Chapter 1

General introduction

Introduction

Anemia is characterized by a decrease in the amount of hemoglobin (Hb) in the blood. Hb is an iron containing protein, which serves as the oxygen binding site of red blood cells and is responsible for the red color. Anemia can be caused by a decreased production of red blood cells, an increased breakdown of red blood cells, or blood loss.

Already at the end of the 19th century it was generally accepted that the rate of red blood cell production was stimulated by hypoxia.[1] Although the idea of a hormonal regulation of this erythropoiesis was proposed 1906 by Carnot and Deflandre,[2] it took several decades before the mechanism was identified and the concept was accepted. Bonsdorff and Jalavisto introduced the name erythropoietin for the hormone, when they demonstrated an increase in red blood cells in rabbits after the injection of plasma from hypoxic patients.[3] In 1949 Oliva *et al.* were the first to show the erythropoietic activity of exogenous erythropoietin in humans.[4] Another important step was made in 1957, when Jacobson *et al.* discovered that erythropoietin was produced by the kidney. Taking everything together, it was concluded that erythropoietin is mainly produced by the kidney in response to hypoxia or anemia to stimulate the production of red blood cells in the bone marrow.[5]

It then became likely that anemia of chronic kidney disease (CKD) was caused by a deficient production erythropoietin by the kidney and that this anemia would respond to treatment with erythropoietin.[1] Unfortunately, erythropoietin is only present in extremely small concentrations in plasma and it proved difficult to purify erythropoietin. After decades of research the group of Goldwasser managed to purify just a few milligrams of erythropoietin from more than 2500 liters of urine from patients with aplastic anemia.[6] The amino acid sequence enabled to identify and clone the gene and

eventually to produce recombinant human erythropoietin. Finally, in 1986 Winearls et al, and a year later Eschbach et al, successfully treated anemic patients with CKD with recombinant human erythropoietin in clinical trials.[7;8]

The development of recombinant human erythropoietin and the introduction into clinical practice has been a major breakthrough in the anemia management of patients with CKD. In addition to recombinant human erythropoietin (epoetine alfa and beta), other types of erythropoiesis-stimulating agents (ESAs) have been developed, such as darbepoetin. Darbepoetin has similar properties but a longer halflife, and was introduced in 2001. Nowadays, as much as 90% of dialysis patients are treated with ESAs, based on Hb levels.[9] Although responsiveness to ESAs varies among patients, a major reduction in the need for red blood cell transfusions was achieved.[8] Systematic reviews also showed that ESA treatment improved energy, fatigue and physical function in patients with CKD.[10-12] Aside from an improved quality of life, ESA treatment was even associated with a decrease in cardiovascular mortality.[8;13;14]

In an attempt to further improve quality of life and achieve survival benefit, target Hb with ESA treatment slowly increased over the years, and therewith ESA doses.[15] Several randomized controlled trials were conducted to evaluate the effect of higher Hb target levels with ESA treatment. The results of these anemia-correction trials were, however, unexpected. In 1998, results of the NHCT were published, in which chronic HD patients with cardiac comorbidity were randomly assigned to ESA treatment with a high Hb target of 13-15 g/dl or a low Hb target of 9-11 g/dl.[16] The study was halted early and reported a 1.3- fold increased rate of death and myocardial infarction in patients assigned to the high Hb target arm. In 2006 two large trials in pre-dialysis patients (CREATE and CHOIR) were published. CHOIR also reported a 1.3-fold increased risk of the composite endpoint of death,

myocardial infarction, congestive heart failure hospitalization and stroke.[17] CREATE showed a non-significant trend towards a higher rate of a first cardiovascular event in the group with an Hb target of 13-15 g/dl compared with 10.5-11.5 g/dl.[18] The largest trial (TREAT) comprised pre-dialysis patients with type 2 diabetes mellitus and was published in 2009.[19] This trial compared darbepoetin-alfa treatment with a target Hb of 13 g/dl with placebo treatment, in which rescue ESA therapy could be given when Hb fell below 9 g/dL. Although no difference in the primary composite outcome of death and cardiovascular events was found, a significant 1.9-fold higher stroke rate was reported with ESA treatment. In addition, meta-analysis showed only weak evidence of a small and clinically irrelevant improvement in health-related quality of life with high hb targets compared to lower Hb targets.[20]

In summary, the anemia-correction trials indicated that ESA treatment with higher Hb targets increased the risk of death or stroke.[21;22] These trials therefore 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. It has been hypothesized that ESAs increase arterial blood pressure, alter endothelial function and may result in pro-thrombotic changes,[23] all contributing to a higher risk of cardiovascular events. However, the trials were designed to identify the optimal target Hb and not to evaluate the safety of ESA treatment. The NHCT reported that mortality rates decreased with increasing hematocrit values in both arms of the trial and no relationship between erythropoietin dose and mortality was found.[16] Secondary analyses of CHOIR also indicated that higher achieved Hb levels were not associated with worse outcomes.[24] But they also showed that within each arm, patients receiving high ESA doses had a 6% higher event rate, than patients with low ESA doses. Although the role of ESAs was thus not entirely clarified, it all resulted in a series of warnings in the ESA label by the US Food and drug administration (FDA), starting at the end of 2006.

