The Possible Role of Testosterone in the Pathogenesis of an Unexplained Anaemia: 
An Analysis of the Heinz Nixdorf Recall Study Cohort

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1 Introduction

1.1 Preface

Anaemia is a condition in which either the total count of blood cells or the haemoglobin content of the blood is lower than normal (WHO, 1968) and thus insufficient to meet the physiological needs of the body. Individual needs vary depending on gender, age, residential altitude, smoking status and stage of pregnancy (WHO, 2011). The physiological details of anaemia will be discussed later, however in brief the essence is that the body cannot develop and perform optimally if the red blood cells don’t supply the metabolism with oxygen sufficiently (Stevens, 2013). Previous research has established that anaemia is a risk factor for poor health, cognitive impairment and mortality (Culleton et al., 2006; Denny et al., 2006; Riva et al., 2008). The causes for anaemia are diverse and include nutritional deficiencies of iron, vitamin B12 and folate acid (due to e.g. malnutrition, chronic inflammatory bowel disease or adverse effects of gastric and intestinal surgery), chronic inflammation and chronic kidney failure. However, particularly in older men and women, a considerable share of anaemia cases cannot be attributed to any of the recognised causes for anaemia and has thus been labelled unexplained. Low testosterone levels have been assigned a potential role in the pathogenesis of this - so far - unexplained anaemia. While anaemia in children and women has been intensively examined by various studies globally, it is only in recent years that anaemia in the elderly has become a popular research topic. The objective of this study is therefore to quantify anaemia in an elderly representative population in Germany, to identify possible underlying causes and to explore whether low testosterone is a risk factor for developing anaemia.
1.2 Related Literature

1.2.1 Epidemiology of Anaemia

The World Health Organisation (WHO) has defined anaemia in the year 1968 as present when blood level haemoglobin is lower than 12 g/dl for women and 13 g/dl for men - an old but still used definition in present research. As iron deficiency, one of the main causes of anaemia, is the most widespread nutritional disorder worldwide and very often affects children, iron deficiency anaemia has been examined in the past as global health issue (WHO, 2000). With the publication of the WHO nutritional report in 1968, anaemia became a popular topic on the global health agenda. Various studies have shown that anaemia in infancy adversely affects development and results in cognitive and motor deficits, which may persist later in life (Carter et al., 2010). Between 1993 and 2005, one out of four people was affected by anaemia globally (McLean, 2008). Splitting the countries according to development status\(^1\), 42.7% of people in all low-income countries taken together were anaemic. In medium-income countries 25.6% of the people were anaemic and in high-income countries only 9.1%. Globally, children and women are at greatest risk (McLean et al., 2013). Subsequently, the World Health Assembly has defined a 50% reduction in anaemia in women as the second of six global nutrition targets to be fulfilled by the year 2025 (WHO, 2014). On the basis of this target, a lot of studies on anaemia have been conducted in the last years. However, little data on anaemia in the high-income countries have been collected. Information on the aged dependency of prevalence was added by Kassebaum et al. in 2013 who used publicly available data from 1990 to 2010 to quantify anaemia cases in 187 countries. Prevalence was again lowest in high-income countries (in all cases less than 25%). Interestingly, age patterns of anaemia were qualitatively similar in all countries and

\(^1\)Level of development, as classified by the United Nations Development Programme (UNDP) Human Development Index (HDI): high (HDI score: >0.80), medium (HDI score: 0.50–0.80), low (HDI score: <0.50)
for most causes: Kassebaum et al. (2013) found that prevalence among women was high in younger ages and decreased with age, while prevalence for men was low in younger ages and increased in older age groups above the level of women. The recognition that the anaemia prevalence among men not only increases with age but also exceeds women’s anaemia prevalence in the elderly was important. So far, it was believed that particularly women and children were vulnerable to anaemia. Further information was added by 2013 Stevens et al. (2013) who compared haemoglobin data from 107 countries from the years 1995 with 2011. In contrast to almost all other countries, there was no upward trend and no improvement of anaemia prevalence seen in the high-income countries compared to low-income countries. The prevalence of anaemia remained on the same level in 2011 as it was in 1995 (11% in children <15 years, 16% in non-pregnant women aged 15-49, 22% in pregnant women) (Stevens et al., 2013). It is particularly worrisome that the existing prevalence of 22% in pregnant women had not decreased at all over a period of 15 years. Since the study did not target elderly people no statement could be made about the development of prevalence in older age groups. However, in the National Health and Nutrition Examination Survey III (NHANES III), Guralnik et al. (2005) found the prevalence of anaemia to be rising with age so high, that they stated a “public health crisis in haematology with need for attention and solutions.

1.2.2 Demographic Change and Challenge in Germany

In high-income countries anaemia affects, according to McLean et al. (2008) 9.1% of the general population. Among the elderly (≥ 60 years) anaemia prevalence in these countries was found to rise over 12%. Germany, like most other high-income countries reflects an ageing society. Significantly, 26% (21.49 million people) of the German population were aged 60 years and older in 2010 (Statistisches Bundesamt, 2011). According
to estimations of the federal bureau of statistics, 39% of the German population will be 60 years and older by the year 2050 (Statistisches Bundesamt, 2018). If anaemia is a menace for older people, as proposed by various studies, the prevalence and consequences in Germany need to be examined and included into health care planning.

1.2.3 Adverse Effects - Research on Morbidity and Mortality of Anaemia

As briefly touched upon above, the perception of anaemia has changed over the last years in terms of being no longer seen solely as a symptom for underlying diseases (Roig et al., 2005). Anaemia on its own might be a risk factor for heart failure, first cardio-vascular hospitalisation, first all-cause hospitalisation, cognitive impairment and mortality (Culleton et al., 2006; Denny et al., 2006; Riva et al., 2008). Grammer et al., 2014, found that low haemoglobin and iron depletion were both independently associated with coronary artery disease (CAD) (Grammer et al., 2014). In a study with people aged 66 and older with normal kidney function, the risk for death increased threefold in anaemic patients and the risk for mortality was lowest with a haemoglobin level between 13 - 15 g/dl for women and 14 - 17 g/dl for men (Culleton et al., 2006). In an intensive care monitored patients study the nadir haemoglobin $< 9$ g/dl was found to be an independent predictor of increased mortality and length of hospitalisation (Fraenkel et al., 2015). In a study on physical performance and muscle strength, anaemic people displayed a higher number of disabilities, worse physical performance and lower strength (hand-grip and knee-extensor strength) compared to non-anaemic participants (Penninx et al., 2004). Thus, if anaemia exhibits a risk factor for morbidity and mortality (Penninx et al., 2006), it can contribute to increased private and societal costs and needs to be further investigated (Smith, 2010).
1.2.4 Testosterone and Anaemia

Erythrocytosis is a long known adverse effect of testosterone therapy and there is evidence that the dose dependence of this effect increases with age (Coviello et al., 2007). Specifically, Coviello et al. found that the older the person, the higher the influence of the same dose of testosterone on erythropoiesis. However, the mechanisms of testosterone impact on erythropoiesis are not fully understood yet (Sperling et al., 2006).

Testosterone in the adult seems to have a stimulating impact on erythropoietin (EPO) synthesis in the kidneys and on erythropoiesis in the bone marrow. However, evidence is not clear (Bachman et al., 2013). Bachman et al. (2013) found that EPO levels increase initially at the beginning of testosterone therapy, however decline soon after. A right shift of the EPO/haemoglobin graph could be observed during testosterone treatment and indicates a more effective activity of erythropoietin under higher testosterone levels (Bachman et al., 2013). What remains unclear is the pathway and whether or not a lack of testosterone plays a particular role in the pathogenesis of unexplained anaemia.

As the discovery of hepcidin was important to understand the pathomechanisms of relative iron deficiency, it might as well play an important role in the connection of testosterone and erythropoiesis. Bachman et al. (2013) found after 20 weeks of treatment with supraphysiological doses of testosterone in healthy men, that hepcidin was suppressed and suppression increased with dosage of testosterone, while changes of EPO levels were equal for all doses of testosterone (Bachman et al., 2013). Older men had higher hepcidin levels at baseline and a relatively higher response rate to testosterone, meaning that hepcidin levels decreased more in old than in young men. Maggio et al. (2013) applied testosterone patches for 36 months on 96 older men and measured a significant increase of haemoglobin while EPO levels remained stable, indicating that the haematopoietic effect of testosterone is not mediated by the quantity of EPO. Studies with elderly mice showed similar results and found a suppression of hepcidin, upreg-
ulation of ferroportin and no effect on EPO in female, male and castrated male mice after testosterone treatment (Guo et al., 2014). Roy et al. (2017) conducted a randomised controlled trial with 788 men 65 years or older of whom 126 were anaemic and applied testosterone gel or placebo for 12 months. A reduction of anaemia cases in the testosterone group could be observed compared to the control group. The study gave evidence that a testosterone screening in the elderly might be beneficial for the prevention of cases of unexplained anaemia in the elderly (UAE), whether the mechanism is known or not. One known difference in testosterone metabolism between younger and older males is the loss of the circadian rhythm in older men (Bremner et al., 1983). While in young men serum testosterone levels are highest in the morning and decline throughout the day, there is evidence that serum testosterone levels are lower in old men, however remain stable throughout the day. This difference is important to consider when interpreting results and comparing serum levels between people. Ferrucci et al. (2006) used data from the InCHIANTI study and included non-anaemic people 65 years and older with GFR > 30ml/min in a longitudinal analysis. The research group hypothesised that low testosterone levels were more strongly associated with UAE than with anaemia of a known cause. Relative risks for development of anaemia associated with total and bioavailable testosterone levels were calculated in the lowest compared to the other quartiles of testosterone levels. Independent of age, the prevalence of unexplained anaemia was significantly higher in the lowest testosterone quartile. The study concluded that older people with low testosterone levels were more likely to have anaemia and have a higher risk of developing anaemia after a three year follow-up. Furthermore the testosterone-anaemia association was stronger in women than in men, however the mechanisms of testosterone influence still remained unclear (Ferrucci et al., 2006). According to Bachman et al. (2013) there are four possible pathways of testosterone effect on erythropoiesis:
(i) Inhibition of hepcidin synthesis in the liver

(ii) Increased EPO production in the kidney

(iii) Increased EPO activity

(iv) Increased estradiol level (due to more transformation from testosterone) down-regulating the hepcidin transcription

The researchers concluded that testosterone increases the bioavailability of iron and the biological activity of EPO, meaning that there is a new EPO "set point" at a higher physiological level of haemoglobin, mediated through testosterone.

Between the fourth and the seventh decade, a yearly testosterone reduction of 1% in men can be observed (Sperling et al., 2006). Other authors assume a total reduction of about 30% between the age of 25 and 75 years (Diemer, 2005). The norm values usually applied are 11 to 40 nmol/l testosterone in serum. 20% of men aged 60 to 80 years display a diagnosed hypogonadism with testosterone serum levels below 12 nmol/L (Diemer, 2005). The definition of the testosterone deficiency syndrome (TDS) / secondary/age-associated hypogonadism includes the monitoring of testosterone, sexual hormone binding globulin and symptoms included in the Ageing Males’ Symptoms scale (AMS) (Lenk, 2005). However, exact prevalence of TDS still remains vague, as there is reason to believe that each person contains his or her own set-value needed for his or her well-being (Sperling et al., 2006). In addition, the extent to which decline in testosterone results from ageing remains controversial (Harman et al., 2001). For women, literature has not converged to a common understanding if testosterone rises or decreases with age (Reckelhoff et al., 2005). Lattrich et al. (2014) state that testosterone does not decline substantially in menopause compared to premenopause. According to their concept less sexual hormone binding globulin (SHBG) is built in the liver due to oestrogen deficiency and thus less testosterone bound to it. Through this mechanism, the share of free and
biologically effective testosterone increases and can lead to an androgenisation often observed in elderly women (Lattrich et al., 2014). Another opinion was reported by Jiroutek et al. (1998) who found a significant rise of 3.8% per year of androstenedione and testosterone in postmenopausal women. Overlie et al. (1999) found fluctuations in the testosterone levels around menopause with a decline before and slight increase after.

1.3 Pathophysiology of Anaemia

Anaemia is the clinical manifestation of reduced erythrocyte mass present in the body and is detected through measurement of low haemoglobin, low haematocrit or low quantity of erythrocytes (Herold, 2016). This study used the commonly used WHO definition, which defines the anaemia cut-off level of haemoglobin at lower than 13 g/dl for men and at lower than 12 g/dl for women (WHO, 1986). In haematology, diagnosis consists of a detailed examination of the erythrocyte morphology (such as the mean corpuscular volume (MCV) and the mean corpuscular haemoglobin concentration (MCHC)), the assessment of the haematocrit and haemoglobin, as well as a blood count (Halwachs-Baumann, 2011). As this study defines anaemia according to the WHO criteria, only haemoglobin will be of importance in this analysis.

