Health-related quality of life in thyroid and blood disorders

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CHAPTER 10

Summary, general discussion and perspectives

Summary

Chapter 1 provides a general introduction and defines the aims of the present thesis. In short, healthy aging will be one of the major challenges in the near future and thyroid and blood disorders are prevalent in older individuals. The aim of this thesis is to provide insight in health outcomes including health-related quality of life (HRQoL) in individuals with thyroid and blood disorders. To this end, we use data from the large Dutch Lifelines Cohort Study.

The first part describes the physiology of thyroid hormone regulation and points out several potential mechanisms for the decreased well-being which is present in a substantial part of individuals with primary hypothyroidism, despite adequate treatment according to current guidelines, i.e. a thyroid stimulating hormone level (TSH) in the normal range.

The second part summarizes the characteristics of the aging hematopoietic system and focuses on blood count abnormalities and their association with negative health outcomes including HRQoL.

Part I - Thyroid disorders

Epidemiology of thyroid disorders

In **Chapter 2** we evaluated and compared the prevalence of thyroid disorders in population-based studies including the Lifelines Cohort Study. In Lifelines, the prevalence of known active thyroid disorders (defined as use of thyroid medication) appeared to be 3.1%. The majority used levothyroxine (>95%). Despite guidelines for the clinical management of hypothyroidism, mentioning to aim for a TSH level in the normal range, and the knowledge that both under- and overtreatment of levothyroxine are associated with adverse health outcomes (1-3), less than 60% of the levothyroxine users had a TSH level in the normal range.

Besides known thyroid disorders, undetected thyroid disorders were also frequently present, especially subclinical hypothyroidism (defined as a TSH 4.01 - 10.0 mU/L) which had a prevalence of 9.4%. Both overt hypothyroidism (defined as a TSH level ≥ 10.0 mU/L) and hyperthyroidism (defined as a TSH level <0.4 mU/L) had a prevalence of 0.7%.

By comparing our Lifelines data with data from the National Health and Nutrition Examination Survey (NHANES) and the Dutch general practitioner morbidity registry, we concluded that the currently used unstructured questionnaires about new thyroid disorders in Lifelines severely underestimated the actual incidence of hypothyroidism and therefore proved to be not reliable. This emphasizes the need for both more structured questions as well as linking to general practitioners and pharmacists' data to improve the completeness and reliability of data (on thyroid disorders) in Lifelines.

Finally, we indicated that the large group of individuals with subclinical hypothyroidism might provide an excellent possibility to prospectively study the natural course of this common disorder.

Hypothyroidism and health-related quality of life

As presented in **Chapter 2**, the prevalence of thyroid dysfunction increases with age, with 15-20% of those above 60 years having subclinical or overt hypothyroidism. Thyroid hormone replacement with synthetic levothyroxine is the standard treatment of primary hypothyroidism, according to current guidelines. The goals are to provide resolution of hypothyroid signs and symptoms, to normalise TSH levels and to avoid overtreatment (1).

Unfortunately, a substantial part of the patients continues to experience symptoms and disturbed well-being, as well as lower neurocognitive functioning, despite the fact that they are biochemically euthyroid (4, 5).

Based on literature demonstrating that certain genetic polymorphisms of deiodinases may be associated with variation in thyroid hormone levels and that carriers of the Thr92Ala polymorphism in deiodinase type 2 (DIO2) exhibit increased expression of genes associated with oxidative stress and inflammation in brain tissue (6), which could contribute to the neurocognitive symptoms of affected carriers, we hypothesized that homozygous carriers of the DIO2-92Ala allele are characterized by a low free triiodothyronine (FT3) to free thyroxine (FT4) ratio, a lower HRQoL and impaired cognitive functioning in comparison to subjects who are heterozygous Thr / Ala or homozygous Thr / Thr carriers.

The cross-sectional study described in **Chapter 3** showed that participants using levothyroxine had a lower FT3 and a higher FT4, while their TSH levels were comparable to the general population, confirming the results of previous studies. We demonstrated that female levothyroxine users had a lower HRQoL in six out of the eight domains of the RAND-36 questionnaire (including physical functioning, vitality, mental health, social functioning, bodily pain and general health). In contrast, we did not observe a difference in executive functioning, as part of cognitive functioning, which was assessed with the Ruff Figural Fluency Test. Due to the low number of male levothyroxine users, no conclusions about males could be drawn. As opposed to our hypothesis, the DIO2 Thr92Ala polymorphism appeared not to be associated with any of the thyroid parameters, HRQoL or cognitive functioning, neither in the general population nor in subjects on thyroid hormone replacement therapy.

