Chapter 9

Summary and general discussion




The general aim of this thesis was to investigate the potential risks associated with erythropoiesis-stimulating agent (ESA) use and to explore some possible mechanisms. A summary of the main results is presented in this chapter. Furthermore, methodological considerations, the context of the results with potential explanations and clinical implications are discussed.

Summary of main findings

Causal inference based on observational data requires careful consideration of confounding. **Chapter 2** explains the basic concepts of Directed Acyclic Graphs (DAGs) and their application in the identification of confounding. It is shown that DAGs can help to identify the presence of confounding and can serve as a visual aid in the scientific discussion. Specially in more complex research questions, DAGs could be preferable to the traditional method to identify confounding. With DAGs it is possible to identify a minimum set of factors to adjust for and to recognize potential collider-stratification bias with certain adjustments. Further on in this discussion, DAGs are used to illustrate time-dependent confounding.

As this thesis aimed to identify possible risks associated with ESA use, more insight in anemia management in patients with chronic kidney disease (CKD) was needed. Last decade, several FDA warnings and changes in anemia guideline changes were issued in response to results of anemia-correction trials, raising questions about the safety of ESAs. **Chapter 3** provides an overview of the developments since 2008. With these in mind, the trends in hemoglobin (Hb), ESA and iron use in stable hemodialysis (HD) patients and patients with CKD stage 3b-5 in Sweden from 2008-2013 were described. The study demonstrates that Swedish nephrologists adapted their anemia management practices in both CKD non-dialysis (CKD-ND) and HD patients. ESA use decreased in both patient groups and less ESA-treated patients had an Hb above 12 g/

dL. Furthermore, decreases in ESA dose were shown in HD patients and a decline in Hb was observed mainly in CKD-ND patients.

One of the proposed mechanisms of ESA-related cardiovascular events is through the elevation of blood pressure (BP) by ESA treatment. In **chapter 4** it is shown that ESA- treated pre-dialysis patients in the Netherlands received more antihypertensive agents than patients without ESA, confirming the hypertensive effect of ESA. Also, within ESA-treated patients, a trend towards a higher BP with high ESA doses is indicated. Patients with high ESA doses had an adjusted 3.7 mmHg higher SBP than those with a low ESA dose. However, no relevant difference in routinely measured BP was observed between patients with and without ESA treatment. It was therefore concluded that the hypertensive effect of ESAs could be controlled to the same extent as patients without ESAs in clinical practice. It seems thus unlikely from our results that this effect could sufficiently explain the increased cardiovascular risk associated with ESA use in pre-dialysis patients.

So far, an increased risk of cardiovascular thrombotic events has mainly been inferred from composite endpoints of anemia-correction trials in CKD patients. In **chapter 5** the relation of ESA treatment with myocardial infarction, ischemic stroke and venous thrombosis was investigated in dialysis patients in the Netherlands. No excess of thrombotic events was shown in ESA-treated dialysis patients compared to patients without ESA treatment. Also, no evident ESA dose-response effect was present and the association of ESA treatment with ischemic strokes seemed even protective. Thus in contrast to the general hypothesis, we could not confirm a higher thrombotic risk in our cohort of dialysis patients.

Whereas in CKD patients ESA treatment is mainly associated with cardiovascular events, in hemato-oncology patients ESA treatment is mostly related to venous thrombosis. **Chapter 6**

examines the relation between ESA treatment with myocardial infarction, stroke and venous thrombosis in patients with multiple myeloma (MM) and myelodysplastic syndrome (MDS) from Denmark. In MM patients, ESA use was associated with a higher risk of all cardiovascular events, with hazard ratios ranging from 1.38 for myocardial infarction to 1.81 for stroke. MDS patients with ESA treatment had an almost twofold increased risk of myocardial infarction and stroke, the hazard ratio for VTE was 1.10. Further investigation should aim to elucidate the mechanism of the ESA-related events and identify patients who will benefit most from ESA treatment.

The anemia-correction trials indicated that CKD patients with ESA treatment targeted to higher Hb levels, had a higher mortality risk. These results have raised the question whether it was just the higher target Hb that caused more mortality, or the on average higher ESA doses used to treat these patients. **Chapter 7** therefore investigates the effect of high ESA doses on mortality in Dutch dialysis patients. Estimating this relation from observational research is challenging because of the strong interplay between ESA dose, patient's health status and time-dependent confounding of Hb. High ESA dose was defined as above 6,000 units/week and two analytical approaches were used to handle time-dependent confounding. The sequential Cox model estimates the effect of starting with a high ESA dose compared to patients that had not been treated with a high ESA dose. The marginal structural model estimates what would happen if everyone was always treated with a high ESA dose versus never. The estimated effect of high ESA doses on mortality was 1.2- to 1.5- fold. Although confidence intervals were wide, this indicates a harmful effect of high ESA doses and supports the current guidelines to use the lowest ESA dose possible to avoid blood transfusions.

Alternatively, it has been proposed that the association between high ESA dose and mortality is a reflection of the need to administer high ESA doses in patients with an

inadequate hematopoietic response and is thus caused by ESA resistance. **Chapter 8** confirms that ESA resistance is associated with increased mortality in both HD and peritoneal dialysis (PD) patients. The hazard ratio was 1.37 in HD and 2.41 in PD patients. As ESA resistance is related to disease severity and comorbidities, it is notable that adjustments for these confounding factors did not eliminate the effect of ESA resistance.