The causes and clinical manifestations of anaemia are manifold. To further categorise the genesis of anaemia, it is helpful to briefly understand the different phases of erythropoiesis: Erythropoiesis describes the production and maturation of erythrocytes which after birth occurs mainly in the red bone marrow and endures for eight days (Halwachs-Baumann, 2011). After 120 days the red blood cell loses its function and is decomposed, mainly in the spleen and liver. The most important hormone in this process is erythropoietin, which is produced in the peritubular fibroblasts in the kidneys and stimulated by low renal partial pressure of oxygen (O₂) (Eckardt, 1998). In
the early erythropoietin dependent period of erythropoiesis, the hormone stimulates the doubling and maturation to proerythroblasts. In this phase, the cells have a narrow time slot to allocate a large quantity of nucleotides and need a high amount of cobalamin (vitamin B12) and folate in order to do so (American Journal of Kidney Diseases, 2006). In the late, iron-dependent period, haemoglobin is synthesised and reticulocytes, the direct precursors of erythrocytes, are released into the plasma. As mentioned above, haemoglobin is the main oxygen transporting protein and changes colour from light red to dark red when deoxygenated (Dörner, 2013).

One main component of haemoglobin is iron. The human body contains 50 mg/kg body weight and 35 mg/kg body weight iron for men and women, respectively. The organism’s iron stock can be divided into haem-iron (70%), depot iron bound intracellular to ferritin or hemosiderin (18%), functional iron, such as myoglobin and enzymes (12%) and transport iron bound to transferrin (0.1%). One can differentiate between absolute iron deficiency and functional iron deficiency, where iron stocks are normal, however mobilisation is not working and iron sequestration in macrophages can be observed. The latter process may be mediated by inflammation (Lefebvre, 2017) and will be discussed in the section on Anaemia of Chronic Inflammation (ACI).

Apart from the availability of the components, the production and stock of haemoglobin depends on other factors, such as smoking and the body mass index.

Herold (2016) defined four different origins for anaemia, that is

(i) formation problem, meaning malfunction of erythropoiesis, disorder in DNA or haemoglobin synthesis or lack of erythropoietin,

(ii) increased cellular decomposition of erythrocytes, due to different kinds of malfunction of the cells or immunological reactions,

(iii) bleeding, or
The aetiology categories examined in this study all belong to the group of formation problems (i): Renal anaemia, iron deficiency anaemia (IDA), vitamin B/ folic acid deficiency, anaemia of chronic inflammation (ACI) and unexplained anaemia (UA). In the following, I will outline the definitions and hypotheses on causes for these formation problems. The exact laboratory definitions used in this study to classify the aetiology categories will be described in the methods section, Chapter 2.3.

1.3.1 Renal Anaemia

Renal anaemia (RA) is a multifactorial process, the primary cause being erythropoietin deficit. In otherwise healthy but anaemic subjects EPO levels rise above normal when the blood haemoglobin is low and stimulates erythropoiesis. However, in cases of chronic kidney disease EPO levels remain low (Klinke et al., 2010). The result is a normochromic and normocytic but hyporegenerative anaemia, which evolves in the course of chronic renal insufficiency (CKD) (Serum creatinine > 3.5 mg/dl or creatinine clearance < 30 ml/min). The prevalence of anaemia rises significantly with decreasing glomerular filtration rate (GFR) and was reported to be 44.1% in the NHANES III study among people with an estimated GFR between 15 and 29 mL/min (Astor et al., 2002). Apart from kidney failure, other factors, such as iron deficiency, inadequate dialysis, aluminium overload or bone marrow fibrosis due to hyperparathyroidism, can also play a role in the development and treatment of renal anaemia.

1.3.2 Iron Deficiency Anaemia (IDA)

(Absolute) Iron deficiency anaemia (IDA) is the most frequent anaemia, with an overall prevalence of 10% in Europe and a prevalence of more than 50% in women of reproductive age in Europe (Herold, 2016). Worldwide 80% of anaemia cases are due to
Iron deficiency (WHO, 2001). IDA is characterised as a hyporegenerative, microcytic, hypochromic anaemia.

In the following, I will briefly introduce the main actors of iron metabolism with regard to blood production that will be relevant for this analysis. Further information can be found in Klinke et al. (2010) and Behnisch et al. (2016).

Orally ingested, bivalent iron is absorbed into the duodenal and jejunal enterocytes and released into the portal vein by the transport protein ferroportin. After oxidation, the trivalent iron is bound to transferrin and circulates through the body. Normally, 15 to 45% of transferrin are saturated with iron. A transferrin saturation lower than 16% suggests an iron deficiency (Thomas et al., 2005). The transferrin receptor then absorbs the iron bound transferrin into the cell, where iron is separated from transferrin and processed in the cytoplasm or distributed further to the mitochondria. In situations of low iron, the transferrin receptors are upregulated and can be measured in the serum in form of elevated soluble transferrin receptors. Excessive iron is bound to ferritin and serves as storage. Thus, a low ferritin signifies low storage iron. It has to be considered that ferritin is an acute phase protein and thus a high value of ferritin does not automatically imply a sufficient iron metabolism. The ferritin index is defined as the ratio of soluble transferrin receptor and the logarithm of ferritin. It is high when iron is deficient and has proven itself to be a useful indicator for the iron supply of erythropoiesis (Thomas et al., 2005).

A very sensitive parameter to detect early stages of iron deficiency is the reticulocyte haemoglobin content. In combination with the ferritin index, it can help to distinguish between absolute and functional iron deficiency and thus between iron deficiency anaemia and anaemia of chronic inflammation (which will be explained below), especially when biochemical markers such as ferritin and transferrin saturation are not informative (Thomas et al., 2005).
A key role in the systemic iron homoeostasis is played by hepcidin. It down regulates the iron supply by decomposing ferroportin and inhibits the release of iron reservoirs (e.g. from macrophages). Loss-of-function mutations in genes which regulate the expression of hepcidin have been associated with iron overload syndromes (Ganz, 2011). However, data on hepcidin was not available in this study and will thus not be considered in the statistical analysis.

Globally, the main reason for inadequate iron supply is malnutrition (WHO, 2001). The recommended daily iron intake is about 12 mg for men, 15 mg for menstruating women and 30 mg for pregnant women. However, only 1 to 2 mg are absorbed in the jejunum and duodenum (Thomas et al., 2005). This corresponds to the normal daily loss resulting from ex-foliated epithelium and bleeding. In Europe, 80% of IDA is caused by iron loss, most frequent in women with the main cause being menorrhagia (Herold, 2016). Other reasons for loss of iron include intestinal bleeding, operations, trauma, haemodialysis and other bleeding. When there is no loss of iron and oral supply is adequate, malabsorption (condition after gastric surgery, chronic inflammatory intestinal disease, celiac disease) or increased metabolism (e.g. pregnancy and growth) can also be the reason for iron deficiency.

Symptoms stretch from atrophy of mucous membrane and angular cheilitis to general symptoms like paleness, hair loss and fatigue, as well as dyspnoea and tachycardia. Furthermore, neurological symptoms, such as headache, reduced concentration capacity and restless legs can be observed (Herold, 2016).

1.3.3 Vitamin B12 Deficiency/Folic Acid Deficiency

Vitamin B12 and folic acid deficiency anaemia, just as iron deficiency anaemia, belongs to the group of nutritional anaemias. As explained above, vitamin B12 (cobalamin) and folate acid are coenzymes needed for the synthesis of deoxyribonucleic acid (DNA)
of e.g. proerythroblasts. If lacking, the maturation of the cells stops and ineffective early erythropoiesis reduces the amount of produced cells. The few cells produced are loaded with more haemoglobin than normally, resulting in a macrocytic hyperchromic anaemia.

Apart from general anaemia symptoms, such as fatigue, paleness and tachycardia, neurologic and psychiatric symptoms can be seen when deficiency in vitamin b12 is severe (Herold, 2016). When folic acid is lacking in pregnant women, the risk for neural tube defects increases. Consequently, the German institute for risk assessment recommends the substitution of folic acid in pregnant women or women with a desire for children (Bundesinstitut für Risikobewertung, 2014).

1.3.4 Anaemia of Chronic Inflammation (ACI)

Chronic inflammation is, after iron deficiency, the second leading cause for anaemia in Germany (Herold, 2016). Anaemia of chronic inflammation (ACI) refers to a type of anaemia that occurs in chronic diseases with chronic inflammatory stages, such as cancer, chronic infection or autoimmune diseases. Recent data indicates that moderate to severe inflammation occurs in conjunction with obesity, ageing, kidney failure or critical illness and with it anaemia, so that the former name “Anaemia of Chronic Disease” (ACD) is superseded by ”Anaemia of Chronic Inflammation” (Fraenkel et al., 2015). Similar to IDA, ACI is defined as a hyporegenerative anaemia due to iron deficiency, however with the difference, that the iron deficiency is functional and not absolute. This means that enough iron is stored in the body (mainly in macrophages), however the metabolism fails to release it rapidly enough to keep up with the demands of erythropoiesis (Fraenkel et al., 2015). ACI is thus characterised by a status of low serum iron level without evidence of low iron stores. This corresponds to decreased intestinal iron absorption and encouraged iron sequestration in macrophages in inflammatory
states (Ganz, 2011). Recent studies provide evidence that this effect could be mediated by hepcidin (Ganz, 2011). For example, the synthesis of hepcidin was found to be induced by IL-6 (Lefebvre et al., 2017). Through its effect on ferroportin, hepcidin was shown to control the main iron inflows: it reduces dietary absorption in enterocytes and inhibits the release of macrophage’s and hepatocyte’s iron store (in form of ferritin) from recycled senescent erythrocytes (Ganz et al, 2011).

However, the exact control mechanisms between hepcidin und iron metabolism are currently not fully understood and there is evidence that EPO has a connecting role. In the Invecchiare in Chianti study (InCHIANTI), a study conducted with 1270 persons in Chianti, Italy, the number of elevated inflammatory markers (IL-6, IL-1beta, CRP, TNF-alpha) in non-anaemic person was associated with progressively higher EPO levels (Ferrucci et al., 2005). Conversely, in anaemic people, inflammation was associated with lower EPO levels. The haemoglobin level at which the relationship between EPO level and inflammation reversed was close to 13 g/dl. These findings suggest that higher levels of EPO are needed to maintain normal erythropoiesis in inflammatory states (Ferrucci et al., 2005). In anaemic individuals with chronic inflammation this regulation might be dysfunctional.

1.3.5 Unexplained Anaemia (UA)

Anaemia is a common haematological problem of the elderly and incidence rises with each age-decade (Herold, 2016). In elderly people it is commonly mild and thus often overlooked by physicians (Makipour et al., 2008). Nevertheless, it is not part of the normal ageing process as anaemia in the elderly is associated with poor health and increased mortality (Riva et al., 2009). Root causes are multifactorial and include deficiencies, chronical diseases and chronic kidney disease. However, parameters derived from the exploration of erythrocytes are not exclusive, causes overlap and about one
third of anaemia cases in the elderly remain unexplained (Woodman, 2005). The Unexplained Anaemia of the Elderly (UAE) is thus an exclusion diagnosis. Beyond that, there has been much debate among experts in the last years concerning a revision of WHO cut-off values in the elderly (Merchant et al., 2011) which would create an even higher prevalence in the elderly than already reported.

So far the causal connection between anaemia and its adverse effects, such as increased mortality and worsened cognition could not be identified clearly. Some authors claim that anaemia could possibly "just" be a sensitive marker for other pathologic processes independently causing these adverse effects (Merchant et al., 2011). As every study provides different criteria for the "explained" subtypes of anaemia the classifications are not consistent in literature. The subgroup UAE is difficult to aggregate and examine across different studies and not enough is known about the peculiarities of this group. Merchant et al. propose a range of potential factors involved in the development of anaemia in the elderly:

Firstly, EPO levels have been shown to rise with age in healthy people with normal haemoglobin. This suggests that the impact of EPO on erythropoiesis declines with age. The theory of a pro-inflammatory state in ageing people independent of disease, is widely accepted. Studies have found evidence of elevated inflammatory markers such as ferritin, hepcidin and erythrocyte sedimentation rate (ESR) independently of other evidence of inflammation (CRP<3, no IL-6,) in UAE people compared to non-anaemic people of the same age. Thus an inflammation-induced reduction of EPO synthesis might play a role in the development of UAE (compare Chapter 1.3.4).

Secondly, androgens have long been known to promote erythropoiesis (Shahidi, 1973). Low testosterone has been associated with the development of anaemia in older men and women (Ferrucci, 2006), but the mechanisms remain unclear and several researchers came to the conclusion that the association between low testosterone and anaemia need
further investigation (Eisele et al., 2013; Ferrucci et al., 2006; Guo et al., 2013). The impact of testosterone on haematopoiesis is the main topic of this study and will be discussed in the subsection on Testosterone and Anaemia. Other hormones mentioned by Merchant et al. (2011), such as oestrogen and thyroid hormone, have been shown to impact on the blood production but will not be discussed in this study.