As presented in **Chapter 3**, the DIO2 Thr92Ala polymorphism appeared not to be an explanation for the decreased HRQoL in individuals treated for hypothyroidism. In **Chapter 4** we focused on the prevalence of comorbidity in individuals with hypothyroidism (defined as use of thyroid hormone) and on the joined impact of hypothyroidism and comorbidity on HRQoL. Data from structured questionnaires concerning current and past medical disorders, verified medication

use, physical examination, biochemical measurements and the Mini-International Neuropsychiatric Interview were collectively used to determine whether an individual had comorbidity.

We demonstrated that comorbidity was more frequently present in individuals with hypothyroidism compared to the general population. The co-existence of other chronic medical conditions in subjects with hypothyroidism led to further lowering of HRQoL, the effect of which was generally additive. Focusing on hypothyroid individuals with an impaired HRQoL, we showed that these individuals had more comorbidity than those without impaired HRQoL. Especially mental disorders and musculoskeletal diseases were more prevalent. This study emphasizes the role of comorbidity in individuals with hypothyroidism concerning HRQoL.

Part II - Blood disorders

Blood count abnormalities, adverse health outcomes and health-related quality of life

Despite the fact that anaemia is a major health problem (7), large scale studies examining the association between anaemia, survival and HRQoL were lacking. In **Chapter 5**, we presented these associations. In Lifelines, the prevalence of anaemia, defined according to the World Health Organization (WHO) criteria, was 4.0% in the total cohort whereas the prevalence was 2.8% among subjects of 60 years and older. This is consistent with findings of other European population-based studies. In about half of the older individuals with anaemia no specific cause could be identified (unexplained anaemia).

Anaemia was associated with worse overall survival and was an independent risk factor for impaired HRQoL in individuals older than 60 years, but not in younger individuals. The most pronounced associations between anaemia and HRQoL were observed in the domains of the RAND-36 related to physical health. Although consensus on the subclassification of anaemia is lacking, our data suggest that particularly anaemia of chronic inflammation is associated with reduced overall survival and is an independent risk factor for impaired HRQoL in individuals older than 60 years.

We hypothesized that in various age cohorts the optimal haemoglobin concentrations in perspective of optimal HRQoL are not synonymous with currently used WHO reference values (<13.0 g/dL (~8.0 mmol/L) in adult males and <12.0 g/dL (~7.5 mmol/L) in adult, non-pregnant females. We observed that females (but not males) of 60 years and older with a haemoglobin concentration in the lower normal WHO range already experience a decreased HRQoL. This suggests that the definition of anaemia in these older females, in perspective of HRQoL, should be altered to a haemoglobin concentration <13.0 g/dL (~8.0 mmol/L), which is comparable to the definition of anaemia in males.

As presented in **Chapter 5**, about one half of the older anaemic cases could not be explained by nutrient deficiency or by anaemia of chronic inflammation. Myelodysplastic syndromes (MDS) are one group of the potential underlying pathologies of unexplained anaemia in older individuals. In current guidelines for symptomatic lower-risk MDS patients with anaemia, treatment modalities which aim to increase haemoglobin levels are the first choice (8), although there is conflicting data on the effect of increasing haemoglobin values concerning its impact on HRQoL (9-12).

In **Chapter 6** the first study assessing the anaemia-independent effect of lower-risk MDS on HRQoL is described. To achieve this, data from a large number of newly diagnosed lower-risk MDS patients (derived from the EUMDS registry) and the large general population-based Lifelines cohort were linked and evaluated. HRQoL was measured in the EUMDS Registry with the EQ-5D questionnaire. RAND-36 domain scores from Lifelines participants were converted to EQ-5D scores to enable comparisons between both cohorts. In this study, we confirmed the previously described overall negative impact of lower-risk MDS on HRQoL, but our data indicated that the majority of this negative impact, particularly in the EQ-5D dimensions mobility, usual activities, self-care and anxiety / depression, was not mediated via anaemia. These data suggest that treatment in lower-risk MDS should not only focus on increasing haemoglobin levels. Attention to underlying pathogenic mechanisms (e.g. inflammation) with possible opportunities for treatment might be integrated with haemoglobin increasing treatment strategies.

Erythrocytosis, the other end of the spectrum of red blood cell count abnormalities, is studied in **Chapter 7**. We defined erythrocytosis in two ways. First, according to the 2008 WHO classification of myeloproliferative neoplasms and the British Committee for Standards in Hematology (BCSH) as a haemoglobin concentration >18.5 g/dL (~11.5 mmol/L) or a haematocrit ≥ 52% in males and a haemoglobin concentration >16.5 g/dL (~10.2 mmol/L) or a haematocrit ≥ 48% in females (strict criteria) (13, 14). Second, according to the renewed 2016 WHO classification of myeloproliferative neoplasms as a haemoglobin concentration >16.5 g/dL (~10.2 mmol/L) or a haematocrit >49% in males and a haemoglobin concentration >16.0 g/dL (~10.0 mmol/L) or a haematocrit >48% in females (wide criteria) (15). In our study population 185 males (0.3%) and 223 females (0.3%) met the strict criteria for erythrocytosis, whereas 4868 males (7.6%) and 309 females (0.4%) met the wide criteria. Lifelines data were linked to the nationwide network and registry of histo- and cytopathology in the Netherlands to assess the incidence of haematological malignancies, and to the national death statistics registry to perform cause-specific survival analyses. Erythrocytosis, only when defined using strict criteria, turned out to be associated with a medical history of cardiovascular events, cardiovascular mortality and all-cause mortality, independent of conventional risk factors.

