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Hematolymphoproliferative malignancies – the impact of lifestyle, organ transplantation and genetic susceptibility

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To my parents and my godchildren

1 ABSTRACT

During the past decades Non-Hodgkin lymphoma (NHL) has demonstrated an immense increase in incidence globally, among almost all age and race groups as well as in both genders. In the search for causes, none of the known risk factors can alone or together entirely explain this trend. This thesis aims to answer a number of research inquiries with regard to NHL but also other hematolymphoproliferative malignancies. Firstly, we sought to estimate the influence of various forms of tobacco use and body mass index on the risk of NHL, Hodgkin lymphoma (HL), leukemia and multiple myeloma. Secondly, we aimed to disentangle the relative importance of putative determinants of NHL risk in the organ transplant setting, including characteristics of donor and recipient, immunosuppressive medications and infectious complications. Thirdly, our objective was to explore the role of genetic variation in translocation breakpoint genes (*BCL2, CCND1),* the protooncogene *MYC* and immunoregulatory genes (*TNF, IL-10*) in the etiology of NHL.

In a large cohort of more than 330,000 construction workers attending regular health check-ups we employed prospectively gathered exposure information on tobacco use and BMI to analyze the incidence rate ratio (IRR) of NHL and HL in a Cox proportional hazards regression model. Tobacco smoking, oral moist snuff use and BMI were all unrelated to NHL. However, among long term users of oral moist snuff (>30 years), an indication of an excess risk of HL was observed (IRR 3.78, 95% confidence interval [95% CI] 1.23-11.6).

In a subsequent study of the construction workers cohort we computed IRR for acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphatic leukemia (ALL) and multiple myeloma (MM) in relation to tobacco use and BMI, applying essentially the same methods. In this investigation, the study population was restricted to male workers and attained age was used as the time scale in the Cox model. Current smoking was associated with a 50% increased risk of AML (95% CI 1.06-2.11).

Within a cohort (n= 5391) of organ transplant recipients (1970-1997) we designed a nested case control study comprising 37 cases of NHL and 97 controls. Odds ratios (OR) were estimated in a conditional logistic regression model. Treatment with antithymocyte globulin (ATG) conferred a five-fold elevated risk of NHL (95% CI 2.17-14.3). The excess risk was more pronounced for a high average daily dose of ATG. Further, having a herpes virus group infection was associated with a five-fold excess risk of NHL (OR 4.89, 95% CI 1.88-12.7), most likely to be driven by Epstein Barr virus.

In a case-control study of 2410 incident cases of NHL and 1963 matched controls selected from the Swedish and Danish population registers, subjects took part in a telephone interview and provided blood specimens for genotyping. OR and 95% CI were computed in multivariate logistic regression analyses. No relationship between investigated polymorphisms *in BCL2, CCND1* or *MYC* and NHL could be discerned. *TNF* rs1800629 minor allele homozygocity was associated with a 50% greater risk of NHL (OR 1.52, 95% CI, 1.06-2.18) as well as a two-fold increased risk of T-cell lymphoma and Mantle cell lymphoma, respectively. *IL10* rs1800890 minor allele homozygocity conferred a higher risk of diffuse large B-cell lymphoma (OR 1.45, CI 1.10-1.90) and Mantle cell lymphoma (OR 1.83, 95% CI 1.08-3.12).

2 LIST OF PUBLICATIONS

I. Fernberg P, Odenbro Å, Bellocco R, Boffetta P, Pawitan Y, Adami J.
Tobacco use, body mass index and the risk of malignant lymphomas - A nationwide cohort study in Sweden.
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II. Fernberg P, Odenbro Å, Bellocco R, Boffetta P, Pawitan Y, Zendehdel K, Adami J.
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III. Fernberg P, Adami J, Odenbro Å, Bellocco R, Tufveson G, Höglund P, Lindelöf B, Pawitan Y, Ekström Smedby K.
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Genetic variation in chromosomal translocation breakpoint and immune function genes and risk of non-Hodgkin lymphoma.
Manuscript submitted

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4 LIST OF ABBREVIATIONS

ALG	Antilymphcyte Globulin
ALL	Acute Lymphocytic Leukemia
AML	Acute Myeloid Leukemia
ASR	Age Standardized Rate
ATG	Antithymocyte Globulin
BMI	Body Mass Index
CHL	Classical Hodgkin Lymphoma
CI	Confidence Interval
CLL	Chronic Lymphocytic Leukemia
CML	Chronic Myeloid Leukemia
CMV	Cytomegalo Virus
CNS	Central Nervous System
CRP	C-Reactive Protein
DLBCL	Diffuse Large B-Cell Lymphoma
EBV	Epstein Barr Virus
FL	Follicular Lymphoma
HIV	Human Immunodeficiency Virus
HL	Hodgkin Lymphoma
HLA	Human Leucocyte Antigen
HSV	Herpes Simplex Virus
HTLV-1	Human T-cell Lymphotropic Virus-1
IGF-1	Insulin Growth Factor-1
IKK-β	Inhibitor-of-NF-κ β Kinase-β
IRR	Incidence Rate Ratio
MALT	Mucosa Associated Lymphoid Tissue
NF-κ β	Nuclear Factor-κ β
NHL	Non-Hodgkin Lymphoma
NK-cell	Natural Killer Cell
NLPHL	Nodular Lymphocyte Predominant Hodgkin Lymphoma
NRN	National Registration Number
OR	Odds Ratio
PTLD	Post Transplant Lymphoproliferative Disroder
RR	Relative Risk
SCALE	Scandinavian Lymphoma Etiology Study
SIR	Standardized Incidence Ratio
SLE	Systemic Lupus Erythematosus
SNP	Single Nucleotide Polymorphism
VZV	Varicella Zoster Virus

5 INTRODUCTION

In 1832 the pathologist Thomas Hodgkin became the first to describe and characterize Hodgkin lymphoma (HL) [1]. Posteriorly, additional forms of lymphoma were detected. An agreement on the classifications of Hodgkin lymphoma was settled rather promptly. There was however a long dissent in terms of categorizing the residual sizable diverse group of diseases. Many years followed of several more or less widely accepted classification schemes. A consensus could not be reached until 1982 when the Working formulation [2] was published and the denotation non-Hodgkin lymphoma (NHL) was instituted.

The immune system is a complex but well organized system comprising a range of white blood cells, including B-cells, T-cells, natural killer cells and antigen presenting cells. It recognizes any enemy individually, and produces a set of molecules and cells that identifies and eliminates that particular adversary. Moreover, the immune defense can discriminate foreign matter from bodily-specific cells, hence specifically attack the unfamiliar substance. The immune system is further adaptive in the sense that the defense is activated only during an infection. Lastly, it possesses memory of previously encountered infectious agents, enabling a more rapid and forceful response to that particular agent for subsequent confrontations.

A malignant lymphoma arises when a single B- or T-lymphocyte is arrested in a specific stage of cell differentiation, leading to malignant transformation and clonal expansion [3].

The astonishingly rapid increase of NHL during the past decades has puzzled epidemiologists and the cause behind this development has not yet been unraveled. Known and alleged causal factors can only be ascribed to a minor part of the incidence trend, which just as inexplicably has started to level off. Presently, NHL is the tenth most frequently diagnosed neoplasm globally and positions seventh in developed countries [4].

Well established risk factors for NHL include states of strong immune perturbation such as HIV/AIDS, rare hereditary immunodeficiency disorders and some autoimmune disorders. The immune system may also be influenced by immunosuppressive medications administered to organ transplanted patients in order to prevent the immune cells to attack and reject the transplanted organ. It is well established that organ transplant recipients are at higher risk of NHL, but the exact biological mechanisms are still not fully clarified. Tobacco as well as pronounced excess weight are both known to affect the immune system. Several studies have investigated these exposures with regard to risk of developing hematolymphoproliferative malignancies, but results have been conflicting. Reports of an excess risk of hematopoietic neoplasias in first degree relatives of patients with NHL indicate a role for genetic susceptibility in NHL. More recently, a number of genetic polymorphisms have been proposed as potential factors of importance for the susceptibility to lymphoma, of which TNF and IL10 as of yet have been the most promising candidate susceptibility genes.

This thesis aims to disambiguate the role of tobacco use and body mass index in the etiology of hematopoietic neoplasms. Furthermore, we set out to answer the much-disputed issue of what determines risk of post-transplant NHL and to assess the effect of variation in chromosomal breakpoint and immunoregulatory genes on NHL susceptibility.

6 BACKGROUND

6.1 CLASSIFICATION OF HEMATOLYMPHOPROLIFERATIVE MALIGNANCIES

6.1.1 Classification of Lymphomas

Non-Hodgkin lymphoma (NHL) is first and foremost distinguished as a unit by excluding Hodgkin lymphoma, which is less common and comprises about 10% of all malignant lymphomas [5]. NHL is probably the most heterogeneous of all cancer forms. Arising from lymphocytes, NHL can appear within primary lymphoid tissue such as lymph nodes as well as other organs, for instance the gastrointestinal tract, skin and central nervous system. Based on immunophenotypic characteristics NHL is grouped in two broad categories; B and T-cell lymphomas, where mature B-cell neoplasms account for more than 85% worldwide [6] and T-cell together with NK-cell malignancies constitute approximately 12% [7].

Hodgkin lymphoma (HL) is generally divided into two categories: nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) and Classical Hodgkin lymphoma (CHL) [6]. NLPHL originates from B-cells and accounts for about 5% of all HL. Histologically, Lymphocytic and/or histiocytic Reed Steinberg cells is a distinguishing feature. Classical HL comprises approximately 95% of Hodgkin lymphomas. The tumor consists of multinucleated Reed Steinberg cells together with mononuclear Hodgkin cells. Classical Hodgkin lymphoma is further subdivided into four histological groups: lymphocyte rich CHL, nodular sclerosis Hodgkin lymphoma, mixed cellularity Hodgkin lymphoma and lymphocyte depleted Hodgkin lymphoma. These subtypes differ mainly in their clinical characteristics while immunophenotypic and genetic features are indistinguishable [6].

Histological categorization of lymphomas has been inconsistent over time and by geographical region. Early classification systems were based on appearance in light microscopy, according to growth patterns [8] and immunology [9], respectively. In 1982, the Working Formulation [2] utilizing solely morphologic criteria was proposed, mainly as a tool to translate between the different systems. This system preponderated in the United States, whereas the Kiel Classification [10-12] was more often applied in Europe. A subsequent effort to achieve unity was made in the Revised European American Lymphoma classification (REAL) in 1994 [13], integrating the two major classification systems and was adopted by the World Health Organization in the year 2000. The WHO classification distinguishes 36 subtypes of NHL, whereof 21 of B-cell origin and 15 arising from T-cells [6].

Table 1. World Health Organization classification of lymphoid neoplasms

B-cell Neoplasms

Precursor B-cell neoplasms

- Precursor B-lymphoblastic leukemia/lymphoma (precursor B-cell acute lymphoblastic leukemia)

Mature (peripheral) B-cell neoplasms

- B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma
- B-cell prolymphocytic leukemia
- Lymphoplasmacytic lymphoma
- Splenic marginal zone B-cell lymphoma
- Hairy cell leukemia
- Plasma cell myeloma/plasmacytoma
- Monoclonal gammopathy of undetermined significance (MGUS)
- Solitary plasmacytoma of bone
- Extraosseus plasmacytoma
- Primary amyloidosis
- Heavy chain diseases
- Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT-lymphoma)
- Nodal marginal zone B-cell lymphoma
- Follicular lymphoma
- Mantle cell lymphoma
- Diffuse large B-cell lymphoma
  - Mediastinal (thymic) large B-cell lymphoma
  - Intravascular large B-cell lymphoma
  - Primary effusion lymphoma
- Burkitt lymphoma/leukemia

B-cell proliferations of uncertain malignant potential

- Lymphoid granulomatosis
- Post-transplant lymphproliferative disorder, polymorphic

T and NK-Cell Neoplasms

Precursor T-cell neoplasms

- Precursor T-lymphoblastic lymphoma/leukemia (precursor T-cell acute lymphoblastic leukemia)

Mature (peripheral) T-cell neoplasms

- T-cell prolymphocytic leukemia
- T-cell granular lymphocytic leukemia
- Aggressive NK-cell leukemia
- Adult T-cell lymphoma/leukemia
- Extranodal NK/T-cell lymphoma, nasal type
- Entreopathy-type T-cell lymphoma
- Hepatosplenic T-cell lymphoma
- Subcutaneous panniculitis-like T-cell lymphoma
- Mycosis fungoides
- Sezary syndrome
- Primary cutaneous anaplastic large cell lymphoma
- Lymphomatioid papulosis
- Angioimmunoblastic T-cell lymphoma
- Peripheral T-cell lymphoma, not otherwise characterized
- Anaplastic large cell lymphoma, T/null cell, primary systemic type.

Hodgkin lymphoma (Hodgkin's disease)

- Nodular lymphocyte predominance Hodgkin lymphoma
- Classical Hodgkin lymphoma
  - Nodular sclerosis classical Hodgkin lymphoma
  - Mixed cellularity Hodgkin lymphoma
  - Lymphocyte-rich classical Hodgkin lymphoma
  - Lymphocyte depleted classical Hodgkin lymphoma

Source: World Health Organization Classification of Tumours Pathology and Genetics of Tumours of Hematopoietic and Lymphoid Tissues. Lyon (France). International Agency for Research on Cancer Press, 2001.

6.1.2 Classification of Leukemia and Multiple myeloma

Arising from the diverse cell lines of the hematopoietic system leukemia is a heterogeneous neoplasm. In a broad categorization, four major types of leukemia have been defined: Acute lymphocytic leukemia (ALL), Acute myeloid leukemia (AML), Chronic lymphocytic leukemia (CLL) and Chronic myeloid leukemia (AML). However, in recent years Chronic lymphocytic leukemia has been regarded as a NHL according to the WHO classification [6]. ALL is more commonly diagnosed in children and generally has a good prognosis while the chronic forms of leukemia affect adults [6].
Similar to leukemia, Multiple myeloma (MM) is a neoplasm originating from the hematopoietic cell line. Normal plasma cells convert into malignant MM cells that throng the bone marrow and produce monoclonal immunoglobulin (M protein), characteristic of multiple myeloma. Multiple myeloma if often described according to which type of light chain (kappa or lambda) or immunoglobulin is generated by the neoplastic plasma cell. IgG and IgA myeloma are the most frequent types [14].

6.2 DESCRIPTIVE EPIDEMIOLOGY

6.2.1 Incidence

6.2.1.1 Non Hodgkin lymphoma (NHL)

Globally, NHL is the tenth most frequently diagnosed form of cancer. The highest incidence is observed among white people in the United States and Canada (about 14 per 100,000 person-years) whereas China and Thailand report the lowest incidence (2 to 3 per 100,000 person-years) worldwide [4].

The most prevalent NHL subtypes are Diffuse Large B-cell lymphoma (DLBCL) and Follicular lymphoma (FL), constituting 31% and 22% of NHL respectively. In the western world Follicular lymphoma is widespread but in South America, Asia, Africa and Eastern Europe FL is more limited. In contrast Burkitt lymphoma is highly prevalent in Africa while it only accounts for 1-2% of NHL cases in the western world [6]. NK/T-cell neoplasms are at large more common in Asia as well as in endemic areas of HTLV-1 such as south western Japan along with the Caribbean's [6].

NHL incidence varies by gender and is by and large higher among men compared to women. This also applies for most NHL subtypes; however male preponderance has been shown to be more marked for Burkitt lymphoma in whites and for follicular lymphoma as well as for angioimmunoblastic T-cell lymphoma in the black and Asian population [15]. Other than the exception of B-cell and T-cell lymphoblastic lymphoma/leukemia which is most often diagnosed in children, NHL incidence rises with age autonomously of sex and race [4, 15, 16]

In Sweden, malignant lymphomas account for 3.2% of all incident cancers. Analogous to other parts of the world NHL has a slight male predominance. Recent figures from 2006 report an incidence of 17.3 per 100,000 person years in males and 12.2 per 100,000 in females (age adjusted to the population 2000). This corresponds to a total of 1349 new cases of NHL, diagnosed in that year, whereof 735 in men and 614 in women [17].

6.2.1.2 Hodgkin lymphoma (HL)

There is a substantial diversification in the incidence of HL worldwide. China has the lowest incidence rates of 0.3 per 100,000 person-years and 0.1 per 100,000 person-years for men and women respectively [18] and it varies up to 4.2 per 100,000 person-years in males in the Valais region of Switherland and 3.3 in Israeli women of Jewish ethnicity [19]. Moreover, in most age groups Hodgkin lymphoma is more common in males compared with females. The only subtype devoid of male predominance is Nodular Sclerosis Hodgkin lymphoma [6].

Independently of sex, nodular lymphocyte predominant Hodgkin lymphoma appears to have black preponderance [15]. Classical Hodgkin lymphoma typically displays a bimodal incidence curve by age with a first peak at 15-35 years of age and a subsequent max out in late life. The mixed cellularity subtype is observed more often in the developing world as well as among persons carrying an HIV infection [6].

In the Swedish population, incidence rates of Hodgkin lymphoma per 100,000 person-years are 2.1 among men and 1.4 in women (age adjusted to the population 2000). In the year 2006 a total of 160 new cases were diagnosed constituting 0.3% of all cancer cases in Sweden during that time period [17].

6.2.1.3 Leukemia and Multiple Myeloma

Altogether, leukemia comprises approximately 3% of the global cancer burden [20] By geographic area there is a moderate variability in incidence rates ranging from five to sevenfold. Leukemia is least frequent in sub-Saharan Africa and the highest rates are seen in North America together with Australia and New Zealand [20]. Acute Lymphocytic leukemia almost exclusively affects children whereas myeloid leukemia and chronic lymphocytic leukemia are more frequent in the adult and elderly population, hence resulting in an early incidence peak and a more gradual rise thereafter [16]. Ordinarily, incidence rates are higher in males than females. A total of 576 cases of lymphocytic leukemia and 447 incident cases of myeloid leukemia were reported in Sweden in 2006 accounting for 2.3% of all newly diagnosed malignancies [17].

About 86,000 new cases of multiple myeloma are diagnosed yearly, representing 0.8% of all malignancies globally. It is uncommon that the disease appears in individuals under the age of 40. Incidence rates are higher in the Western world in comparison to Asia [20]. Multiple myeloma is twice as frequent in the African-American population of the United States than that of the white population and individuals of Japanese or Chinese decent have lower rates [20]. Among Swedish men the incidence rate of multiple myeloma is 7.6 per 100,000 person-years and for women 5.0 per 100,000 person-years. Out of all diagnosed cancer cases in 2006, multiple myeloma comprised 1.1% [17]

6.2.2 Time trends

6.2.2.1 Non-Hodgkin lymphoma

Commencing in the 1970s there has been a marked yearly increase in NHL incidence of approximately 2-4% [21-23]. This rapid rise in incidence has been observed in both genders, across diverse racial back grounds and in almost all age groups [21, 24]; the exemption being children under the age of 15 where stable NHL incidence has been described [25-27]. Nevertheless, incidence rates have been consistently higher in males than females and in the white population [21, 24, 28]. In terms of disease subtypes, High-grade NHL doubled among females and tripled in men during 1978-83 and 1990-1995, mainly driven by a fast increase in immunoblastic NHL. Small lymphocytic NHL rose by 36-44% in all races and both genders while follicular lymphoma was constant among African-American women but increased in whites and black men. In addition a 30-40% augment was detected for diffuse NHL [29]. Morton et al. [15] reported a considerable rise in incidence rates for marginal zone lymphoma, mantle cell lymphoma and Burkitt lymphoma/leukemia along with a reduction for diffuse large B-cell lymphoma and chronic lymphocytic leukemia/small lymphocytic lymphoma

between 1992-2001. Furthermore, a rise in incidence of Burkitt's/Burkitt-like lymphoma together with immunoblastic NHL have been observed in countries with high prevalence of HIV/AIDS [4, 30, 31]

The swift augment in NHL incidence is largely unexplained. After accounting for the impact of HIV and other viruses, familial factors, correctness of diagnoses along with environmental and occupational exposures, Hartge et al. [32] concluded that, of the rise in NHL incidence amongst all Caucasian men, 80% remained unexplained, while among those aged 0-64 the corresponding figure was 42%. Banks et al. estimated that 10-15% of cases which according to traditional classification would have been labeled as Hodgkin lymphomas may nowadays well be diagnosed as NHL due to changes in classification schemes over time. However, the increment in reported NHL incidence could not be explained by alterations in diagnostic standards [33]. In addition, another study found the observed 9-10% drift/five year period unlikely to be ascribed to artifactual changes in diagnostic methodology [34]. Although the HIV epidemic has contributed to a part of the increase in NHL this fraction was estimated in 1992 to approximately 10% [35].