Thirdly, medication such as chemotherapy, immunosuppressive drugs, drugs against the human immunodeficiency virus (HIV) or other viral diseases, as well as alcohol can influence haematopoiesis. Multi-medication is common among elderly people and thus play an increasing role in an ageing society.

Fourthly, haematopoietic stem cell (HSC) function has been shown to decline with age, since the capacity for self-renewal and differentiation diminishes. The stem cell ageing might be of importance in the development of UAE.

Lastly, nutrient deficiencies and chronic inflammation are frequent in the elderly. As these have been shown to be risk factors this puts this demographic group at special risk for the development of anaemia.

The mentioned factors are diverse and the interdependencies remain speculative and implicate various issues that have yet to be discussed and explored.

This study focused on the impact of testosterone on the pathogenesis of UAE. The linkage between testosterone and anaemia is discussed in Section 1.2.4.

1.4 Objectives of Research

While the prevalence of anaemia and unexplained anaemia in particular has been shown to rise with age, the insufficient basis of data in Germany and other industrialised countries on anaemia might underestimate the effects of anaemia on individuals and society. Because of the demographic development in Germany anaemia might even become a more important topic in the future. Furthermore, only few studies have examined the
link between the development of unexplained anaemia and low testosterone. An increase in haematocrit is a recognized side-effect of testosterone therapy in hypogonadal men. Meanwhile there are relatively few studies on whether or not a testosterone level within the physiological range modulates the haemoglobin (Hb) level when hypogonadism or testosterone supplementation is absent (Yeap et al., 2009). Therefore the aim of the present study is:

(1) to examine how important the issue of anaemia in Germany is, how high the prevalence in specific age groups is and how the anaemia cases in Germany are distributed to the aetiology subgroups.

(2) whether or not testosterone is a risk factor for the development of anaemia in the elderly.

As data on the overall prevalence is scarce so far (Gaskell et al., 2008), the dataset used in this study contributes to the quantitative and qualitative examination of anaemia in Germany.

2 Material and Methods

2.1 The Heinz Nixdorf Recall Study

2.1.1 Study Design

The data used in this study were collected within the Heinz Nixdorf Recall Study (HNRS), a population-based cohort study conducted between the years 2000 and 2016 in three adjacent cities in the former coal and steel industrial Ruhr area in Germany (Essen, Mülheim and Bochum). The study’s name Recall is an acronym for „Risk Factors, Evaluation of Coronary Calcium and Lifestyle“ and indicates the initial objective of this study: to assess whether new diagnostic methods (such as the assessment of
coronary calcium, novel risk factors and psychological environment) improve the risk prediction for coronary events (Schmermund et al., 2002). Long established cardiovascular risk factors such as smoking, arterial hypertension and low-density lipoprotein (LDL cholesterol) have been measured as well as new potential risk factors. An accurate summary of conception, examinations, hypotheses and objectives of the HNRS can be found in Schmermund et al. (2002) and Erbel et al. (2012).

Data were collected in three waves: baseline (t0), 5-year follow-up (t1) and 10-year follow-up (t2). The baseline assessment was conducted between 2000 and 2003 and consisted of physical examination, assessment of medical history, questionnaires with focus on socio-economic status and laboratory analyses. Detailed Standard Operating Procedures (SOPs) were worked out for each measurement process and certified according to DIN EN ISO 9001:2000/2011. The 5-year follow-up was conducted from 2006 to 2008 and consisted of the same comprehensive examination. The 10-year follow-up proceeded from 2011 to 2016. The HNRS thus consists of versatile data from a representative German cohort at three points over a period of ten years.

In addition to the initially collected data, laboratory measurements were subsequently taken for all anaemic people in the HNRS within a study founded by the Jackstaedt foundation. Further information can be found in Eisele et al. (2012) and Eisele et al. (2010). In the course of the present study these data were merged with the initially collected Recall data.

2.1.2 Participants

The participants aged 45 to 75 were randomly selected from mandatory registries of residence and contacted via invitational letter and phone call. Of all contacted people, 4814 were included in the HNRS. The response-rate for baseline examination was 55.6% (Stang et al., 2005). Since this study was designed to represent the general German
population, exclusion criteria only applied for a few people and due to feasibility issues. These were institutionalised people, people not willing or able to give informed consent or to participate in interviews (e.g. due to linguistic barriers, deaf- or muteness, severe illness, psychiatric disorders, illegal substance abuse) and pregnant women (Schmermund et al., 2003). For the five and ten year follow-up, all participants from t0 were contacted again.

The participants’ educational information and declaration of consent was developed based on the International Ethical Guidelines for Biomedical Research Involving Human Subjects (WHO, 1993). Data protection occurred according to the federal state of North Rhine-Westphalia and health data protection laws (Gesundheitsschutzgesetz und Datenschutzgesetz Nordrhein-Westfalen). All participants gave written informed consent. The study was approved by the required institutional local ethical committees from the Universities of Essen and Witten/Herdecke. The data protection concept was monitored by the university hospital’s legal experts.

2.2 Variables of Interest

*Biological Samples*

On average eight participants per day were examined in the study centre in Essen. Blood samples were taken according to Standardised Operating Procedures (SOP) and processed immediately in the central laboratory of the university hospital of Essen. In addition, serum and plasma samples were stored at -80°C for future analyses. In the course of the Jackstaedt study mentioned above, the stored biobank was used to measure several laboratory variables retrospectively for a subsample of all HNRS participants. The measured laboratory variables included anaemia specific variables, such as vitamin B12, ferritin, iron, transferrin, soluble transferrin receptor and folate. Erythropoietin
was measures for the complete cohort. In t1 the stored aliquots were of reduced quantity and the additional analyses could only be completed for a diminished sample.

For the analyses, HNRS data from t0, t1 and t2 were supplemented by data measured in the Jackstaedt study for t0, t1 and t2. Units of measurement were not the same for every measuring time. I standardised the units according the most frequently used in literature. When measurement procedures changed over the time periods, I compared the results with a Bland-Altman plot.

**Anaemia-Related Measures**

Haemoglobin (Hb) (g/dl) was measured in EDTA-stabilised blood with the Coulter STKR in t0. At t1 and t2 the Sysmex XE 5000 was used. Data were available for 4761 participants at t0, 4104 at t1 and 3044 at t2. Since haemoglobin is the main outcome variable, its availability restricted the dataset at each examination time.

Iron (µg/dl) was measured in blood serum with the Siemens ADVIA 1650 and 2400 (ferrozine method). At t0 data for 4781, at t1 for 4125 and at t2 for 3061 participants were available.

Vitamin B12 (pg/ml) was measured with the Siemens Immunoassay Systems ADVIA Centaur (t0) and ADVIA Centaur XP (t1, t2). Data were available at t0 for 379, at t1 for 251 and at t2 for 264 participants.

Folic acid (ng/ml) was measured with a competitive immunoassay, using the Siemens ADVIA Centaur for samples of 392 people at t0, and the ADVIA Centaur XP at t1 for 259 samples and at t2 for 242 samples.

Erythropoietin (EPO) (IU/L) was measured with a solid-phase, enzyme-labelled chemiluminescent immunometric assay, using the Siemens Immulite at t0 and Immulite 2000 XPi at t1 and t2. At t0 4213, at t1 327 and at t2 2782 EPO values were available.

Ferritin (µg/L), Soluble Transferrin Receptors (sTfR) (mg/L) and Transferrin (g/L)
were measured by nephelometry with the BN II at t0 and at t1 and t2 with the Dimension Vista® 1500. At t0 ferritin data for 4488, at t1 for 274 and at t2 for 2156 participants exist. Data on sTfR were available at t0 for 4481, at t1 for 273 and at t2 for 2118 participants. Transferrin was available for 4457 at t0, for 274 at t1 and for 2118 at t2.

I calculated the ferritin-index (findex) as follows: \( \text{Findex} = \frac{\text{Transferrin}}{\log(\text{Ferritin})} \). At t0 findex was available for 4449, at t1 for 273, and at t2 for 2118 participants.

Creatinine (mg/dl) was measured using the Jaffé-Reaction with the ADVIA 1650 at t0 and ADVIA 2400 at t1 and t2. Data can be found at t0 for 4784 participants, at t1 for 4125 and at t2 for 3061 participants.

I estimated the glomerular filtration rate (EGFR) (ml/min) according to the Cockcroft-Gault formula for each proband and time of investigation. For men I used

\[
\text{EGFR(male)} = \frac{(140-\text{Age}) \cdot \text{Weight}}{72 \cdot \text{Creatinine}}
\]

and for women

\[
\text{EGFR(female)} = 0.85 \cdot \text{EGFR(male)}.
\]

At t0 the EGFR could be calculated for 4723, at t1 for 4088 and at t2 for 3016 participants.

**Hormone Measurement**

Testosterone (nmol/L) was measured at t0 and t1 with the Roche Cobas E170. At t0 4296 samples and at t1 3900 were measured. The Siemens ADVIA Centaur® XP was used at t1 and t2. With this method, data for 3401 participants at t1 and 3055 at t2 were measured. There are thus overlapping data from both methods at t1 and I compared both measurements using a Bland-Altman plot. Differences were small and the methods were assessed as comparable. For t0 results from the Roche Cobas E170
and for t1 and t2 the ADVIA Centaur measurement was used. For the comparison of different testosterone levels in the study cohort, I computed age group and sex specific testosterone quartiles and assigned the cohort to four different quartile levels (Q1 being the lowest quartile group and Q4 the highest). The dummy variable for a testosterone level in Q1 is called lowtesto in the analyses.

**Anthropometric and Personal Data**

Weight was measured with the personal scale type SECA 709. The function was controlled daily and calibration was tested every three months. The measurement range is from 5 to 150 kg. Height was measured with the height rod of the type SECA 221, integrated in the scale. Height and weight were captured with a precision of 0.1 kg and 0.1 cm. Weight was measured for 4735 people at t0, 4143 at t1 and 4092 at t2. The Body mass index (BMI) was calculated according the following formula:

\[ \text{BMI} = \frac{\text{Weight(}kg\text{)}}{\text{Height}^2\text{(}m^2\text{)}} \]

At t0 4777, at t1 4092 and at t2 3063 results for BMI were available. Specification on sex was taken from the data delivered by the municipal registration offices.

**Comorbid Conditions**

Detailed information on smoking habits were taken from self-report questionnaires and in this study classified into three groups: Non smoker, ex-smoker, current smoker. I created a comorbidity score, using 13 self-reported chronic diseases in t0, including diabetes, thyroid dysfunction, osteoporosis, arthrosis, coronary heart disease, heart insufficiency, heart defect, peripheral arterial occlusive disease or atherosclerosis, stroke, emphysema, asthma, cancer and arterial hypertension. Death certificates were collected and families and general practitioners of the deceased were interviewed if possible (Erbel et al., 2010). The numbers of deaths reported in this study include all cases reported
until March 2017. According to the dates reported on the death certificates, I allocated the deaths to the assessment periods.

2.3 The Aetiology of Anaemia and Applied Definitions

The identification of anaemia in the study cohort was conducted according to the WHO definition from 1968: Hb < 12 g/dl in women and Hb < 13 g/dl in men was classified anaemic. I defined five different aetiologies for anaemia in this study and allocated anaemic persons to these groups according to their laboratory parameters. I defined the aetiologies according to publications from Guralnik et al. (2005) and Eisele et al. (2013). The groups were: 1. Renal Anaemia (RA), 2. Iron Deficiency Anaemia (IDA), 3. Anaemia of Chronic Inflammation (ACI), 4. Vitamin B12/Folic Acid Deficiency and 5. Unexplained Anaemia (UA). The criteria used were as follows: Cases with a glomerular filtration rate (Cockcroft-Gault formula) corresponding to chronic kidney failure stadium IV and V (KDOQI) (GFR<30 ml/min) were classified as renal anaemia (RA). Cases with ferritin index > 1.5 or ferritin < 12 ng/mL were considered IDA (Guralnik et al., 2005). ACI was defined as low serum iron without evidence of iron deficiency: iron < 60 µg/dl and ferritin index ≤ 1.5 and ferritin ≥ 12 ng/mL (Guralnik et al., 2005). Vitamin B12 < 200 pg/ml and folic acid < 2.2 ng/ml were defined deficient (Eisele, 2013). Due to the small number of cases, vitamin B12/folic acid deficiency and iron deficiency anaemia were combined to nutritional anaemia in the multivariate and logistic regressions. All other anaemia cases that did not match the described criteria were judged as unexplained anaemia (UA).

Adjustment for altitude was found to be necessary after 1000 metres above sea level (WHO, 2011) and since the Ruhr Area is flat and low no adjustment was made. Concerning smoking status the WHO proposes a haemoglobin adjustment of -0.03 g/dl for
smokers. This would have changed the anaemia status of one person in t0 and two people in t2 without influence on the outcome, so that this adjustment was not included into the analyses.