Methodological strengths and limitations

Before the results of this thesis can be interpreted properly, some methodological considerations should be taken into account. Specific strengths and limitations have already been presented in different chapters of this thesis. In this section, more general topics are discussed.

Confounding by indication

A major challenge in observational research is to address confounding. The ideal design to study treatment effects is a randomized controlled trial. Randomly allocating ESA treatment aims to create exchangeability, thus creating groups of patients with and without ESAs that are comparable with respect to prognosis. In clinical practice, ESAs are certainly not prescribed randomly. In fact, doctors are trained to prescribe the treatment to those patients that they expect to benefit most. As also shown by the baseline characteristics in several chapters of this thesis, it results in differences between the ESA treatment groups. Factors that influence both treatment and outcome cause confounding. Treatment decisions are a result of patients' and physicians' considerations, based on several patient characteristics and prognostic factors. As indicated in **chapter 2**, it is only possible to adjust for measured confounding factors. If unknown or unmeasured factors exist on which treatment decisions are based that

are related with prognosis, it will lead to confounding by indication.

In general, all patient characteristics and physicians' considerations that influence treatment decisions are hard to capture in data. The overall impression of a patient's condition is influenced by the patient's appearance, a hand shake, or even the ability to joke. If the measured confounding factors did not adequately reflect the patient's overall health status and thus prognosis, and if the more subtle unmeasured factors highly influenced the treatment decision and studied outcome, it could result in invalid conclusions. It is however important to separate research for intended effects from research for unintended effects. Confounding by indication occurs when the patient's estimated chance of the outcome is weighted in the treatment decision, thus any intended treatment effect becomes inextricably intermingled with prognosis.[1] However, adverse effects are always unintended. Furthermore, adverse effects of most medications are unpredictable and are therefore generally not weighted in treatment decisions. Unintended effects mostly have different risk factors than the intended effects. If that is the case, there should be no confounding by indication when investigating unintended effects, since the prescribing of ESAs is independent of the chance for the adverse event of interest. However, especially when risk factors for the unintended and intended effects overlap, confounding could still be present, both measured and unmeasured.

In the case of ESA treatment, the intended effect of ESAs would be to raise Hb level, hoping to improve energy, physical functioning and quality of life. Furthermore, before results of the anemia-correction trials were published, alleviating anemia was expected to improve survival, most possibly by decreasing the risk for cardiovascular events. In our studies, we investigated the effect of ESAs on BP, cardiovascular events and mortality. Elevation of BP by ESAs was an often described side effect, mainly in the early days of ESA

treatment, and could be regarded as an adverse, unintended effect. The primary decision for ESA treatment is based on the presence of anemia and related symptoms, but it might be that physicians were more cautious with ESAs in patients with pre-existing high BP or other comorbidities. In **chapter 4** however, BP was similar in patients with and without ESA treatment, which does not support this theory. The effect of ESAs on thrombotic events, as studied in **chapter 5**, was mainly unknown during the NECOSAD study. Although there had been reports, mainly of vascular access thrombosis, the anemia-correction trials were set up to identify a possible beneficial effect of ESAs on cardiovascular events, and specially in patients with more cardiovascular comorbidities. Of course, the NHCT was published at the beginning of the NECOSAD study in 1998,[2] but this only raised some survival concerns for patients with higher ESA doses and cardiac comorbidity and did not result in guideline changes. After the publication of two additional anemia-correction trials at the end of 2006,[3;4] the ESA guidelines and FDA labels warned for stroke and death in ESA-treated patients to higher Hb levels. As new patients were included in the NECOSAD study until the end of 2006, these warnings could only have affected ESA treatment practices during the last years of follow-up of a minority patients. The patients' expected risk of thrombotic events therefore probably minimally affected ESA prescribing during the NECOSAD study. In **chapter 6**, we included MM and MDS patients from 2004-2011, thus mainly after the public debate was started. In this patient population, ESAs were mainly prescribed to improve quality of life. Cardiovascular events would still be an unintended effect, but in this time period not unknown any more, and could therefore have been weighted in treatment decisions. If this risk of cardiovascular events was estimated by different factors than the measured comorbidities and medical history and influenced treatment decisions, this could have resulted in residual confounding by indication and influenced our results. If patients with a higher estimated cardiovascular risk were less likely to be treated

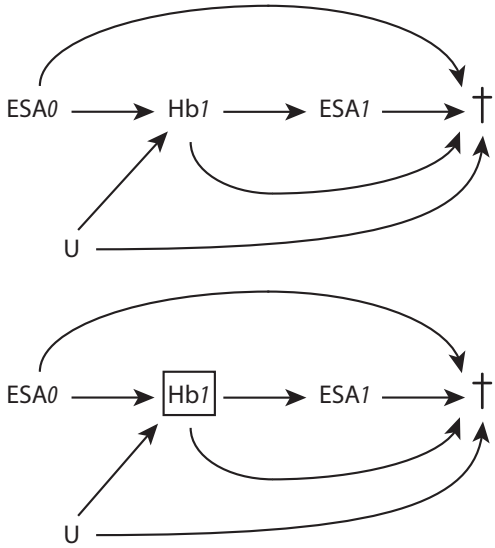
with ESAs, this could underestimate a possible harmful ESA effect. However, most important prognostic factors for future cardiovascular events (age, sex, comorbidities and history of cardiovascular events) were accounted for in the analyses.