Out of the 408 individuals with erythrocytosis defined using the strict criteria, six were diagnosed during follow-up with a haematological malignancy of myeloid origin, as compared to eleven out of 4995 individuals with erythrocytosis defined according to the wide criteria.

Furthermore, we performed next generation sequencing on a subset of individuals with erythrocytosis (using the strict criteria) and concurrent thrombocytosis and / or leucocytosis who were matched with individuals with isolated erythrocytosis. This showed an unexpected high proportion of clonal haematopoiesis (38%), with a comparable proportion in both groups. Clonal haematopoiesis appeared to be associated with cardiovascular morbidity. The JAK2 V617F mutation with a variant allele frequency >5%, was present in seven out of 133 sequenced individuals, all having erythrocytosis with concurrent thrombocytosis and / or leucocytosis. We detected a remarkable high proportion of individuals with BCOR / BCORL1 mutations, of which the role in haematopoiesis is currently unknown.

Not only large-scale studies examining the association between anaemia and HRQoL were lacking, also data about the association between abnormalities in white blood cell and platelet count and HRQoL were scarce. In **Chapter 8**, we revealed an association between inflammatory (leucocytosis) and myeloid-skewed (e.g. neutrophilia and a high neutrophil to lymphocyte ratio) blood cell counts and inferior HRQoL in community-dwelling adult individuals of all ages, independent of potential confounders, like anaemia. These blood cell abnormalities might be used as a marker for inferior health outcomes. Further, leucocytosis proved to be a better marker for impaired HRQoL as compared to high-sensitivity C-reactive protein levels in this study.

Finally, in **Chapter 9** we examined the effect of smoking on the mean corpuscular volume of erythrocytes in two large population-based studies. The Lifelines Cohort Study, in which smoking was assessed by means of a self-administrated questionnaire and the Prevention of Renal and Vascular EndStage Disease (PREVEND) cohort study, in which smoking was assessed by 24-hour urinary cotinine excretion levels. In both cohorts, smoking was an important determinant of mean corpuscular volume levels and macrocytosis, independent of prominent known causes such as alcohol intake, liver disease, and vitamin B12 and folic acid deficiency. Accordingly, smoking should be included in current guidelines regarding known causes of an elevated mean corpuscular volume.

General discussion and perspectives

Part I - Thyroid disorders

Hypothyroidism and health-related quality of life

A substantial part of the individuals who are adequately treated for hypothyroidism, experience persistent symptoms and decreased well-being. In a study of 381 euthyroid levothyroxine users, who were treated for at least four months, and 535 matched controls, levothyroxine users were more likely to have an abnormal score on the Thyroid Symptom Questionnaire for psychological well-being (48.6% vs. 35.0%) (4). In a Dutch study, well-being scores of 141 euthyroid levothyroxine treated patients, based on the Symptom Check List-90 and the mental health and vitality domains of the RAND-36, appeared to be lower than reference scores from the general population (5).

Given the high prevalence of hypothyroidism, which is increasing with age, and the considerable proportion of patients having persistent complaints and impaired HRQoL, it is relevant to elucidate underlying factors which might contribute to this phenomenon. In this thesis, we could not demonstrate a role of the DIO2 Thr92Ala polymorphism. Comorbidity appeared to impair HRQoL in thyroid hormone users in an additive manner and was more frequently present in thyroid hormone users with an impaired HRQoL as compared to those not experiencing an impaired HRQoL. This also applied to the subgroup of thyroid hormone users with a TSH level in the normal range. Therefore, comorbidity may contribute to the impaired HRQoL in individuals treated for hypothyroidism. Other possible explanations will be discussed in the following paragraphs.

First, ongoing complaints and impaired HRQoL may be the result of ineffective levothyroxine supplementation, meaning under- or overtreatment of patients. Despite guidelines mentioning to aim for a TSH level in the (low)normal range, several studies, including ours, report that only around 60% of individuals treated for hypothyroidism are biochemically euthyroid (1). Undertreatment, which appears to be more prevalent than overtreatment, has previously been reported to be associated with poorer HRQoL (16). Additionally to impaired HRQoL, both under- and overtreatment are associated with adverse events. For example, undertreatment is associated with increased risk of cardiovascular disease and arrhythmias, whereas overtreatment results in an increased risk of atrial fibrillation, osteoporosis and fractures (1, 2). Furthermore, duration of both under- and overtreatment has been shown to be associated with increased all-cause mortality (3).