2.4 Software Used

The statistical analyses were done with SAS 9.4 (The SAS Institute, Cary, NJ) and Stata 13.1 (StataCorp, 2015, Stata Statistical Software: Release 14, College Station, TX: StataCorp LP). Graphs were created with SAS 9.4, Stata 13.1 and Microsoft Excel 2013 (Microsoft Corporation, 2013, USA). The tables in this study were made with \LaTeX (Texmaker and Miktex) and Microsoft Excel. The flow chart was made with the freeware YeD Version 3.17.1 (yWorks, 2017, Germany).

2.5 Statistical Methods

In this section the statistical analyses and procedures used in this study will be described. Summary statistics (SAS MEANS and FREQ procedures) provide information for a set of 18 variables for the study population (chapters 3.1 to 3.3). To present the prevalence of anaemia by age groups, I used the SAS PROC FREQ statement and drew according bar charts with Microsoft Excel. The aetiology box plots were done with the vbox statement in SAS PROC SGPLOT and the pie charts were made with SAS PROC GCHART.

The analysis was based on four different estimations: First, the examination of how testosterone levels differ across age, anaemia status and aetiology in the cross section. Second, the development of these differences over time. Third, the examination of how haemoglobin levels differ across testosterone levels in a longitudinal analysis. And fourth, the estimation of odds ratios in a binary dependent variable model to predict
if the risk for the development of anaemia changes with the testosterone level. In the following, the models used will be explained in detail:

Testosterone in the Cross Section

In order to show how the testosterone levels Testo\textsubscript{i}, for each individual i, differ across age, I regressed testosterone on age and BMI in a cross sectional multivariate analysis. According to Equation (1), the SAS procedure PROC REG was used:

\[ \text{Testo}_i = \alpha + \beta_1 \cdot \text{age}_i + \beta_2 \cdot \text{BMI}_i + \varepsilon_i \] (1)

The analysis was done for each aetiology and each gender category separately in cross section for t0, t1 and t2. I used a normal-weight fixed BMI = 25 kg/m\(^2\) for the calculation of the predicted testosterone and drew according graphs with SAS PROC SGPLOT, including the predicted graphs (series) and data points of the actually measured testosterone levels (scatter) (see Figure 8 for men in t2). All graphs were drawn for men and women and each period t0, t1 and t2, separately and can be found in Appendix C.1 to C.5.

Testosterone in the Longitudinal Section

For a first overview on how testosterone levels differ across age in the longitudinal section, I used Stata to draw a smoothed two way locally weighted scatter plot (twoway lowess) with testosterone plotted on the ordinate and age plotted on the abscissa. The stata lowess graph compares the testosterone levels over age of people who never suffered from anaemia with people who at least once during the study had an unexplained anaemia (UA). The graph was made for men and women separately (see Figure 9 and 10, respectively). The smoothing was done according to Cleveland (1979).

In the following models, I used the centred age (variable transformation of age, sub-
tracting 63 of each observation) for easier interpretability (Williams, 2015). As centre I used 63 years, this means that the coefficients shown in the regression tables are the effects for a 63 year old person. I converted the data from wide format to long format and generated a new aetiology variable, this time only with three different subgroups: nutritional anaemia (that is iron deficiency, vitamin B12 deficiency and folic acid deficiency combined), anaemia of chronic inflammation and unexplained anaemia. The proband’s identification number (ProbID) was used for the random effects statement for both, the regression on testosterone and the regression on haemoglobin, to address serial correlation. A change in the covariance matrix did not have influence on the results and I used the UNSTRUCTURED matrix (UN).

To quantify the differences in testosterone levels between the anaemia subgroups, I regressed testosterone (Testo) on age, BMI and the subtypes of aetiology in a mixed model with SAS PROC MIXED (see Equations (2) - (4)). I controlled for ProbID as random and age and BMI as fixed effects. The estimations were made according to the following equations, with Testo$_{it}$ being the testosterone level of individual $i$ at time $t$, with $t= (t_0, t_1, t_2)$. In these models $\mu$ was the average testosterone level for the whole cohort with $u_i \sim N(0, \sigma_u^2)$ and $\varepsilon_{it} \sim N(0, \sigma_e^2)$ as individual-level random effects:

\[ \text{Testo}_{it} = \mu + u_i + \beta_1 \cdot \text{age}_{it} + \beta_2 \cdot \text{aetiology}_{it} + \varepsilon_{it} \]  

(2)

\[ \text{Testo}_{it} = \mu + u_i + \beta_1 \cdot \text{age}_{it} + \beta_2 \cdot \text{aetiology}_{it} + \beta_3 \cdot \text{age}_{it} \cdot \text{aetiology}_{it} + \varepsilon_{it} \]  

(3)

\[ \text{Testo}_{it} = \mu + u_i + \beta_1 \cdot \text{age}_{it} + \beta_2 \cdot \text{aetiology}_{it} + \beta_3 \cdot \text{age}_{it} \cdot \text{aetiology}_{it} + \beta_4 \cdot \text{BMI}_{it} + \varepsilon_{it} \]  

(4)

These models, again were estimated for men and women separately. In model (3) and (4) I included an interaction term of age and aetiology to find out whether the influence of the anaemia aetiology changes with age. The coefficient of interest was the coeffi-
cient for aetiology and for the interaction term of aetiology and age. The regression coefficients are displayed in Table 10. I calculated the predicted testosterone for model (4) with a normal-weight fixed BMI = 25 kg/m^2 and drew the according graphs with SAS PROC SGPLOT (see Figure 11 and 12).

**Haemoglobin in the Longitudinal Section**

To evaluate the connection of testosterone on haemoglobin, I again used a mixed model to regress haemoglobin on testosterone and controlled for BMI and age, for men and women separately. I used an interaction term of age×testosterone to examine whether the influence of testosterone rises with age and a square term of age×age to find whether age has a linear or quadratic influence on haemoglobin. The coefficient of interest was testosterone. The Equations (5) to (7) show the models used with \(hb_{it}\) as haemoglobin level of individual \(i\) at time \(t\) and \(t=(t_0, t_1, t_2)\). \(\mu\) was the average haemoglobin level for the whole cohort with \(u_i \sim N(0, \sigma_u^2)\) and \(\varepsilon_{it} \sim N(0, \sigma_e^2)\) as individual-level random effects:

\[
hb_{it} = \mu + u_i + \beta_1 \cdot \text{age}_{it} + \beta_2 \cdot \text{Testo}_{it} + \beta_3 \cdot \text{bmi}_{it} + \varepsilon_{it} \tag{5}
\]

\[
hb_{it} = \mu + u_i + \beta_1 \cdot \text{age}_{it} + \beta_2 \cdot \text{Testo}_{it} + \beta_3 \cdot \text{BMI}_{it} + \beta_4 \cdot \text{Testo}_{it} \cdot \text{age}_{it} + \varepsilon_{it} \tag{6}
\]

\[
hb_{it} = \mu + u_i + \beta_1 \cdot \text{age}_{it} + \beta_2 \cdot \text{Testo}_{it} + \beta_3 \cdot \text{BMI}_{it} + \beta_4 \cdot \text{Testo}_{it} \cdot \text{age}_{it} + \beta_5 \cdot \text{age}_{it}^2 + \varepsilon_{it} \tag{7}
\]

The regression coefficients are shown in Table 11.

**Odds Ratios for Anaemia and Unexplained Anaemia**

To evaluate the risk of developing anaemia and unexplained anaemia in particular, when having low testosterone, I used a generalised mixed model with binary outcome to estimate odds ratios. Analogously to the generalised mixed models used in the regressions before I used the SAS procedure GLIMMIX. The parameter \(\text{lowtesto}\) is a binary
variable which takes the value one if the testosterone level is within the lowest testosterone quartile in the proband’s age and sex group and zero else. The variable ProbID was included into the random effects statement as to control for serial correlation. I regressed the dummy for anaemia status on the lowtesto dummy in a crude (unadjusted) and an adjusted model: The ProbID was included as random and BMI and age as fixed effects. As outcome variable I used the anaemia status (anaemia\(_{it}\)) in the first model and unexplained anaemia (UA\(_{it}\)) in the second model. The assumption of rare prevalence holds in this study, so that the odds ratio numerically resembles the rate ratio (Lee, 1994). The estimations were done with SAS according to Kuss (2001) and repeated again in Stata with the logistic command (clustering the standard errors at the level of ProbIDs), according to Equation (8). To examine the connection between low testosterone and the development of anaemia in the future I included a time lag of one period with logistic in Stata (compare Equation (10)).

I used the anaemia status anaemia\(_{it}\) and testosterone status lowtesto\(_{it}\) of individual \(i\) at time \(t\), with \(t = (t_0, t_1, t_2)\), with \(\mu\) as regression intercept and \(u_i\) as random intercept adjustments at the patient level with variance \(\sigma_u^2\). The disturbance term \(\varepsilon_{it}\) was modelled to follow a logistic distribution \(\varepsilon_{it} \sim \text{Logistic}(0, \sigma_e^2)\). Consequently, the regression models are described by the following equations:

\[
\Pr[\text{anaemia}_{it} = 1] = \Pr[\mu + u_i + \beta_1 \cdot \text{lowtesto}_{it} + \beta_2 \cdot \text{age}_{it} + \beta_3 \cdot \text{BMI}_{it} + \varepsilon_{it} > 0] = \text{Logit}[\mu + u_i + \beta_1 \cdot \text{lowtesto}_{it} + \beta_2 \cdot \text{age}_{it} + \beta_3 \cdot \text{BMI}_{it}] \quad (8)
\]

\[
\Pr[\text{UA}_{it} = 1] = \Pr[\mu + u_i + \beta_1 \cdot \text{lowtesto}_{it} + \beta_2 \cdot \text{age}_{it} + \beta_3 \cdot \text{BMI}_{it} + \varepsilon_{it} > 0] = \text{Logit}[\mu + u_i + \beta_1 \cdot \text{lowtesto}_{it} + \beta_2 \cdot \text{age}_{it} + \beta_3 \cdot \text{BMI}_{it}] \quad (9)
\]
In the time-lagged version, the corresponding equations are:

\[
Pr[\text{anaemia}_{it2} = 1] = Pr[\mu + u_i + \beta_1 \cdot \text{lowtesto}_{it1} + \beta_2 \cdot \text{age}_{it2} + \beta_3 \cdot \text{BMI}_{it2} + \epsilon_{it2} > 0] = \text{Logit}[\mu + u_i + \beta_1 \cdot \text{lowtesto}_{it1} + \beta_2 \cdot \text{age}_{it2} + \beta_3 \cdot \text{BMI}_{it2}]
\] (10)

\[
Pr[\text{UA}_{it2} = 1] = Pr[\mu + u_i + \beta_1 \cdot \text{lowtesto}_{it1} + \beta_2 \cdot \text{age}_{it2} + \beta_3 \cdot \text{BMI}_{it2} + \epsilon_{it2} > 0] = \text{Logit}[\mu + u_i + \beta_1 \cdot \text{lowtesto}_{it1} + \beta_2 \cdot \text{age}_{it2} + \beta_3 \cdot \text{BMI}_{it2}]
\] (11)

### 2.6 Sensitivity Analysis

All analyses were made for women and men separately, since estimations were expected to vary widely for the different sexes. In order to validate the robustness of the models used and the SAS processes selected, the multivariate regressions were repeated with Stata \texttt{areg} (with the specifications \texttt{cluster(Probid) absorb(Probid)}) and the logistic regressions were repeated with Stata \texttt{logistic} and SAS \texttt{PROC GENMOD}. The results of both programmes came to the same conclusions. With the random effects statement in SAS and the cluster statement in Stata I accounted for the correlation of repeated measures. Furthermore, the analyses were made in both, longitudinal and cross sectional data.

### 3 Results

#### 3.1 Participants

The analysis group was composed of all participants of the HNRS with available haemoglobin values from at least one measuring time. Figure 1 illustrates the eligibility of study participants. Of all HNRS participants, nine were excluded from the whole study, since no data on haemoglobin levels were measured. As a result, a pop-
ulation of 4805 people (2391 male, 2414 female) was the basis for this study. At t0, there were data on haemoglobin for 4761 participants aged 45 to 76.

The periods between the assessment points endured on average five years (95%CI 5.13 - 5.14 years between t0 and t1; 95%CI 5.27 - 5.3 years between t1 and t2) and the overall duration of study per person was on average ten years (95%CI 10.39 - 10.42 years).

![Study Cohort Composition Diagram]

**Figure 1:** Composition of the Study Cohort. Each Box Indicates the Number of Available Haemoglobin Samples in Period t0, t1 and t2.

Between the baseline assessment and t1 134 participants died and 515 participants failed to appear in the 5-year follow-up. At t1, 4156 participants were examined and haemoglobin values for 4104 participants (2037 male, 2067 female) aged 50 to 80 years
were available. Between \( t_1 \) and \( t_2 \) 295 participants died and 771 did not show up for the next follow-up. Seven participants which had failed to appear in \( t_1 \) participated in \( t_2 \) again and the study group in the 10-year follow-up consisted of 3097 people (1512 male, 1585 female) with 3044 haemoglobin values. After the last examination, follow-up questionnaires were sent out annually. Until the last data update in March 2017 183 people from the participants in \( t_2 \) had died.