In a similar manner, NHL mortality has showed upward trends in Europe as well as in North America. Levi et al. [36] reported mortality rates rising to 6/100,000 in men and 4/100,000 in women in the United States in 1995-98. Correspondingly, mortality rates in the European Union reached 4.4/100,000 in men and 2.8 in women respectively, and a less pronounced rise in mortality rates was seen in Japanese men 3.7/100,000 and women 1.9/100,000 during the same time period.
Within Europe, NHL incidence increased in almost all countries in an investigation including 20 European countries [37]. The highest change in rates was detected for Slovakia and Slovenia (1992-1997) whereas incidence trends were more auspicious in northern European countries (Sweden, Denmark, Finland and the Netherlands).

Interestingly, after two decades of an almost epidemic increase in NHL, the incidence started to level off in the 1990s and decreased subsequently in the US [25] , in most European countries [37] including the Nordic countries [38], although in the US there is evidence of a persistent increase in NHL incidence among whites aged 15-24 years, 25-54 year old women, blacks ≥ 55 years [25] as well as for 60-79 year old men and women in the Nordic countries [38].

Incidence of NHL rose in both men and women from the initiation of the Swedish Cancer Registry in 1958 up until the beginning of the 1990s. Then a succeeding plateau phase was seen from the mid 1990s and onwards [17, 37, 38]. The annual percent change in NHL incidence over the last ten year period (i.e. 1996-2006) was -0.1 in men and -0.8 among women [17].

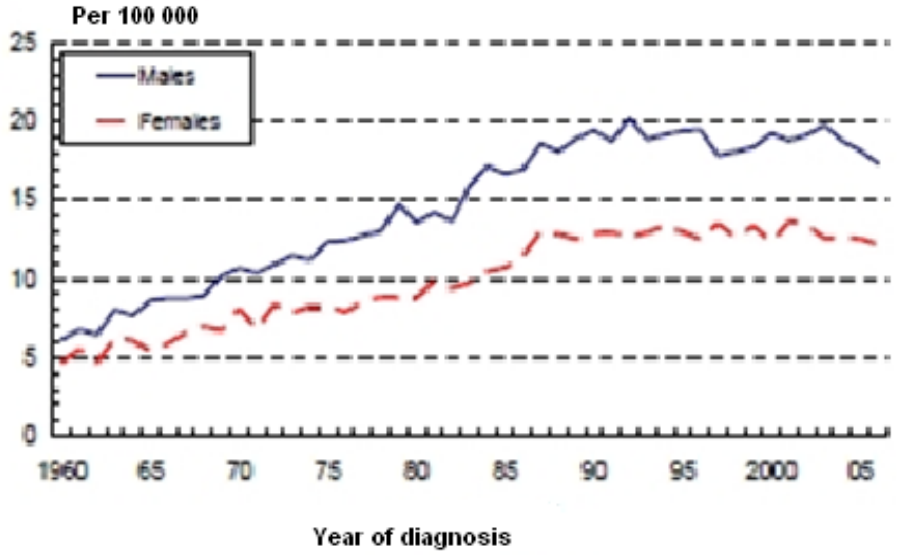


Figure 1. Non-Hodgkin lymphoma (NHL) incidence 1960- 2006 in males and females, age standardized to the Swedish population in the year 2000 (ICD7:200).
Source: Cancer Incidence in Sweden 2006

6.2.2.2 Hodgkin lymphoma

By contrast with the clear rise in NHL incidence and mortality during the late 1990s a decline in Hodgkin lymphoma has been observed in numerous sceneries [5]. A comparison on time trends in Hodgkin lymphoma incidence reported a decrease of age standardized rate (ASR) among men in all registries under study in Europe, Asia and United states, apart from Nagasaki [24]. Stable Hodgkin lymphoma rates overall was reported during 1992 to 2001 in United States by Morton et al. [15]. Furthermore, a 6% reduction of mixed cellularity/lymphocyte depleted HL was discerned, but incidence of nodular sclerosis HL remained unchanged. Age specific incidence trends demonstrate grand variability in the Nordic countries, with decreasing incidence in age groups older than 40 years but increased 4.6% annually in adolescents aged 10-19 years and the increment was present but less pronounced in 20-29 year olds, hence showing a dislocation of the bimodal incidence curve [39].

6.2.2.3 Leukemia and multiple myeloma

A rather stable incidence pattern has been perceived for leukemia. Recently some indication of a vague rise in incidence has been discerned; however it is not clear if this is an actual increase or whether it is due to improvements in diagnostics and /or registration practice [40].

MM incidence has not changed considerably in East Asia whereas in Europe a slight increase has been observed among males. In the United States a clear rise in incidence has been distinguished in the African-American population. In addition, white males also showed more modest increment in MM incidence [41].

6.2.3 Clinical Synopsis

6.2.3.1 Non-Hodgkin lymphoma

Typically, NHL presents with painless swelling of one or several lymph nodes. The disease may also have extra nodal involvement of lymphoid tissue of the gut, skin, along with bone marrow and tonsils. Additional symptoms (B symptoms) include night sweats, fever and weight loss. Infections due to neutropenia as well as fatigue caused by anemia may also be signs of NHL. High and intermediate grade (aggressive) NHL are commonly localized at diagnosis while the low grade (indolent) type more often presents as generalized disease with involvement of spleen, liver and bone marrow. Of lymphomas affecting children close to 40% are of the lymphoblastic group[1]. However, for the most part NHL is a disease of middle-aged and elderly individuals.

Diagnosis is based upon tissue biopsy which is further used for histological classification and staging by a pathologist. Bone marrow aspiration from the sternum or pelvis is central in the investigation and in patients with a known bone marrow involvement or testis lymphoma, a lumbar puncture is performed in order to exclude meningeal disease. Flow cytometry, immunohisochemistry, cytogenetic or molecular genetics make sub typing of NHL possible. Imaging of the thorax and abdomen with Computer Tomography is useful for localizing primary cancers and to quantify the extent of tumor spreading [4]. For clinical staging of the disorder, the Ann Arbour classification is applied. This staging scheme ranges from stage I (involvement of single lymph node region or lymphoid structure) to stage IV (generalized disease including involvement of extranodal sites) [6].

Watchful waiting with regular health check-ups until symptoms appear is usually the treatment option for indolent lymphomas. NHL localized to either a limited number of lymph nodes or a single extra nodal organ can be treated with radiotherapy. Chemotherapy, in some instances in combination with radiotherapy is used for more aggressive and generalized disease [1, 4]. Throughout time the prognosis for NHL has improved significantly. Nowadays the 5-year relative survival rate is in the region of 50% in adults and 80-90% in children [4]. Nevertheless, 5-year survival has been shown to fluctuate by histologic subtype; varying from less than 30% (peripheral T-cell lymphoma, precursor T lymphoblastic lymphoma, mantle cell lymphoma) up to more than 70% (follicular lymphoma, marginal zone B-cell lymphoma of MALT type, anaplastic large T/null-cell lymphoma [7].

6.2.3.2 Hodgkin lymphoma

Similar to NHL, the majority of patients with Hodgkin lymphoma have painless swollen lymph nodes. Clinical features diverge to some extent depending on disease subtype. Nodular lymphocyte predominant Hodgkin lymphoma typically presents with localized peripheral lymphadenopathy and the disease progression is tardy. A large fraction of patients diagnosed with Nodular sclerosis Hodgkin lymphoma are asymptomatic on presentation but might have lymph adenopathy in the neck region [1]. B symptoms are common in mixed cellularity Hodgkin lymphoma and is this subtype commonly at a more advanced stage (Ann Arbor III or IV) at the time of diagnosis [6].

6.2.3.3 Leukemia and multiple myeloma

Clinically acute leukemias are distinguished from the chronic forms by their more abrupt onset and disease is fatal within months if treatment is not initiated. Moreover, bone marrow malfunction is not as distinct in chronic leukemias compared with the acute types. Anemia occurs in both acute and chronic types of leukemia, although it can be more severe in patients with acute leukemia. In contrast, enlargement of the spleen liver or lymph nodes is more pronounced among individuals diagnosed with chronic leukemia [42].

Multiple myeloma is characterized by pain in the back and the ribs, pathological fractures, fatigue as a result of anemia and reduced immunoglobulin production makes the patient more susceptible to repeated infections. Renal failure can come about in some cases [6].

6.3 ETIOLOGY OF HEMATOLYMPHOPROLIFERATIVE MALIGNANCIES

6.3.1 Risk factors for Non-Hodgkin lymphoma

6.3.1.1 Tobacco

About 1.1 billion people, accounting for close to 30% of the world population aged 15 years or older smoke on a daily basis [43]. Smoking is least frequent in Latin- America, the Caribbean and the largest fraction of regular tobacco users (38%) reside in East Asia and the Pacific [43]. In Europe, approximately 25-30% of all deaths due to cancer is estimated to be caused by tobacco smoking [44]. In Sweden, the prevalence of tobacco smoking among men is one of the lowest in Europe [45], whereas the consumption of oral smokeless tobacco is one of the highest per capita globally [46]. Swedish women on the other hand have tobacco habits comparable to most other European countries [45, 47]. Smoking prevalence in Sweden has decreased during the past decades, and in 2007 roughly 12% among men and 16% among women aged 18-84 were current smokers on a daily basis [48].

Although epidemiological evidence regarding tobacco smoking and NHL risk has been contradictory, a large fraction of cohort [49-53] and case-control [54-59] studies do not support a causal relationship between with NHL and smoking status (never, previous, current). However, some studies have reported a slightly increased risk of NHL [60-64] whereof one a two-fold elevated risk confined to women who were current smokers [64]. A number of investigations [56, 62, 65] have found a positive association between smoking intensity (no. of cigarettes per day) and/or number of pack-years of smoking and NHL. Among non-Hodgkin lymphoma subtypes, a positive relation with smoking has most consistently been reported for follicular lymphoma [51, 52, 55, 60, 61, 65].

In a pooled analyses of nine case-control studies by the InterLymph Consortium [65], including 6,594 cases and 8,892 controls a slightly increased risk of NHL was observed in ever smokers vs. never smokers (OR = 1.07 95% CI = 1.00-1.15). Furthermore, NHL risk was associated with smoking duration (years) (p trend < 0.01) as well as no. of pack-years of cigarette smoking (p trend = 0.01). Smoking duration of more than 36 years conferred a 16% excess risk of NHL and heavy smokers (≥36 pack – years) were at 21% excess risk of NHL development. In analyses stratified by non-Hodgkin lymphoma subtype, an increased risk of follicular lymphoma was observed in ever (OR = 1.15, 95% CI = 1.02-1.29) and current smokers (OR = 1.31, 95% CI = 1.12-1.52), respectively. Moreover, current heavy smoking (≥36 pack–years) conferred an increase in risk of NHL development (OR = 1.45, 95% CI = 1.15-1.82). In a Danish-Swedish case-control study encompassing 3,055 incident non-Hodgkin lymphoma cases and 3,187 controls Shöllkopf et al. [58] reported an association between follicular lymphoma and current smoking in women but not in men (p heterogeneity = 0.03), but failed to confirm any association between NHL overall and smoking history, intensity or duration of smoking. A recent large prospective cohort study [50] comprising 1,304 cases of NHL and 417,111 non cases did not provide any evidence of an association between smoking status, intensity and NHL overall or follicular lymphoma. Nevertheless, heavy smokers (≥20 cigarettes/day) were at a two-fold increased risk of diffuse large B-cell lymphoma.

Two main biological pathways through which smoking may give rise to an elevated risk of non-Hodgkin lymphoma have been postulated. Firstly by means of an immunomodulatory effect. Cigarette smoke has been shown to induce leukocytosis [66, 67] along with decreased immunoglobulin levels, reduced number of Natural Killer (NK) cells and a decrease of CD4+ /CD8+ ratio [68]. Diminished NK-cell activity and possibly also the proliferative response of lymphocytes are other observed effects on the immune system by cigarette smoke [69]. Secondly, tobacco smoke may act through direct carcinogenic effects. For instance, the chromosomal translocation, t(14;18) in the bcl-2 gene is well known and common in some NHL tumors. This translocation is present in approximately 70-95% of FL [6] and about 35% of germinal centre B-cell like DLBCL [70, 71] The translocation puts the bcl-2 locus on chromosome 18 next to the immunoglobulin heavy chain joining region gene [72, 73] of chromosome 14 ensuing in an anomalous over expression of the bcl-2 gene with consequent protracted survival of B-cells as apoptosis is deferred.

Table 2. Smoking status and risk of non-Hodgkin lymphoma *Subset analyses of men ** Subset analyses of women

Reference	Study type	No. of exposed cases	Relative Risk/Odds Ratio (95% Confidence Interval)
Adami et al. 1998 [49]	Cohort	504	Former smoker 1.0 (0.8-1.3) Current smoker 1.1 (0.9-1.3)
Nieters et al. 2008 [50]	Cohort	772	Former smoker 1.12 (0.98-1.28) Current smoker 0.93 (0.79-1.09)
Herrinton et al. 1998 [51]	Cohort	388	Former smoker 1.1 (0.9-1.3) Current smoker 1.1 (0.9-1.3)
Parker et al. 2000 [52]	Cohort	67	Former smoker 1.0 (0.8-1.5) Current smoker 1.0 (0.7-1.5)
Fernberg et al 2006 [53]	Cohort	679	Former smoker 1.01 (0.85-1.21)* Former smoker 0.55 (0.13-2.32)** Current smoker 1.00 (0.87-1.15) * Current smoker 0.75 (0.38-1.47)**
Besson et al. 2003 [64]	Case-control	115	Former smoker 0.71 (0.38-1.33)* Former smoker 2.00 (0.88-4.54)** Current smoker 0.64 (0.33-1.22)* Current smoker 2.40 (1.19-4.84)**
Stagnaro et al. 2001 [60]	Case-control	854	Former smoker 1.2 (0.99-1.5) Current smoker 1.2 (1.0-1.5)
Stagnaro et al. 2004 [61]	Case-control	381	Former smoker (blond tobacco) 1.5 (1.1-1.9) Current smoker (blond tobacco) 1.3 (1.1-1.7)
Brown et al. 1992 [63]	Case-control	425	Former smoker 1.2 (0.9-1.6) Current smoker 1.5 (1.1-2.0)
Fabbro-Peray et al. 2001 [74]	Case-control	223	Ever smoker 0.9 (0.7-1.2)
Bracci et al. 2005 [59]	Case-control	295	Former smoker 1.2 (0.94-1.5) * Former smoker 0.93 (0.72-1.2)** Current smoker 0.98 (0.74-1.2)* Current smoker 1.1 (0.82-1.4) **
Morton et al. 2003 [55]	Case-control	331	Former smoker 1.1 (0.8-1.4) Current smoker 0.9 (0.6-1.2)
Talamini et al. 2005 [56]	Case-control	134	Former smoker 0.87 (0.55-1.39) Current smoker 1.33 (0.87-2.03)
Zahm et al. 1997 [57]	Case-control	726	Former smoker 0.9 (0.7-1.1) Current smoker 1.1 (0.9-1.3)
Shöllkopf et al. 2005 [58]	Case-control	1,623	Former smoker 0.95 (0.84-1.08) Current smoker 0.99 (0.86-1.13)
Freedman et al. 1998 [62]	Case-control	812	Former smoker 0.94 (0.79-1.14) Current smoker 1.17 (0.97-1.40)
Besson et al. 2006 [54]	Multi-centre case-control	979	Former smoker 1.04 (0.88-1.22) Current smoker 1.07 (0.91-1.27)
Morton et al. 2005 [65]	Pooled analysis of nine case-control studies	3,765	Ever smoker 1.07 (1.00-1.15) Former smoker 1.10 (1.00-1.20) Current smoker 1.06 (0.98-1.15)

During the past decades, the consumption of oral moist snuff in Sweden more than doubled [75]. In 2004, approximately 22% of Swedish men and 3% of women used snuff on a daily basis [76].

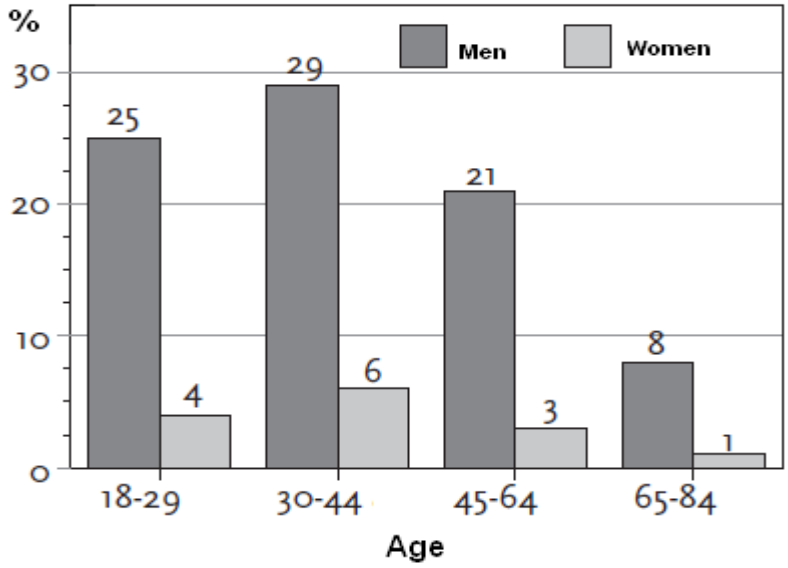


Figure 2. Proportion of daily users of oral moist snuff by age groups, 2004
Adapted from: The national public health survey Health on equal terms, Swedish National Institute of Public Health

Smokeless tobacco has previously been associated with other forms of cancer [75]. Boffetta et al. [75] estimated 10.7% of esophageal cancer and 13.8% of pancreatic cancer cases among Swedish men to be attributable to smokeless tobacco. Moreover, the proportion of oral cancers caused by smokeless tobacco ranged from 1.6% in Canadian men to 68.2% among men in Sudan. However, studies investigating the risk of NHL in relation to smokeless tobacco are sparse, but the majority have reported null findings [49, 63, 77] Conflicting evidence of a four-fold elevated risk (95%CI= 1.3-12) of NHL among male exclusive users of snuff or chewing tobacco has been presented in a US population-based case-control study by Bracci et al [59]. However, comparisons of results are hampered by the different compositions of diverse smokeless tobacco products [78].

Smokless tobacco products contains alkaloids (whereof nicotine comprises 85-95%) as well as over 30 carcinogenes, such as nitrosamines, nitrosamino acids and polycyclic aromatic hydrocarbions [75]. One possible carcinogenic pathway postulated is formation of DNA adducts in the tissue, resulting if unrepaired in irreversible DNA mutations. Specifically, mutations in the RAS (an oncogene) or P53 (a tumor suppressor) genes are particularly hazardous and may lead to cancer development, as these genes regulate normal cell growth [75]. Other potential factors contributing to carcinogenesis from smokeless tobacco include oxidative stress, inflammation and viruses [75].

