Liothyronine"[All Fields] OR "Cytomel"[All Fields])

Appendix 3.

Figure 1. Mean (95%CI) annual change in eGFR (mL/min/1.73m²) across different thyroid function groups as calculated by pooling of patient specific betas[1]



1. Regression lines were fitted on the repeated measurements for each individual separately. Then these betas were pooled within the different thyroid hormone groups (as specified in the methods section) and compared by means of a one-way ANOVA test. Mean annual changes were not different between groups (p=0.149). Adjusted for sex, DM, smoking, cardiovascular disease, malignancies, and amiodarone treatment.

Appendix 4.

Figure 1. Crude (A) and adjusted (B)[2] mean (95%CI) eGFR (mL/min/1.73m²) in those not using thyroid medication at age 85 across different thyroid hormone groups*

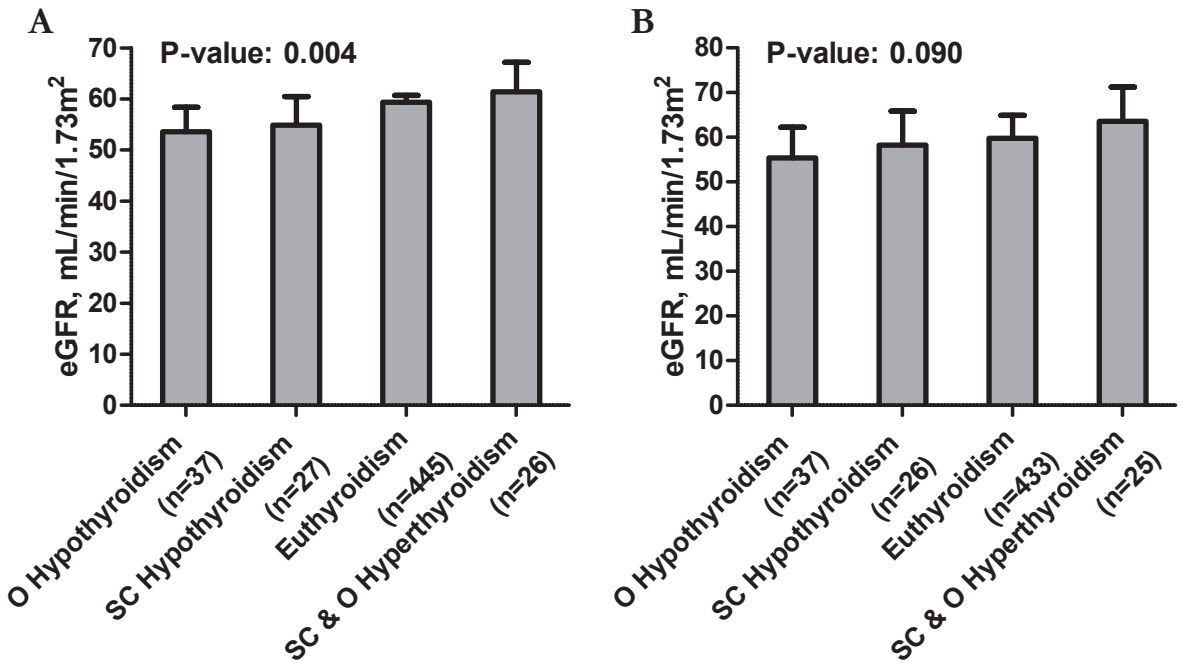
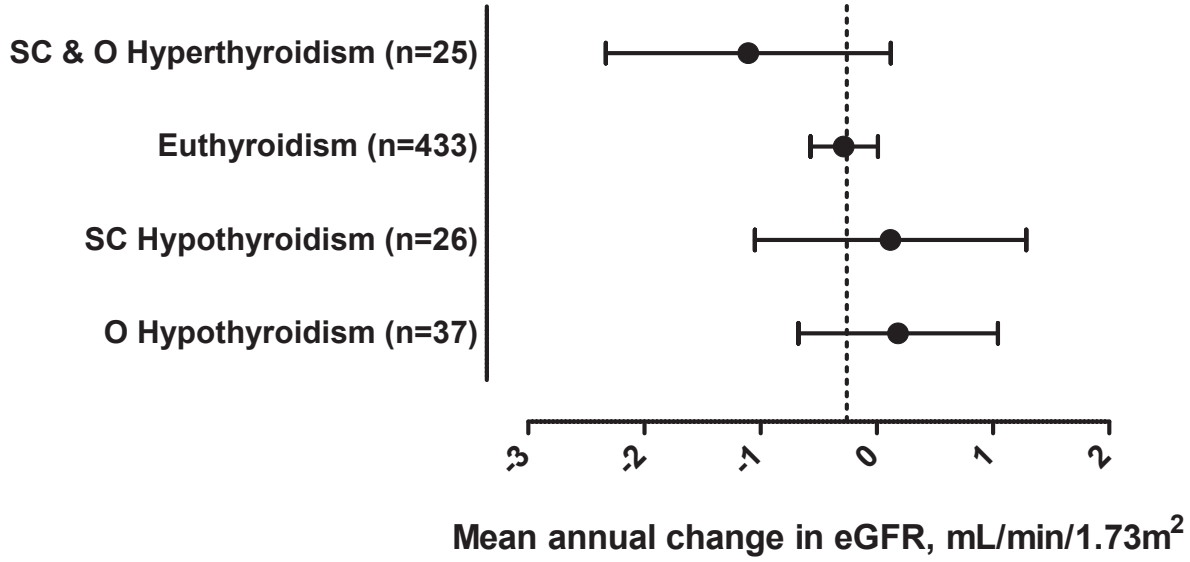