### 3.2 Characteristics of the Study Population

The main characteristics of the study population will be presented in the following form: mean ± standard deviation or median (first quartile; third quartile). The median age of the baseline cohort was 60 years (53; 66). Of these, 2391 (49.76\%) were female and 2414 (50.24\%) male. The median body mass index was 27.4 (24.8; 30.3) kg/m\(^2\) and haemoglobin was on average 14.39 ± 1.2 g/dl. The average and standard deviation of haemoglobin was within the defined healthy (not anaemic) range of haemoglobin values. Haemoglobin was lower in all anaemic compared to non-anaemic groups. Anaemic males were on average older than non-anaemic males and the body mass index (BMI) in non-anaemic males and females was slightly higher in non-anaemic compared to anaemic people in \( t_0, t_1 \) and \( t_2 \). Iron, ferritin and sTfR levels were lower and EPO levels were higher in anaemic men and women in all periods. The testosterone levels were lower in anaemic compared to non-anaemic people and contemplated over the periods there was a decline in medians. For all gender and periods, the share of current smokers was higher in the non-anaemic group, while the share of ex-smokers was higher in the anaemic group. In \( t_0 \) 1128 participants (23.52\%) were smokers and a third of the whole cohort ex-smokers. The non-smokers share in \( t_0 \) was 41.9\% and rose to 43.53\% in \( t_2 \), while the share of smokers diminished to 12.69\% in \( t_2 \).
Table 1: Non-Anaemic and Anaemic Men in t0

The Tables 1, 3 and 5 summarise characteristic mean laboratory values for all men in t0, t1 and t2. The characteristic data for women are shown in Table 2 for t0, Table 4 for t1 and Table 6 for t2. To get an impression of the subgroups focused on in this study, the characteristic data were stratified for sex and anaemia-status. It is to be noted that vitamin B12 and folic acid have been measured subsequently for people that had once in the study period been anaemic. Erythropoietin, ferritin and sTfR (soluble transferrin receptor) have also been measured retrospectively when missing in anaemic people. This only corresponds to twelve values of ferritin, 19 values of sTfR and 18 values of EPO but must be considered when interpreting the values.
<table>
<thead>
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<th>Variable</th>
<th>Non-Anaemic Female in t0</th>
<th>Anaemic Female in t0</th>
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<tr>
<td></td>
<td>N</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Age [years]</td>
<td>2302</td>
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<td>BMI [kg/m²]</td>
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<tr>
<td>Ferritin [µg/L]</td>
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<td>112.98 ± 117.48</td>
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<tr>
<td>sTfR [mg/L]</td>
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Table 2: Non-Anaemic and Anaemic Women in t0
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<thead>
<tr>
<th>Variable</th>
<th>Non-Anaemic Male in t1</th>
<th>Anaemic Male in t1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>1950 64.18 ± 7.56</td>
<td>87 68.77 ± 6.75</td>
</tr>
<tr>
<td>Male</td>
<td>1950</td>
<td>87</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>1943 28.47 ± 4.05</td>
<td>86 27.59 ± 4.37</td>
</tr>
<tr>
<td>Hb [g/dl]</td>
<td>1950 15.04 ± 0.97</td>
<td>87 12.03 ± 1.05</td>
</tr>
<tr>
<td>Hct [%]</td>
<td>1950 0.55 ± 2.22</td>
<td>87 0.94 ± 3.85</td>
</tr>
<tr>
<td>MCV [fl]</td>
<td>1950 89.23 ± 3.94</td>
<td>87 88.41 ± 5.82</td>
</tr>
<tr>
<td>Iron [µg/dl]</td>
<td>1950 104.97 ± 33.96</td>
<td>87 74.37 ± 29.98</td>
</tr>
<tr>
<td>Ferritin [µg/L]</td>
<td>68 185.57 ± 233.02</td>
<td>71 151.28 ± 140.51</td>
</tr>
<tr>
<td>sTIR [mg/L]</td>
<td>68 185.57 ± 233.02</td>
<td>71 151.28 ± 140.51</td>
</tr>
<tr>
<td>Ferritin Index</td>
<td>67 0.66 ± 0.2</td>
<td>71 0.92 ± 0.73</td>
</tr>
<tr>
<td>EPO [IU/l]</td>
<td>92 10.61 ± 5.41</td>
<td>78 19.74 ± 16.42</td>
</tr>
<tr>
<td>EGFR [mL/min]</td>
<td>1943 80.42 ± 19.22</td>
<td>86 69.04 ± 21.92</td>
</tr>
<tr>
<td>CRP [mg/dL]</td>
<td>1909 0.27 ± 0.53</td>
<td>83 0.59 ± 1.19</td>
</tr>
<tr>
<td>Testosterone [nmol/l]</td>
<td>1918 16.12 ± 6.09</td>
<td>84 12.55 ± 6.36</td>
</tr>
<tr>
<td>SHBG [nmol/l]</td>
<td>1907 39.91 ± 16.39</td>
<td>83 41.89 ± 18.76</td>
</tr>
<tr>
<td>Folic Acid [ng/ml]</td>
<td>66 11.04 ± 8.66</td>
<td>66 9.27 ± 6.8</td>
</tr>
<tr>
<td>Vitamin B12 [pg/ml]</td>
<td>59 424.95 ± 188.69</td>
<td>68 369.09 ± 136.55</td>
</tr>
<tr>
<td>Non-Smoker</td>
<td>564 (28.98 %)</td>
<td>17 (19.54 %)</td>
</tr>
<tr>
<td>Ex-Smoker</td>
<td>1005 (51.64 %)</td>
<td>56 (64.37 %)</td>
</tr>
<tr>
<td>Smoker</td>
<td>377 (19.37 %)</td>
<td>14 (16.09 %)</td>
</tr>
</tbody>
</table>

Table 3: Non-Anaemic and Anaemic Men in t1
**Table 4: Non-Anaemic and Anaemic Women in t1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-Anaemic Female in t1</th>
<th>Anaemic Female in t1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>1985 64.26 ± 7.61</td>
<td>82 68.3 ± 8.58</td>
</tr>
<tr>
<td>Female</td>
<td>1985 64 (58; 70)</td>
<td>82 69 (62; 75)</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>1981 28.01 ± 5.36</td>
<td>82 27.94 ± 7.32</td>
</tr>
<tr>
<td>Hb [g/dl]</td>
<td>1985 13.85 ± 0.88</td>
<td>82 11.12 ± 0.96</td>
</tr>
<tr>
<td>Hct [%]</td>
<td>1985 0.64 ± 3.02</td>
<td>82 0.34 ± 0.03</td>
</tr>
<tr>
<td>MCV [fl]</td>
<td>1985 88.96 ± 3.81</td>
<td>82 85.3 ± 8.32</td>
</tr>
<tr>
<td>Iron [µg/dl]</td>
<td>1980 97.34 ± 30.74</td>
<td>82 60.96 ± 28.48</td>
</tr>
<tr>
<td>Ferritin [µg/L]</td>
<td>68 84.25 ± 72.3</td>
<td>67 99.09 ± 113.22</td>
</tr>
<tr>
<td>sTfR [mg/L]</td>
<td>68 84.25 ± 72.3</td>
<td>67 99.09 ± 113.22</td>
</tr>
<tr>
<td>Ferritin Index</td>
<td>68 0.84 ± 0.87</td>
<td>67 1.48 ± 1.65</td>
</tr>
<tr>
<td>EPO [IU/l]</td>
<td>82 11.38 ± 4.89</td>
<td>75 26.99 ± 29.43</td>
</tr>
<tr>
<td>EGFR [mL/min]</td>
<td>1977 67.66 ± 17.44</td>
<td>82 61.57 ± 19.68</td>
</tr>
<tr>
<td>CRP [mg/dL]</td>
<td>1937 0.27 ± 0.42</td>
<td>80 0.64 ± 1.34</td>
</tr>
<tr>
<td>Testosterone [nmol/l]</td>
<td>1326 1.55 ± 0.72</td>
<td>56 1.23 ± 0.77</td>
</tr>
<tr>
<td>SHBG [nmol/l]</td>
<td>1457 56.81 ± 30.24</td>
<td>61 62.18 ± 33.8</td>
</tr>
<tr>
<td>Folic Acid [ng/ml]</td>
<td>65 12.76 ± 10.63</td>
<td>62 8.89 ± 8.55</td>
</tr>
<tr>
<td>Vitamin B12 [pg/ml]</td>
<td>60 493.48 ± 319.25</td>
<td>64 419.09 ± 263.86</td>
</tr>
<tr>
<td>Non-Smoker</td>
<td>1105 (55.75 %)</td>
<td>43 (52.44 %)</td>
</tr>
<tr>
<td>Ex-Smoker</td>
<td>558 (28.15 %)</td>
<td>30 (36.59 %)</td>
</tr>
<tr>
<td>Smoker</td>
<td>319 (16.09 %)</td>
<td>9 (10.98 %)</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-Anaemic Male in t2</th>
<th>Anaemic Male in t2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Age [years]</td>
<td>1386</td>
<td>68.53 ± 7.22</td>
</tr>
<tr>
<td>Male</td>
<td>1386</td>
<td></td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>1376</td>
<td>28.46 ± 4.01</td>
</tr>
<tr>
<td>Hb [g/dl]</td>
<td>1386</td>
<td>14.98 ± 0.97</td>
</tr>
<tr>
<td>Hct [%]</td>
<td>1388</td>
<td>0.44 ± 0.03</td>
</tr>
<tr>
<td>MCV [fl]</td>
<td>1386</td>
<td>89.96 ± 4.09</td>
</tr>
<tr>
<td>Iron [µg/dl]</td>
<td>1385</td>
<td>107.06 ± 31.75</td>
</tr>
<tr>
<td>Ferritin [µg/L]</td>
<td>960</td>
<td>206.65 ± 165.66</td>
</tr>
<tr>
<td>sTfR [mg/L]</td>
<td>960</td>
<td>206.65 ± 165.66</td>
</tr>
<tr>
<td>Ferritin Index</td>
<td>960</td>
<td>0.56 ± 0.19</td>
</tr>
<tr>
<td>EPO [IU/L]</td>
<td>1275</td>
<td>9.62 ± 4.93</td>
</tr>
<tr>
<td>EGFR [mL/min]</td>
<td>1374</td>
<td>76.01 ± 18.9</td>
</tr>
<tr>
<td>CRP [mg/dL]</td>
<td>1365</td>
<td>0.24 ± 0.34</td>
</tr>
<tr>
<td>Folic Acid [ng/ml]</td>
<td>30</td>
<td>11.7 ± 9.6</td>
</tr>
<tr>
<td>Vitamin B12 [pg/ml]</td>
<td>30</td>
<td>391.97 ± 200.28</td>
</tr>
<tr>
<td>Non-Smoker</td>
<td>420</td>
<td>(30.39 %)</td>
</tr>
<tr>
<td>Ex-Smoker</td>
<td>764</td>
<td>(55.28 %)</td>
</tr>
<tr>
<td>Smoker</td>
<td>198</td>
<td>(14.33 %)</td>
</tr>
</tbody>
</table>

Table 5: Non-Anaemic and Anaemic Men in t2
<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-Anaemic Female in t2</th>
<th>Anaemic Female in t2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Age [years]</td>
<td>1478</td>
<td>68.51 ± 7.33</td>
</tr>
<tr>
<td>Female</td>
<td>1478</td>
<td>68.51 ± 7.33</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>1466</td>
<td>28.13 ± 5.27</td>
</tr>
<tr>
<td>Hb [g/dl]</td>
<td>1478</td>
<td>13.75 ± 0.89</td>
</tr>
<tr>
<td>Hct [%]</td>
<td>1478</td>
<td>0.41 ± 0.03</td>
</tr>
<tr>
<td>MCV [fl]</td>
<td>1478</td>
<td>89.67 ± 4.05</td>
</tr>
<tr>
<td>Iron [µg/dl]</td>
<td>1477</td>
<td>99.54 ± 29.17</td>
</tr>
<tr>
<td>Ferritin [µg/L]</td>
<td>1003</td>
<td>123.81 ± 117.17</td>
</tr>
<tr>
<td>sTIR [mg/L]</td>
<td>1003</td>
<td>123.81 ± 117.17</td>
</tr>
<tr>
<td>Ferritin Index</td>
<td>1002</td>
<td>0.63 ± 0.28</td>
</tr>
<tr>
<td>EPO [IU/l]</td>
<td>1351</td>
<td>9.36 ± 4.7</td>
</tr>
<tr>
<td>EGFR [mL/min]</td>
<td>1447</td>
<td>64.47 ± 16.47</td>
</tr>
<tr>
<td>CRP [mg/dL]</td>
<td>1454</td>
<td>0.26 ± 0.38</td>
</tr>
<tr>
<td>Testosterone [nmol/L]</td>
<td>1475</td>
<td>1.37 ± 0.79</td>
</tr>
<tr>
<td>SHBG [nmol/L]</td>
<td>1475</td>
<td>1.37 ± 0.79</td>
</tr>
<tr>
<td>Folic Acid [ng/ml]</td>
<td>57</td>
<td>10.64 ± 5.37</td>
</tr>
<tr>
<td>Vitamin B12 [pg/ml]</td>
<td>57</td>
<td>391.89 ± 157.63</td>
</tr>
<tr>
<td>Non-Smoker</td>
<td>836</td>
<td>(56.64 %)</td>
</tr>
<tr>
<td>Ex-Smoker</td>
<td>463</td>
<td>(31.37 %)</td>
</tr>
<tr>
<td>Smoker</td>
<td>177</td>
<td>(11.99 %)</td>
</tr>
</tbody>
</table>

Table 6: Non-Anaemic and Anaemic Women in t2
Table 7 shows the prevalence of 13 comorbidities stratified according to their anaemia status in t0. The diseases were chosen according to the impact directly on anaemia or mediated through inflammation on anaemia. Heart failure concerned 8.87% anaemic and 4.92% non-anaemic people. Coronary heart disease concerned 14.57% anaemic people and 6.83% non-anaemic people.