When studying the effect of ESAs on mortality in **chapter 7**, improving survival could be regarded as an intended effect. However, before the anemia-correction trials it was believed that ESAs could contribute to longer survival, after these trials it was debated whether (high doses of) ESAs could lead to a higher mortality risk. Since the NECOSAD study was partly before and after, but mostly in between the several trials, it is hard to predict in which way this would have affected the physician's treatment decision. If physicians would have been more inclined to give ESAs to patients with a subjectively estimated higher mortality risk, as supported by a higher frequency of measured confounding factors such as comorbidities in our studies, any residual confounding would lead to an overestimation of the harmful ESA effect. In general we believe that the important known confounding factors were measured and taken into account, but we cannot verify this untestable assumption or determine the size of possible residual confounding.

Time-dependent confounding

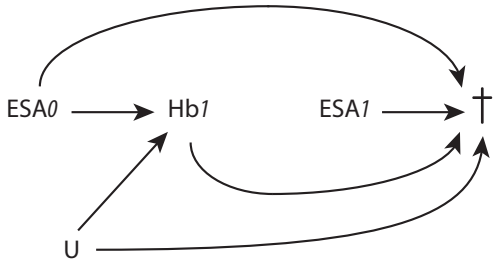
An even more complicated problem in observational research constitutes the presence of time-dependent confounding. As shown in the DAG in Figure 1, the current Hb is a consequence of the previous ESA treatment. Subsequently, the current Hb will also affect the next ESA treatment and the outcome mortality. Following DAG rules as discussed in **chapter 2**, on the one hand we want to condition on (or adjust for) Hb*1* because it confounds the relation between ESA*1* and mortality. On the other hand we do not want to condition on Hb*1*, since it is in the causal path between ESA*0* and mortality. Even more so, in the DAG it is shown that conditioning on Hb*1* could also introduce collider-stratification bias, by opening a

▾ **Figure 1.** Time-dependent confounding by Hb



Conditioning on Hb1 adjusts in the causal path and could introduce collider-stratification bias.

▾ **Figure 2.** Weighting for time-dependent confounding



Weighting creates a pseudopopulation in which no relation between ESA treatment and confounding factors exists.

path from Hb*1* to mortality via unknown confounding factors between Hb1 and mortality, collectively called U. Conditioning on a factor in the causal path usually underestimates the effect of ESAs, because it removes part of the effect that is mediated by Hb*1*. The direction of the possibly introduced collider-stratification bias is unpredictable, particularly since it involves unknown factors. It is important to realize that collider-stratification bias is only introduced when we assume that unknown confounding factors between Hb and mortality exist.[5]

To adequately handle time-dependent confounding, a marginal structural model was used in **chapter 7** to estimate the effect of treatment with high ESA dose on mortality. A marginal structural model uses inverse probability of treatment weights (IPTW) to adjust for confounding. Weighting each patient by the inverse of this patient's probability of the received treatment creates a pseudopopulation in which there is no relation between ESA treatment and confounding factors.[6] Weighting removes the link between Hb*1* (and other confounding factors) and ESA*1* as illustrated by the DAG in Figure 2. After weighting, groups are made exchangeable and no conditioning on confounding with regression is needed to estimate the causal relation between ESAs and mortality. This weighting is performed at each moment in time for the chance of treatment, and also for the chance of censoring with inverse probability of censoring weights (IPCW). This model then estimates the relative hazard if all patients would have always received ESAs versus if all patients would never have been treated with ESAs.

Alternatively, we used a sequential Cox model in which several landmark datasets were created, with consecutive starting moments of follow-up. Patients starting with high ESA treatment were compared to patients that were not treated with high ESA dose, conditional on covariates at start. The sequential Cox model also uses IPCW to adjust for informative

censoring. The different landmark datasets were stacked to obtain an overall result. The advantage of the sequential Cox model is that it only uses IPCWs and not IPTWs, since IPCWs tend to be more stable than IPTWs.[7]

In **chapter 5** we related ESA use and dose to thrombotic events. We did not perform an IPW analysis due to the limited sample size. IPW estimation can become very unstable when the chance of the treatment given covariates becomes very small or even almost zero.[8] This chance of non-positivity becomes higher with smaller sample size.[9] We therefore chose to present time-dependent Cox regression analyses with and without adjustment for Hb. Adjustment for Hb did not affect results substantially. This could indicate that Hb does not have a very strong effect on thrombotic events and the unknown confounding producing collider-stratification bias is minimal. However, theoretically the two effects could have cancelled out each other. In **chapter 6** Hb was not available, which limited our analyses. However, since additional adjustment for Hb did not substantially affect our results in **chapter 5**, we proceeded with time-dependent Cox regression analyses without adjustment for Hb.