6.3.1.2 Body Mass index (BMI)

Overweight and obesity has become a growing public health hazard. In 2004 approximately 34.1% and 32.2% of US residents were overweight and obese, respectively [79]. Correspondingly, in 2007 roughly 55-75% among males and 40-60% of women were overweight or obese in the European Union [80]. As depicted in Figure 3, the prevalence of overweight in Sweden is about 42% among men and 26% in women, while the fraction of obese men is slightly lower than among women, 11% and 14% respectively [76].

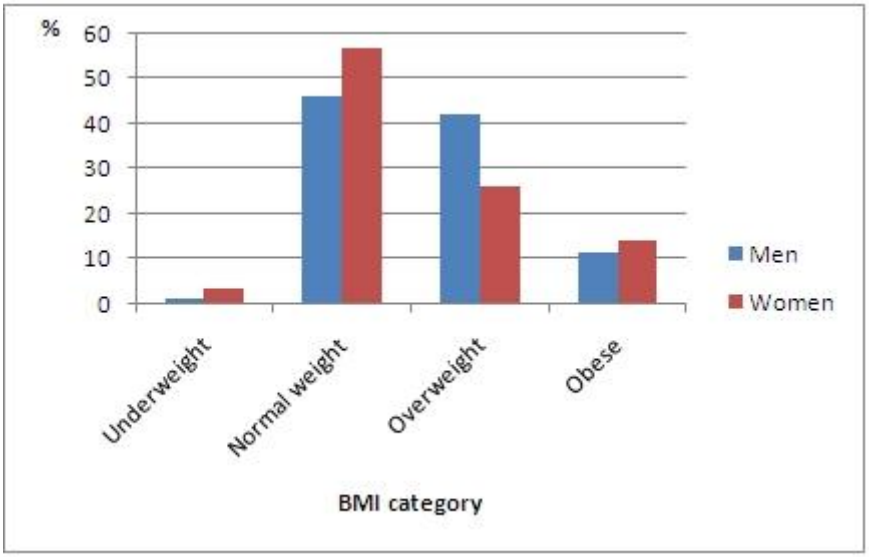


Figure 3. Proportion of men and women with underweight, overweight and obesity, age 16-84, 2007. Standardized by age.

Adapted from: The national public health survey Health on equal terms, Swedish National Institute of Public Health

The synchronous trend of a rapid rise in the prevalence of obesity and NHL in the previous decades initiated a search for a causal link. BMI is defined as body weight (kg)/height^2 (m^2) [81]. Using BMI as a measure of overweight and obesity, some cohort studies [82-87] have provided evidence of a positive association with obesity, while others [53, 88-94] have presented conflicting results. Two population-based case-controls studies [95, 96] observed a 40% and 70% increased risk, respectively, of NHL in obese individuals (BMI ≥30). Chang et al. [97] did not detect any association between risk of NHL overall and overweight or obesity.

A pooled analysis [98] of 18 case-control studies with a total of 10,453 cases of NHL did not show any association between obesity and NHL overall, OR= 0.84 (95% CI 0.72-0.99) for BMI 30-39.99 and OR= 0.63 (95% CI 0.40-0.99) for BMI ≥40, respectively.

A meta-analysis [99] combining data from 10 cohort and six case-control studies reported a 7% excess risk of NHL associated with overweight (BMI 25-29.9) and a 20% risk-increase for obese individuals (BMI) ≥30. Furthermore, obesity was related to a 10% elevated risk per 5kg/m^2 increment in BMI. Analysis of NHL subtypes, although based on few studies, revealed a statistically significant 40% increase in risk of DLBCL associated with obesity. Additional support for a relationship linking BMI to DLBCL has been provided by other investigations [96, 97, 100].

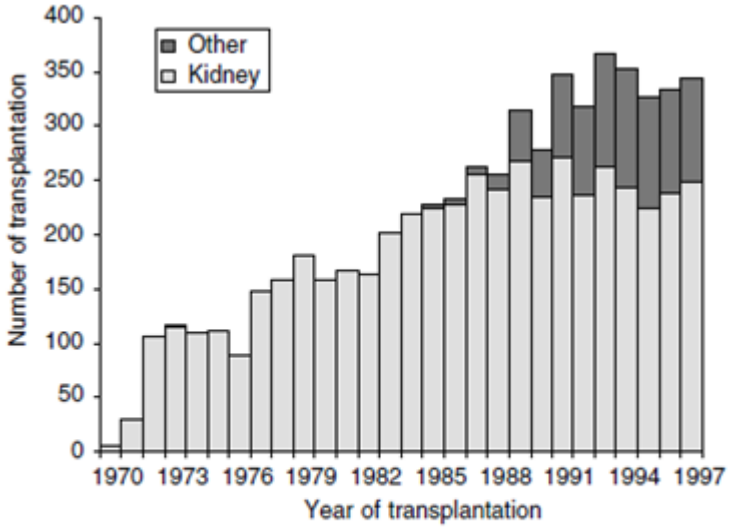
A large multicentre prospective cohort study in Europe [100] reported a two-fold risk increase in women, with BMI ≥28.7. This study did however not detect any association between NHL overall and overweight or obesity. Abdominal fat measured as waist-to-hip ratio or waist or hip circumference did not appear to influence risk neither of NHL overall, nor any of the investigated disease subtypes.

Several mechanisms have been suggested, linking excess body weight to cancer. Insulin resistance is often seen among obese individuals and the growth promoting effects of insulin could possibly contribute to carcinogenesis mainly through elevated levels of free Insulin like growth factor (IGF-I). Moreover, insulin may have a direct effect via the insulin receptor which activates intracellular pathways resulting in mitogenic and antiapoptotic effects [101]. Leptine is an appetite regulating hormone secreted by adipose tissue and levels are proportional to amount of body fat. Leptine has been shown to have mitogenic effects on hematopoietic progenitor cells and can exert anti-apoptotic and pro-angiogenetic effects. Another example is adiponectine, where levels are negatively correlated to BMI. Adiponectine is a tumor inhibitory hormone secreted by mature adipocytes [101]. Obesity is known to induce chronic inflammation by an abnormal cytokine production, increased synthesis of C-reactive protein (CRP) and other acute phase reactants as well as activating pro-inflammatory

pathways [101]. It has been hypothesized that inflammation and cytokine production may elicit their effect by activating the IKK-β resulting in up-regulation of NF-κβ [101].

6.3.1.3 Organ transplantation

In 1954, Dr. Joseph Murray and Dr. David Hume at the Brigham hospital in Boston conducted the first successful living related kidney transplantation between identical twins [102]. Ever since then there has been a tremendous and rapid progress within the field of transplantation surgery. In the 1960's and 70's the chief immunosuppressive regimen to prevent graft rejection was corticosteroids in combination with azathioprine [103]. The introduction of cyclosporine in 1979 had a major improvement on graft as well as patient survival. The 1-year functional survival increased from roughly 50% to over 80% in kidney grafts [103, 104]. Similar result were also seen in liver and heart transplant recipients [105]. In 1995 a microemulsion formulation of cyclosporine, Neoral® was launched. This formulation had superior absorption properties, which resulted in decreased rates of acute rejection in de novo kidney, liver as well as heart transplanted patients [105]. Additionally, advancements in methods for donor recipient HLA (Human leukocyte antigen) matching, pre-transplant serological testing, enhancements in diagnosing acute and chronic rejection along with new drugs for treating steroid resistant rejections such as antithymocyte globuline (ATG), antilymphocyte globulin (ALG) and OKT3/muromonab CD3 further contributed to a better outcome subsequent to organ transplantation. In Sweden, organ transplantation has been in practice since the 1960's. The number of transplantation procedures yearly has increased over time [106] and in the year 2007, 379 kidney, 49 heart, 43 lung and 138 liver transplantations were performed [107] in Sweden.



Total number of patients undergoing organ transplantation in Sweden per year during 1970-1997. The dark bars show number of transplantations other than kidney.

Figure 4. Change in number of solid organ transplantations in Sweden during the period 1970-1997. *Reprinted by permission from Macmillan Publishers Ltd: [British Journal of Cancer], (Adami J, Gäbel H, Lindelöf B et al. Cancer risk following organ transplantation: a nationwide cohort study in Sweden (2003) 89, 1221-1227 [106]), copyright (2003)*

Besides well known side effects, for instance rejections and infectious complications organ transplantation has been related to an elevated risk of NHL [4]. Kinlen et al.[108] reported a close to 60-fold increased risk of NHL (34 cases observed vs. 0.58 expected in a multicenter study in the UK, Australia and New Zealand). In that study, additional observations were made of a very short induction period, as well as a large fraction (44%) of lymphomas involving the brain. Subsequent investigations [106, 109-111] also detected a higher incidence of NHL in organ transplant recipients. In a population-based prospective cohort (n=5931) study in Sweden [106] a striking excess of NHL (SIR 6.0;

95% CI 4.4-8.0) , scquamous cell skin carcinoma (SIR 56.2; 95% CI 49.8-63.2) and lip cancer (SIR 53.3; 95% CI 38.0-72.5) was reported . A higher risk of NHL in patients with a heart, lung or liver graft compared with cadaver kidney transplant recipients was observed by Opelz et al. [110, 111], relative risk 27.6, 58.6, 29.6 and 12.6 respectively [111]. The overall incidence of NHL declined after the first year, although in heart recipients it was persistently higher [110], and the long term rise in incidence was steeper compared to other organs [111].

It is still not fully elucidated what determines the higher risk of NHL or post transplant lymphoproliferative disorder (PTLD) in the organ transplanted population. PTLD ranges from early polyclonal proliferations to monomorphic lymphomas, most often of B-cell origin [6]. Several studies have indicated an importance of immunosuppressive medications, in particular ATG and OKT3 [110-117] . There is also strong evidence of Epstein Barr Virus as an important cofactor in the lymphomagenesis [116, 118-121]. Most investigations were however not able to estimate NHL risk in relation to accumulative doses or mean dose but rather limited to utilizing assessments such as ever/never use, intention to treat guidelines or measurements of administered doses at a few occasions.

6.3.1.4 Genetic factors

Having a family history of hematolymhpoproliferative malignancy is associated with a 2-3-fold excess risk of NHL [122-127]. In a population-based case-control study in Sweden [122], the risk of NHL was increased by 80% for individuals with any first degree relative with a history of hematopoietic cancer. Furthermore, a more pronounced association with DLBCL, FL and T-cell lymphomas was observed for paternal than maternal history of hematopoietic malignancy. Sibling history of hematopoietic neoplasm appeared to be of greater importance than parental history for risk of NHL overall and for several subtypes. A similar pattern of a stronger aggregation for siblings has also been reported by others [126, 127].

Genetic variability which may influence the susceptibility to NHL has been a research field of interest in recent years. A number of single nucleotide polymorphisms (SNP) have been investigated in terms of NHL risk and thus far the genes most consistently associated with NHL risk are *TNF* and *IL-10* [128-133], two genes with the primary function of regulating the inflammatory response. Rothman et al [128] observed a positive association between the TNF rs1800629 GA or AA genotype and NHL overall in a large pooled analysis by the InterLymph Consortium of eight studies from Europe, United States and Canada. The association was even more distinct for DLBCL. Correspondingly, the rs1800890 polymorphism in *IL-10* was related to an elevated risk of both NHL overall as well as DLBCL.

A few case-control studies looking at the influence of folate-metabolizing genes and risk of NHL development [134-137] have yielded to some extent conflicting results, however, evidence has been provided indicating that polymorhpismis in the thymidylate syntase and methionine syntase genes may affect the risk of NHL.

Skibola et al. [96] found an excess risk of NHL, FL and DLBCL associated with the *LEP19G* allele in the leptin gene, which functions in the control of appetite and food consumption. A subsequent large population-based case-control study [138] did not confirm this result, although the GG allele had been used as the referent. However, a positive association was reported between variation in the LEP -2548 SNP NHL overall and FL.

More recently, the estrogen receptor1, vitamin C receptor and matrix metalloproteinase genes have been postulated to comprise susceptibility loci for NHL [139], however additional studies are called for to corroborate these novel findings.

Patients with certain rare hereditary recessive immunodeficiency disorders, namely Wiskot-Aldrich syndrome, Ataxia-telangiectasia, Nijemegen Brekage Syndrome (NBS), X-linked lymphoproliferative disorder, hyper Ig-M syndrome, severe combined immunodeficiency, common variable immunodeficiency and autoimmune lymphoproliferative disorder are well known to more frequently develop lymphomas, most commonly of B-cell origin [6, 140]. For instance, Wiskott-Aldrich syndrome, a recessive X-linked malady has been associated with an increased risk of NHL [141] and the neurodegenerative disorder ataxia telangiectasia has been reported to confer a 250- to 750-fold

elevated risk of lymphoma [142]. The causal link to NHL differs conditionally on type of underlying primary immune defect. An anomalous DNA repair mechanism and loss of T-cell control are putative mechanisms in ataxia telangiectasia and Wiskott-Aldrich syndrome respectively [6].

6.3.1.5 Autoimmune /inflammatory disorders

Non-Hodgkin lymphoma has consistently been associated with autoimmune/inflammatory conditions such as rheumatoid arthritis (RA), Sjögren's syndrome, systemic lupus erythematosus (SLE), celiac disease, dermatitis herpetiformis and Hashimoto thyreoiditis [143]. In addition, psoriasis, Crohn's disease, systemic sclerosis along with sarcoidosis have been suggested to be related to NHL risk, however, results have been inconsistent [143].

Rheumatoid arthritis has been reported to entail a two-fold increase in NHL risk [143]. A meta-analysis [144] based on 16 studies presented a standardized incidence rate ratio (SIR) of 1.95 (95% CI 1.70 to 2.24) for NHL and an even more pronounced risk of Hodgkin lymphoma was detected. However, the extent of the association diverges greatly between diverse autoimmune disease entities and the highest increase in NHL risk (nine- to eighteen-fold) has been observed for Sjögren's syndrome. Celiac disease, SLE and Hashimoto thyreoiditis are linked to a three- to –six-fold excess risk whereas dermatitis herpetiformis confers a two- to ten-fold excess risk of developing NHL [143].

Some specific NHL-subtypes have also been associated with certain autoimmune and/or inflammatory conditions. For instance, in a large pooled analysis [145] of twelve case-control studies Sjögren's syndrome yielded a six-fold increased risk of DLBCL along with a 30-fold elevated risk of mucosa associated lymphoid tissue (MALT) lymphomas. A seven-fold and a close to three-fold increase in risk of marginal zone lymphoma and DLBCL, respectively, were associated with SLE. In addition, celiac disease gave rise to a six-fold excess risk of T-cell NHL, mainly driven by a considerably increased risk enteropathy-type T-cell NHL. Smedby et al. [146] also reported an approximately two-fold higher risk of DLBCL-cell lymphoma and lymphoplasmacytic lymphoma among individuals with rheumatoid arthritis in a population-based case-control study included in the pooled analysis mentioned above.
Interestingly, there has been evidence indicating that severity of the disease may be an important predictor of lymphoma risk [147], although it is difficult to completely disentangle this relationship from observations of an association with disease modifying anti-rheumatic drugs (chiefly methotrexate and azathioprine) frequently reported for NHL, since activity of disease and immunomodulatory therapy are likely to correlate [143].

6.3.1.6 Infectious agents

The number of NHL cases attributable to infection per annum has been estimated to more than 57,000 representing approximately 19% of all NHL cases worldwide [148].

EBV
Infection with Epstein Barr virus (EBV) is omnipresent globally and about 80-100% of the adult population (age ≥30) is seropositive. In the developed world, the primary infection typically occurs during adolescence and roughly 50% of cases present with infectious mononucleosis [149]. EBV has also been linked to African Burkitt lymphoma. In sub-Saharan Africa as much as 95% of Burkitt lymphoma cases have been found to have EBV genome in the tumor cells [148]. Moreover, the EB-virus appears to have a causal role in NHL development in various immunosuppressive states. Among organ transplanted patients the T-cell response to infection is hampered due to immunosuppressive medications administered in order to prevent graft rejection whereby B-cells immortalized by EBV viral proteins primarily LMP-1 and EBNA-1 can progress to a post transplant lymphoroliferative disorder (PTLD) including malignant lymphomas [150, 151]. In the setting of a primary hereditary immunodeficiency disorder such as ataxia telangiectasia and Wiskott-Aldrich syndrome, EBV is interspersed in most lymphoid proliferations [6]. Furthermore, the acquired immunosuppression seen in individuals with HIV/AIDS also creates an environment allowing uncontrolled proliferation of B-cells [152]. Lastly EBV is associated with several types of NK/T-cell lymphomas [4].

HIV

The Human Immunodeficiency Virus (HIV) greatly reduces the amount of $CD4^+$ T- lymphocytes. $CD4^+$ counts less than 200 x 10^6 together with symptoms or an AIDS-defining diagnosis, for instance candidasis, lymphoma or pneumonia are diagnostic criteria's to classify disease progression to AIDS (varies between the US and Europe). Studies have consistently reported an excess risk of NHL in individuals with HIV/AIDS [153-157] and HIV is nowadays a well established risk factor for NHL. A recent large case-control study in South Africa [154] observed a six-fold increased risk of NHL overall (OR 5.9, 95% CI 4.3-8.1) in HIV-1 individuals. An excess risk of NHL in subjects with HIV/AIDS (RR 72.8, 95% CI 70.4-75.3) and (SIR 7.3, 95% CI 6.4-8.4) respectively, has also been reported in two large cohort studies in the US [153, 155] and one prospective cohort study in Switzerland [156] (SIR 76.4, 95% CI 66.5-87.4). The Swiss study [156] further reported an inverse relationship between $CD4^+$ at enrollment and subsequent NHL, and risk of NHL was lower in users of highly active antiretroviral therapy (HAART) in comparison with non users.

The most prevalent forms of NHL among HIV patients are those arising in the central nervous system (CNS), DLBCL and Burkitt lymphoma [152]. A 250-fold increased risk among HIV-infected persons (SIR 250, 95% CI 160-360) of NHL in the CNS was observed by Engels et al. [153]. The CNS lymphomas are almost exclusively EBV positive, however, for HIV related NHL in general about 50% holds EBV [4].

HHV8 & HTLV1

Human herpesvirus 8 (HHV8) is strongly linked to Karposi sarcoma but is also frequently demonstrated in the uncommon subtype primary effusion lymphoma [152]. Human T lymphotropic virus (HTLV-1) is a retrovirus that has an well-known causal relationship with Adult T-cell leukemia/lymphoma which originates from activated helper T-lymphocytes [149].

Hepatitis C virus

Hepatitis C virus might induce proliferation of lymphocytes by means of binding to the CD81 surface receptor of the B-cell [152]. A two- to four-fold elevated risk has been observed in most countries; however a stronger association has been reported in areas with higher prevalence of hepatitis C such as Japan and the Mediterranean region [4].

Other infections

Additional infectious agents have more recently been indicated to have a causal role by chronic antigen stimulation, giving rise to some types of lymphoma. Helicobacter pylori has been associated with MALT lymphoma [158]. Borrelia burgdorferi has been correlated to a higher risk of cutaneous B-cell lymphoma[159-161]. Lastly, evidence has been provided supporting Chlamydia Psittaci as a risk factor for ocular adnexal lymphomas [162]. The hypothesis of these agents playing a role in the etiology of lymphoma is further strengthened by findings of remission of the lymphoma following treatment with antibiotics [158, 163, 164].