Second, a TSH in the reference range, which is one of the aims of thyroid hormone substitution therapy, might not be a reliable marker of an individual's thyroid status and therefore might not be the most suitable target for thyroid hormone substitution therapy (17). Currently, reference values for thyroid hormones,

which are used to determine whether an individual is euthyroid, are defined as the central 95% of the results of healthy individuals (18). Given the large differences in thyroid function in healthy individuals, the reference ranges are relatively wide. It is demonstrated that the width of an individual's 95% confidence interval of thyroid hormones is about half of the 95% confidence interval of the total study cohort (19). The differences in thyroid hormone levels in healthy individuals are caused by both analytical and biological variation. Biological variation can be divided into inter-individual and intra-individual variation. Inter-individual variation is caused by the difference in set point between individuals, whereas intra-individual variation is characterized by seasonal and circadian variance amongst others (20, 21). Whether a reference range derived from population-based data is able to detect an abnormal result for an individual, depends on the ratio of intra-individual to inter-individual variation. A low ratio suggests that the reference range is an insensitive measure, whereas a high ratio suggests that the reference range is an adequate tool. Since thyroid hormones are characterized by small intra-individual variation, compared to inter-individual variation (22), the low ratio indicates that laboratory population-based reference ranges are rather insensitive to deviations from normality in an individual (20).

As a consequence, thyroid parameters of an individual treated with thyroid hormone might be normalised in respect of the used reference range, but might still differ from an individual's set point. TSH, FT4 and FT3 are interrelated through the hypothalamic-pituitary-thyroid axis. Connected pairs of TSH and FT4 define an individual's set point (23, 24). It has been demonstrated that the equilibrium in thyroid hormones differed between individuals treated with levothyroxine and untreated individuals (25). Several mathematical techniques using multiple thyroid hormone measurements to estimate an individual's set point have been described (26, 27). Genetic variability appears to be a main determinant of this set point (28, 29). A recently published meta-analysis of genome-wide association studies for thyroid function (e.g. TSH and FT4) and dysfunction reports an independent association with 109 genetic variants (30). Future studies might explore whether genetic variants are able to predict an individual's set point. Additionally, physiological processes such as aging and pathophysiological processes might also be determinants of the set point (31, 32). With aging, TSH levels increase without a change in FT4 levels. Together, this suggests that the increase in TSH level emerges from an age-related alteration in the TSH set point (31). Up till now, no randomized clinical trials have been performed in which set point targeting is compared with normal replacement treatment. Studies which assessed HRQoL in treated hypothyroid participants using different TSH targets demonstrated contradictory results. In a randomized, double-blind, cross-over study levothyroxine dose adjustments aiming for a low-normal TSH level did not result in beneficial HRQoL compared with target TSH levels in the upper-normal range (33). In contrast, in another study, well-being was assessed when treating with various doses of levothyroxine and it was demonstrated that the

best well-being was achieved in individuals treated with a supra-optimal dose (34). In the context of personalized medicine, future studies might focus on whether a set point can be used as a guide for treatment to improve HRQoL.

Besides HRQoL, an individual's set point may also be involved in adverse health outcomes. Not only overt and subclinical thyroid dysfunction, but also variation within the euthyroid range is described to be associated with adverse outcomes. In studies restricted to euthyroid subjects, variation in TSH and FT4 levels has been demonstrated to be associated with a large number of adverse health outcomes including atrial fibrillation, stroke, metabolic syndrome, dementia and mortality (35-40). Thyroid hormone levels, and specifically FT4 levels, seem to be stronger associated with clinical parameters than TSH levels (41). Future studies might focus on whether a set point can also be used in attempts to define optimal thyroid function based on clinical outcomes (42).

Third, levothyroxine may not reverse hypothyroidism in all tissues. Normally, approximately 20% of the biologically active T3 is secreted by the thyroid, whereas 80% is derived from deiodination of T4 in extra-thyroidal tissue. Treatment of hypothyroidism with levothyroxine monotherapy relies on the capacity of the deiodinases to fully restore T3 levels. However, when adequately treating hypothyroidism with levothyroxine monotherapy, TSH level normalises, but the FT3 / FT4 ratio is reduced. Our study was in accordance with several other large studies demonstrating that FT3 is lower, whereas FT4 is higher in euthyroid individuals treated with levothyroxine compared with euthyroid controls (43, 44).