Selection bias

As shortly mentioned in **chapter 2**, selection bias occurs when we select on a common effect of exposure and outcome. In other words, selection bias arises when the relationship between the exposure and outcome of interest is different for those who participate and those who do not participate in the study, but were eligible.[10] Most chapters in this thesis describe dialysis patients (**chapters 5,7,8**). Dialysis patients are obviously a highly selected group of patients, since only patients with CKD who survive long enough to reach end-stage renal disease and are expected to be strong enough to handle dialysis will start on dialysis. However, starting dialysis is not a common effect of ESAs and mortality (**chapter 7**) or ESAs and thrombotic events (**chapter 5**) on dialysis, since

causes precede their effects. Selecting these survivors is not a problem as long as we realize that our findings apply to dialysis patients. Furthermore, in **chapter 5** and **7** baseline was set at 3 months after start of dialysis and in **chapter 8** at 6 months after start of dialysis. Hence, these results apply to patients surviving 3 and 6 months on dialysis. These patients are probably different from the entire dialysis population, which also includes patients with acute and transient kidney failure and the fragile patients that do not survive the first months on dialysis. Survivor bias, i.e. selecting patients that are still alive, is much more pronounced in studies including prevalent instead of incident dialysis patients. Strength of the NECOSAD study is that patients were followed from start of dialysis and we know exactly which patients do not reach our baseline, and thus to which patients our results do apply. We also performed a sensitivity analysis in **chapter 5** with baseline at start of dialysis, thus including the first months.

The PREPARE study as used in **chapter 4** included incident pre-dialysis patients. This is a selection of the CKD patients that have been referred to a nephrologist for specialized pre-dialysis care. Selection bias might have arisen if patients that used ESAs and with high BP were selectively referred to specialized pre-dialysis care. Then at start of pre-dialysis care an inverse association between ESA use and high BP would have been created, thus patients with ESAs would on average have a lower BP than patients without ESA. In our study, we found no difference in BP between patients with and without ESA treatment. However, since we believe that ESAs have a BP raising effect, and the selection bias would result in a lower BP in ESA-treated patients, in theory the combination counteracts, which would result in no difference in BP between ESA treatment groups. However, since multiple reasons for referral to specialized pre-dialysis care exist, but the eGFR is most important, this probably did not have a large influence on our results. The CKD patients included in **chapter 3** are CKD patients under the care of any nephrologist.

An advantage of Swedish registry data is that recordings are mandatory and part of the routine care. In contrast to cohort studies like NECOSAD and PREPARE, registration of patients does not depend on the patient's willingness to participate, probably resulting in a better reflection of the unselected general CKD population. Even more so, the Danish population based registries, as used in **chapter 6**, registers all diagnosis and covers the total Danish population. This ensures studying patients without selection.

Reverse causation/temporality

Reverse causation is to mistake the cause for the effect and vice versa. Most chapters in this thesis are based on cohort studies. Reverse causation is not a problem when the outcome is an event, such as mortality or thrombosis, that occurs after the exposure. Then, effect automatically follows cause. In chapter 4 however, we used mixed models as repeated cross-sectional analyses. Since cause and effect are then recorded at the same time, in theory it is possible that cause and effect could have been reversed. In our study this would mean that BP had an effect on ESAs and ESA dose instead of the other way around. If BP would have influenced the physicians' prescribing of ESAs, the physician would probably dose ESAs more cautiously in patients with a higher BP. This would lead to an underestimation of the ESA effect. A trend towards lower ESA doses with higher BP would then be expected, and not our observed trend towards higher BP with higher ESA doses. Furthermore in literature the hypertensive effect of ESAs is established after ESA injection. We thus believe that our study represents the effect of ESAs on BP and that a possible influence vice versa is only secondary.

Missing data

Missing data are common in observational studies and all chapters of this thesis describe studies with missing data. The most simple way to handle missing data, and the default method in most statistical packages, is to perform a complete case analyses, thus only including patients without missing data. However, this can result in inefficient analyses and more importantly, can produce biased estimates.[11] In general, three types of missing data can occur. The first is missing completely at random (MCAR), where the probability that an observation is missing is independent of (observed and unobserved) patient characteristics and the patients with missing data are thus a random subset of the complete sample. In this case a complete case analysis will give unbiased estimates and multiple imputation will also give unbiased, but probably more precise estimates. The second is missing at random (MAR), where the probability that an observation is missing is dependent on observed patient characteristics. It is called MAR, because missing data can be considered random conditional on observed patient characteristics and can thus be predicted based on these observed characteristics. When data is MAR, complete case analysis will produce biased estimates, but it has been shown that multiple imputation gives correct results. Third, the observation can be missing not at random (MNAR). This occurs when the probability of an observation being missing depends on unobserved information, like the value of the observation itself. Unfortunately, there is no general method to adequately handle missing data not at random.[11;12]

In most chapters of this thesis, multiple imputation was performed to handle missing data on confounding factors. In **chapter 7** no imputation was performed, due to the complexity of the analysis and computational demands. This implies that for the analyses only patients without missing data at baseline were selected. Our results are therefore only valid in these patients without missings and we must assume missing completely at random to apply our results to the total

dialysis population. Information on exposure and confounding was only updated when new information was available, thus implicitly performing last observation carried forward. As this method almost continuously weights patients according to their patient characteristics, it is virtually impossible to measure or impute these characteristics each moment in time. This extrapolation of data seems fairly reasonable for a short period after the measurement, but might not be adequate months later. Therefore, misclassification of confounding factors could have occurred, resulting inadequate adjustment for confounding, which could lead to residual confounding.