<table>
<thead>
<tr>
<th>Chronic Diseases in t0</th>
<th>n (%) of all</th>
<th>n (%) of anaemic persons</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD</td>
<td>327 (6.83 %)</td>
<td>22 (14.57 %)</td>
</tr>
<tr>
<td>Arthrosis</td>
<td>1886 (50.95 %)</td>
<td>60 (54.05 %)</td>
</tr>
<tr>
<td>Stroke</td>
<td>134 (2.81 %)</td>
<td>7 (4.64 %)</td>
</tr>
<tr>
<td>Emphysema</td>
<td>74 (1.87 %)</td>
<td>1 (0.79 %)</td>
</tr>
<tr>
<td>Asthma</td>
<td>286 (7.22 %)</td>
<td>9 (7.14 %)</td>
</tr>
<tr>
<td>Heart Failure</td>
<td>234 (4.92 %)</td>
<td>13 (8.78 %)</td>
</tr>
<tr>
<td>Heart Defect</td>
<td>192 (4.06 %)</td>
<td>11 (7.38 %)</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>253 (7.04 %)</td>
<td>8 (7.77 %)</td>
</tr>
<tr>
<td>Thyroid Disorder</td>
<td>907 (22.89 %)</td>
<td>31 (25 %)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2707 (56.49 %)</td>
<td>85 (56.29 %)</td>
</tr>
<tr>
<td>PAOD</td>
<td>753 (15.67 %)</td>
<td>32 (21.05 %)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>395 (8.22 %)</td>
<td>20 (13.16 %)</td>
</tr>
<tr>
<td>Cancer</td>
<td>317 (7.94 %)</td>
<td>15 (12 %)</td>
</tr>
</tbody>
</table>

CHD= Coronary Heart Disease  POAD= Peripheral Artery Occlusive Disease
Heart Defect: Congenital and Acquired Heart Defects

Table 7: Comorbidities as Reported in t0 for Anaemic and Non-Anaemic people

3.3 Prevalence and Aetiology of Anaemia in the HNRS Population

The overall prevalence of anaemia increased from 3.19% in baseline to 4.12% in t1 and to 5.91% in t2, as shown in Table 8. For women, the prevalence was 3.76% (n= 90 of 2392) in t0, 3.97% (n= 82 of 2067) in t1 and rose to 4.77% (n=74 of 1552) in t2. The
The prevalence of anaemia in men was 2.63% (n=62 of 2369) in t0, 4.27% (n=87 of 2037) in t1 and 7.1% (n=106 of 1492) in t2.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Frequency</th>
<th>%</th>
<th>Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>t0</td>
<td>152</td>
<td>3.19</td>
<td>4761</td>
</tr>
<tr>
<td>t1</td>
<td>169</td>
<td>4.12</td>
<td>4104</td>
</tr>
<tr>
<td>t2</td>
<td>180</td>
<td>5.91</td>
<td>3044</td>
</tr>
</tbody>
</table>

Table 8: Prevalence of Anaemia in t0, t1 and t2

The prevalence of anaemia in men is illustrated in Figure 2. The bars with the same colour belong to the same cohort (either t0, t1 or t2). The prevalence was relatively low in the younger age groups (around 50 years) and increased with every age group. It was highest among the age group 70+ years (6.12%, n=37 of 605) and lowest between 55 and 59 years (1.84%). For men in t0 in the age group 70 years and older, prevalence was 5% (n=15 of 300) and for women in this group 7.21% (22 of 305).

Figure 3 shows the prevalence of anaemia in women. In contrast to men (Figure 2), the bar chart for women shows a relatively high prevalence of anaemia in the younger age groups (around 50 years) which declined with every age group until it increased again after the age of 65. For men in t1, the highest prevalence was 8.58% in the age group 70 to 74 years. As before in t0 and t1, the prevalence in t2 for men and women combined was highest in the oldest age group (80 years and older), accounting for 10.93% (n=27 of 247). For males in t2 separately, the prevalence remained higher than 10% from age 70 on and rose to 12.07% (n=14 of 116) in men aged 80 years and older. The prevalence in women stayed below 10% with a maximum of 9.92% (n= 12 of 131) in women aged 80 years and older. A comparison of the bar charts for men and women shows that the anaemia prevalence in elderly men was higher than in elderly women.
Prevalence of Anaemia in Men

![Graph showing prevalence of anaemia in men across age groups from t0 to t2.]

Figure 2: Prevalence of Anaemia over Age in Men in t0, t1 and t2

Prevalence of Anaemia in Women

![Graph showing prevalence of anaemia in women across age groups from t0 to t2.]

Figure 3: Prevalence of Anaemia over Age in Women in t0, t1 and t2
In t0 149 of 152 cases of anaemia could be categorised according to the definitions presented before (unexplained anaemia included). Three cases were categorised as renal anaemia, 43 cases as IDA, 32 cases as ACI, three cases as vitamin B12 or folic acid deficiency and 68 cases remained unexplained (UA). In the 5-year follow-up, 163 of 169 cases could be assigned to one of the categories: five renal, 59 IDA, 32 ACI, four vitamin b12/folic acid deficiency and 63 UA. In t2 163 of 180 cases were categorised as follows: eight renal, 31 IDA, 18 ACI, 14 vitamin B12/folic acid deficiency and 92 UA. The most prevalent anaemia category for men was UA for all examination times (57.38% in t0, 42.84% in t1 and 56.70% in t2). For women the most prevalent anaemia categories were IDA and UA. Of all anaemia cases in women in t0 37.50% were UA and 35.23% IDA. In t1 more women had IDA than UA (43.04% IDA, 34.18% UA). In t2 56.06% of anaemic women had UA and 24.24% IDA.

The relation between aetiology and age for both sexes in t0 is illustrated in Figure 4. The length of the boxplots represents the range of age in the subgroups of anaemia in t0. The average age for men and women with unexplained anaemia was higher, compared to the other aetiology groups. The age range for women was very wide, while the age range for men was more compact and located in higher ages. The according Boxplot 13 for t1 and Boxplot 14 for t2 can be found in Appendix A.1 and Appendix A.2.

The pie chart in Figure 5 shows the allocation of the five subtypes of anaemia categorised in this study in t0. The pie charts for t1 and t2 can be found in the appendix (Appendix B.1 and Appendix B.2, Figures 15 and 16). While for women iron deficiency and unexplained anaemia together were the main causes in t0 and t1, for men unexplained anaemia was in all observation periods the most prevalent category and rose from 35 in t0 to 55 cases in t2.

Table 9 shows the frequencies of each aetiology category in the whole observation time at a glance. In total, there were 11408 cases without anaemia (96.00%) and 223 cases
Figure 4: Aetiology Categories and Age in t0: Comparison of No Anaemia, Renal Anaemia, Iron Deficiency Anaemia (IDA), Anaemia of Chronic Inflammation (ACI), Vitamin B12/Folic Acid Deficiency Anaemia and Unexplained Anaemia (UA).

of unexplained anaemia (1.88%) in this study.
Figure 5: Number of Anaemia Cases in Each Aetiology Category in $t_0$ for Men and for Women

<table>
<thead>
<tr>
<th>Aetiology Category</th>
<th>$t_0$</th>
<th>$t_1$</th>
<th>$t_2$</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>male</td>
<td>female</td>
<td>male</td>
<td>female</td>
</tr>
<tr>
<td>No Anaemia</td>
<td>2307 (97.42)</td>
<td>2302 (96.3)</td>
<td>1950 (95.87)</td>
<td>1985 (96.17)</td>
</tr>
<tr>
<td>Renal Anaemia</td>
<td>2 (0.08)</td>
<td>1 (0.04)</td>
<td>4 (0.20)</td>
<td>1 (0.05)</td>
</tr>
<tr>
<td>IDA</td>
<td>12 (0.51)</td>
<td>31 (1.30)</td>
<td>25 (1.23)</td>
<td>34 (1.65)</td>
</tr>
<tr>
<td>ACI</td>
<td>11 (0.46)</td>
<td>21 (0.88)</td>
<td>15 (0.74)</td>
<td>17 (0.82)</td>
</tr>
<tr>
<td>Vitamin B12/Folic Acid Deficiency</td>
<td>1 (0.04)</td>
<td>2 (0.08)</td>
<td>4 (0.20)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>UA</td>
<td>35 (1.48)</td>
<td>33 (1.38)</td>
<td>36 (1.77)</td>
<td>27 (1.31)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2368</td>
<td>2390</td>
<td>2034</td>
<td>2064</td>
</tr>
</tbody>
</table>

Frequency Missing = 2532

Table 9: Frequency of Anaemia by Sex and Period

The development of testosterone over age is visualised in Figure 6 for men and Figure 7 for women: Average testosterone levels in the different age groups were compared between anaemic and non-anaemic participants. Both for men and women, average testosterone levels were lower for almost all anaemic versus non-anaemic age groups. Interestingly, the average testosterone level declined with age in men, while it increased with age in women.
Figure 6: Mean Testosterone Level in Men in nmol/l by Age Groups, Summarised over t0, t1 and t2

Figure 7: Mean Testosterone Level in Women nmol/l by Age Groups, Summarised over t0, t1 and t2
3.4 Analyses

The purpose of this analysis was to examine the possible influence of low testosterone on the pathogenesis of anaemia, particularly on unexplained anaemia, in the elderly. The main variables of interest were the haemoglobin level, the testosterone level and the aetiology of anaemia. The analysis was, as mentioned in the subsection on statistical methods, based on four different estimation approaches: Firstly, differences in testosterone levels across age and dependent on aetiology in the cross section in a multivariate linear regression model. Secondly, differences in testosterone levels across age, depending on the aetiology of anaemia in a mixed model. Thirdly, differences in haemoglobin levels as a function of testosterone in a mixed model and lastly, the estimation of odds ratios to predict the risk associated with low testosterone.

