

Cover Page



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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 noncardiovascular 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- α*) 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 triiodothyronine 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*.

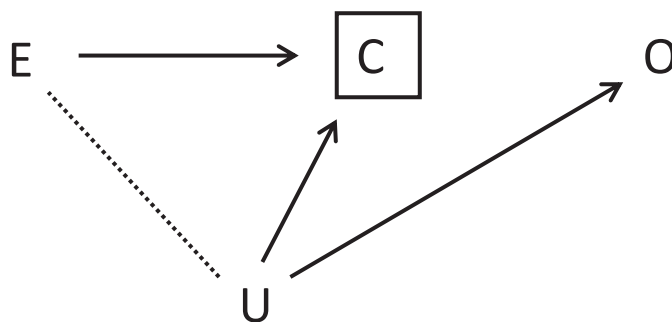


Figure 1. A Directed Acyclic Graph (DAG) illustrating collider-stratification bias. E: exposure, C: collider, U: other (un)known factors, O: outcome of interest.

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.

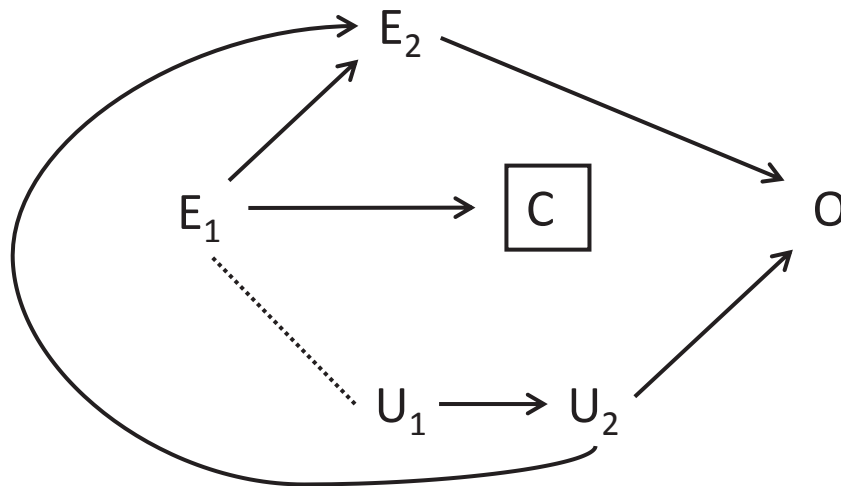


Figure 2. A directed acyclic graph (DAG) illustrating the complexity of collider stratification bias in end-stage renal disease. E1: exposure at timepoint 1, E2: exposure at timepoint 2, C: collider, U1: other (un)known factors at timepoint 1, U2: other (un)known factors at timepoint 2, O: outcome of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