Results

US vs Europe

With the aforementioned methodological issues in mind, we showed in **chapter 7** a 1.2- to 1.5-fold higher mortality risk in patients treated with high ESA doses. Previous observational studies that adequately handled time-dependent confounding reported no increased risk with higher ESA doses to a 30% increased mortality risk.[13-18] However, these analyses were all performed in large cohorts of US patients, who were generally treated with higher ESA doses. Median ESA doses in these studies were 8,900-30,000 units/week. Also, the anemia-correction trials that started the debate on the safety of high ESA doses and form the basis of the FDA warnings and current guidelines, were mostly US studies as well. In US-based trials CHOIR and TREAT, mean ESA doses were 11,215 and 13,577 units/week in the higher Hb target arm respectively.[4;19] To compare, the mean ESA dose of 6,276 in the lower Hb arm of the CHOIR is more similar to ESA doses in Dutch clinical practice. Median ESA dose in Dutch dialysis patients included in NECOSAD was 6,000 units/week and median ESA dose in Dutch pre-dialysis patients included in PREPARE was 4,000 units/week. Similarly, median ESA doses were 8,000 units/week in Swedish HD patients and 4,000 units/week in Swedish

CKD-ND patients. The higher ESA doses in the US could be a reflection of different anemia management practices, whether or not influenced by the former reimbursement system in the US, and is an indication of incomparability in health care between Europe and US.

Aside from differences in ESA dose and analytical techniques used in observational studies, US studies usually have a higher sample size and therefore more power. Sample size and power limitations make it difficult to compare results from US with European anemia-correction trials. In a meta-analysis by Palmer *et al,* most weight is contributed by the 3 largest trials, which included only US or mostly US patients.[20] If estimates of non-US studies would be pooled, the overall result would probably be more neutral. Even more so, De Nicola et al. have shown considerable geographical differences in cardiovascular events in the two arms of the TREAT study, the largest trial that consisted of 60% US patients.[21] Whereas the overall trial showed no difference in the composite of cardiovascular events and mortality with darbepoetin versus placebo, the part of the study that was performed in Western Europe and Australia almost showed a protective effect of ESA treatment with a higher Hb target, with a hazard ratio of 0.66 (0.43-1.01) on the composite endpoint.[22] A very interesting finding, since the TREAT was the main indicator of a higher stroke risk in the higher Hb arm and thus with higher ESA doses. This is in contrast to our findings in **chapter 5**, where the effect of ESAs on stroke seemed even protective in our cohort of Dutch dialysis patients. Differences in health care system, anemia management including ESA-dosing practices and maybe patient population all raise questions about the generalizability of our and other European studies to the US and vice versa.

ESA resistance

The higher ESA doses used in the US could also be a reflection of a different patient population, with more ESA resistance.

Since up until now, no trials exist that randomly assign patients to high and low ESA doses, ESA dose is always a result of the doctor's prescription in reaction to the patient's response to ESAs. In other words, patients with high ESA doses are the patients that fail to respond to lower ESA doses, also called ESA resistance. In **chapter 8** we showed that ESA resistance is associated with mortality in both HD and PD patients. ESA resistance is associated with numerous comorbid conditions as well, among which malnutrition, inflammation, low iron stores and hyperparathyroidism.[23-26] All these conditions are also risk factors for mortality and ESA resistance can therefore be regarded as an indicator of disease severity. The association between high ESA doses and mortality could be all subject to confounding by indication. It is however remarkable that after adjustment for a wide range of comorbidities, the association between ESA resistance and mortality in **chapter 8** is still present. It is also postulated that the ESA resistance itself, thus the high ESA doses in the presence of inflammation, and perhaps high levels of soluble Epo receptor (EpoR), would insufficiently correct anemia while stimulating other possible EpoR on inflammatory cells, thereby inducing an even higher proinflammatory state.[21] This would provide a biological background for ESA resistance and the link with mortality.

Mechanism

A convincing biological mechanism would make a causal interpretation of the association between high ESA dose and mortality more plausible. However, the exact pathway from high ESA dose to the higher mortality or adverse event rate is largely unknown. There are several hypotheses, all of which are no sufficient explanations. First, by raising the patients' Hb, ESAs also increase blood viscosity. Higher viscosity could result in a higher risk of thrombotic events, but this is mainly reported for above normal hematocrit levels, for instance in patients with polycythemia vera.[27] The obtained hematocrit or Hb in ESA-treated patients with

CKD is not supraphysiologic, although in NECOSAD, Hb is measured before the dialysis session and will increase after fluid removal. Anemia or normal Hb levels are not known as risk factors for VTE in the general population.[28] In **chapter 5**, we also showed that higher achieved Hb levels in ESA-treated patients were not associated with an increased risk of thrombotic events and a dose-response effect favoring higher Hb levels was suggested. This is in concordance with results from the anemia-correction trials. The NHCT reported that mortality rates decreased with increasing hematocrit levels in both arms of the trial and secondary analyses of the CHOIR indicated that higher achieved Hb levels were not associated with worse outcomes.[2;29] Other observational studies did not indicate a higher risk of thrombotic events or mortality with higher Hb or hematocrit as well.[30;31] The role of achieved Hb in the pathophysiological mechanism seems therefore limited.