6.3.1.7 Other putative risk factors for NHL

Environmental and occupational pesticide exposure has been postulated as a risk factor for NHL, nevertheless convincing support for such an association has not been provided [28]. It has been suggested that ionizing radiation by causing immunosuppression through reduction of the number of T-cells may increase risk of NHL. Although studies have been inconsistent positive associations have only been observed for almost lethal doses of ionizing radiation [4]. Investigations of the impact of alcohol consumption have mainly reported a protective effect on NHL risk independent of type of alcoholic beverage [28]. Similarly, there is evidence of an inverse relationship between higher levels of physical activity and risk of NHL [4, 28]. A protective effect of exposure to ultraviolet light on risk of NHL has been reported, possibly by inducing immune modulation or stimulating vitamin D-production [4].

6.3.2 Risk factors for Hodgkin lymphoma

6.3.2.1 EBV

As for NHL there is substantial evidence to support the role of EBV as a determinant of risk for HL [152], and in the neoplastic Hodgkin/Reed Steinberg cells of the tumor EBV has been demonstrated [165].

Synchronous with the bimodal incidence curve of HL, the fraction of EBV-positive tumors fluctuates from 80-90% in children younger than 10 and then declines to 20-30% between the ages of 11-45 to steadily rise again to more than 80% [5]. There is also mounting evidence of an association connecting infectious mononucleosis to subsequent development of HL, which is most pronounced among young adults [5]. Hjalgrim et al. [166] found a two-fold higher risk of Hodgkin lymphoma (SIR 2.55, 95% CI 1.87-3.40) in a large Scandinavian cohort study of more than 38,000 patients diagnosed with mononucleosis. Interestingly, the elevated risk had the propensity to increase with age at infectious mononucleosis diagnosis.

6.3.2.2 Immunodeficiency

Furthermore, indigenous as well as acquired immunodeficiency (after organ transplantation and HIV infection) entails an excess risk of HL [5]. In the setting of HIV/AIDS patients Frisch et al. [155] observed an eleven-fold increase in HL risk (RR 11.5, 95% CI 10.6-12.5) and the risk was even higher for the mixed cellularity and the lymphocytic depletion subtypes; RR 18.3 (95% CI 15.9-20.9) and RR 35.3 (95% CI 24.7-48.8), respectively.

6.3.2.3 Social factors

Socioeconomic status also appears to influence HL risk. Birth order has been shown to be inversely associated with risk of developing Hodgkin lymphoma among young adults [167]. In addition, having multiple older (but not younger) siblings is associated with a reduced risk of HL, putatively due to a later exposure to infectious agents in first born children [168].

6.3.2.4 Tobacco

Previously, little evidence has been provided to support a causal link between tobacco use and HL but a more up to date investigation has suggested that tobacco smoking, chiefly current smoking confers an elevated risk of Hodgkin lymphoma and/or the mixed cellularity subtype [169-173].

6.3.3 Risk factors for Leukemia

6.3.3.1 Radiation

A well known risk factor for leukemia is ionizing radiation. Exposure to ionizing radiation can broadly be categorized into high energy exposure such as atomic bomb explosions, nuclear accidents, occupational exposure along with therapeutic exposure i.e. radiation therapy and radioactive iodine therapy. The absorbed dose radiation is measured in Gray (Gy = 1 Joule/kg) [174]. A four-fold elevated risk of acute myeloid leukemia (AML) has been reported among survivors of the bombings of Hiroshima and Nagasaki [40]. A two-fold higher risk of leukemia was reported in an investigation of over 80,000 women treated with radiation therapy for cervical cancer [175] as well as in a cohort study of over 27,000 medical x-ray workers in China [176]. However there is no persuasive evidence for a an association between non-ionizing radiation, for instance electric and magnetic fields and non-ionizing electromagnetic radiation and leukemia risk [176].

6.3.3.2 Benzene

A number of occupations including shoe, leather and rubber industry work as well as refinery occupations, printing and painting entail exposure to Benzene. A more substantial part of the total benzene exposure comes from cigarette smoke and automobile exhaust [40]. Benzene is a well established risk factor for leukemia, mainly acute myeloid leukemia (AML) [40].

6.3.3.3 Tobacco

Although not a fully established as a risk factor of leukemia, there is substantial evidence suggesting that smoking tobacco may cause myeloid leukemia, [177-183]. However no apparent relationship linking tobacco use to lymphoid leukemia has been detected [40]. A population-based case-control study in Italy [60] found no clear association between smoking and leukemia. Current or previous smoking was unrelated to risk of ALL (OR current 1.2, 95% CI= 0.54- 2.5) (OR previous= 1.5, 95% CI= 0.66-3.6) and AML (OR current= 0.93, 95% CI= 0.61- 1-4) (OR previous= 1.2, 95% CI= 0.81-1.9). Furthermore, smoking status was not associated with CML, but a negative dose-response relationship with the number of

cigarettes smoked daily was observed. A large prospective study [184] of a cohort of 248,000 United States veterans, reported an excess risk of leukemia among current cigarette smokers compared with non-smokers (RR 1.53, 95% CI 1.36-1.70) and there was a significant dose-response relationship with number of cigarettes smoked, RR 1.63 (95% CI 1.35-1.97) for the highest level of smoking intensity (>20 cigarettes/day). In contrast, another large cohort study by Adami et al. [49] , did not find any evidence of a significant association linking smoking status, smoking intensity (cigarettes/day) or duration of smoking and leukemia. Analyses of myeloid and acute leukemia as separate entities did not reveal any excess risk in relation to smoking.

6.3.3.4 Body Mass Index

In recent years a number of studies have indicated that excess body weight might be a risk factor for leukemia [83, 95, 177, 185-188]. Conflicting evidence has been provided by one cohort study of more than 780,000 Korean men [85], and a Swedish cohort of 362,552 males [189]. A Norwegian cohort study of more than two million persons [83] observed an increased risk of ALL among overweight (RR 1.49, 95% CI 1.01-2.19) and obese (RR 2.77, 95% CI 1.49-5.2) men but not women. Furthermore, having a BMI >30 was associated with an elevated risk of CML (RR 1.65, 95% CI 1.18-2.31) in men. Larsson et al [185] reported an excess risk of leukemia associated with overweight (summary RR 1.14, 95% CI 1.03-1.25) and obesity (summary RR 1.39, 95% CI 1.25-1.54) in a meta analysis of nine cohort studies. Subtype specific associations with ALL, AML and AML were observed, however, these analyses were limited to four of the studies, hence statistical power was reduced.

6.3.4 Risk factors for Multiple myeloma

The risk factors for multiple myeloma (MM) are largely unknown. Exposure to ionizing radiation has been shown to confer an elevated risk of MM, but more recent evidence is conflicting [190]. Other factors postulated to play a role in the etiology of MM include occupational exposures (metals, pesticides, petroleum rubber wood products etc.), chronic infections (ostomyelitis) chronic antigenemia as well as dietary factors along with obesity [190]. With regard to tobacco use, most studies have found no association with MM [49, 60, 63, 191]. Nevertheless, a population- based case-control study, current smoking conferred a two-fold elevated risk of MM in both genders [192]. A few other studies have also indicated a role of cigarette smoking in the etiology of MM.

7 AIMS

The overall objective of this thesis was to achieve better knowledge about the impact of putative risk factors for non-Hodgkin lymphoma and other hematolymphoproliferative malignancies, focusing on lifestyle factors and genetic susceptibility. Our aim was also to disentangle the relative importance of determinants for the established excess risk of non-Hodgkin lymphoma in solid organ transplant recipients.

Specifically, the aims were:

- To investigate if current or previous tobacco smoking or usage of oral moist snuff is associated with an elevated risk of developing NHL, HL, leukemia or MM, and to test if risk of hematolymphoproliferative malignancies is effected by dose and/or duration of tobacco use (studies I and II).

- To study if high BMI, classified as overweight or obesity, confers an increased risk of developing NHL, HL, leukemia or MM (studies I and II).

- To study the relative importance of, and the interplay between, donor/recipient characteristics, immunosuppressive medications and post-transplant infectious complications in risk of NHL in the setting of organ transplanted individuals (study III).

- To investigate whether genetic variation in the chromosomal translocation breakpoint genes *BCL2* and *CCND1*, *MYC* as well as the immunoregulatory genes *TNF* and *IL10* are associated with increased risk of non-Hodgkin lymphoma and/or specific subtypes (study IV).

8 SUBJECTS AND METHODS

8.1 REGISTRY SOURCES

8.1.1 Cancer Registers

8.1.1.1 Sweden

The nationwide Swedish cancer register was initiated in 1958 and receives reports of all incident cancers. Notification to one of six regional registers associated with the oncological center in every medical region is mandatory by law for clinicians as well as pathologists and cytologists. Malignancies are coded according to present ICD standards and also translated for assessment over time to a national revision of ICD7. Subsequent to coding and quality checks, the data is fused at the national register annually. Approximately 99% of all incident diagnosed malignancies are reported to the registry [17] and the fraction of cases with morphological verification has enhanced over time and is today close to 99%. Histological type is classified according to the WHO C24 system that has been in practice since the commencement of the register. From 2005 and onwards the system has applied ICD O/3 (International Classification of Diseases for Oncology. Third Edition, WHO Geneva 2000) [17]. An evaluation of registrations (for all cancer sites) by Mattson et al. [193] demonstrated roughly 96% completeness. A more recent report presented a underreporting of 3.7% of individuals with malignant disease in the Swedish Cancer register [194]. Furthermore, the underreporting was higher in older age groups, specifically among individuals over 70 years of age. In addition, approximately 70% of cases not reported were deficient in information from a pathological or cytological report.

8.1.1.2 Denmark

Commencing in 1943 the Danish Cancer Registry makes record of diagnosed cancers in Denmark. Initially reporting was voluntary but became mandatory by law in 1978. All malignancies are coded pursuant to the ICD-O. The Danish Cancer Register has almost 100% coverage for most types of malignancies[195].

8.1.2 The Swedish Cause of Death Register

Since 1958 the Swedish National Cause of Death Registry records information on deaths of all Swedish residents, although the completeness as well as the quality of the data was improved after 1961. At each death in Sweden a death certificate is issued by a physician and for classification of causes of death the International Classification of Diseases (ICD) is employed. The record comprises data on the main underlying cause of death, up to 20 contributing causes of death together with the date of death. From 1997 and onwards, reporting of event of death is 100%. However, for a small proportion of deaths (0.7%) information on the cause of death is lacking [196].

8.1.3 The Swedish National Patient Register

Data on all individual hospital discharges is brought together in the Swedish National Patient Register (Swedish Inpatient Register) which was started in 1964-1965. The register was at the outset regional, but reporting became compulsory in 1984 and in 1987 the register was nationwide. At present the register includes 50 million discharges for the period 1965-2006. For every hospitalization a form is filled out containing data such as national registration number (NRN), date of admission, date of discharge, main and secondary diagnosis, external cause of injury and poisoning, surgical procedures (coded according to the Swedish Classification of Operations and Major Procedures) along with anesthesiological processes. The NRN assigned to all Swedish citizens enables follow-up of patients between hospitals and over time. The information is delivered annually by the 21 county councils to the National Board of Health and Welfare. Until 1968 the 7th revision of ICD was utilized, the 8th revision from 1969 to 1986, the 9th revision between 1987 and 1996 and the 10th revision thereafter. For somatic short time care during the period 1987 to 1991 the total number of drop-outs has been approximated to less than 2 percent. Diagnostic validity of 88-90% overall has

been signified in a validation study (in 1990) of discharge information from 1000 hospitalizations for somatic short time care [197].

8.1.4 The Swedish Register of Population and Population Changes

The Swedish Register of Population and Population Changes is sustained by Statistics Sweden. The register holds official Swedish census data in computerized form ever since 1960. The current address of residents alive at the end of each year, and the NRN are included. Starting in 1969 the registry also takes record of emigrations.

8.1.5 The Danish National Lymphoma Registry Organization

The Danish national Lymphoma Registry Organization (LYFO) was started in 1983. Since then, clinical data on all Danish patients with lymphoma diagnosis is gathered in the register [198]. The register encompasses information on data of cancer diagnosis, clinical staging of the disease pursuant to the Ann Arbor system[199] together with involvement of specific organs. Furthermore a panel of expert hematopathologists continuously reviews a random sample of 10% out of all incident LYFO-registered lymphoma cases in the nation.

8.1.6 The Danish Pathology Register

The Danish Pathology Register is a nationwide databank initiated in 1999. The register functions as a diagnostic tool for all pathologists in Denmark, enabling easily accessible information regarding all previous pato-anatomical investigations and diagnoses. Aside from the clinical use, the data is further utilized for research purposes. A close to 100% coverage of the register has been estimated [200].

8.1.7 Record linkage – national registration number (NRN)

All since 1947 every Swedish citizen is assigned a ten digit national registration number unique to each individual. The first six digits is made up of the person's date of birth. Thereafter, a three digit serial number follows, together with a fourth check-digit which was added to the NRN in 1967. The NRN is widely used within health care, by authorities, businesses and insurance companies. Accordingly, it may also be applied for record linkage between health registers and population registers, or other data sources [201].

8.2 STUDY DESIGN

8.2.1 Study I and II - Lifestyle and risk of hematolymphoproliferative malignancies

The aims of study I and II was first to investigate if diverse types of tobacco use (cigarettes, pipe, cigar and oral moist snuff) are associated with an increased risk of developing NHL, HL (study I), leukemia or MM (study II). Secondly, to examine if dose and/or duration of tobacco use was linked with elevated risk of these malignancies. Thirdly, we wanted to test the hypothesis that overweight or obese individuals are at excess risk of hematolymphoproliferative cancers.

Hence, we implemented a cohort study based on more than 330,000 construction workers in Sweden. Regular health check-ups offered by the Contruction Industry's Organization for Working Environment Safety and Health to all blue and white collar workers within the building industry; taking place at mobile and stationary clinics all over Sweden between 1969 and 1993 (On average, each worker had 2,6 health check-ups) [202]. This project "Bygghälsan" was a joint venture between the construction trade unions and the employers' associations. Altogether, the cohort comprises 386,000 individuals visiting the clinic from 1971 through 1992.

During 1971 to 1975 exposure information on tobacco use, occupational exposures, medical history, prescribed drugs and symptoms of disease was obtained by a self-administered 200-item questionnaire double-checked by a nurse in order to prevent misinterpretations. This was

complemented by a staff-administered form containing information on job code as well as clinical measurements such as height, weight, pulse and blood pressure. From 1978 until 1992, the self-administered form was replaced by a more comprehensive questionnaire, completed by nurses.

In 1975 to 1977, no data on tobacco exposure use was collected as the self-administered form was not in use. Consequently, we barred all subjects only visiting the clinics during this period. We also excluded from analyses all subjects with a history of cancer before entry, incorrect NRN, inconsistencies during record linkage, female gender (study II) and missing information on Body Mass Index (BMI) (study II).

Entry into the cohort was defined as the primary visit to the clinic. The NRN was utilized for follow up by linkage to the National Causes of Death Register, Migration Register and Cancer Register. All members of the complete cohort were followed from date of entry until emigration, death, date of cancer diagnosis or end of follow up (December 31st 2000 and 2004 respectively), whichever occurred first. The final study cohort of study I comprised 335,612 persons, whereof less than 5% (n=17,691) were women. The cohort used for analyses in study II included 336,381 subjects.

8.2.2 Study III – Solid organ transplantation and risk of non-Hodgkin lymphoma

The purpose of study III was to shed further light on factors of importance for the well known elevated risk of NHL in recipients of organ grafts.

A nested case-control study was conducted within a cohort of all patients hospitalized for solid organ transplantation (n=6,457) in Sweden between 1970 to1997. In a preceding cohort study [106] these subjects were identified by linking the NRN to the nationwide National Patient Register. After exclusion of patients with prior history of cancer, cancers reported within 30 days following transplantation, mismatching transplantation dates or unknown transplantation codes, a total of 5,931 subjects were eligible for follow-up. Incident cases of NHL were ascertained by linkage to the Swedish Cancer Register using the NRN.

From the remaining cohort, three controls were randomly selected per each incident case of NHL, and matched on age at transplantation (within 5-year intervals) and calendar period of transplantation (5-year intervals). Controls were required to be free of cancer and alive at the time of lymphoma diagnosis of the corresponding case.

We excluded seven subjects due to erroneous transplantation codes, five because of loss to follow-up due to death or emigration, and one control for the reason of an un-registered squamous cell skin carcinoma diagnosis. Informed consent to take part in the study was refused by one individual and we failed to position and collect information from medical records of three cases and 22 controls. Moreover, two cases and three controls had to be discarded because there was no corresponding case or control left in their matched set and these subjects could not be fitted with any existing matched set resulting in 37 cases and 97 controls for statistical analyses.

A wide-ranging protocol was designed in order to assemble information in a standardized way from medical records of the study participants. Data on patient and donor characteristics (sex, type of transplanted organ, number of transplantations, pre-transplant serology, and HLA antigens) was extracted. Futhermore, information on infectious complications, rejection episodes and explantation of organs along with re-transplantation and reversion to dialysis as well as comprehensive information on exact daily doses, dates of dose changes and administration forms of immunosuppressive medications were brought together.

8.2.3 Study IV - Genetic susceptibility to non-Hodgkin lymphoma and subtypes

In study IV the object was to explore whether genetic variability in the chromosomal translocation breakpoint genes (*BCL2*, *CCND1*), *MYC* and immunoregulatory genes (*TNF* and *IL10*) has an impact on the susceptibility to NHL overall or any of the major subtypes.

The aim was addressed in a population-based case-control study called SCALE (Scandinavian lymphoma etiology) [203], comprising inhabitants between the ages of 18 to 74 in Denmark and Sweden. We excluded all subjects with a history of organ transplantation, HIV infection and other hematopoietic malignancies, or with insufficient language proficiency in Swedish/Danish.

All subjects with a primary recently diagnosed NHL, identified through a rapid case ascertainment system were selected as case patients. Controls were sampled at random from the nation-wide population registers every six months all through the duration of the study, and were frequency matched to the cases by age (in 10-year intervals) and sex within each country. All eligible study subjects were requested to participate in a telephone interview grasping a wide range of conceivable environmental risk factors for lymphoma, such as smoking habits and history of autoimmune disease, as well as to provide a blood specimen. Among those interviewed, about 85% of the cases (n=2,595) and 65% of the controls (n=2,085) supplied blood. The present study covers all participants in SCALE that were interviewed and gave blood, and whose both parents were born in Sweden, Denmark or other Nordic countries (Iceland, Norway, Finland). Review of tumor material was performed by expert cytologists and pathologists in Sweden and in Denmark by the national lymphoma registry organization (LYFO) [198]. The WHO classification[6] of malignant lymphomas was employed in both nations.

From the biological samples (blood specimens) genomic DNA was isolated. For all Danish study participants and Swedish case patients genomic DNA was available, but for most Swedish controls DNA was amplified from dried whole blood spots on filter paper at a later stage. A small number of samples (n=20) were lost during storage, the DNA content was too low in 12 samples, and an additional 8 failed in quality controls. Previously validated SNPs were selected from genetic databases. A total of 14 SNPs were chosen. Out of these, two were not in Hardy Weinberg equilibrium and one was barred consequent to low success rate. Genotyping was conducted applying matrix-assisted laser desorption/ionization-time of flight mass spectrometry [204] at the Mutation Analysis Facility, Karolinska Institutet Huddinge. All genotypes were corroborated by two independent scorers.

8.3 STATISTICAL ANALYSES

All data management and statistical analyses were conducted using Intercooled STATA 8.2 (StataCorp. 2003. Stata Statistical Software: Release 8.2 College Station, TX: Stata Corporation), or later versions.

8.3.1 Study I and II: Cox regression

8.3.1.1 Cox proportional hazards model

Under the proportional-hazards model the hazard h(t) is modeled as follows:

$h(t) = h_0(t)\exp(\beta_1 x_1 + \cdots + \beta_k x_k)$

$x_1 \ldots x_k$ = independent variables
h_0 = Baseline hazard at time *t* corresponding to the hazard for an individual with the value 0 for all independent variables in the model.