*Testosterone in the Cross Section*

The first step of this analysis was to look at the data in the cross section. The testosterone level was regressed on aetiology and controlled for age and BMI, as both are known to influence testosterone in different ways. I used a multivariate linear model as shown in Equation (1). The regression was estimated for the different subgroups of anaemia and for men and women separately at each observation period. Figure 8 sets out the estimated regression lines and the measured testosterone values for men in different colours for each subtype of aetiology for t2 and a set BMI= 25 kg/m². While non-anaemic men (light blue line) show only a gentle descent and start at a comparatively high intercept, UA (purple) and IDA (orange) show a steeper slope and ACI (green) a very low intercept. The graphs for t0 and t1 for men (Figure 17 and 18) and for women (Figure 19, 20 and 21) can be found in Appendix C.1 to C.5. The testosterone values from non-anaemic people were excluded for better transparency.
Figure 8: Testosterone and Age in Men in t2

Testosterone in the Longitudinal Section

To further inspect the development of testosterone over age in conjunction with the anaemia category, I transposed the data into a longitudinal section. Figure 9 shows the development of testosterone by age in men and Figure 10 in women. The smoothed plots compare people who never suffered from anaemia (blue graph) with people who at least once were classified to have an unexplained anaemia (red graph). Men and women with unexplained anaemia had a lower testosterone level compared to never-anaemic men and women. UA men show a steep decrease of testosterone level after the age of 70 compared to never-anaemic participants. UA women show a decelerated increase in testosterone after 75 years of age compared to never-anaemic women. While in men the testosterone level decreased with age, it increased in women when growing older.
Figure 9: Testosterone and Age in Men in a Stata Lowess Graph: Comparing Never-Anaemic Men (blue) ith at Least Once Unexplained Anaemic Men (red)

Figure 10: Testosterone and Age in Women in a Stata Lowess Graph: Comparing Never-Anaemic Women (blue) with at Least Once Unexplained Anaemic Women (red)
Table 10 shows the regression coefficients of the mixed model with testosterone as dependent variable. In column (1) I regressed male testosterone on age and aetiology and included BMI in column (2) which did not change the magnitude of the other coefficients. The model indicates age and BMI and all subtypes of anaemia have a negative influence on testosterone for men. Coefficient estimates in Table 10, column (3) indicate that testosterone levels in healthy men decrease by 0.11 nmol/l annually. For all men suffering from any type of anaemia (except ACI) that decrease is larger. Testosterone levels of men suffering from UA decrease approximately at an annual rate that is 145% of that of healthy individuals. Column (4), (5) and (6) of Table 10 show the according results for the female sample. Coefficient estimates in column (6) indicate that in healthy women testosterone increase by 0.01 nmol/l per year and that BMI has a positive influence on testosterone. All aetiologies of anaemia have a negative impact on testosterone while the effect on the annual testosterone development is not clear. The coefficients for renal anaemia are to be treated with caution because of the few cases in this study. The predicted testosterone according to model(3) for men is illustrated in Graph 11. It shows the predicted testosterone level for men when including interaction terms into the regression. From age 60 years on all anaemia categories had a lower testosterone level than the non-anaemic group. While IDA had the highest testosterone levels in the younger ages, the regression line also had the steepest negative slope (compare Table 10). ACI had the lowest testosterone level in the younger ages but the decline over age was rather mellow, comparable to the non-anaemic group. For UA, the group of interest in this study, the decline of serum testosterone level over age was bigger than for ACI and testosterone was lower than for nutritional anaemia from the beginning on, so that predicted serum testosterone levels in this group were lowest from age 75 years on. The according graph for women is illustrated in Graph 12. Women with unexplained anaemia had lower testosterone levels compared to women
with no anaemia. In contrast to men testosterone levels increased with age in all groups except for nutritional anaemia.

<table>
<thead>
<tr>
<th></th>
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<tr>
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</tr>
<tr>
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<td>-0.50</td>
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<td>-0.46</td>
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<td>0.00</td>
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<td>[-1.01, -0.14]</td>
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<td></td>
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<tr>
<td>Intercept</td>
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<td>15.3</td>
<td>29.77</td>
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</table>

95% Confidence Interval in Square-Brackets.

Table 10: Linear Regression: Testosterone, Centered Age and Different Types of Anaemia
Figure 11: Predicted Testosterone in Men: Mixed Model with Interaction of Aetiology and Age (Table 3)

![Graph](image)

Figure 12: Predicted Testosterone in Women: Mixed Model with Interaction of Aetiology and Age (Table 6)

![Graph](image)
Haemoglobin in the Longitudinal Section

Table 11 shows the results of the mixed model, in which I used the panel data to evaluate the influence of testosterone on the level of haemoglobin. I regressed haemoglobin on testosterone and controlled for BMI and age, for men and women separately. In all models for men and for women, age had a negative coefficient. Column (1) indicates that haemoglobin decreases on average by 0.02 mg/dl for men. Accordingly, column (5) indicates an annual decrease by 0.01 mg/dl in haemoglobin for the female sample. The testosterone coefficient was positive and higher for women than for men. The 95% confidence interval of testosterone for men was [95%-CI 0.03, 0.04] and for women [95%-CI 0.08, 0.14] and did not change when including BMI or the interaction between testosterone and age. For men the BMI coefficient was positive and of the same size as testosterone [95%-CI 0.03-0.05]. For women the BMI coefficient was half the size of the men’s [95%-CI 0.01, 0.03]. The coefficients of the interaction between age and testosterone and the square term of age were very close to statistically indistinguishable. Column (4) and (8) indicate that the effect of testosterone on haemoglobin was constant over age for both sexes.
### Table 11: Linear Regression: Haemoglobin, Centered Age and Testosterone

**Odds Ratios for Anaemia and Unexplained Anaemia**

To evaluate the effect of low testosterone on the development of anaemia and unexplained anaemia in particular, I used a logistic regression to determine odds ratios. The coefficients in Table 12 show the odds ratios for being anaemic in the same period as belonging to the lowest testosterone quartile. For men the adjusted odds ratio was 2.50 [95%-CI 1.86, 3.34] and for women 2.04 [95%-CI 1.53, 2.72]. Table 13 shows the odds ratios for belonging to the lowest testosterone quartile when having an unexplained anaemia in the same period. For men, the adjusted odds ratio was 2.43 [95%-CI 1.67, 3.54] and for women 1.94 [95%-CI 1.26, 3.00].

<table>
<thead>
<tr>
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<td>(3) Haemoglobin (g/dl)</td>
<td>(4) Haemoglobin (g/dl)</td>
<td>(5) Haemoglobin (g/dl)</td>
<td>(6) Haemoglobin (g/dl)</td>
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95% Confidence Interval in Square-Brackets
Table 12: Logistic Regression with a Generalised Mixed Model using SAS PROC GLIMMIX: Odds Ratio of Anaemia and Lowest Testosterone Quartile

<table>
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<tr>
<th>Parameter</th>
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<th>95% Confidence Limits</th>
<th>women</th>
<th>95% Confidence Limits</th>
</tr>
</thead>
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<td>2.17</td>
<td>[1.63, 2.90]</td>
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<td>lowtesto **</td>
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<td>[1.92, 3.39]</td>
<td>2.22</td>
<td>[1.67, 2.97]</td>
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</table>

*crude estimation  **estimation adjusted for age and BMI

Table 13: Logistic Regression with a Generalised Mixed Model using SAS PROC GLIMMIX: Odds Ratio of Unexplained Anaemia and Lowest Testosterone Quartile

<table>
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<tr>
<th>Parameter</th>
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<th>95% Confidence Limits</th>
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<th>95% Confidence Limits</th>
</tr>
</thead>
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<td>lowtesto *</td>
<td>2.74</td>
<td>[1.91, 3.94]</td>
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<td>[1.32, 3.17]</td>
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<tr>
<td>lowtesto **</td>
<td>2.51</td>
<td>[1.71, 3.67]</td>
<td>2.09</td>
<td>[1.34, 3.24]</td>
</tr>
</tbody>
</table>

*crude estimation  **estimation adjusted for age and BMI

Table 14 shows the results of the odds ratio estimations with one period time lag for anaemia in general and Table 15 for the odds ratio estimations with one period time lag for unexplained anaemia. For men, the odds ratio for anaemia in t2 when having low testosterone in t1 was 1.97 [95%-CI 1.25, 3.12] and for women 2.24 [95%-CI 1.10, 4.54]. For unexplained anaemia, the odds ratio for men was 1.79 and the 95% confidence level encompassed the one [95%-CI 0.96, 3.34]. For women, the odds ratio for unexplained anaemia was 2.69 [95%-CI 1.09, 6.64].
Table 14: Logistic Regression with Time Lag: Odds Ratio of Anaemia in t2 and Lowest Testosterone Quartile in t1

*crude estimation    **estimation adjusted for age and BMI

<table>
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<tr>
<th>Parameter</th>
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</thead>
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<td></td>
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<td>Anaemia t2 (=1) 95%</td>
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<tr>
<td>lowtesto t1*</td>
<td>1.81 [1.18, 2.79]</td>
<td>2.35 [1.17, 4.74]</td>
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<tr>
<td>lowtesto t1**</td>
<td>1.97 [1.25, 3.12]</td>
<td>2.24 [1.17, 4.54]</td>
</tr>
</tbody>
</table>

Table 15: Logistic Regression with Time Lag: Odds Ratio of Unexplained Anaemia in t2 and Lowest Testosterone Quartile in t1

*crude estimation    **estimation adjusted for age and BMI

<table>
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<tr>
<th>Parameter</th>
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<th>women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UA t2 (=1) 95%</td>
<td>UA t2 (=1) 95%</td>
</tr>
<tr>
<td>lowtesto t1*</td>
<td>1.88 [1.04, 3.40]</td>
<td>3.07 [1.26, 7.48]</td>
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<tr>
<td>lowtesto t1**</td>
<td>1.79 [0.96, 3.34]</td>
<td>2.69 [1.09, 6.64]</td>
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</table>

4 Discussion

4.1 Characteristics of the Study Population

In Chapter 3.2, the mean and median of the main laboratory parameters were compared between non-anaemic and anaemic participants (see Tables 1 to 6). As expected, mean haemoglobin values were lower for anaemic people than for non-anaemic people. Interestingly, independent of the aetiology, anaemics had on average lower iron stores (lower iron, ferritin and sTfR and higher ferritin index) and higher EPO levels than non-anaemic people. Furthermore, anaemic people had on average a worse kidney function than the non-anaemic participants which is in agreement with Salive et al. (1992) and Culleton et al. (2006). Anaemic males were on average older than non-anaemic males which matches the expectations from previous studies (Gaskell et al., 2008).
The share of smokers was higher in the non-anaemic groups, while ex-smokers were more frequent in the anaemic groups. This might be explained by the anaemia masking effect which is attributed to smoking (Leifert, 2008) and suggests that the group of non-anaemic smokers might contain “hidden” anaemic persons.

The exploration of comorbidities showed similar results for anaemic and non-anaemic people for diseases with a high prevalence in the general population, such as arthrosis and hypertension (compare Table 7). In comparison, disease with a comparably lower prevalence in the general population, such as coronary heart disease, heart failure and stroke showed a higher prevalence in anaemic compared to non-anaemic people. The prevalence of heart failure in this study was twice as high for anaemic as for non-anaemic people which is in line firstly with the clinical expectations and secondly with results from other studies. In a population based study from Italy, Riva et al. (2009) found similar results with regard to prevalences of comorbidities and the distribution of diseases between anaemic and non-anaemic people.

4.2 Prevalence and Aetiology of Anaemia

An initial objective of the project was to identify and characterise the prevalence of anaemia in a German representative cohort. In the presented study, the prevalence of anaemia increased with age and rose for men older than 70 years and women older than 75 years over 10%. Until the age of 55 years, women had a higher anaemia prevalence than men, while after the age of 70 years the prevalence in men superseded the women’s prevalence (compare Figure 2 and Figure 7). These results correspond to the expectations based on previous research on anaemia in elderly people (Gaskell et al., 2008) and identifies elderly men and women as a group vulnerable to anaemia.

The prevalence of 152/4761 cases in t0 is in line with the results from Eisele et al. 2013 who also determined prevalence and aetiology of anaemia in the HNRS. However,
the present study had a higher classification rate than Eisele et al. (2013), because they required joint observability of the possibly defining laboratory values, even when the satisfaction of only one criterion was needed for classification. In t1 Eisele et al. (2013) found 153 anaemia cases in HNRS, compared to Dlugaj et al. (2016) who found 163 cases and the present study, that identified 169 cases in the HNRS population. These differences can be explained by the fact that some laboratory variables needed for classification had been measured subsequently and were thus not available when the first studies took place.

In total the present study identified 501 anaemia cases in the study cohort. Of these, the applied definitions could categorise 94.81% (475 of 501 cases) - unexplained anaemia included. Most anaemia cases could not be attributed to an ”explained” cause. The prevalence of anaemia and its causes was comparable to other studies with an overall UA share of 46.95% compared to 34% in NHANES III (Guralnik et al., 2004) and 36.7% in InCHIANTI (Ferrucci et al., 2013), nutritional anaemia 32.42% compared to 34% in NHANES III 40.6% in InCHIANTI, ACI 17.26% compared to 32% in NHANES III (chronic illness and renal failure) and 15.6% in InCHIANTI and renal anaemia 3.37% compared to 7% in InCHIANTI.

4.3 Testosterone and Anaemia

Association Between Testosterone and the Subgroups of Anaemia

The mixed model with testosterone as dependent variable (compared Table 10) served to quantify the lifetime development of testosterone and the association between testosterone and unexplained anaemia. Age had a negative influence on testosterone for men and the predicted decrease per year of 0.11 nmol/l was of comparable magnitude per year as reported by Harman et al. (2001), but not as high as reported e.g. by Morley et al. (1997) who found a yearly decline of 0.4 nmol/l in a far smaller cohort. For women,
the serum testosterone levels were significantly lower than for men which is consistent with general expectations. The testosterone levels increased as women aged, with a yearly increase of 0.01 nmol/l with a comparable trend as e.g. Jiroutek et al. (1998) examined. These results enhance the evidences in the understanding of postmenopausal testosterone development.

The mean testosterone level in this study was 15.31 nmol/l ± 5.94 nmol/l in men and 1.24 nmol/l ± 0.86 nmol/l in women. The testosterone level for a non-anaemic 63 year old would be, according to the results shown in Table 10, 15.51 nmol/l for men and 1.24 nmol/l for women (without adjustment for BMI). Consequently, the testosterone level would decrease by 9% (to 14.11 nmol/l) for a 73 year old non-anaemic man and increase by 8% (to 1.34 nmol/l) for a 73 year old non-anaemic women just because of ageing and is thus of clinical relevant magnitude.