Another hypothesis is that ESAs stimulate thrombus formation, mainly through an increase in platelet counts and activation. Also in the context of high hematocrit, platelets will circulate more along the side of the blood vessels, favoring platelet and endothelial activation.[32;33] Reports are however inconsistent. Indeed, higher platelet reactivity and production in response to ESAs are reported in dogs and rats.[34;35] Increased thrombopoiesis, platelet reactivity and endothelial activation was reported in healthy men[36] and a shortening of bleeding time, transient rise in platelet count and platelet aggregation, decline in protein C and S, and rise in antithrombin III activity was reported in HD patients.[37] However, no difference was detected in platelet count and activity in healthy volunteers[38] and a reduction in platelet reactivity with darbepoietin was reported in mice.[39] The exact coagulant effect of ESAs therefore remains uncertain.

In addition, ESA treatment has been reported to induce elevated BP since the introduction late 1980s. Hypertension would be caused by reducing the hypoxia-associated

vasodilation and a modification of endothelial function, for instance resulting in a decrease in the vasodilating nitric oxide and an increase in the vasoconstrictive endothelin-1.[32;40;41] On the one hand, results in **chapter 3** confirm the hypertensive effect of ESAs, since ESA-treated patients more often receive antihypertensive medication and a trend towards a higher BP with high ESA dose is suggested. There was however, no difference in routinely measured BP between patients with and without ESAs and it is therefore questionable that the ESA-mediated BP effect could contribute to an increased cardiovascular risk.

The main proposed mechanisms would all lead to a higher risk of thrombotic or cardiovascular events. Indeed in **chapter 6** a relation between ESA treatment and cardiovascular events was shown in MM and MDS patients. However, **chapter 5** showed that in our population of dialysis patients from the Netherlands, there was no excess of thrombotic events in ESA-treated patients and no dose-response effect was present within ESA-treated patients. This lack of consistency could indicate that association is not causation in this case. However, different results of different analyses cannot disprove original evidence.[42] It could also be possible that ESAs have indeed different effects in different groups of patients, maybe through the interaction with comorbidities or other medications.

Furthermore, the outcome of the anemia-correction trials does not lead us to more understanding in the mechanism. These trials were designed to identify a cardiovascular benefit with ESA treatment towards higher Hb targets, as illustrated by their acronyms: 'Trial to Reduce cardiovascular events with Aranesp therapy (TREAT)' and 'Cardiovascular risk reduction by early treatment with epoetin beta (CREATE)'. Although overall higher Hb targets resulted in more of the composite cardiovascular endpoints, the difference between the two arms, if any, was not consistently caused by the same event. In the NHCT the total number of myocardial infarctions in

both groups were the same and the difference was caused by all-cause mortality.[2] CREATE assessed several events and the higher event rate in the higher Hb arm was distributed amongst transient ischemic attacks, peripheral vascular diseases and sudden death.[3] The difference in the CHOIR was mainly due to death and hospitalisations for congestive heart failure and there was no difference in myocardial infarction or stroke.[4] Last, no significant effect on the composite endpoint was found in the TREAT.[19] However, a higher stroke rate was reported in the darbepoietin treated group. Again, the lack of specificity hinders us to draw causal conclusions without hesitation, but does not prove there is none.[42] If ESA would have a harmful effect, this would merely imply a very divers and multifactorial pathway, not only limited to the hematopoietic or thrombotic effects, and probably not limited to the currently proposed mechanisms.

In cancer patients, ESA treatment irrespective of dose is also associated with a shorter survival. Aside from the increase in thrombotic events with ESA use, two other mechanisms have been proposed that could promote tumor progression and thereby decrease survival in this specific patient population.[43] Firstly, ESAs would induce angiogenesis, allowing the tumor to spread and grow. Secondly, ESAs could activate EpoRs that exist on the surface of various cancer cells. Stimulation of these EpoRs could also possibly stimulate tumor growth. However, the exact purpose of EpoRs and the ESA-induced pathway still need to be elucidated. Up until now, antibodies detecting EpoR were non-specific and cross-reacted with multiple other proteins.[32] The EpoCan consortium has developed novel specific EpoR antibodies, which provides a very important basis for future studies to unravel the effects of ESAs.[44]

In fact, EpoR plays a crucial role in one final hypothesis. Treatment with ESA results in an unphysiologic rapid rise in plasma erythropoietin levels and a subsequent fast decline.[45]

The effects of these spikes of erythropoietin in patients with CKD or cancer are virtually unknown.[33] It has been described that the EpoR in the bone marrow, responsible for the hematopoietic effects of erythropoietin, is different from the EpoR found in other tissues, such as the myocardium, brain, retina and vascular endothelium.[46] It has been proposed that the first is a homodimer that can be activated with very low concentrations of ESAs. However, the second would be a low-affinity heterodimeric receptor that is only activated with high ESA doses. Since the exact location and action of these EpoRs remains to be elucidated, the clinical consequences are unknown. Thus, it is not impossible that the non-hematopoietic effects could play a central role in the adverse events with high ESA doses.[33;47]

Implications and recommendations

Taking everything together, the one consistent finding of the anemia-correction trials in CKD patients is a similar to worse overall mortality and event rate with higher Hb targets. Since achieving survival benefit seems illusory and evidence for further improvement in quality of life is weak, higher Hb targets should not routinely be aimed for. However, since achieved higher Hb levels do not seem to be related with adverse events, it seems unlikely that the Hb levels are the cause of the unexpected higher event rate in the higher Hb arm of the anemia-correction trials. Therefore, the higher ESA doses or ESA resistance remain a subject of debate. The current guidelines recommend to treat with the lowest ESA dose possible to avoid blood transfusions. Given the first positive experiences with low dose ESA treatment and especially the risk of inducing alloantibodies with recurrent blood transfusions in patients possibly waiting for a kidney transplant, this seems like a wise strategy. The main challenge will be to discriminate the patients with a positive from a negative risk-benefit balance. Meanwhile, randomized trails such as the C.E. DOSE trial[48] should establish whether high