The Cox model is designed for analysis of time to event or time between events. The Cox regression model allows for assessment of the hazard ratio of the primary variable at the same time as controlling for the effect of other covariates. However, the model assumes that the hazard ratio of the primary exposure remains constant over time [205].

8.3.1.2 Study- I and II

In both study I and II information was restricted to that collected at first visit with regard to tobacco smoking, use of oral moist snuff along with height and weight used to calculate BMI.

Incidence Rate Ratios (IRR) and 95% confidence intervals (CI) were estimated by a Cox proportional Hazard regression model. In study I all models were adjusted for age at entry and follow up time in years (time-on-study) was used as the time scale. Age is a strong determinant for NHL. When using time-on-study as the time scale in a Cox regression model, bias may be present for non-exponentially distributed age to disease onset. Instead, age has been recommended as a preferable alternative [206]. Thus, in study II attained age (in years) was employed as the time scale and all point estimates were implicitly adjusted for age. The predictor variable BMI was adjusted for tobacco use and correspondingly all tobacco covariates were adjusted for level of BMI (studies I and II). In paper II we discarded from analyses subjects with missing BMI as well as female workers.

The effect of the covariate of interest was tested using a likelihood ratio test (study I). We also performed trend tests for amount of tobacco using the mean (study I)/median (study II) value of each stratum as the score. In both study I and II subjects were stratified on age by five year intervals and BMI was classified as underweight (18.5 kg/m^2), normal weight (18.6-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obese (≥30.0 kg/ m^2).

We approximated one cigarette to contain one gram, and one cigar roughly six grams of tobacco. Date of entry (first visit for each individual) defined smoking status (never-ever, and never, former or current smoker) as well as snuff use category (ever, never) and duration. The WHO classification from 2000 [6] categorizes chronic lymphocytic leukemia (CLL) as an NHL-subtype and hence CLL was analyzed together with NHL and as a separate outcome. Given that results changed only marginally, data from the combined analyses were presented.

NOTE: When datasets were updated for study II a reevaluation of the original registry data sent to us from the Swedish National Migration Registry was performed. It was discovered that the cohort-id number assigned to each individual was erroneously created in that dataset. Consequently, in the original study cohort of 386,000 persons we found that the number of individuals excluded due to inconsistencies in their national identification number decreased from 5,627 to 570 when the correct migration data was employed. Moreover, in study I we had used date of diagnosis of the hematolymphoproliferative malignancies (not any cancer) under study when censoring subjects, thus some individuals later developing other cancer forms would contribute with additional person-time. The analyses for study I was repeated using the correct migration data, as well as date of any diagnosis of any cancer for censoring. Estimates did not change substantially and the interpretations of our results remained the same; hence, no erratum has been submitted to the journal.

8.3.2 Study III: Conditional logistic regression

8.3.2.1 Conditional logistic regression

As study III had a matched set design the preferable model to apply in the statistical analyses was the conditional logistic regression, designed to work with individually matched subjects or repetitive measures.

8.3.2.2 Study III

As an estimate of relative risk, conditional logistic regression was utilized to compute odds ratios (OR) and 95% confidence intervals (CI). We compared the null model to a model of interest with the Likelihood Ratio Test (LRT). When analyzing exposure covariates with small frequencies (n<5) exact logistic regression was used.

A total of eight controls lacked a corresponding case due to loss to follow up or exclusion of their matched case. Out of these, five controls were matched with an existing set employing less rigid matching criteria (ten years difference in age and date of transplantation and 14 days deviation in follow-up time). Given that the results were essentially unchanged after insertion of these controls,

the data presented were from analyses including these individuals. Spearman's rank correlation coefficient (r_s) [207] was applied to test for correlation between independent variables

We classified total accumulated dose and average dose of immunosuppressive drugs consistent with the quartile distribution among controls; however, the median was used as cutoff for a few covariates. Orally and intravenously administered drugs were analyzed individually and combined. Combined results were presented since results were only marginally dissimilar. Average daily dose comprised only orally administered drugs. ATG, and OKT3 are not given orally, hence average dose calculations were based on intravenously administered doses. Diversity in biological availability and potency of the medications were taken into account when analyzing orally and intravenously administered drugs together by multiplying the corresponding factor in order to assess equivalent doses. Because of insufficient power, a full multivariate logistic regression model including all potential confounding factors simultaneously was not feasible. As an alternative, we adjusted the analysis for each of the covariates associated with NHL in the univariate model one by one.

8.3.3 Study IV: Unconditional logistic regression

8.3.3.1 Unconditional logistic regression

Logistic regression is employed to analyze a binary dependent variable as a function of continuous or categorical independent variables. Logistic regression models the odds of the occurrence of a certain event, typically death or disease. Hence, the effect of the independent variables on the outcome is usually expressed as odds ratios.

The multiple logistic-regression model is given by:

$$\text{logit}(p) = \ln\left(\frac{p}{1-p}\right) = \alpha + \beta_1 x_1 + \cdots \beta_k x_k$$

Where p= probability of a disease (y) with a binary outcome

$x_1, \cdots x_k$ = independent variables [205]

8.3.3.2 Study IV

The unconditional logistic regression model was applied to calculate odds ratios (OR) and corresponding 95% confidence intervals to approximate relative risk. All models were adjusted for country of residence. The single nucleotide polymorphisms (SNPs) were categorized according to Wild type (referent), minor heterozygote allele and minor homozygote allele, coded 0, 1, 2 respectively. Subset analyses were performed stratified by country of residence. We conducted Cochran-Armitage trend tests by fitting models with SNPs considered as continuous variables. We also tested for possible interaction between SNPs, tobacco smoking or autoimmune disease history by using a likelihood ratio test, i.e. the fit of a main effects model was judged against the fit of a model which also included the interaction product. In these analyses we excluded subjects with missing data on smoking (n=1) or autoimmune disease (n=8). Moreover, we tested for gene-gene interactions between polymorphisms in *BCL2* and *MYC* or *CCND1* and *MYC*.
Lastly, for each gene (*BCL2, MYC, CCND1*) we used a likelihood ratio test to compare a model including all investigated variants to a null model without covariates, that is, a model including country of residence as the sole independent variable. This multi-locus tests of association has been proposed as a powerful haplotype test of association in closely linked regions [208].

8.4 RESULTS

8.4.1 Study I

At the end of the study period 1,309 morphologically verified incident cases of NHL and 205 cases of Hodgkin lymphoma had been diagnosed. The total accumulated person time of observation contributed by the cohort was 6,804,539 person-years with an average of 19.1 years ranging from 0-31 years. Mean age at first visit (entry) was 34.4 (range, 14 to 82 years). The majority of male subjects (85%) had at some point used some tobacco product. Approximately 58% were ever users of smoking tobacco while ever use of oral moist snuff was reported by 28% of the males. Cigarettes were the most common form of smoking tobacco used (30%). However, about 23% of male workers had used more than one tobacco product (mixed users). Similarly, cigarettes were the most prevalent tobacco product among women.

Among men previous (IRR 1.01, 95% CI 0.85-1.21) or current smoking (IRR 1.00, 95% CI 0.87-1.15) was not related to an elevated risk of developing NHL. Exclusive use of cigarettes, pipe, cigars or mixed tobacco use did not confer an excess risk of NHL development (Table 3). In addition, individuals reporting to be ever snuff dippers were not at higher risk of NHL (IRR 0.77, 95% CI 0.59-1.01). Furthermore, neither having a BMI <18.5 (underweight) nor being classified as overweight or obese entailed an elevated risk of NHL in comparison with subjects of normal weight.

Smoking status was not associated with risk of HL, IRR 1.18, 95% CI 0.70-1.98 for former and IRR 1.32, 95% CI 0.91-1.90 for current smokers respectively. Pure use of cigarettes, cigars, pipe, or oral moist snuff was unrelated to HL risk (Table 3). High (overweight, obese) or low (underweight) levels of body mass index was not associated with risk of HL development.

The amount of cigarettes (cig/day), pipe tobacco (g/w) or cigars (cgr/day) smoked was not related to risk of NHL (Table 4). Moreover, number of years of tobacco smoking as well as time since cessation was not associated with NHL risk (Table 4). Correspondingly, neither quantity of tobacco smoked nor smoking duration and time since recency of smoking was associated with risk of HL (Table 4). Among users of oral moist snuff for more than thirty years, a statistically significant close to four-fold excess risk was detected. However, the number of exposed cases was low (n=4) and 95% confidence limits were wide (Table 4).

Among female workers, previous or current smoking did not influence risk of NHL. Analyses of pure smoking tobacco products were hampered by small numbers or in some instances no exposed cases. Overweight or obesity did not entail any increase in NHL risk. In the same way, analyses of HL resulted in null findings.

TABLE 3: Estimated incidence rate ratios (IRR) together with corresponding 95% confidence intervals (95% CI) for non-Hodgkin lymphoma (including chronic lymphocytic leukemia) and Hodgkin lymphoma (HL)*, ** among men.

TOBACCO USE and BMI Type/level	Number of individuals	Number of person years, accumulated (in millions)	Number of cases NHL including CLL	IRR (95% CI) Age adjusted NHL including CLL	Number of cases Hodgkin lymphoma	IRR (95% CI) Age adjusted Hodgkin lymphoma
Never tobacco users (reference)	102,443	1.98	337	1.0	49	1.0
Smoking tobacco						
Ex	33,721	0.80	209	1.01 (0.85-1.21)	22	1.18 (0.70-1.98)
Current	107,552	2.29	455	1.00 (0.87-1.15)	73	1.32 (0.91-1.90)
Cigarette smoker						
Ever	99,530	2.04	357	1.00 (0.86-1.16)	66	1.32 (0.91-1.91)
Cigar smoker						
Ever	1,972	0.05	13	0.82 (0.46-1.45)	3	2.48 (0.77-8.02)
Pipe smoker						
Ever	17,097	0.40	146	1.08 (0.88-1.31)	11	1.07 (0.55-2.09)
Snuff dipper						
Ever	40,981	1.81	66	0.77 (0.59-1.01)	15	0.88 (0.49-1.58)
Mixed users						
Ever	76,689	0.08	348	0.98 (0.84-1.14)	53	1.24 (0.83-1.83)
BMI						
<18.5	4, 779	0.09	5	0.57 (0.23-1.36)	1	0.38 (0.05-2.73)
18.6-24.9	210, 529	4.24	651	1.0	126	1.0
25-29.9	102, 033	2.11	528	1.05 (0.93-1.18)	54	0.81 (0.58-1.12)
=>30	15, 404	0.31	78	0.95 (0.75-1.21)	15	1.48 (0.86-2.57)

*Adjusted for tobacco use (BMI data) and BMI (smoking data) **All Likelihood Ratio tests were non-significant

TABLE 4: Estimated age- and body mass index-adjusted incidence ratios (IRR), together with corresponding 95% confidence intervals (CI) for non-Hodgkin lymphoma (including Chronic Lymphocytic Leukemia) and Hodgkin lymphoma (HL) among men [1, 2.]

[1]Adjusted for tobacco use (BMI data) and BMI (smoking data) [2] All Likelihood ratio tests were non-significant

	Person years (millions)	**Number of cases NHL including CLL**	**IRR (95% CI) NHL including CLL Age and BMI adjusted**	**Number of cases Hodgkin lymphoma**	**IRR (95% CI) Hodgkin lymphoma**
Never tobacco users (reference)	1.98	337	1.0	49	1.0
Dose cigarettes					
<10	1.36	276	1.02 (0.87-1.20)	31	0.95 (0.60-1.49)
11-20	0.96	179	1.04 (0.86-1.25)	40	1.73 (1.14-2.63)
>20	0.15	22	0.72 (0.47-1.11)	5	1.34 (0.53-3.38)
Dose pipe g/w					
<80	0.84	262	1.06 (0.90-1.26)	21	0.93 (0.54-1.61)
>80	0.03	13	1.38 (0.79-2.4)	2	2.34 (0.56-9.76)
Dose cigars/day					
>=1	0.10	28	0.86 (0.58-1.27)	3	1.16 (0.35-3.82)
Years of smoking					
<=15	1.57	184	0.97 (0.80-1.16)	51	1.39 (0.93-2.07)
16-25	0.77	191	1.09 (0.91-1.31)	21	1.08 (0.64-1.83)
26-max	0.67	279	0.97 (0.82-1.16)	22	1.22 (0.68-2.20)
Time since cessation of smoking (years)					
Smoke stop<=10	0.51	123	1.06 (0.86-1.30)	16	1.29 (0.73-2.29)
Smoke stop >10	0.22	81	0.94 (0.73-1.21)	6	1.01 (0.41-2.49)
Years snuffer					
1-30	0.64	49	0.81 (0.60-1.11)	11	0.70 (0.36-1.37)
>30	0.04	16	0.69 (0.41-1.15)	4	3.78 (1.23-11.60)

8.4.2 Study II

During the total follow up of 7,475,628 person-years, 47 cases of ALL, 224 of AML, 101 of CML and 520 with a diagnosis of MM were ascertained. In this study cohort about 16% reported a history of past smoking and close to 40% were current smokers. A total of 62.5% were of normal BMI, whereas 1.3% were underweight, 31.5% overweight and 4.8% obese, respectively.

Current smoking was associated with a 50% increase in risk of AML (IRR 1.50, 95% CI 1.06-2.11). Former or current smoking status was not related to risk of developing ALL, CML or MM (Table 5).
Smoking intensity of >20 gram daily conferred a twofold, albeit not statistically significant excess risk of ALL (IRR 2.29, 95% CI 0.71-7.73) and no trend could be discerned (p_{trend}= 0.11). Furthermore, intensity of smoking did not influence risk of AML (Table 6), CML or MM. Pure use of cigarettes, pipe (Table 6) or snuff (Table 7) was not associated with any of the malignancies under study. In terms of BMI, compared with subjects of normal BMI, overweight and obese individuals were not at an increased risk of developing ALL, AML, CML or MM respectively (Table 8).

Table 5: Incidence Rate Ratios for tobacco smoking of leukemia subtypes and MM

	Smoking status		
	Never tobacco users (reference)	Ex-smokers	Current smokers
No. individuals	101,959	33,374	106,264
Person-years, accumulated (in millions)	2.24	0.78	2.40
Number of cases ALL	10	7	19
IRR (95% CI)	Reference	1.56 (0.58-4.20)	1.80 (0.83-3.90)
Number of cases AML	52	30	92
IRR (95% CI)	Reference	0.94 (0.60-1.48)	1.50 (1.06-2.11)
Number of cases CML	35	10	28
IRR (95% CI)	Reference	0.64 (0.32-1.32)	0.69 (0.42-1.14)
Number of cases MM	143	102	168
IRR (95% CI)	Reference	1.11 (0.86-1.43)	0.96 (0.77-1.20)

NOTE: When analyzing smoking, we restricted to never snuff dippers.
Adjusted for age and BMI.

Table 6: Incidence Rate Ratios for smoking intensity and use of specific types of tobacco of acute leukemia.

			ALL		AML	
TOBACCO USE	No. individuals	Person years, accumulated (in millions)	No. cases	IRR (95% CI)	No. cases	IRR (95% CI)
Never tobacco users (reference)	101,959	2.24	10	Reference	52	Reference
Current smokers						
<10 gram/day*	49,465	1.39	5	1.00 (0.34-2.96)	50	1.58 (1.07-2.34)
10-20 gram/day	33,607	1.05	9	2.66 (1.07-6.64)	25	1.21 (0.75-1.96)
>20 gram/day	20,429	0.62	4	2.29 (0.71-7.37)	16	1.59 (0.90-2.79)
P for trend				0.11		0.59
Pure cigarette smoker	98,183	2.20	18	1.94 (0.89-4.21)	64	1.29 (0.89- 1.86)
Pure pipe smoker	16,988	0.38	2	0.84 (0.18-3.92)	25	1.38 (0.85-2.24)
Mixed users	76,381	1.81	12	1.41 (0.61-3.29)	69	1.38 (0.96-1.98)

NOTE: Analyses of smoking intensity as well as specific types of smoking tobacco were adjusted for age and BMI. We equated one cigarette to contain one gram and one cigar six grams of tobacco on average. Pipe smoking was measured in grams per week. Mixed users were defined as users of at least two tobacco products, either snuff and smoking tobacco, or more than one type of smoking tobacco.

* Analyzes of smoking intensity was restricted to current smokers. The number of cases for amount of smoking does not add up to that of current smokers for all outcomes due to missing information on amount.

Table 7: Incidence Rate Ratios for use of snuff of leukemia subtypes and MM.

	Snuff status	
	Never tobacco users (reference)	Pure snuff dippers
No. individuals	101,959	40,932
Person years, accumulated (in millions)	2.24	0.80
No. cases ALL	10	4
IRR (95% CI)	Reference	1.24 (0.39-4.01)
No. cases AML	52	10
IRR (95% CI)	Reference	0.81 (0.41-1.60)
No. cases CML	35	12
IRR (95% CI)	Reference	1.17 (0.60-2.28)
No. cases MM	143	26
IRR (95% CI)	Reference	0.92 (0.61-1.40)

NOTE: Analyses of snuff use was restricted to pure users of snuff.
Adjusted for age and BMI

Table 8: Incidence Rate Ratios for Body Mass Index of leukemia subtypes and multiple myeloma.*

	Level of Body Mass Index		
	18.5-25	25.1- 30	>30
No. individuals	210,081	105,793	16,260
Person years, accumulated (in millions)	4.74	2.31	0.33
No. cases ALL	25	19	3
IRR (95% CI)	Reference	1.43 (0.76-2.69)	1.46 (0.43-4.98)
No. cases AML	112	94	18
IRR (95% CI)	Reference	1.07 (0.80-1.42)	1.30 (0.77-2.17)
No. cases CML	66	27	8
IRR (95% CI)	Reference	0.69 (0.43-1.09)	1.35 (0.64-2.84)
No. cases MM	256	236	27
IRR (95% CI)	Reference	1.04 (0.86-1.24)	0.70 (0.46-1.06)

NOTE: Due to the small number of subjects (n= 4,247) and number of cases in the stratum BMI less than 18.5 is not presented in the table.
Adjusted for age and tobacco use.

8.4.3 Study III

Among cases, median time to NHL diagnosis was 22 months range (2.7-193). The most frequently transplanted type of organ was kidney, constituting 78% (Table 9). Donor characteristics i.e. age, sex and vital status were unrelated to NHL risk. Compared to kidney recipients, patients receiving a heart or lung-graft were at a 30- and 20-fold increased risk of post transplantation NHL, while liver recipients had a 17-fold but non-significant excess risk. Moreover, more than two HLA-mismatches conferred a significant four-fold higher risk of NHL in univariate analysis (Table 10).

A high total accumulated dose or a high average daily dose of corticosteroids, azathioprine or cyclosporine was not statistically significantly related to risk of NHL (Table 11). However, a high total accumulated dose of ATG entailed a statistically significant five-fold increased risk of NHL (OR 5.28, 95% CI 1.62-17.2) and the association was even more pronounced for a high average daily dose (OR 8.49, 95% CI 1.88-38.3). Patients treated with Tacrolimus were at a ten-fold higher risk of NHL compared to subjects who were never users, and ever use of OKT3 was associated with a five-fold albeit not statistically significant excess risk (Table 11).

Pre-transplant serology (EBV and CMV) was not associated with risk of NHL, but the interpretation these results was impeded by missing data. A total of seven recipients had an EBV-infection following organ transplantation (2 primary infections and 5 reactivations). No point estimate could be assessed because all seven patients with an EBV infection were cases, suggesting a causal relation to NHL. In analyses of herpes group virus infections (including EBV, CMV, VZV and HSV), a statistically significant five-fold excess risk was observed (OR 4.89, 95% CI 1.88-12.7). Fungal infections as well as multiple infectious complications conferred a four-fold elevated risk of NHL (OR 2.93, 95% CI 1.31- 6.55) and (OR 4.25, 95% CI 1.08-21.7), respectively. In contrast, bacterial infections were unrelated to NHL risk.