This phenomenon can be explained by two characteristics of DIO2. DIO2 is a membrane protein on the endoplasmic reticulum. It has a variable half-life time, which depends on the concentration of its substrate T4. First, DIO2 is ubiquitinated and degraded after interaction with T4. So, the half-time (1 hour) of DIO2 becomes shorter (20 minutes) with increasing T4 levels. Conversely, lower T4 levels lead to a prolonged half-time of DIO2, which results in an increase of extra-thyroidal T3 production. Second, DIO2 in the hypothalamus is less sensitive to ubiquitination, compared to the rest of the body (45, 46). As a consequence, in individuals treated with levothyroxine, more peripheral DIO2 activity is lost compared to DIO2 activity in the hypothalamus. Accordingly, levothyroxine use results in relatively greater central T3 than peripheral T3 production. The dose required to normalise serum TSH levels is therefore lower than the dose that is required to normalise serum FT3 (47). It has been hypothesized that a two-hit theory might explain why a substantial part, but not all levothyroxine treated patients have persistent complaints. The relatively low FT3 levels together with individual patient factors, may exhaust the ability of an individual to compensate (47).

The lower FT3 levels and higher FT4 levels in combination with the fact that some patients might be less responsive to levothyroxine monotherapy, led to multiple trials in which levothyroxine was combined with liothyronine (combination therapy). The majority of these trials have failed to demonstrate a beneficial effect of combination

therapy (48, 49). However, it could be argued whether these studies included the appropriate subjects. Many studies did not specifically include subjects with residual complaints. However, some studies included subpopulations with a small number of participants with complaints. These studies were not able to demonstrate a benefit of combination therapy (50-53). The Dutch multicenter double-blind randomized placebo-controlled trial with levothyroxine / liothyronine combination therapy for hypothyroidism (T3-4-Hypo trial), will assess the effect of combination therapy compared to levothyroxine monotherapy on HRQoL in individuals with Hashimoto thyroiditis with persisting complains despite being euthyroid (54).

Fourth, autoimmunity might be another contributing factor. Hashimoto thyroiditis, the most common cause of hypothyroidism, is characterized by gradual thyroid failure, due to autoimmune-mediated destruction of the thyroid. Nearly all patients have high serum levels of antibodies against one or more thyroid antigens (thyroid peroxidase (TPO) and / or thyroglobulin) (55). Several studies demonstrated that in individuals with Hashimoto thyroiditis the presence of antibodies against thyroid antigens are associated with impaired HRQoL, independent of thyroid function (56 - 58). Additionally, a recently published randomized, open-label, controlled trial studied the effect of a thyroidectomy in individuals with Hashimoto thyroiditis with anti-TPO titers >1000 IU/mL, having persistent symptoms despite adequate thyroid hormone replacement. Compared to the control group, in individuals treated with thyroidectomy, HRQoL and fatigue improved, while anti-TPO titers decreased (59). However, it should be noted that no sham procedure was performed, so a placebo effect cannot be excluded. The expected five years follow-up data from this trial will therefore provide additional information. In contrast to studies concerning thyroid antibodies, in individuals with Hashimoto thyroiditis, in large general population-based studies no association was observed between anti-TPO levels and depressive mood (60-62). Taken together, this raises the hypothesis that thyroid autoimmunity, independent of thyroid function, impacts on HRQoL only in individuals with Hashimoto thyroiditis.

With the assumption that most participants using thyroid hormone in our study have underlying Hashimoto thyroiditis, we confirmed that these individuals are at significantly increased risk of having additional autoimmune diseases. In a British study, 14.3% of individuals with Hashimoto thyroiditis had at least one other autoimmune disease. The risk was especially increased for pernicious anaemia, systemic lupus erythematosus, Addison's disease, celiac disease, and vitiligo (63). In another study, there was a significant higher prevalence of auto-immune disorders in individuals with autoimmune thyroid diseases (19.5%) compared to healthy controls (3.9%). Five disorders had a prevalence larger than 1% (autoimmune gastritis (2.8%), vitiligo (2.7%), rheumatoid arthritis (2.4%), polymyalgia rheumatica (1.4%) and celiac disease (1.3%)) (64). The presence of auto-immune disorders is known to be associated with decreased HRQoL (65, 66).

The other way around, thyroid autoimmunity is also frequently present in other autoimmune diseases. For example the prevalence of concurrent autoimmune thyroid disease (thyroiditis, Hashimoto disease or Graves' disease) in populations with other autoimmune diseases was 23% in systemic sclerosis, 21% in rheumatoid arthritis, 18% in systemic lupus erythematosus and 9% in multiple sclerosis (67). In contrast to prevalence of auto-immune diseases, other studies assessed the prevalence of non-thyroid auto-antibodies in individuals with Hashimoto thyroiditis (68). In a cohort of 359 individuals with Hashimoto's thyroiditis, adrenal autoimmunity had a prevalence of 9.0%, β-cell autoimmunity of 25.4%, celiac autoimmunity of 1.2% and gastric autoimmunity of 23.4% (69). The elevated thyroid antibody levels may therefore be seen as marker of the altered immunity (58).