ESA doses indeed cause more mortality or adverse events.
As indicated in this thesis, the assumed mechanism of ESA-induced adverse events is far from complete. Further research should first focus on identifying the expression of EpoR in various tissues. Then, when expression of EpoR is identified, the effect of stimulation of this receptor by ESAs needs to be established. Furthermore, it should be evaluated whether the effect of ESAs is dose-dependent or maybe has a certain threshold in different tissues. In addition, the presence of soluble EpoR should be verified and quantified in both healthy volunteers and patients with CKD. In patients with CKD, it should be investigated whether ESA-resistant patients have higher levels of soluble EpoR and if inflammation and ESA administration affect the level of soluble EpoR.

References

1. Vandenbroucke JP. Why do the results of randomised and observational studies differ? BMJ 2011; 343: d7020
2. Besarab A, Bolton WK, Browne JK et al. The effects of normal as compared with low hematocrit values in patients with cardiac disease who are receiving hemodialysis and epoetin. N Engl J Med 1998; 339: 584-590
3. Drueke TB, Locatelli F, Clyne N et al. Normalization of hemoglobin level in patients with chronic kidney disease and anemia. N Engl J Med 2006; 355: 2071-2084
4. Singh AK, Szczech L, Tang KL et al. Correction of anemia with epoetin alfa in chronic kidney disease. N Engl J Med 2006; 355: 2085-2098
5. Robins JM, Hernan MA. Estimation of the causal effects of time-varying exposures. In: Fitzmaurice G, Davidian M, Verbeke G, Molenberghs G, eds. Longitudinal Data Analysis. Chapman & Hall/CRC, Boca Raton: 2009; 553-597
6. Hernan MA, Robins JM. Inverse Probability Weighting. Causal Inference. 2012; 20-24
7. Gran JM, Roysland K, Wolbers M et al. A sequential Cox approach for estimating the causal effect of treatment in the presence of time-dependent confounding applied to data from the Swiss HIV Cohort Study. Stat Med 2010; 29: 2757-2768
8. Greenland S. Introduction to Regresison Modeling. In: Rothman KJ, Greenland S, Lash TL, eds. Modern Epidemiology. Lippincott Williams & Wilkins, Philadelphia: 2008; 444-446
9. Cole SR, Hernan MA. Constructing inverse probability weights for marginal structural models. Am J Epidemiol 2008; 168: 656-664
10. Rothman KJ. Biases in Study Design. Epidemiology: An Introduction. Oxford University Press, New York: 2002; 96-98
11. Donders AR, van der Heijden GJ, Stijnen T, Moons KG. Review: a gentle introduction to imputation of missing values. J Clin Epidemiol 2006; 59: 1087-1091
12. de Goeij MC, van Diepen M, Jager KJ, Tripepi G, Zoccali C, Dekker FW. Multiple imputation: dealing with missing data. Nephrol Dial Transplant 2013; 28: 2415-2420

13. Zhang Y, Thamer M, Cotter D, Kaufman J, Hernan MA. Estimated effect of epoetin dosage on survival among elderly hemodialysis patients in the United States. Clin J Am Soc Nephrol 2009; 4: 638-644
14. Zhang Y, Thamer M, Kaufman JS, Cotter DJ, Hernan MA. High doses of epoetin do not lower mortality and cardiovascular risk among elderly hemodialysis patients with diabetes. Kidney Int 2011; 80: 663-669
15. Weinhandl ED, Gilbertson DT, Collins AJ. Association of mean weekly epoetin alfa dose with mortality risk in a retrospective cohort study of medicare hemodialysis patients. Am J Nephrol 2011; 34: 298-308
16. Wang O, Kilpatrick RD, Critchlow CW et al. Relationship between epoetin alfa dose and mortality: findings from a marginal structural model. Clin J Am Soc Nephrol 2010; 5: 182-188
17. Bradbury BD, Do TP, Winkelmayer WC, Critchlow CW, Brookhart MA. Greater Epoetin alfa (EPO) doses and short-term mortality risk among hemodialysis patients with hemoglobin levels less than 11 g/dL. Pharmacoepidemiol Drug Saf 2009; 18: 932-940
18. Yang W, Joffe MM, Feldman HI. Exploring the effect of erythropoietin on mortality using USRDS data. Pharmacoepidemiol Drug Saf 2013; 22: 593-606
19. Pfeffer MA, Burdmann EA, Chen CY et al. A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease. N Engl J Med 2009; 361: 2019-2032
20. Palmer SC, Navaneethan SD, Craig JC et al. Meta-analysis: erythropoiesis-stimulating agents in patients with chronic kidney disease. Ann Intern Med 2010; 153: 23-33
21. de Nicola L, Locatelli F, Conte G, Minutolo R. Responsiveness to erythropoiesis-stimulating agents in chronic kidney disease: does geography matter? Drugs 2014; 74: 159-168
22. National Kidney Foundation. KDOQI Clinical Practice Guideline and Clinical Practice Recommendations for anemia in chronic kidney disease: 2007 update of hemoglobin target. Am J Kidney Dis 2007; 50: 471-530
23. Gunnell J, Yeun JY, Depner TA, Kaysen GA. Acute-phase response predicts erythropoietin resistance in hemodialysis and peritoneal dialysis patients. Am J Kidney Dis 1999; 33: 63-72