The association between ATG and NHL sustained adjustment for other covariates that came out significant in univariate analyses with the exception for type of organ (Table 12). Similarly, aside from when adjusted for ATG estimates for organ type were essentially unchanged. As displayed in Table 13, some of the independent covariates, such as ATG and EBV correlated. Furthermore, after adjustments for other covariates, risk of NHL in relation to herpes virus infections persisted while estimates for fungal infections were diluted (Table 12).

The excess risk of NHL associated with a high total accumulated dose of ATG was apparent for early-onset lymphomas arising within a year subsequent to organ transplantation as well as for late onset NHL (after 12 months), while the association with herpes virus group infections was stronger and statistically significantly increased only with regard to early-onset NHL

Table 9: Selected characteristics at solid organ transplantation of cases with non-Hodgkin lymphoma (NHL) and controls

	Cases (n=37)	Controls (n=97)
Age of recipient (years), median (range)	49 (14-65)	50 (13- 68)
Transplantation center (n, %)		
Uppsala	3 (8)	14 (14)
Huddinge	6 (16)	17 (18)
Göteborg	22 (60)	46 (47)
Malmö/Lund	6 (16)	20 (21)
Calendar period of transplantation (n, %)		
1970-1974	2 (5.4)	4 (4.1)
1975-1979	1 (2.7)	2 (2.1)
1980-1984	5 (14)	15 (16)
1985-1989	7 (19)	21 (22)
1990-1994	16 (43)	39 (40)
1995-1997	6 (16)	16 (17)
Follow-up time (months), median (range)	22 (2.7-193)	15 (2.2-193)
Underlying disease (n, %)*		
Kidney recipients		
Chronic glomerulonephritis	9 (24)	27 (28)
Diabetes nephropathy	1 (2.7)	14 (14)
Cystic kidneys	3 (8.1)	13 (13)
Chronic pyelonephritis	1 (2.7)	10 (10)
Other**	6 (16)	19 (20)
Liver recipients		
Sclerosing cholangitis	1 (2.7)	0 (0.0)
Liver cirrhosis	1 (2.7)	3 (3.1)
Heart recipients		
Cardiomyopathy	9 (24)	5 (5.2)
Transposition	1 (2.7)	0 (0.0)
Lung recipients		
COPD/emphysema	2 (5.4)	3 (3.1)
Primary pulmonal hypertension	1 (2.7)	1 (1.0)
Cystic fibrosis	1 (2.7)	0 (0.0)
Essential pulmonal fibrosis	1 (2.7)	0 (0.0)
Fibrosing alveolitis	0 (0)	1 (1.0)
Time in dialysis (days), median (range) kidney recipients only	229 (0-1864)	244 (0-1918)

* One individual had missing information on underlying disease.

** IgA nephritis (n=4), amyloidosis (n=2), vasculitis (n=1), unspecified kidney disease (n= 18)

Table 10: Relative Risk* of non-Hodgkin Lymphoma (NHL) by recipient and donor characteristics at the time of the first solid organ transplantation and number of rejection episodes and re-transplantations during follow-up.

	Cases (n, %)	Controls (n, %)	OR (95%CI)*	LRT p-value†
Recipient characteristics				
Gender of recipient				
Men	27 (73)	54 (56)	ref	
Women	10 (27)	43 (44)	0.48 (0.21-1.10)	0.06
No of Tx**				
1	33 (89)	94 (97)	Ref	
≥ 2	4 (11)	3 (3.1)	6.50 (0.49-354)	0.10
Organ Tx1				
Kidney	20 (54)	84 (87)	Ref	
Liver	2 (5.4)	3 (3.1)	17.1 (0.76-1168)	
Heart	10 (27)	5 (5.2)	31.0 (3.49-275)	
Lung	5 (14)	5 (5.2)	19.4 (1.75-214)	0.0001
Donor characteristics				
Donor status***				
Deceased donor	30 (81)	69 (71)	ref	
Living related donor	6 (16)	25 (26)	0.51 (0.14-1.58)	
Living unrelated donor	1 (2.7)	2 (2.1)	1.41 (0.02-118)	0.49
Donor age (years)				
<50	25 (68)	47 (49)	ref	
>50	7 (19)	32 (33)	0.39 (0.14-1.09)	0.06
Missing	5 (13)	20 (19)		
Donor gender				
Male	11 (30)	31 (32)	ref	
Female	15 (41)	27 (28)	2.46 (0.73-8.29)	0.13
Missing	11 (30)	39 (40)		
HLA-mismatches				
HLA-A				
No mismatch	8 (22)	30 (31)	ref	
One mismatch	11 (30)	33 (34)	1.35 (0.45-4.06)	
Two mismatches	12 (32)	20 (21)	3.07 (0.96-9.86)	0.12
Missing	6 (16)	14 (14)		
HLA-B				
No mismatch	5 (14)	23 (24)	ref	
One mismatch	11 (30)	31 (32)	2.25 (0.58-8.71)	
≥ 2 mismatches	15 (41)	29 (30)	4.05 (1.04-15.8)	0.08
Missing	6 (16)	14 (14)		
HLA-DR				
No mismatch	6 (16)	22 (23)	ref	
One mismatch	10 (27)	34 (35)	1.29 (0.32-5.22)	
≥ 2 mismatches	9 (24)	19 (20)	2.01 (0.54-7.49)	0.55
Missing	12 (32)	22 (23)		

(Table 10 continued)

Rejection				
No	8 (22)	37 (38)	ref	
Yes	29 (78)	60 (62)	2.08 (0.88-4.95)	0.09
Total No of rejection episodes				
0	8 (22)	37 (38)	ref	
1	10 (27)	26 (27)	1.73 (0.60-4.96)	
≥2	19 (51)	34 (35)	2.34 (0.91-6.02)	0.19
Transplantectomy				
No	31 (84)	90 (93)	ref	
Yes	6 (16)	7 (7.2)	2.72 (0.70-10.6)	0.15

* Odds Ratios (OR) and 95% confidence intervals (CI) computed in a conditional logistic regression model.

** Exact logistic regression was used to get more accurate results for covariates with small frequencies (n<5)

*** Missing information on one individual

† p-value of the association computed with the likelihood ratio test (LRT)

Table 11: Relative Risk* of non-Hodgkin lymphoma (NHL) by ever use, total accumulated dose and average daily dose during treatment periods of immunosuppressive medications***

	Cases (n, %)	Controls (n, %)	OR (95%CI)	LRT p-value†
Corticosteroids**				
Accumulated corticosteroid dose (iv,o)				
1st Q	6 (16)	25 (26)	ref	
2nd Q	8 (22)	24 (25)	1.35 (0.40-4.53)	
3rd Q	7 (19)	24 (25)	1.59 (0.37- 6.83)	
4th Q	16 (43)	24 (25)	4.50 (0.85-23.8)	0.32
Average dose corticosteroids (o)				
1st Q	11 (30)	22 (23)	ref	
2nd Q	11 (30)	23 (24)	1.04 (0.34-3.18)	
3rd Q	7 (19)	26 (27)	0.59 (0.14-2.52)	
4th Q	8 (22)	26 (27)	0.67 (0.17-2.67)	0.83
Azathioprine (iv,o)				
Never	3 (8.1)	20 (21)	ref	
Ever	34 (92)	77 (79)	2.84 (0.75-16.0)	0.12
Accumulated Azathioprine dose (iv,o)				
No Azathioprine	3 (8.1)	20 (21)	ref	
1st Q	12 (32)	19 (20)	4.35 (1.05-18.1)	
2nd Q	5 (13.5)	20 (21)	1.73 (0.35-8.61)	
3rd Q	5 (13.5)	19 (20)	1.52 (0.29-7.99)	
4th Q	12 (32)	19 (20)	5.25 (0.69-39.9)	0.10
Average dose Azathioprine (o)				
No Azathioprine	3 (8.1)	20 (21)	ref	
1st Q	10 (27)	20 (21)	2.89 (0.67-17.6)	
2nd Q	10 (27)	18 (19)	3.70 (0.78- 24.6)	
3rd Q	5 (14)	19 (20)	1.36 (0.20-10.9)	
4th Q	9 (24)	20 (21)	3.35 (0.68-22.8)	0.27
Cyclosporine (iv, o)				
Never	6 (16)	13 (13)	ref	
Ever	31 (84)	84 (87)	0.91 (0.15-5.76)	0.92
Accumulated Cyclosporine dose (iv,o)				
No Cyclosporine	6 (16)	13 (13)	ref	
1st Q	10 (27)	21 (22)	2.36 (0.24-23.5)	
2nd Q	5 (14)	21 (22)	0.49 (0.04-5.65)	
3rd Q	6 (16)	21 (22)	0.51 (0.06-4.39)	
4th Q	10 (27)	21 (22)	1.18 (0.14-10.2)	0.34
Average dose Cyclosporine (o)**§**				
No Cyclosporine	6 (16)	13 (13)	ref	
1st Q	7 (19)	22 (23)	0.35 (0.04-3.37)	
2nd Q	8 (22)	21 (22)	0.47 (0.05-4.17)	
3rd Q	4 (11)	25 (26)	0.27 (0.03- 2.59)	
4th Q	12 (32)	16 (17)	2.48 (0.31-19.9)	0.03
Muromonab CD3 (OKT3)				
Never	32 (87)	94 (97)	ref	
Ever	5 (14)	3 (3.1)	5.97 (0.89-66.9)	0.03
Antithymocyte Immunoglobulin (ATG)				
Never	16 (43)	74 (76)	ref	
Ever	21 (57)	23 (24)	5.57 (2.17-14.3)	0.0001
Accumulated ATG dose (iv)				
No ATG	16 (43)	74 (76)	ref	
<50th percentile	12 (32)	13 (13)	5.82 (1.90-17.8)	
>50th percentile	9 (24)	10 (10)	5.28 (1.62-17.2)	0.0005

(Table 11 continued)

Average dose ATG (iv)				
No ATG	16 (43)	74 (76)	ref	
<50th percentile	14 (38)	15 (16)	4.96 (1.84-13.4)	
>50th percentile	7 (19)	8 (8.3)	8.49 (1.88-38.3)	0.0004
Tacrolimus (FK506)				
No	32 (87)	96 (99)	ref	
Yes	5 (14)	1 (1.0)	10.6 (1.16- 512)	0.02
Cyclofosphamide (Sendoxan)				
Never	36 (97)	95 (98)	ref	
Ever	1 (2.7)	2 (2.1)	1.30 (0.02-25.4)	1.0
Combined treatment regimens				
Steroids +Azathioprine	6 (16)	13 (13)	ref	
Steroids + Azathioprine + Cyclosporine	17 (46)	36 (37)	1.99 (0.17-31)	
Steroids+Cyclosporine	2 (5.4)	9 (9.3)	0.70 (0.04-12.3)	
Azathioprine+ Cyclosporine	12 (32)	39 (40)	0.74 (0.07-10.6)	0.35

* Odds Ratios (OR) and 95% Confidence intervals (CI) computed in a conditional regression model
** All subjects received steroids, hence OR for ever versus never use of corticosteroids could not be estimated
*** Dose levels were categorized according to the quartile distribution among the controls. For ATG, the 50^{th} percentile was used as a cut-off. Total accumulated dose was the total dose during the entire follow-up period. Average daily dose was the average dose per day administered during treatment periods. Analyses of average dose (apart from ATG and OKT3 that are exclusively intravenous drugs) were restricted to orally administered drugs, since intravenous drugs were often given during much shorter periods and with much higher doses. Biological availability of oral medications has been adjusted for in the combined analyses of orally and intravenously administered drugs. Furthermore, varying efficacy of different steroids is adjusted for (see statistical analyses).
† p-value of the association computed with the likelihood ratio test (LRT)
§ Including cyclosporine microemulsion

Table 12: Adjusted relative risk* of non-Hodgkin lymphoma (NHL) by selected transplant-related characteristics and immunosuppressive treatment

	OR (95%CI)	LRT P-value†
HLA-B mismatch (1, and 2 or more versus none)		
Adjusted for ever use of ATG	1.55 (0.36- 6.72)	
	3.71 (0.78-17.6)	0.17
Adjusted for type of organ	2.36 (0.54-10.3)	
	2.74 (0.61-12.2)	0.35
Ever use of Azathioprine (versus never)		
Adjusted for ever use of ATG	0.94 (0.18- 6.30)	0.93
Adjusted for type of organ	0.97 (0.17- 6.79)	0.98
Adjusted for HLA-B mismatch	2.85 (0.54-28.42)	0.15
Adjusted for herpes virus group infections	1.59 (0.38- 9.44)	0.42
Adjusted for fungal infections	2.06 (0.51-11.9)	0.26
Ever use of ATG (versus never)		
Adjusted for type of organ	2.54 (0.79-8.18)	0.12
Adjusted for HLA-B mismatch	8.77 (2.29-33.6)	0.0002
Adjusted for herpes virus group infections	4.57 (1.69-12.4)	0.002
Adjusted for fungal infections	5.28 (1.98-14.0)	0.0003
Type of organ (versus kidney)		
Adjusted for ever use of ATG		
liver	13.5 (0.57-954)	
heart	14.4 (1.43-145)	
lung	11.2 (0.87-146)	0.02
Adjusted for herpes virus group infections		
liver	17.8 (0.67-1419)	
heart	23.6 (2.73-205)	
lung	22.0 (1.28-379)	0.0008
Herpes virus group infections (ever vs. never)		
Adjusted for ever use of ATG	3.77 (1.38- 10.3)	0.006
Adjusted for type of organ	3.98 (1.34-11.7)	0.008
Adjusted for HLA-B mismatch	4.01 (1.35-11.9)	0.008
Adjusted for fungal infections	3.85 (1.41- 10.6)	0.005
CMV infection (ever vs. never)		
Adjusted for ever use of ATG	2.30 (0.89-5.92)	0.08
Adjusted for type of organ	1.89 (0.70-5.08)	0.21
Adjusted for HLA-B mismatch	2.03 (0.76-5.44)	0.16
Adjusted for fungal infections	2.31 (0.93-5.72)	0.07
Fungal infections (ever vs. never)		
Adjusted for ever use of ATG	2.60 (1.09- 6.17)	0.03
Adjusted for type of organ	2.16 (0.85- 5.43)	0.10
Adjusted for HLA-B mismatch	2.17 (0.87-5.39)	0.09
Adjusted for herpes virus group infections	1.99 (0.81- 4.87)	0.13

* Odds ratios (OR) and 95% Confidence intervals (95%CI) in a conditional logistic regression model
† p-value of the association computed with the likelihood ratio test (LRT) comparing the adjusted model to the univariate model.

Table 13: Correlation coefficients for covariates of interest

	Type of organ	EBV serology recipient	EBV infection	Total number of rejections	Mismatch HLA-B	CMV infection	ATG yes/no	OKT3 yes/no	FK506 yes/no	Azathioprine yes/no	Cyclosporine yes/no
Type of organ	1.00	0.05	0.61	0.18	0.43	0.26	0.69	-0.09	0.08	0.24	0.27
EBV serology recipient	0.05	1.00	-0.25	-0.18	0.08	-0.04	-0.03	-0.14	-0.38	-0.20	0.20
EBV infection	0.61	-0.25	1.00	-0.05	0.32	0.17	0.55	-0.16	0.24	0.18	0.19
Total number of rejections	0.18	-0.18	-0.05	1.00	0.27	0.28	0.30	0.28	0.33	0.40	-0.17
Mismatch HLA-B	0.43	0.08	0.32	0.27	1.00	0.13	0.39	0.08	0.12	0.09	0.23
CMV infection	0.26	0.04	0.17	0.28	0.13	1.00	0.39	0.07	0.30	0.12	-0.18
ATG yes/no	0.69	-0.03	0.55	0.30	0.39	0.14	1.00	0.25	0.19	0.32	0.35
OKT3 yes/no	-0.09	-0.14	-0.16	0.28	0.08	0.07	0.25	1.00	0.39	0.13	0.14
FK506 yes/no	0.08	-0.38	0.24	0.33	0.12	0.30	0.19	0.39	1.00	0.11	0.12
Azathioprine yes/no	0.24	-0.20	0.18	0.40	0.09	0.12	0.32	0.13	0.11	1.00	-0.15
Cyclosporine yes/no	0.27	0.20	0.19	-0.17	0.23	-0.18	0.35	0.14	0.12	-0.15	1.00

8.4.4 Study IV

The investigated SNPs are presented in Table 14 together with minor allele frequencies among the study control subjects. Table 15 presents selected characteristics of cases and controls. Polymorphisms under study in *BCL2* (rs1801018, rs1893806, rs194423), *CCND1* (rs603965, rs649392, rs678653, rs7178) and *MYC* (rs4645959, rs10110283) were not related to risk of NHL overall (Table 16). No association sustainable to multiple testing could be discerned between variation in *BCL2, CCND1* or *MYC* genes and risk of specific NHL subtypes (Table 17).

No clear heterogeneity was observed when testing for gene-gene (*BCL2-MYC, CCND1-MYC*) interaction. Similarly, when allowing the genetic effect to vary by smoking category (ever vs. never tobacco smokers) and history of autoimmune disorders (rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome and celiac disease) there was no clear evidence of effect measure modification in relation to NHL overall, diffuse large B-cell lymphoma (autoimmunity) or follicular lymphoma (smoking). Nonetheless, there was an indication of interaction between the *CCND1* rs603965 polymorphism and autoimmune disease that not unlikely may be due to chance alone. Moreover, multi-locus tests of association were not linked to risk of NHL overall for *BCL2* (p= 0.06), *CCND1* (p= 0.31) or *MYC* (p= 0.20).

In comparison to major allele homozygosity of the *TNF* rs1800629, minor allele AA homozygosity conferred a 50% elevated risk of NHL over all (OR 1.52, 95% CI 1.06-2.18). In addition, subtype specific analyses revealed a two-fold excess risk of mantle cell lymphoma as well as with T-cell lymphoma (Table 17). A 14% higher risk of NHL overall was detected among minor allele AA homozygotes of the *IL10* rs1800890, although the result did not reach statistical significance at the α-0.05 level. This association was more pronounced for DLBCL (OR 1.45, 95% CI 1.10-1.90) as well as mantle cell lymphoma (OR 1.83, 95% CI 1.08-3.12). With regard to *TNF* and *IL10*, our results did not indicate any effect measure modification by smoking status, history of autoimmune disease (the latter perhaps consequent to few exposed individuals) or country of residence.