The BMI was, like age, negatively correlated with testosterone in men and positively correlated with testosterone in women. These results reflect those of Overlie et al. (1999) with regard to women and of Harman et al. (2001) with regard to men. The results are also in line with the established theories on the physiological link between body fat and testosterone. Apart from its activity in the gonads and adrenal glands, the enzyme aromatase is expressed in the body fat and responsible for the conversion of androgen into oestrogen, hence the more body fat the more testosterone is converted into oestrogen (Klinke et al., 2010). A difference in BMI of 1 kg/m² explains a 0.5 nmol/l difference in testosterone in men. All other factors equal, an overweight man with BMI 30 kg/m² would have 25.5% (5 nmol/l) less testosterone than a slim man with BMI 20 kg/m². Making this comparison for women, an overweight female would have 8% more testosterone (0.1 nmol/l) than a slim women.

The coefficient for unexplained anaemia was -2.48 for men and -0.23 for women (without adding an interaction term). Belonging to the group of unexplained anaemia thus
reduces the testosterone level by 16% (from 15.51 nmol/l by 2.48 nmol/l) for men and by 17.7% (from 1.24 nmol/l by 0.22 nmol/l) for women. The magnitude of the effect of UA on testosterone is of clinical significance. The Graphs 11 and 12 visualise concisely the differences in longitudinal effects of ageing on testosterone between the subgroups of anaemia for men and women, respectively. Interestingly, all male anaemia groups had lower testosterone levels with a stronger decrease per year than the comparison group without anaemia. In contrast to men, testosterone levels in women increased with age in all groups except for nutritional anaemia. These results suggest a linkage between low testosterone and anaemia. Moreover, compared to the other anaemia groups, unexplained anaemia displayed the lowest testosterone level in higher ages, which indicates a stronger relationship of low testosterone with unexplained anaemia than with the other anaemia subtypes.

Association between Testosterone and Haemoglobin

The coefficient estimates from table 11 indicate that age had a negative influence on haemoglobin. This effect was higher for men than it was for women. The mean haemoglobin level in the cohort was 14.93 g/dl ± 1.12 g/dl for men and 13.72 g/dl ± 1.03 g/dl for women. While a 63 year old men loses 0.2 g/dl haemoglobin until his 73th birthday just by getting ten years older, a women only loses 0.1 g/dl haemoglobin in ten years. BMI had, according to the estimation, a positive influence on haemoglobin. Using the example from before, an overweight man’s haemoglobin level would be 0.4 g/dl higher, than a slim man’s. For an overweight woman, BMI would account for a 0.2 g/dl higher haemoglobin level, compared to a slim woman. Dlugaj et al. (2015) who used data of the same cohort also came to the conclusion that anaemic people had on average an lower BMI compared to persons without anaemia.

For men the coefficients size for testosterone was about the same as for BMI. Testos-
terone had a standard deviation of 5.94 nmol/l and two hypothetical men with testos-

terone values close to the upper and lower confidence level with a testosterone difference

development of e.g. 10 nmol/l would have a haemoglobin difference of 0.3 g/dl. Analogously, a testos-
terone difference of 1.5 nmol/l in women (coefficient 0.11 ± 0.86 nmol/l) could account
for a haemoglobin difference of 0.16 g/dl between a high and a low testosterone women.

Odds Ratios for Anaemia and Unexplained Anaemia

Men with low testosterone had a 2.5 times increased chance of being anaemic and a
2.43 times increased chance of having unexplained anaemia. For women, being in the
lowest testosterone quartile, the chance was 2.04 times higher for being anaemic and
1.94 times higher for having unexplained anaemia. Computing the risk ratios again with
a time lag of one period supports the possibility of causation by proving temporality
(Hill, 1965). Men with low testosterone had a 1.97 fold increased chance of becoming
anaemic after 5 years and a 1.79 fold increased chance of developing an unexplained
anaemia after 5 years. For women, the risk was even higher with a chance of 2.24
for developing anaemia and 2.69 for developing unexplained anaemia. Ferrucci et al.
(2013) came to similar conclusions with an increased risk of 1.4 for men and 2.5 for
women. Their estimations made with bioavailable, instead of total testosterone level
reached even higher risk scores.

Independent of age and BMI, men and women in the lowest total testosterone quartile
had a higher risk of being anaemic, higher risk of having unexplained anaemia and
higher risk of developing anaemia and unexplained anaemia over a five year period,
compared to men and women in the highest quartile. The contemporary correlation
of anaemia status and low testosterone was slightly higher for men than for women.
Conversely, correlation between low testosterone and future anaemia was a stronger
predictor for women than for men. This finding is in line with Ferrucci et al. (2013),
who found the relationship between testosterone and anaemia to be stronger for women than for men.

4.4 Strengths and Limitations

A strength of this study is the population-based approach, which provides a variety of detailed laboratory and socio-economic data for a comparatively large cohort over three examination periods in ten years. However, the Heinz Nixdorf Recall Study was not initially designed for the research on anaemia or unexplained anaemia. Some laboratory anaemia specific variables, such as serum albumin, hepcidin and reticulocyte haemoglobin would be of special interest for haematological diagnostics, and might allow to attribute a known cause to some of the so far unexplained anaemia cases.

As mentioned before, the definition of anaemia and its subgroups is inconsistent in literature and cut-off values for haemoglobin levels are the subject of an ongoing debate. The application of the WHO definition could underestimate the true anaemia prevalence in this study cohort. In order to compare the herein presented results with other studies, it is important to consider the definitions and criteria used.

This study’s findings encourage a biologically plausible explanation for a disease which so far has not been explained satisfactorily by any other approach. The longitudinal odds ratio estimations suggest a temporal relationship of the association between low testosterone and anaemia or unexplained anaemia. This provides evidence for a causal connection between low testosterone and anaemia/ unexplained anaemia. It is the second study which could provide evidence for the causation between low testosterone and unexplained anaemia and suggests consistency of the findings. The study by Ferrucci et al. (2013) was carried out in a different country with a different cohort and in a different time and still came to the same conclusions.

Nevertheless, the association with low testosterone was stronger for unexplained anaemia
but not exclusive and could also be shown for the general diagnosis anaemia. Also, multi-causation is likely and this study did not consider other diagnosed diseases as possible cause for anaemia in the statistical analyses.

Furthermore, only laboratory variables were used to classify the aetiology of anaemia. Diseases, such as cancer or myelodysplastic syndrome, as well as drugs affecting the haematopoiesis were not considered in the causation of anaemia and might in some cases deliver a different underlying cause for anaemia as assumed in this study.

As erythrocytosis is a known adverse effect of testosterone therapy, the discovered association between low testosterone and anaemia is consistent with the physiological characteristics of blood production so far.

The majority of studies that have examined the relationship between testosterone and haemoglobin so far excluded women from their analyses. This results in a lack of knowledge on this topic with regard to women. Since in this study independent analyses were made for both sexes, it contributes to the understanding of the influence of testosterone on haemoglobin in elderly men and women.

4.5 Outlook

Although data on medication and diagnoses were collected, they were not used in this study. Particularly medication, that affects or treats anaemia and drugs, that affect the testosterone level would be of special interest for further investigations in this topic. This study supplement the results of Eisele et al. (2013), who provided for the first time estimates of prevalence, incidence and aetiology of anaemia in the general population in Germany. Much more information is still to be extracted from this comprehensive study and the still ongoing follow-up examinations. The subsequent measuring of hepcidin, albumin, IL6, reticulocyte haemoglobin and so on could still enrich the knowledge about anaemia in Germany. Furthermore the economic implications for the health
care system would be of interest in order to implement targeting diagnosis tools and preventive measures.

The findings encourage the theory that a low testosterone level is a risk factor for the development of anaemia in older men and women. However, this should be confirmed in further studies. The underlying pathomechanism is still unclear and the question of how testosterone effects the haematopoiesis remains open.

Ferrucci et al. (2006) showed that the association of the testosterone level and anaemia was stronger for bioavailable than for total serum testosterone. In this study, I only looked at total serum testosterone. Particularly in the margins of testosterone level cut-offs, it could be interesting to look at the results when using bioavailable testosterone (Vermeulen et al., 1999).

Further questions concerning clinical implications remain. The study of Roig et al. (2017) gave evidence that a testosterone screening and a consequential low dose testosterone treatment in the elderly might be beneficial for the prevention of cases of unexplained anaemia in the elderly. However, the effects and the side-effects of testosterone treatment need closer examination. Apart from low dose androgen therapy, anti-inflammatory therapy could potentially be a therapeutic approach to improve anaemia in the elderly, which needs further examination.
5 Summary

Over the past decade, it has been observed that anaemia is not just a symptom but also a risk factor for heart failure, first all-cause hospitalisation, cognitive impairment and mortality. Existing research recognised the prevalence to be increasing with age, while a high rate of anaemia could not be allocated to a specific cause. In previous studies on unexplained anaemia, low testosterone has been found to play a potential role in the pathogenesis of anaemia in the elderly.

The present study sets out with the aim of quantifying anaemia in the elderly in Germany and of assessing whether low testosterone is a risk factor for developing anaemia. For this cause, a population-based cohort study with men and women between the ages 45 and 85 years was used. The criteria for the definition and classification of anaemia were aligned to the World Health Organisation guidelines and to previous research. The prevalence of anaemia was found to be rising with age to over 10% in the oldest age group which identified elderly men and women as a group vulnerable to anaemia. Furthermore, the prevalence of older men was higher than of older women. Among all anaemia cases, 46.95% remained unexplained. Anaemic people had on average lower iron stores, were older and had worse kidney function than non-anaemic people. Testosterone levels were lower in anaemic than in non-anaemic people and while the testosterone in men declined with age, it rose with age in women.

Independent of age and body mass index, low testosterone was found to be a risk factor for developing anaemia and unexplained anaemia in the future for men and women. Moreover, the relationship between testosterone and anaemia was stronger for women than for men. The findings suggest that low testosterone might be a potential cause or co-cause in the development of unexplained anaemia, a disease that particularly rises with older age and has shown severe consequences on cognition, overall health and mortality.
6 References


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7 Appendix

7.1 Figures

A.1

Figure 13: Aetiology Categories and Age in t1.
IDA = Iron Deficiency Anaemia, ACI = Anaemia of Chronic Inflammation UA = Unexplained Anaemia
Figure 14: Aetiology Categories and Age in t2

IDA = Iron Deficiency Anaemia, ACI = Anaemia of Chronic Inflammation UA = Unexplained Anaemia
B.1

Figure 15: Number of Anaemia Cases in Each Aetiology Category in t1
IDA = Iron Deficiency Anaemia, ACI = Anaemia of Chronic Inflammation UA = Unexplained Anaemia

B.2

Figure 16: Number of Anaemia Cases in Each Aetiology Category in t2
IDA = Iron Deficiency Anaemia, ACI = Anaemia of Chronic Inflammation UA = Unexplained Anaemia
Testosterone and Age in Men with BMI = 25 kg/m² in t0

Figure 17: Testosterone and Age in Men in t0

IDA = Iron Deficiency Anaemia, ACI = Anaemia of Chronic Inflammation UA = Unexplained Anaemia
Testosterone and Age in Men with BMI = 25 kg/m² in t1

Figure 18: Testosterone and Age in Men in t1

IDA = Iron Deficiency Anaemia, ACI = Anaemia of Chronic Inflammation UA = Unexplained Anaemia
Figure 19: Testosterone and Age in Women in t0

IDA = Iron Deficiency Anaemia, ACI = Anaemia of Chronic Inflammation UA = Unexplained Anaemia
Figure 20: Testosterone and Age in Women in t1
IDA = Iron Deficiency Anaemia, ACI = Anaemia of Chronic Inflammation UA = Unexplained Anaemia
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7.4 Abbreviations

DRG ......... German Diagnosis Related Groups System
WHO ........ World Health Organisation
UNDP ...... United Nations Development Programme
HDI ........ Human Development Index
NHANES .... National Health and Nutrition Examination Survey
CAD ........ Coronary Artery Disease
MCV ........ Mean Corpuscular Volume
MCHC ...... Mean Corpuscular Haemoglobin Concentration
EPO .......... Erythropoietin
Hb ........... Haemoglobin
BMI .......... Body Mass Index
CKD .......... Chronic Kidney Disease/ Chronic Renal Insufficiency
GFR .......... Glomerular Filtration Rate
IDA .......... Iron Deficiency Anaemia
InCHIANTI . The Invecchiare in Chianti Study
ACI .......... Anaemia of Chronic Inflammation
UA ........... Unexplained Anaemia
UAE ........ Unexplained Anaemia of the Elderly
ProbID ...... Proband Identification Number
HNRS ....... The Heinz Nixdorf Recall Study
RECALL ..... Risk Factors, Evaluation of Coronary Calcification, and Lifestyle
LDL .......... Low Density Lipoprotein
8 Curriculum Vitae

Der Lebenslauf ist in der Online-Version aus Gründen des Datenschutzes nicht enthalten.