Several observational studies were therefore conducted to estimate the effect of high ESA doses on mortality, but results were inconclusive. Some studies indeed associated high ESA doses with higher mortality rates.[25;26] However, this association seems highly sensitive to the analytic method and the adequacy of handling time-dependent confounding.[27] But even recent studies with advanced analytic techniques reported conflicting results. Effect estimates ranged from a beneficial effect of ESAs in severely anemic patients, no effect of high ESA doses, to an increase in mortality up to 1.3-fold.[28-33] Meta-regression analyses of anemia-correction trials concluded that higher ESA dose might be associated with all-cause mortality and cardiovascular complications.[34]

Since no trials have been published that randomly assigned patients to a high or low ESA dose, it is still unknown whether the high ESA dose itself or the need for treatment with high ESA doses is related to adverse outcomes. Patients with an inadequate hematopoietic response require higher ESA doses and are referred to as ESA-resistant. A major cause of ESA resistance is iron deficiency, since iron is an essential part of Hb. Iron status should therefore always be evaluated in ESA-resistant patients and supplemented if needed. In addition, ESA-resistant patients generally have more comorbidities, inflammation and malnutrition[35;36] and thus a worse prognosis. The association between high ESA doses and higher adverse event rates might therefore be attributed to confounding by indication. On the other hand, it is speculated that higher ESA doses could induce a release of inflammatory cytokines especially in the ESA-resistant patients, causing mortality and morbidity.[37]

Meanwhile, ESAs were also used for the treatment of chemotherapy associated anemia in oncology patients. Again, decreased transfusion rates and some evidence of improved quality of life with ESA treatment were reported.[38;39] However, several trials identified mortality and tumor progression

risks in chemotherapy or radiation therapy treated cancer patients compared to patients without ESA. Meta-analyses indicated mortality risks up to 1.17-fold[40;41] and VTE risk up to 1.69-fold.[38;41;42] Nowadays, ESA treatment is therefore not recommended in cancer patients with potentially curative disease.[43;44] ESA treatment is restricted to patients with palliative chemotherapy and to certain patients with hematologic malignancies, and can be started when Hb is below 10 g/dl.[45]

For patients with CKD, the FDA now recommends the lowest dose of ESAs sufficient to prevent red blood cell transfusions and ESA treatment should only be considered when Hb is less than 10 g/dL.[46] These recommendations are affirmed by the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guideline for anemia in CKD patients.[47] A European Renal Best Practice (ERBP) position statement mostly agrees with the published KDIGO guidelines, although it suggests a more liberal target Hb range between 10-12 g/dL, the target that has been removed from the FDA label.[48] It also proposes more flexibility in starting ESA treatment at Hb levels above 10 g/dL, tailored at the individual patient. In this decision, the risks of ESA therapy but also the patient's comorbidities, ischemic symptoms and expected benefit in quality of life should be taken into account. The current Dutch anemia guideline for CKD is based on these KDIGO and ERBP communications.[49] None of the guidelines provide any firm restriction to the administered ESA dose, although caution is warranted in patients hyporesponsive to ESA treatment. One of the strongest recommendations concerns CKD patients with active malignancy or a history of malignancy or stroke. ESA therapy should be used with great caution in these patients, if used at all.

Aim and outline of this thesis

Taking everything together, the debate about the safety of ESAs and especially high doses of ESAs remains. In 2011, A European FP-7 project named EpoCan was therefore funded to address these safety concerns. The EpoCan consortium combined researchers in erythropoietin physiology, cell signalling, mouse models, hemostasis and oncology with epidemiologists and physicians. The research presented in this thesis is mostly performed as part of the EpoCan project.

The main aim was thus to investigate the potential cardiovascular and mortality risks related to ESA use. As the studies described in this thesis are all observational, the presence of confounding should be carefully considered in order to make causal inferences. **Chapter 2** therefore gives an introduction to the use of Directed Acyclic Graphs (DAGs) as a tool in the identification of confounding. Before investigating the ESA-related cardiovascular risks, more insight in the anemia management of patients with CKD in the last years was needed. Hence, trends in Hb and ESA use are described in Swedish hemodialysis and CKD patients in **chapter 3**. Subsequently, proposed ESA-related cardiovascular events were investigated. ESA-induced hypertension has already been reported at the introduction of ESA therapy late 1980s and is one of the hypothesized mechanisms causing the ESA-associated cardiovascular events and mortality. However, anemia management has changed over the years and aimed at a more gradual increase in Hb with slow ESA dose escalations to reduce this side effect. The effect of ESAs on blood pressure in a recent cohort of pre-dialysis patients is examined in **chapter 4**. The hypothesized ESA-induced elevation of blood pressure, together with endothelial changes and platelet activation with ESA use, has been proposed to lead to a higher risk of thrombotic events. The ESA-associated risk of arterial thrombosis is mainly inferred from composite endpoints of anemia-correction trials. A higher risk of venous thrombosis

with ESA use has only been reported in oncology patients. **Chapter 5** therefore examines the relation between ESA use and myocardial infarction, ischemic stroke and venous thrombosis in dialysis patients. In addition, **chapter 6** examines the relation between ESA use and myocardial infarction, stroke and venous thrombosis in patients with multiple myeloma and myelodysplastic syndrome. A higher risk of thrombotic events with ESA treatment is also the main explanation for a higher mortality risk with ESA use. However, secondary analyses of anemia-correction trials did not consistently indicate that higher ESA doses were associated with mortality and results from observational studies are inconclusive. **Chapter 7** thus investigates the effect of high ESA dose on mortality in dialysis patients. Alternatively, it has been proposed that the association between high ESA dose and mortality is a representation of the need to administer high ESA doses in patients with an inadequate hematopoietic response and is thus caused by ESA resistance. The association between ESA resistance and mortality is investigated in hemodialysis and peritoneal dialysis patients and shown in **chapter 8**. Finally, in **chapter 9** all results are summarized and discussed.

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