Table 14: List of selected genes and single nucleotide polymorphisms (SNPs) with reference SNP ID, location, nucleotide substitution, minor allele frequency and p-values for tests of Hardy-Weinberg equilibrium (HWE)

Gene	Reference SNP ID	Location	Nucleotide substitution	Minor allele frequency (mean)	HWE Pearson χ^2 Sweden (controls)	HWE Pearson χ^2 Denmark (controls)
BCL2	rs1801018	intron2	T→C	0.44	0.90	0.46
	rs1893806*	intron2	A→C	0.45	0.96	0.01
	rs1944423	chr18: 59141498 upstream	G→A	0.28	0.25	0.94
CCND1	rs603965	exon4	G→A	0.46	0.83	0.55
	rs649392	intron4	A→G	0.45	0.63	0.71
	rs678653	exon5	G→C	0.33	0.05	0.36
	rs7178	exon5	A→C	0.09	0.06	0.05
MYC	rs4645959*	exon2	A→G	0.03	0.21	0.19
	rs10110283*	intron2	G→A	0.03	0.02	0.39
TNF	rs1800629	chr6: 31651010 upstream	A→G	0.18	0.53	0.54
IL10	rs1800890	chr1: 20505988 downstream	A→T	0.39	0.87	0.93

* p-values computed with the exact test for Hardy-Weinberg equilibrium

Table 15: Characteristics of participating Non-Hodgkin Lymphoma (NHL) cases and controls

	Controls (n=1936) n (%)	Cases (n=2410) n (%)
Country of residence		
Denmark	765 (39)	784 (33))
Sweden	1,198 (61)	1,626 (67)
Sex		
Male	1,069 (54)	1,458 (61)
Female	894 (46)	952 (40)
Age (years)*		
18-24	41 (2)	21 (1)
25-34	63 (3)	58 (2)
35-44	128 (7)	145 (6)
45-54	338 (17)	454 (19)
55-64	611 (31)	804 (33)
65-75	782 (40)	928 (38)
Median (range)	61 (18-75)	61 (18-75)
Ethnicity **		
Both parents born in Denmark/Sweden	1,883 (96)	2,314 (96)
Either or both parents born in another Nordic country	76 (4)	92 (4)
Tobacco smoking†		
Ever	1,109 (57)	1,392 (58)
Never	853 (43)	1,018 (42)
History of autoimmune disorder§		
Yes	79 (4)	93 (4)
No	1,878 (96)	2,315 (96)

* Age at diagnosis (cases); age at interview (controls)
** Missing information on birth country of parents for 8 individuals
† Missing information on smoking for 1 individual
§ Missing information on history of autoimmune disease (rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome and celiac disease) for 8 individuals

Table 16: Frequencies and odds ratios (OR) with 95% confidence intervals (CI) for associations between genotypes of investigated SNPs and risk of non-Hodgkin lymphoma (NHL) overall

Gene/Genotype		Controls n (%)	Cases n (%)	OR (95% CI)	P trend
BCL2	**rs1801018**				
	TT	543 (32)	723 (32)	ref	
	CT	855 (50)	1,070 (48)	0.94 (0.82-1.09)	
	CC	318 (19)	442 (20)	1.05 (0.87-1.26)	0.78
	rs1893806				
	AA	409 (28)	683 (31)	ref	
	AC	758 (52)	1,103 (50)	0.87 (0.75-1.02)	
	CC	290 (20)	431 (19)	0.89 (0.73-1.08)	0.17
	rs1944423				
	GG	808 (51)	1,092 (53)	ref	
	AG	630 (40)	833 (40)	0.98 (0.86-1.13)	
	AA	135 (9)	149 (7)	0.82 (0.64-1.06)	0.24
CCND1	**rs603965**				
	GG	527 (30)	655 (29)	ref	
	AG	879 (49)	1,105 (49)	1.02 (0.88-1.17)	
	AA	374 (21)	498 (22)	1.08 (0.91-1.29)	0.39
	rs649392				
	AA	496 (28)	703 (32)	ref	
	AG	872 (50)	1,076 (48)	0.87 (0.75-1.00)	
	GG	379 (22)	452 (20)	0.83 (0.70-1.00)	0.04
	rs678653				
	GG	818 (46)	1,011 (45)	ref	
	CG	744 (42)	970 (43)	1.06 (0.93-1.21)	
	CC	211 (12)	264 (12)	1.01 (0.83-1.24)	0.64
	rs7178				
	AA	1,235 (83)	1,791 (82)	ref	
	AG	235 (16)	385 (18)	1.08 (0.90-1.30)	
	GG	11 (0.7)	15 (0.7)	1.01 (0.46-2.24)	0.42
MYC	**rs4645959**				
	AA	1,572 (95)	2,202 (95)	ref	
	AG	73 (4)	128 (6)	1.30 (0.96-1.75)	0.160
	GG	2 (0.1)	0 (0.0)	…….	
	rs10110283				
	GG	1,761 (94)	2,207 (96)	ref	
	AG	99 (5)	101 (4)	0.81 (0.61-1.08)	
	AA	4 (0.2)	2 (0.1)	0.36 (0.07-1.98)	0.08
	rs1800629				
TNF					
	GG	1,003 (67)	1,464 (66)	ref	
	AG	430 (29)	668 (30)	1.06 (0.91-1.22)	
	AA	46 (3)	100 (4)	1.52 (1.06-2.18)	0.06
IL10	**rs1800890**				
	TT	688 (38)	831 (37)	ref	
	AT	860 (47)	1,073 (47)	1.05 (0.91-1.20)	
	AA	273 (15)	371 (16)	1.14 (0.95-1.38)	0.16

Table 17: Frequencies and odds ratios (OR) with 95% confidence intervals (CI) for associations between genotypes of investigated SNPs and risk of Non-Hodgkin lymphoma subtypes

Gene /Genotype	Diffuse large B-cell lymphoma			Chronic lymphocytic leukemia			Follicular lymphoma		
	Cases n (%)	OR (95% CI)	P trend	Cases n (%)	OR (95% CI)	P trend	Cases n (%)	OR (95% CI)	P trend
BCL2									
rs1801018									
TT	192 (33)	ref		167 (30)	ref		119 (31)	ref	
CT	282 (49)	0.93 (0.75-1.16)		282 (50)	1.07 (0.86-1.34)		187 (48)	1.00 (0.78-1.29)	
CC	102 (18)	0.91 (0.69-1.20)	0.45	110 (20)	1.13 (0.85-1.49)	0.38	81 (21)	1.16 (0.85-1.59)	0.40
rs1893806									
AA	160 (29)	ref		181 (33)	ref		135 (33)	ref	
AC	287 (52)	0.99 (0.78-1.24)		263 (48)	0.80 (0.64-1.01)		202 (49)	0.81 (0.63-1.04)	
CC	108 (19)	0.95 (0.71-1.27)	0.74	109 (20)	0.85 (0.64-1.13)	0.17	76 (18)	0.78 (0.57-1.07)	0.10
rs1944423									
GG	270 (52)	ref		262 (53)	ref		222 (53)	ref	
AG	216 (42)	1.03 (0.84-1.27)		201 (40)	0.99 (0.80-1.22)		165 (39)	0.96 (0.76 -1.21)	
AA	33 (6)	0.74 (0.49-1.10)	0.41	35 (7)	0.80 (0.54-1.19)	0.42	32 (8)	0.86 (0.57-1.30)	0.49
CCND1									
rs603965									
GG	169 (29)	ref		171 (30)	ref		117 (30)	ref	
AG	287 (50)	1.02 (0.82-1.27)		271 (48)	0.95 (0.76-1.18)		182 (46)	0.93 (0.72-1.21)	
AA	123 (21)	1.03 (0.79-1.35)	0.80	121 (22)	1.00 (0.77-1.31)	0.96	95 (24)	1.15 (0.85-1.55)	0.44
rs649392									
AA	183 (32)	ref		165 (30)	ref		132 (34)	ref	
AG	282 (49)	0.87 (0.70-1.08)		271 (49)	0.93 (0.74-1.16)		171 (44)	0.74 (0.57-0.95)	
GG	115 (20)	0.81 (0.62-1.06)	0.11	116 (21)	0.91 (0.69-1.20)	0.48	82 (21)	0.81 (0.60-1.10)	0.11
rs678653									
GG	260 (44)	ref		233 (43)	ref		182 (46)	ref	
CG	264 (45)	1.12 (0.92-1.36)		240 (44)	1.13 (0.92 -1.39)		168 (43)	1.02 (0.81-1.28)	
CC	66 (11)	0.98 (0.72-1.33)	0.69	75 (14)	1.25 (0.92-1.69)	0.10	44 (11)	0.94 (0.65-1.35)	0.83
rs7178									
AA	440 (82)	ref		458 (84)	ref		341 (81)	ref	
AG	94 (18)	1.08 (0.83-1.41)		84 (15)	0.92 (0.70-1.21)		75 (18)	1.15 (0.86-1.53)	
GG	2 (0.4)	0.62 (0.14-2.84)	0.77	2 (0.4)	0.59 (0.13-2.69)	0.42	2 (0.5)	0.74 (0.16-3.37)	0.46
MYC									
rs4645959									
AA	557 (95)	ref		545 (95)	ref		413 (95)	ref	
AG	30 (5)	1.26 (0.81-1.96)		31 (5)	1.29 (0.84-1.99)		23 (5)	1.28 (0.79-2.08)	
GG	0 (0.0)	……	0.41	0 (0.0)	……	0.36	0 (0.0)		0.42
rs10110283									
GG	569 (95)	ref		552 (96)	ref		390 (95)	ref	
AG	27 (5)	0.84 (0.54-1.30)		20 (4)	0.64 (0.39-1.05)		19 (5.0)	0.87 (0.52-1.43)	
AA	1 (0.2)	0.69 (0.08-6.18)	0.39	1 (0.2)	0.77 (0.09-6.89)	0.09	0 (0.0)	…….	0.39
TNF									
rs1800629									
GG	364 (65)	ref		365 (65)	ref		292 (70)	ref	
AG	170 (31)	1.10 (0.89-1.37)		170 (30)	1.10 (0.88-1.36)		113 (27)	0.92 (0.72- 1.18)	
AA	22 (4)	1.32 (0.78-2.25)	0.22	24 (4)	1.44 (0.86-2.40)	0.16	12 (3)	0.89 (0.47-1.72)	0.49
IL10									
rs1800890									
TT	200 (34)	ref		215 (38)	ref		151 (38)	ref	
AT	278 (47)	1.13 (0.91-1.39)		258 (46)	0.95 (0.77-1.17)		187 (47)	0.99 (0.78-1.25)	
AA	112 (19)	1.45 (1.10-1.90)	0.01	94 (17)	1.10 (0.83-1.46)	0.60	60 (15)	1.00 (0.72-1.40)	1.00

(Table 17 continued)

Gene/Genotype	T-cell lymphoma			Mantle cell lymphoma		
	Cases n (%)	OR (95% CI)	P trend	Cases n (%)	OR (95% CI)	P trend
BCL2						
rs1801018						
TT	53 (34)	ref		34 (29)	ref	
CT	70 (45)	0.84 (0.58-1.22)		58 (49)	1.09 (0.70-1.68)	
CC	32 (21)	1.03 (0.65-1.64)	0.95	27 (23)	1.35 (0.80-2.29)	0.27
rs1893806						
AA	48 (33)	ref		33 (28)	ref	
AC	70 (48)	0.80 (0.54-1.18)		62 (53)	1.04 (0.67-1.61)	
CC	27 (19)	0.79 (0.48-1.29)	0.29	22 (19)	0.93 (0.53-1.64)	0.85
rs1944423						
GG	75 (56)	ref		61 (53)	ref	
AG	48 (36)	0.82 (0.56-1.20)		42 (37)	0.89 (0.59-1.34)	
AA	10 (8)	0.79 (0.40-1.57)	0.29	12 (10)	1.17 (0.62-2.24)	0.96
CCND1						
rs603965						
GG	45 (28)	ref		41 (34)	ref	
AG	75 (47)	1.00 (0.68-1.47)		50 (41)	0.73 (0.48-1.12)	
AA	38 (24)	1.19 (0.76-1.88)	0.47	30 (25)	1.04 (0.63-1.69)	0.95
rs649392						
AA	49 (32)	ref		40 (33)	ref	
AG	74 (48)	0.85 (0.58-1.24)		55 (45)	0.78 (0.51-1.18)	
GG	32 (21)	0.85 (0.53-1.35)	0.45	26 (21)	0.84 (0.50-1.41)	0.44
rs678653						
GG	72 (46)	ref		59 (49)	ref	
CG	66 (42)	1.01 (0.71-1.43)		50 (41)	0.93 (0.63-1.38)	
CC	19 (12)	1.02 (0.60-1.73)	0.93	12 (10)	0.79 (0.42-1.49)	0.47
rs7178						
AA	109 (75)	ref		96 (81)	ref	
AG	34 (23)	1.58 (1.05-2.39)		22 (19)	1.16 (0.71-1.88)	
GG	3 (2)	3.74 (0.95-12.8)	0.007	0 (0.0)	……..	0.80
C-MYC						
rs4645959						
AA	148 (94)	ref		119 (94)	ref	
AG	9 (6)	1.39 (0.68-2.85)		7 (6)	1.35 (0.61-3.02)	
GG	0 (0.0)	……	0.44	0 (0.0)	…….	
rs10110283						0.53
GG	152 (96)	ref		118 (95)	ref	
AG	6 (4)	0.70 (0.30-1.62)		6 (5)	0.90 (0.39-2.10)	
AA	0 (0.0)	……	0.32	0 (0.0)	…….	0.67
TNF						
rs1800629						
GG	94 (63)	ref		75 (64)	ref	
AG	45 (30)	1.14 (0.78-1.66)		33 (28)	1.05 (0.69-1.61)	
AA	11 (7)	2.56 (1.28-5.12)	0.04	10 (7)	2.87(1.39-5.93)	0.06
IL10						
rs1800890						
TT	63 (41)	ref		35 (29)	ref	
AT	78 (50)	1.00 (0.71-1.41)		61 (50)	1.41 (0.91-2.16)	
AA	13 (9)	0.57 (0.31-1.03)	0.14	25 (21)	1.83 (1.08-3.12)	0.02

9 DISCUSSION

9.1 METHODOLOGICAL CONSIDERATIONS

9.1.1 Study design

9.1.1.1 Cohort studies (studies 1 and II)

A cohort is defined as a group of individuals with similar distinguishing qualities or experience. The cohort study is a standard way to evaluate an exposed group (index group) to an unexposed comparison group. A cohort can broadly be divided into three categories: the open/dynamic cohort in which individuals can enter or leave regardless of point in time; a fixed cohort, where a certain event characterizes the study population; and a closed cohort that differs only to the fixed cohort by that it does not have any loss to follow up.

The cohort study design is preferable when investigating unusual exposures and if the study aim is to estimate multiple effects of an exposure. A major strength of the cohort study is that in enables the investigator to directly measure disease incidence or risk. A prospective cohort design is further considered to have superior exposure information and to be less inclined to bias compared to a retrospective approach. Nevertheless, the high financial cost of a cohort study has to be taken into consideration, and in terms of rare outcomes cohort studies tend to be an inefficient study design.

In the two studies of the construction workers cohort (study I and II) the choice of a cohort design was natural given that in Sweden nationwide register based data is provided which can easily be linked using the NRN. The record linkage further makes a close to complete and very long-term follow up feasible. Moreover, in this setting it was possible to investigate all the hematolymphopoietic malignancies of interest in the same study population. Using a retrospective study design, but with prospectively gathered exposure information on tobacco use along with measured height and weight we achieved two things. Firstly, the study became more time efficient as we had both exposure and outcome data available. Secondly, the study should be less prone to recall bias (see page 50).

9.1.1.2 Case-control studies (studies III and IV)

In contrast to cohort studies, in a case-control study setting subjects are chosen on the basis of the outcome/disease. Next, a sample of the source population that brought about the cases serves as the control group and hence gives information on the distribution of the exposure under investigation, in the study base that generated the cases.

A case-control study design is advantageous when data on exposure is arduous to obtain since it saves time and money. Also, in the instances when the disease/outcome is rare, a case-control study is more efficient compared to a cohort design, and a case-control study saves time compared to a prospective cohort study if the disease/outcome has a long induction and latency period. Other circumstances where a case-control study most likely would be the better choice include when the study population is dynamic and if little is known about the disease because it permits concurrent evaluation of several hypotheses.

In study III we employed a nested case-control design within a cohort of solid organ transplant recipients. The source for case identification was the Swedish National Cancer Register (see page 20) with high quality control and close to 100% completeness. This along with the use of ICD codes gave us well defined inclusion criteria and valid and close to complete assessment of incident cases of NHL.

9.1.1.3 Genetic association studies (study IV)

Genetic association studies aspire to uncover associations between genetic polymorphisms and a trait or disease. Study design as well as methods of data analysis is essentially the same as in classic epidemiological studies. Unless the disease is prevalent, a case-control design will be superior to a cohort study because the cohort study sample would yield only a small number of cases. Moreover, the case-control design has the benefit that the investigator avoids the arduousness of gathering biological matter from a large number of

study subjects, and following the cohort for a long time period. Genetic association studies are typically aimed at detecting gene variants with low to moderate relative risks; which requires sample sizes of thousands of individuals [209]. Table 18 displays the sample size needed in relation to the occurrence of the exposure together with effect size. The total number of NHL cases in study IV (n=2,410) would, for example, roughly be sufficient to detect an allelic odds ratio of approximately 1.5 for an allele frequency of 5% at the alpha 0.001 level of significance.

Table 18: Approximate sample sizes necessary to detect significant association (power=90%, two sided α= 0.001 by effect size and allele frequencies for predisposing allele.

	Frequency of susceptibility allele in controls					
γ (allelic odds ratio)	1%	5%	10%	20%	30%	40%
1·1	221 927	46 434	24 626	13 987	10 759	9505
1·2	58 177	12 217	6509	3730	2896	2581
1·3	27 055	5702	3051	1763	1380	1240
1·5	10 604	2249	1213	712	566	516
2·0	3193	687	377	229	188	177
4·0	598	134	78	52	46	47

Calculations assume multiplicative effect on disease risk (ie, homozygous susceptibility genotype has penetrance that exceeds that of heterozygote by factor γ, the genotype relative risk, and that of wild-type homozygote by γ^2). Under such model, each allele has independent effects on disease risk, and allelic odds ratio is also equal to γ. Sample sizes presented are total number of cases needed in case control study where controls are present in equal numbers. These sample size derivations assume best-case scenario in which susceptibility variant itself (or a perfect proxy) has been typed.

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9.1.2 Validity

When methodical errors, confounding along with random errors (see page 53) have been excluded, the results of a study can be regarded to have high *internal validity*. The internal validity is a presupposition for the generalizability of the study findings to populations beyond the study subjects, also called *external validity*.

9.1.3 Bias

Within epidemiology the definition of bias is a systematic error in the design or conduct of a study resulting in an erroneous estimate of the measure of association. Bias can come about in all forms of epidemiological investigations and at any stage (data compilation, analysis or publication).

9.1.3.1 Selection bias

Selection bias will arise if there are systematic differences in characteristics between individuals enrolled in a study and those who are not.

In study I and II there may be some self-selection bias if construction workers with a positive history of tobacco smoking or snuff dipping would be either more or less inclined to attend the voluntary health check-ups than never tobacco users. This is however unlikely considering the high participation rate, about 80-90% of the workers took part at least once [202]. Nonetheless, one potential source of selection bias in this study would be differential surveillance of smokers and non smokers, i.e. due to other harmful health

effects caused by smoking [210, 211], tobacco users may well have been under medical care more frequently than never tobacco users. This could result in an overdiagnosis or shorter time to diagnosis of subclinical malignancies. If true, such bias would result in an overestimation of a true relationship. Furthermore, a general concern with regard to occupational cohort studies is the "healthy worker effect" i.e. rates of death and disease are typically lower in a working population than in the general population. However, given that in study I and II, internal comparisons within the cohort were applied, this form of selection bias should not have any influence on our results.

In the organ transplantation study (study III), all study participants were identified in the national population-based patient register (see page 20). Controls were selected from the same base population of transplanted patients that generated the cases, hence reducing the risk of selection bias. In addition, participation rates were high for both cases and controls. Taken together, the non-participation, exclusions and loss to follow up described previously (see page 22) is unlikely to have resulted in selection bias as reasons for non-participation were probably unrelated to the outcome.

In study IV conditions known to be related to NHL (HIV-infection and organ transplantation) were excluded. Also, individuals with insufficient proficiency of the Swedish/Danish language were barred from the investigation. Given that language skills would not be expected to be associated with the outcome it is therefore not likely to introduce selection bias. Cases were enrolled by means of a rapid case ascertainment system which is a time efficient and reliable way of gathering information on outcome. This method has the advantage to record linkage alone that severely ill case patients can be approached while they are still well enough to participate, hence achieving a more complete enrollment of cases and possibly diminishing the peril of selection bias. This study might however to some extent suffer from some selection bias because a lower percentage of eligible controls than cases participated in the interview and provided a blood sample. However, for this to occur, genotype and non-participation would have to be related.