Taken together, these data indicate that autoimmunity and / or the presence of other autoimmune disorders might contribute to the decreased HRQoL in a subset of patients and that screening for these disorders is justified in individuals with persistent complaints. To extend knowledge on this topic, more extensive phenotyping for example by measurement of anti-TPO antibodies in the Lifelines Cohort is indicated.

Part II - Blood disorders

Reference intervals and clinical decision limits

Biochemical measurements play an important role in clinical decision making. Reference intervals and clinical decision limits provide information on whether results can be classified as normal or abnormal. Reference intervals describe the typical distribution, usually the central 95% interval, of results seen in a healthy reference population. In contrast, a clinical decision limit is a value for which a score above or below is associated with a significantly higher risk of adverse clinical outcomes, or is of diagnostic value for the presence of a particular disease. Clinical decision limits are mainly based on clinical outcome studies (e.g. prospective cohort studies and meta-analysis) (70). Clinical decision limits based on longitudinal or interventional studies can rarely be repeated in a local setting, which emphasizes the requirement of a high degree of standardization across different laboratories. Theoretically, clinical decision limits are the most important and reference intervals can be used as a surrogate for them (71).

Thresholds for defining anaemia and erythrocytosis

The current haemoglobin thresholds for the definition of anaemia recommended by the WHO are <13.0 g/dL (~8.0 mmol/L) in adult males and <12.0 g/dL (~7.5 mmol/L) in adult, non-pregnant females. They were proposed

in 1968 and are based on data from five studies including 2712 participants from predominantly white populations in Europe and North America. The ages of the included participants ranged from 15 to 64 years. Documentation of methodology of the specific studies as well as data from other countries and races, were not available (72). It was doubted whether it was the intention of the Expert Committee in 1968 to set up a general standard. Since then, several papers have proposed criteria with thresholds ranging from 13.2 to 14.2 g/dL (~8.2 to ~8.8 mmol/L) in males and 11.6 to 12.3 g/dL (~7.2 to ~7.6 mmol/L) in females, based on 95% confidence intervals (73). For example, based on a large number of subjects (over 32000) without diabetes, renal failure, elevated inflammatory markers, decreased or increased levels of ferritin or transferrin in the NHANES and Scripps-Kaiser database, cut-off values of 13.7 g/dL (~ 8.5 mmol/L) for white adult males younger than 60 years, 13.2 g/dL (~8.2 mmol/L) for white males of 60 years and older and 12.2 g/dL (~7.6 mmol/L) for white adult females were proposed, using the lowest 2.5th percentile. For black individuals slightly lower cut-off values were proposed (73). Especially in older individuals, it could be wondered whether using the lowest 2.5th percentile to generate cut-off values is the best method, since the generally used definitions of anaemia do not always address the complex relationship between haemoglobin levels and health outcomes in these individuals (74). A different approach might be to base the definition on haemoglobin concentrations relevant to clinical outcomes, the above-mentioned clinical decision limit. It has been proposed that anaemia might be better defined with respect to a haemoglobin range associated with most favourable health outcomes, even if such a definition results in a much larger proportion of individuals being classified as anaemic (74).

In this light, the WHO is currently revisiting its haemoglobin thresholds taking in mind physiological, environmental, and genetic factors. A panel of, among others, international experts in anaemia research, and organizations (e.g. American Society of Hematology) were asked to identify key questions. Questions covered diverse themes, including differences in thresholds between individuals of different gender, age and burden of anaemia. One of the specific key questions was: *'Should anaemia be defined by physiologic, clinical or functional consequences of low haemoglobin?'* (75). Our data might contribute to answering this question and suggest that the definition of anaemia in males, a haemoglobin concentration <13.0 g/dL (8.0 mmol/L), might also be applied for females older than 60 years in perspective of HRQoL. We were not able to study the optimal definition of anaemia in perspective of mortality, hampered by the limited number of deaths in our cohort. Multiple studies demonstrated different cut-off levels in perspective of mortality. For example, Martinsson et al. reported in a Swedish population, which included participants between 44 and 73 years of age, a diagnostic cut-off of 14 g/dL (~8.7 mmol/L) in males and 13 g/dL (~8.1 mmol/L) in females, below which excess 10-years mortality was present (76). Comparable cut-offs were demonstrated in a Canadian study including individuals of 66 years and older with a median follow-

up of 3.2 years (77). Finally, in a very large Korean study of 292194 participants aged above 40 years with a mean follow-up of 7.8 years, increased mortality was observed in males with a haemoglobin concentration <14 g/dL (~ 8.7 mmol/L) and <11 g/dL (~6.9 mmol/L) in females (78).