Figure 2. Adjusted mean (95%CI) annual change in eGFR (mL/min/1.73m2) in those without thyroid hormone medication[1,2]



1. The association between thyroid hormone groups and renal function was studied in those without medication influencing thyroid function (thyroid hormone supplementation or antithyroid medication).
2. Adjusted for sex, DM, smoking, cardiovascular disease, malignancies, and amiodarone treatment.

Appendix 5.

Figure 1. Crude (A) and adjusted (B)[2] mean (95%CI) eGFR (mL/min/1.73m^2) at age 88 across different thyroid hormone change groups[1]

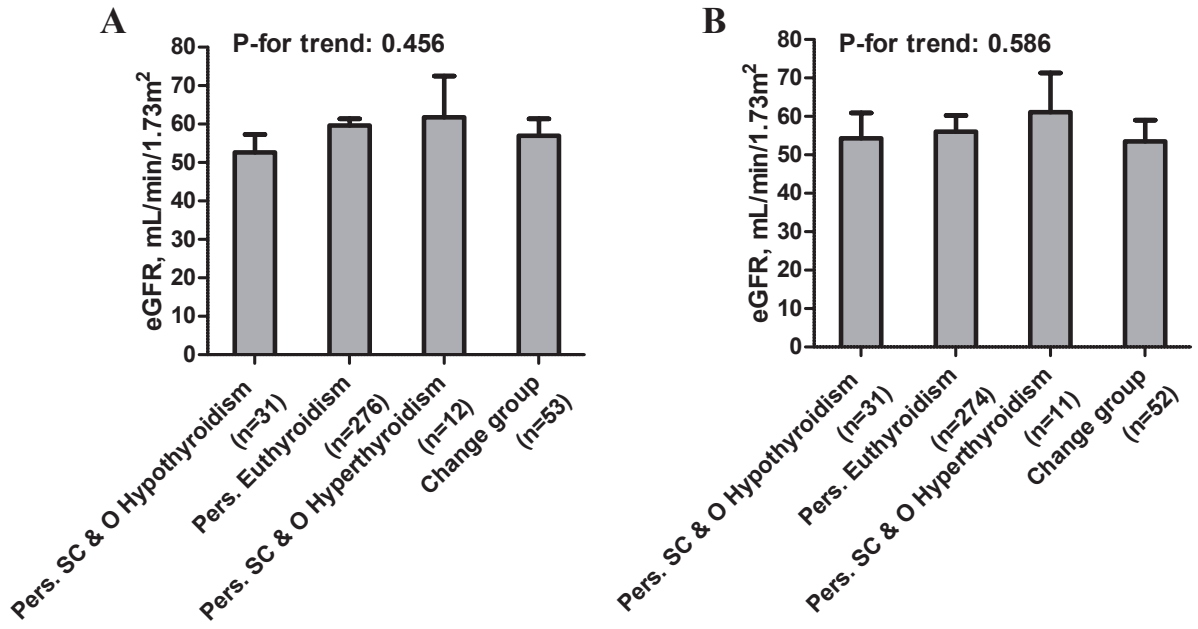
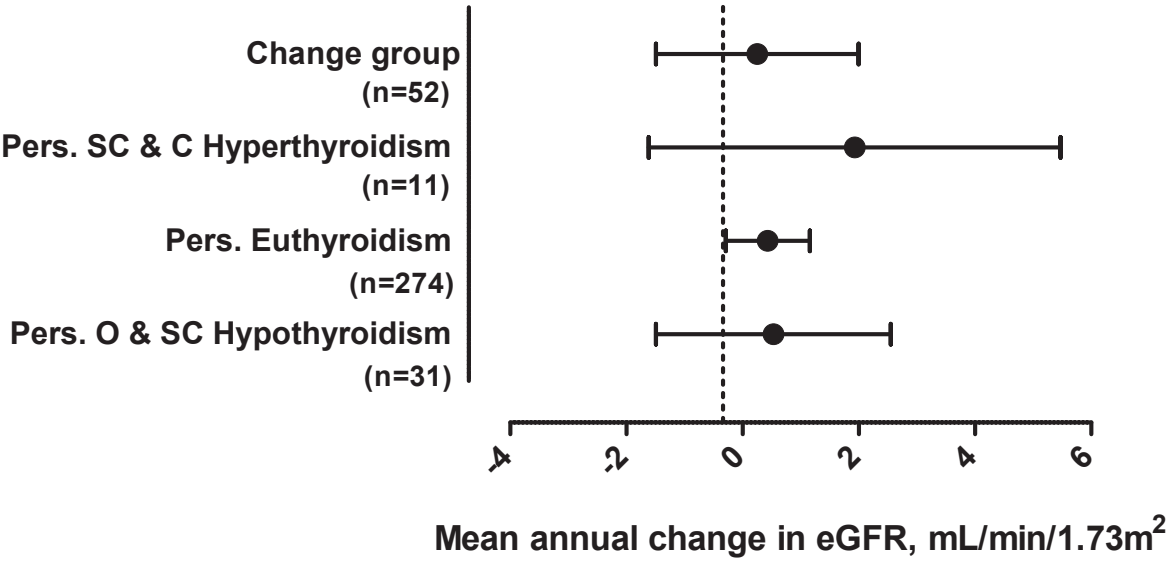


Figure 2. Adjusted mean (95%CI) annual change in eGFR (mL/min/1.73m^2) after age 88 accross thyroid hormone change groups[1,2]



1. Change groups were based on categorization at 85 and 88 years (see above). Pers. = Persistent. eGFR in mL/min/1.73m^2.
2. Adjusted for sex, DM, smoking, cardiovascular disease, malignancies, and amiodarone treatment.

Chapter 12

Acknowledgements

Bij de totstandkoming van dit proefschrift gaat mijn erkenning uit naar vele mensen die mij aldoor de onuitputtelijke steun gaven om deze taak te volbrengen. In het speciaal noem ik een aantal mensen.

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afdeling Cardiologie in het UMC Utrecht voelde ik mij tot in het hoogste uitgedaagd. Ik dank al mijn collega's en stafartsen voor het warme onthaal en de prachtige tijd. Beste Hans in het speciaal, dank voor jouw voorbeeld en het vertrouwen wat je me geeft. In mijn huidige werk op de afdeling interne geneeskunde in het Meander Medisch Centrum (Amersfoort) ben ik mijn collega's en stafartsen erkentelijk voor de geweldige tijd die we mogen ervaren.