24. Kalantar-Zadeh K, McAllister CJ, Lehn RS, Lee GH, Nissenson AR, Kopple JD. Effect of malnutrition-inflammation complex syndrome on EPO hyporesponsiveness in maintenance hemodialysis patients. Am J Kidney Dis 2003; 42: 761-773
25. Gaweda AE, Goldsmith LJ, Brier ME, Aronoff GR. Iron, inflammation, dialysis adequacy, nutritional status, and hyperparathyroidism modify erythropoietic response. Clin J Am Soc Nephrol 2010; 5: 576-581
26. Kalantar-Zadeh K, Lee GH, Miller JE et al. Predictors of hyporesponsiveness to erythropoiesis-stimulating agents in hemodialysis patients. Am J Kidney Dis 2009; 53: 823-834
27. Kwaan HC, Wang J. Hyperviscosity in polycythemia vera and other red cell abnormalities. Semin Thromb Hemost 2003; 29: 451-458
28. Rezende SM, Lijfering WM, Rosendaal FR, Cannegieter SC. Hematologic variables and venous thrombosis: red cell distribution width and blood monocyte count are associated with an increased risk. Haematologica 2014; 99: 194-200
29. Szczech LA, Barnhart HX, Inrig JK et al. Secondary analysis of the CHOIR trial epoetin-alpha dose and achieved hemoglobin outcomes. Kidney Int 2008; 74: 791-798
30. Ma JZ, Ebben J, Xia H, Collins AJ. Hematocrit level and associated mortality in hemodialysis patients. J Am Soc Nephrol 1999; 10: 610-619
31. Regidor DL, Kopple JD, Kovesdy CP et al. Associations between changes in hemoglobin and administered erythropoiesis-stimulating agent and survival in hemodialysis patients. J Am Soc Nephrol 2006; 17: 1181-1191
32. Jelkmann W, Elliott S. Erythropoietin and the vascular wall: the controversy continues. Nutr Metab Cardiovasc Dis 2013; 23 Suppl 1: S37-S43
33. Singh AK. What is causing the mortality in treating the anemia of chronic kidney disease: erythropoietin dose or hemoglobin level? Curr Opin Nephrol Hypertens 2010; 19: 420-424
34. Wolf RF, Peng J, Friese P, Gilmore LS, Burstein SA, Dale GL. Erythropoietin administration increases production and reactivity of platelets in dogs. Thromb Haemost 1997; 78: 1505-1509
35. Berridge MV, Fraser JK, Carter JM, Lin FK. Effects of recombinant human erythropoietin on megakaryocytes and on platelet production in the rat. Blood 1988; 72: 970-977

36. Stohlawetz PJ, Dzirlo L, Hergovich N et al. Effects of erythropoietin on platelet reactivity and thrombopoiesis in humans. Blood 2000; 95: 2983-2989
37. Malyszko J, Malyszko JS, Borawski J et al. A study of platelet functions, some hemostatic and fibrinolytic parameters in relation to serotonin in hemodialyzed patients under erythropoietin therapy. Thromb Res 1995; 77: 133-143
38. Roest M. Personal Communication during EpoCan meeting. 2013.
39. Lindenblatt N, Menger MD, Klar E, Vollmar B. Darbepoetin-alpha does not promote microvascular thrombus formation in mice: role of eNOS-dependent protection through platelet and endothelial cell deactivation. Arterioscler Thromb Vasc Biol 2007; 27: 1191-1198
40. Vaziri ND. Mechanism of erythropoietin-induced hypertension. Am J Kidney Dis 1999; 33: 821-828
41. Briet M, Barhoumi T, Mian MO et al. Effects of recombinant human erythropoietin on resistance artery endothelial function in stage 4 chronic kidney disease. J Am Heart Assoc 2013; 2: e000128
42. Hill AB. The Environment and Disease: Association or Causation? Proc R Soc Med 1965; 58: 295-300
43. Oster HS, Neumann D, Hoffman M, Mittelman M. Erythropoietin: the swinging pendulum. Leuk Res 2012; 36: 939-944
44. Maxwell P, Melendez-Rodriguez F, Matchett KB et al. Novel antibodies directed against the human erythropoietin receptor: creating a basis for clinical implementation. Br J Haematol 2014;
45. Fishbane S, Besarab A. Mechanism of increased mortality risk with erythropoietin treatment to higher hemoglobin targets. Clin J Am Soc Nephrol 2007; 2: 1274-1282
46. Arcasoy MO. The non-haematopoietic biological effects of erythropoietin. Br J Haematol 2008; 141: 14-31
47. Brines M. The therapeutic potential of erythropoiesis-stimulating agents for tissue protection: a tale of two receptors. Blood Purif 2010; 29: 86-92
48. Strippoli GF. Effects of the dose of erythropoiesis stimulating agents on cardiovascular events, quality of life, and health-related costs in hemodialysis patients: the clinical evaluation of the dose of erythropoietins (C.E. DOSE) trial protocol. Trials 2010; 11: 70