In contrast to anaemia, the WHO definition of myeloproliferative neoplasms has been revised in 2016 (15). According to the 2008 classification of the WHO and the BCSH, polycythaemia vera should be considered in individuals with a haemoglobin concentration >185 g/L (~11.5 mmol/L) in males and >165 g/L (~10.2 mmol/L) in females or with a haematocrit >52% in males and >48% in females (13, 14). Concerned with the possibility of underdiagnosing polycythaemia vera, in the revised 2016 WHO classification the haemoglobin threshold levels were decreased to >16.5 g/dL (~11.5 mmol/L) for males and >16.0 g/dL (~10.0 mmol/L) for females and haematocrit thresholds were introduced (>49% for males and >48% for females). These concerns were based on studies which demonstrated that a substantial part of individuals with polycythaemia vera had lower haemoglobin and haematocrit levels than the WHO 2008 and BCSH thresholds and that these individuals had a higher risk of thrombosis and a decreased survival (79).

A disadvantage of the revised criteria is the large increase of cases with erythrocytosis where one would consider polycythaemia vera, compared with the stricter criteria (3000-fold increase in males and 100-fold increase in females) (80). Consequently, the specificity and positive predictive value for diagnosing polycythaemia vera is much lower using the revised criteria of erythrocytosis. Although the criteria are only proposed for diagnosing polycythaemia vera, they are also integrated in guidelines concerning erythrocytosis in general for clinical practice (81). As a consequence, this may lead to an increase in possible unnecessary diagnostic tests including bone marrow biopsies. Therefore, it has been suggested that a diagnostic work-up of polycythaemia vera is (only) indicated in cases with borderline haemoglobin or haematocrit concentrations in combination with clinical or laboratorial features associated with myeloproliferative neoplasms (80).

Our results support the perspective of clinical decision limits in erythrocytosis. They suggest that the lower haemoglobin / haematocrit thresholds in the revised WHO classification might be useful to diagnose masked polycythaemia vera, but might be too low as screening tool for secondary erythrocytosis, given the absence of associations with negative health outcomes. Additionally, our results emphasize the value of concurrent leucocytosis and or thrombocytosis in the diagnostic work-up.

Inflammaging

The increasing prevalence of chronic diseases is a reflection of the growing aging population observed worldwide. It is assumed that there is a large overlap in mechanisms driving aging and age-related diseases. One of these underlying mechanisms is inflammation. This chronic, sterile (occurring in the absence of

infection), low- grade inflammation that occurs during aging is called 'inflammaging' (82, 83). The inflammatory state, characterized by increased circulating markers of inflammation, including pro-inflammatory cytokines, can potentially accelerate the onset of age-related diseases. It has been linked to a number of chronic conditions, such as cardiovascular disease and cancer and it is also associated with mortality (84, 85). Several sources or stimuli of inflammation have been described, including accumulation of cell debris of damaged and / or dead cells and organelles, accumulation of senescent cells and changes in gut microbiota (86).

In multiple population-based studies, pro-inflammatory cytokines (tumour necrosis factor-α and interleukin-6), acute-phase proteins (C-reactive protein and fibrinogen) and erythrocyte sedimentation rate were associated with decreased HRQoL or subjectively perceived health (87-99). This is in accordance with our study in which we showed that inflammatory and myeloid-skewed blood cell counts were associated with decreased HRQoL and therefore may be used as markers for inferior health outcomes. Furthermore, we demonstrated that especially anaemia of chronic inflammation was associated with impaired HRQoL and survival in older individuals. Given these associations, it makes one wonder if treatment targeting (low-grade) inflammation might be beneficial.

Anaemia of inflammation is characterized by low serum iron levels with preserved iron storage in marrow, splenic and hepatic macrophages. So, anaemia of inflammation is primarily a disorder of iron distribution. A liver-derived peptide, hepcidin, plays a central role in iron homeostasis. Hepcidin inhibits the iron-exporting activity of ferroportin (the iron transporter which exports iron from the iron storage to the blood). Hepcidin production is upregulated in case of iron overload and inflammation, whereas it is downregulated by iron deficiency. Treatment modalities which aim to reverse hypoferremia by decreasing hepcidin concentration (e.g. by hepcidin binding or by reducing hepcidin production) are under development (100, 101).

In recent years, dysregulation of the immunological environment is considered one of the important factors in the pathogenesis of MDS (102, 103). For instance, inflammatory and auto-immune diseases have been shown to increase the risk of developing MDS (103). Furthermore, it is known that MDS patients do have higher levels of pro-inflammatory cytokines (tumour necrosis factor-α, interleukin-6 and interleukin-8) as compared to individuals without MDS (104). We demonstrated that the majority of the negative impact of lower-risk MDS on HRQoL is not mediated via anaemia. Inflammation might be a contributing factor to the decreased HRQoL in MDS. Elevated levels of pro-inflammatory cytokines in MDS were associated with worse HRQoL (105). Treatment options which aim to reduce inflammation have been shown to reduce fatigue in individuals with psoriasis and rheumatoid arthritis (106). In MDS, treatment with lenalidomide, which modulates different components of the immune system, among which altering cytokine production, has been demonstrated to be associated with improved HRQoL (107-110). Other treatment modalities targeting the immune system are currently under study (111).