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Chapter 13

Curriculum Vitae & List of publications

Curriculum Vitae

Christiaan Lucas Meuwese was born as part of a twin in Zeist, the Netherlands, on the 2nd of September, 1986. From 1999 to 2005 he attended the Christelijk Lyceum (secondary school) in Zeist where he passed the gymnasium. In 2005 he commenced medical studies at the Leiden University Medical Center (LUMC). During his second year of studies he participated in an exchange programme and spent half a year studying at the Karolinska Institute in Stockholm, Sweden. After his return to the Netherlands (Christmas 2006), he obtained a scholarship for excellent students and consulted prof. dr. F.W. Dekker at the department of Clinical Epidemiology in Leiden. From this time on he participated in research as an addition to his medical studies. In 2008 a collaboration was set up with the Renal Department at the Karolinska Institute (dr. J.J. Carrero and prof. dr. P. Stenvinkel). In 2009 Christiaan applied for, and obtained the Willem Kolff Grant from the Dutch Kidney Foundation and the Jo Keur Grant from the LUMC to pay another longer term visit to Stockholm.

After graduating from medical studies in 2011 (cum laude for both the theoretical and clinical part), he started working as a resident at the Cardiology Department at the University Medical Center of Utrecht (UMCU) for half a year. During this period, he confirmed his interest in cardiology and received a training place. After this, the author went back into research for one more year to complete his PhD thesis. He was enabled to do so by means of a personal grant which he obtained from the LUMC (MD-PhD grant). During this final year, he assisted in the design and organization of the Dutch part of a new European study project (EQUAL: A European QUALity Study on treatment in advanced chronic kidney disease). Also, he gave several presentations at international congresses, was engaged in teaching (bio)medical students and followed courses in epidemiology and statistics himself whereby he completed his training to receive the title of epidemiologist. The current thesis is the result from a fruitful 5 year collaboration between the LUMC and the Karolinska Institute.

The author currently lives in Utrecht, and has since 2013 started his clinical training to become a cardiologist (Dr. J.H. Kirkels, UMC Utrecht).

List of Publications

2014 **Meuwese C.L.**, Gussekloo J., de Craen A.J.M., Dekker F.W., den Elzen W. Thyroid hormone status and renal function in an elderly community based population. *Journal of Clinical Endocrinology & Metabolism*. Epub ahead of print. 2014

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2011 **Meuwese C.L.**, Carrero J.J., Stenvinkel P. Recent insights in inflammation-associated wasting in patients with chronic kidney disease. *Contributions to Nephrology.* 171, 120-126 (2011).

2010 **Meuwese C.L.**, Snaedal S., Halbesma N., Stenvinkel P., Dekker F.W., Qureshi A.R., Barany P., Heimburger O., Lindholm B., Krediet R.T., Boeschoten E.W. and Carrero J.J. Trimestral variation patterns of C-reactive protein, interleukin-6 and tumor necrosis factor-α are similarly associated with survival in hemodialysis patients. *Nephrology Dialysis & Transplantation.* 26, 1313-1318 (2011).

2010 **Meuwese C.L.**, Halbesma N., Stenvinkel P., Dekker F.W., Molanaei H., Qureshi A.R., Barany P., Heimburger O., Lindholm B., Krediet R.T., Boeschoten E.W., Carrero J.J. Variations in C-reactive protein during a single haemodialysis session do not associate with mortality. *Nephrology Dialysis & Transplantation.* 25, 3717-3723 (2010).

2010 **Meuwese CL**, Euser AM, Ballieux BE, van Vliet HA, Finken MJJ, Walther WJ, Dekker FW, and Wit JM. Growth-restricted preterm newborns are predisposed to functional adrenal hyperandrogenism in adult life. *European Journal of Endocrinology.* 163, 681-689 (2010).