Besides anaemia and inflammation, other contributing factors to fatigue, which is strongly associated with HRQoL in MDS, are proposed, including oxidative stress, sleep disorders, physical deconditioning, psychosocial factors and complications related to treatment of MDS (106). Since improving HRQoL is one of the primary treatment aims in lower-risk MDS, future studies examining these factors and their relationship with fatigue / HRQoL, are indicated.

Methodological considerations and perspectives

The studies described in this thesis were mainly based on data from the Lifelines Cohort Study and health-related quality of life was measured with the RAND 36-Item Health Survey. The major strength of the Lifelines Cohort is its large sample size, often resulting in our studies to be the largest, presenting an association. Given the cross-sectional design, no conclusions about causality of the observed outcomes (health outcomes including HRQoL) and disorders of interest (thyroid or blood disorders) can be drawn. Nevertheless, the observed associations in our studies might be useful for formulating hypotheses and provide directions for future studies.

To determine whether the results of the studies presented in this thesis can be generalized to other populations, it is important to assess the risk of selection bias. Although participating individuals of the Lifelines Cohort Study were more often female, middle aged, married, living in a semi-urban place and Dutch native than the total population living in the three Northern provinces of The Netherlands, the Lifelines Cohort was reported to be broadly representative for inhabitants of the North of the Netherlands (112). However, it should be taken in mind that the primary aim of the Lifelines Cohort Study was to elucidate interactions between environmental and genetic risk factors in the development of multifactorial diseases using an unique three generation design. Therefore, confirming our results with data from other (population-based) cohort studies, as for example those which participate in the Biobank Standardisation and Harmonisation for Research Excellence in the European Union (BioSHaRE-EU) will strengthen our findings (113). Additionally, since data from population-based studies might not perfectly represent real-life, comparing our results with registries using actual healthcare-related data as implemented in for example Spain (Catalonia) and Sweden, is indicated (114, 115). Whether our results could be generalized to other areas in the world could be argued. Especially the studies concerning anaemia should be interpreted with care, given the possible different underlying pathology (e.g. thalassemia) of anaemia in other parts of the world.

The health-related data were based on a large number of questionnaires in Lifelines. For example, data on health behaviour, past and current diseases were generated from both structured and non-structured questions. Therefore, the quality of the data largely depends on the accuracy of the participants answers.

As a consequence, under- or overestimation of specific diseases as well as health behaviour might be present. To improve this, self-reported diseases were, if possible, validated by medication use, biochemical data and data from clinical measurements. However, it was not possible to validate all self-reported disorders. In **Chapter 2** we demonstrated that the non-structured questions concerning past and current diseases appeared not to be reliable. This emphasizes the need for both more structured questions as well as linking Lifelines data to general practitioners and pharmacists' data to improve the completeness and reliability of the data in future studies.

HRQoL was measured using the RAND 36-Item Health Survey (116), which is the Dutch version of the Short-Form-36 Health Survey (SF-36). This is a disease-generic instrument. The SF-36 is widely used in various clinical and healthy populations and has been proven to be valid and reliable. The disadvantage of a generic instrument is that it is often less sensitive to clinically important disease-specific changes and that disease-specific symptoms or consequences are not measured. As an example, a thyroid specific HRQoL questionnaire is the thyroid-related quality of life instrument ThyPRO. This might provide additional information on aspects identified as important by participants as for example the ThyPRO scales 'hypothyroid symptoms' and 'cognitive problems', for which no equivalent SF-36 domain exists (117). Therefore, it is recommended to perform add-on studies evaluating the specific impact of (subclinical) thyroid disorders on HRQoL using ThyPRO in the Lifelines cohort. Additionally, this will enable to compare ThyPro and SF-36 scales. The same applies to future studies concerning anaemia in which for example the Functional Assessment of Cancer Therapy -Anemia (FACTAn) questionnaire can be used.

Conclusions

The data in this thesis extend the current knowledge on the association of thyroid and blood disorders with health outcomes, including health-related quality of life. Overall, our findings suggest that in both thyroid dysfunction as well as in some blood disorders, the optimal laboratory values in perspective of clinical outcomes, might not be synonymous with currently used reference ranges. Therefore, our data provide supporting evidence for re-evaluation of these frequently used ranges. In the context of personalized medicine, future research assessing whether the use of clinical decision limits and individual treatment goals could be beneficial, seems warranted. Lastly, our data emphasize the need for studying the impact of underlying pathogenic mechanisms of these prevalent disorders on health-related quality of life.

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