9.1.3.2 Observation/information bias

In the case of a systematic difference between compared groups in the manner that the exposure or outcome is gauged, observation bias (also denoted information bias) may arise. In distinction to selection bias, which is more predisposed to come about in retrospective studies, observation bias can arise in prospective as well as retrospective study designs. Below diverse forms of observation bias are discussed.

Recall bias

In a case-control study setting, recall bias occurs if cases are more or less likely than controls to remember and report preceding exposures, whereas in a cohort study it will arise if exposed and unexposed diverge in recollection and reporting of ensuing disease.

In study I and II exposure information on various types of tobacco use was collected by self-administered questionnaires along with face to face interviews by nurses prior to any outcome/diagnosis of cancer occurred (see page 21). Given the prospective nature of the exposure data, there should be no differential recollection of smoking and snuff use habits between workers who later developed lymphoma, leukemia or MM, and those who did not. Nonetheless, the more common difficulty of correctly remembering and notifying exposures influences all persons to a certain degree, but would be liable to be non-differential between cases and non-cases.

The exposure information in study III was obtained from patient medical records by research assistants that were blinded to case/control status. In addition, a structured protocol was used to assure exposure ascertainment to completeness and accuracy. Therefore recall bias ought not to be an issue in this investigation.

In study IV the data on smoking and autoimmunity was gathered by means of telephone interviewes. As this study has a case-control design it would be more prone to be influenced by recall bias. However, in this study, participants had not been informed beforehand about the nature of the questions to be asked, in order to minimize prepared responses and disparity in reporting. In general, subjects with knowledge of the study hypothesis are assumed to be more prone to selection bias, and taking into account that the hypothesis of a causal relationship between tobacco smoking and NHL or any disease subtype is not common knowledge in the general population makes it less credible that cases would have over reported their smoking habits. On the other hand, subjects might be aware of more established associations linking

tobacco smoking to other types of malignancies, such as lung cancer [211]. If so, there could be some concern for differential recollection and reporting of tobacco usage which would lead to an overestimation of a true association between exposure and outcome.

Interviewer bias
Interviewer bias arises in studies employing telephone or in-person interviews. That is, if the interviewer is aware of the disease status/outcome of the study participants and therefore inquires cases and controls in different ways. Conversely, in a cohort study interviewer bias occurs when the interviewer knows the exposure status and is inconsistent when querying exposed and unexposed about the disease.

In study IV interviewers were not blinded to case control status, hence there would be concern for interviewer bias to arise. To avoid this, a standard questionnaire with easily understandable questions was applied. Furthermore, interviewers were instructed to treat cases and controls in the same manner as far as possible as well conducting interviews with the same number of cases and controls.

Survivor bias
Differential survival is another source of selection bias in epidemiological studies. Although in study IV a rapid case ascertainment system (see page 23) was utilized and enabled us to approach cases of NHL shortly after diagnosis, which most certainly gave higher participation rates in comparison to the sole use of record linkage to the national cancer register, SCALE did not incorporate 100% of eligible case patients. A minor part of cases might have been too ill to take part and/or passed away before the interview could take place. Hence, if subjects who died prior to interview diverge from those who participated concerning disease severity or prognosis and this in turn is related to the SNPs under investigation results may be biased.

Loss to follow up
A possible issue that may occur in cohort studies is loss to follow up. This can become a problem in the instances when power to detect an association that actually is present is abridged due to a reduction in sample size obtainable for data analyses. In the two cohort studies (study I and II), loss to follow up was negligible as complete national registers with a very high level of coverage was used to follow the participants of the "Bygghälsan" program.

Misclassification bias
Misclassification (measurement error) is an error in the classification of the exposure or the disease. For instance, if an exposed individual is classified as unexposed or vice versa. This type of bias may occur in cohort or case-control studies consequent to inaccurate recall of exposures by study subjects or when wide-ranging exposure definitions are applied. Disease misclassification can arise if the classification is excessively broad or if the method of verification is imprecise. Misclassification bias can further be divided into differential and non-differential misclassification, contingent on whether the measurement error of either exposure or disease is related to the other axis (disease or exposure).

In study I and II there is some possibility of exposure misclassification with regard to smoking history and snuff use. Between the years 1971-1975 this information was obtained by means of a self administered questionnaire, and subjects may have been more or less thorough when answering the questions. For example, never smokers were instructed to pass over questions about smoking tobacco and proceed to question 71. However, this question was at the bottom of a page and stated "Have you ever used oral moist snuff?" and might therefore accidently have been missed by some exclusive users of snuff, which would then be misclassified as never tobacco users. Moreover, since the workers did not have to actively negate ever having smoked some current or previous smokers might have been misclassified as never smokers. However, in both cases this should not comprise many individuals and the misclassification is most likely to be non-differential, thus bias results towards the null. The accuracy of self-reported history of tobacco use has been evaluated in comparison to biochemical validation and indicates high levels of sensitivity (87%) and specificity (89%) for self-report [212]. Misclassification of dose and duration of tobacco use due to differential recollection should not be an issue in study I and II, given the prospectively gathered exposure information, though some non-differential misclassification might be present, and if so, estimates would as a general rule be biased towards the null.

Another source of misclassification bias may be caused by change in smoking habits over time. During the course of follow-up, a proportion of current smokers would be expected to give up smoking, or transition to

oral moist snuff. In contrast, the number of never tobacco users commencing smoking would in all probability be rather small; since the mean age at entry of our study cohort was 34 years, and smokers typically start the habit during adolescence. Between the initial and sixth visit, about 14% of previous smokers in the cohort had resumed smoking and among current smokers roughly 40% had quit smoking. Thus, the distinction between previous and current smokers was somewhat diluted and might have hampered our ability to discern any difference in risk between these groups. Sensitivity analyses performed in another investigation of the same cohort by Zendehdel et al [213] showed that 6.7% of subjects classified as never tobacco users at entry were later on denoted as current or former tobacco smokers in one subsequent record as a minimum. Nonetheless, the possibility of self-selection bias among subjects attending the clinic multiple times should be taken into account when reviewing the numbers presented above. With regard to height and weight for BMI calculations, these variables would be less sensitive to misclassification because they were measured. Height is rather constant over time whereas weight generally fluctuates over time. Among workers with repeated measures between 70% of workers categorized as normal weight (BMI categories as defined on page 24) and 80% of those overweight, were stable in these categories. About 30% of subjects of normal weight at entry were registered as overweight at the fifth visit, while 21% obese individuals lost weight and had a BMI categorized as overweight. Again, comparison of diverse BMI strata would be impeded by a non-differential bias which possibly could have obscured a minor positive association between level of BMI and NHL.

Exposure misclassification may have been introduced in study III when approximating unknown doses of immunosuppressive medications and dates for dose change. This misclassification would however in all probability be non-differential considering that a prearranged system was employed and all subjects were treated equally due to blinding of case control status. Moreover, misclassification might be present in our estimates of the effect of HLA and infectious complications respectively, on the risk of NHL. In course of time and between hospitals there has been variation in methods for HLA typing with different sensitivity and specificity, and therefore misclassification of this exposure variable may be present. However, since this misclassification would probably be unrelated to the outcome it would result in a non-differential bias towards the null. Although patients almost solely had the medical surveillance at specialized clinics (G. Tufveson, personal communication), some infections occurring during follow up might not have been recorded. Thus, the relationship between infections and NHL may perhaps be slightly weakened due to the misclassification of some exposed individuals as unexposed.

Another potential source of misclassification bias would be if research assistants extracting data interpreted lab results or notes in medical records slightly inconsistently. In order to avoid this all research assistants were given instructions on how to define a positive serology, viral or bacterial infections and any misclassification ought to be non-differential and therefore bias estimates towards the null. With regard to bias attributable to outcome misclassification, such systemic error was curtailed by the morphological verification of all cases by a pathologist or cytologist.

In study IV, misclassification of the main exposure could be caused by the diverse methods used for DNA extraction. For the majority of Swedish controls, DNA could not be prepared from whole blood samples. Hence, DNA was prepared posteriorly from dried whole blood spots on filter paper. The very fact that this method was confined to Swedish controls solely gives rise to concern for differential misclassification bias. Nevertheless, concordance checks of both materials were conducted and resulted in 99% overall concordance of outcome between materials, arguing against exposure misclassification. The approach of two autonomous scorers corroborating all genotypes enhanced the validity of the exposure categorization; and since classification of NHL and subtypes was performed by skilled experts the influence of outcome misclassification is small. Importantly, results were similar in Denmark and Sweden, and in Denmark, the source of DNA was the same among cases and controls.

9.1.4 External validity

Once internal validity of a study has been established, the issue of external validity needs to be reflected on. A high external validity implies that inference about causal relationships on the basis of a particular investigation can further be generalized beyond the study population.

In study II we restricted analyses to male workers, hence our findings will not be generalizable to women. One could also argue that construction workers might have different habits of tobacco use in comparison

with the general population which would limit the external validity of the results. The occurrence of smoking and oral moist snuff use was however similar for the construction workers in our cohort and the Swedish population for the corresponding time period [76]. International generalizability may perhaps be restricted since tobacco smoking among men is less common and snuff dipping is more prevalent in Sweden than in most other countries [46, 47].

In study III we investigated determinants of importance for NHL development among organ transplant recipients specifically; hence our findings are not generalizable to a non-transplanted population. Additionally, as our study sample was comprised of adult individuals, one should be careful when making inference to transplanted children.

Provided that alternative explanations, i.e. random error, bias and confounding do not have considerable influence on results presented in study IV, the findings should be applicable to other Caucasian populations outside Sweden and Denmark. Notwithstanding, generalizability to groups of non-Caucasian origin is doubtful.

9.1.5 Precision

Random error may arise consequent to sampling alterability or measurement error. Precision can be defined as lack of random error which brings about an erroneous association between the exposure and disease by chance alone. Three principal ways of increasing precision can be used by the epidemiologist: firstly, to increase the sample size of the study; secondly, by repeating a measurement within the investigation or replicate the complete study; thirdly, to maximize the quantity of information acquired for a set sample size by utilizing an efficient study design. In epidemiological studies random error can be quantified by estimating p-values and confidence intervals. The confidence limit is generally set to 95%.

Although in the two cohort studies of construction workers (study I and II) sample size in was large, the number of events was few among women in general (study I) and in some levels of categorical covariates among males (study I and II), thus impairing statistical precision in face of the impressive size of the originating cohort.

In study III, three control subjects per case were selected with the objective to acquire better precision. Notwithstanding, the major limitation of this study is the low statistical precision due to few cases along with the small sample size. However, the positive associations reported are not likely to be a consequence of chance since they are in line with preceding investigations and p-values were highly significant at the alpha 0.05 level despite the small sample size.

In the genetic association study (study IV) within SCALE the statistical precision of the data was enhanced by the large sample size. However, low frequency of certain genotypes as well as low numbers in subtype specific analyses diminished our ability to obtain stable point estimates of association.

When examining numerous hypotheses in a dataset of considerable size, the probability of a false positive finding due to chance alone increases [209], and random error can therefore not be ruled out. In the light of a high likelihood of chance findings in genetic association studies, adjustments for multiple testing were taken into account (though not performed) when interpreting the results in study IV.

9.1.6 Confounding

The mixing of effects between an exposure, an outcome and a third variable distorting the relationship is the basic concept of confounding. More specifically, to be defined as a confounder the variable has to be associated with the exposure in the population that yielded the cases. Furthermore, the variable should be independently associated with the outcome. Lastly, a confounding variable must not be an intermediate in the causal pathway linking exposure and outcome.

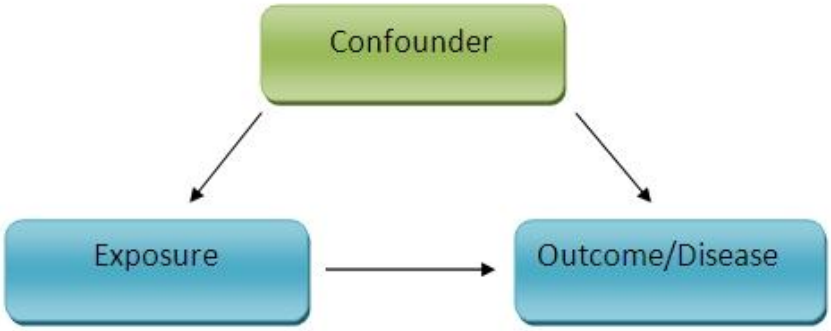

Figure 5. Confounding by a third variable on the association between the exposure and disease.

There are three main ways to adjust for confounding when designing an epidemiologic study: randomization (clinical trials), restriction and matching. However, in a case-control study matching simply gives an enhanced precision for an adjusted analysis. In the analysis phase the investigator can further control for confounding by means of stratification (i.e. subset analyses), multivariate methods and standardization.

The extensive 200-item questionnaire filled out by the construction workers in connection with health check-ups provided the opportunity to control for various putative confounders. Thus, the investigation of the construction workers cohort is not limited to a great extent by lack of information on potential otherwise common in register-based cohorts. However, information on some known risk factors for lymphoma such as HIV/AIDS, EBV and immunosuppression and autoimmune conditions were lacking or insufficient. We were therefore unable to control for possible confounding by these factors. However, aside from EBV infection, these possible confounders have low prevalence and subjects with rheumatoid arthritis would probably not be able to continue laboring as construction workers for very long due to progression of the disease.

By restricting analysis to men (study II) the variability due to gender was eliminated. In study I confounding by gender was controlled for by stratified analyses, also enabling evaluation of homogenous categories of gender. Moreover, in the Cox proportional hazards regression model other known and potential confounders such as age, BMI and tobacco use where controlled for.

In the design phase of the nested case-control study (study III) of solid organ transplant recipients cases and controls were frequency matched on categories of age and calendar period of transplantation within five-year intervals, thereby achieving a better precision for the conditional logistic regression analysis conditioning on the matching factors. Additionally, covariates that came out statistically significant in the univariate models were adjusted for in multivariate conditional logistic regression model. In this study, the interplay between a wide range of variables is very complex and consequently extensive information on a large number factors was extracted during data collection to allow for evaluation of confounding by these factors in the analysis phase. Figure 6 depicts the interwoven relations between determinants and putative causal pathways leading to NHL development in the setting of organ transplanted patients.

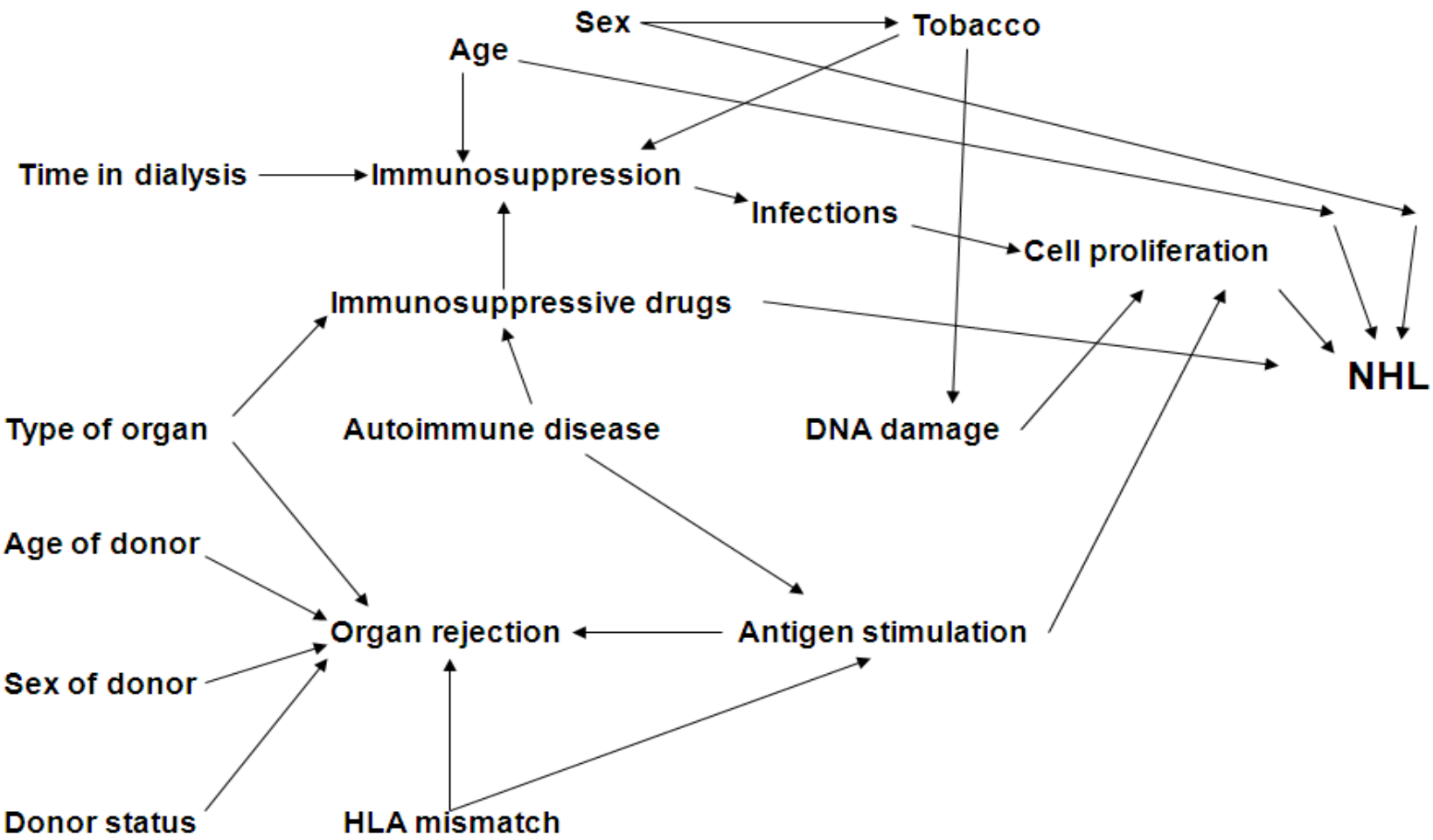

Figure 6. Directed Acyclic Graph of casual pathways leading up to NHL in the setting of organ transplanted patients.

In study IV we chose a genetically homogeneous study population encompassing persons of Scandinavian/Nordic country decent, and since both regions were equally represented within cases and controls (Table 15), we have reduced the chance of confounding resulting from population stratification i.e. dissimilarity in allele frequencies between cases and controls caused by systematic differences in ancestry rather than genes related to disease [214]. Frequency matching of controls was employed for age and sex by 10-year intervals within country of residence. In the logistic regression model, we only adjusted for country of residence, since the selection of controls based on age and sex is irrelevant for genotype.

Nevertheless, generally even when all known confounders have been controlled for, residual confounding may persist. This can arise in the instances when information on the confounder is not at hand, when controlling for confounders in excessively broad categories or if the confounder suffers from measurement errors. Seeing that not many risk factors for malignant lymphomas and leukemia and MM have been firmly, established residual confounding attributable to some yet unknown confounder which distorts the relationship between exposure and outcome may perhaps be present in our results.

9.2 FINDINGS AND IMPLICATIONS

9.2.1 Study I

In study I our findings did not provide any evidence of a casual role for tobacco smoking in the development of NHL overall. Nor did duration or intensity of tobacco smoking appear to be related to subsequent NHL development. Furthermore, ever use of oral moist snuff as well as mixed usage (more than one tobacco product) did not confer any excess risk of NHL. No support was observed for an association between having a BMI classified as overweight or obese and NHL. For HL results were analogous with the exemption of a close to four-fold increase in risk related to long term use of oral moist snuff (>30 years). This result should however be interpreted with caution as it was based on very few cases.

With regard to tobacco smoking, our results are consistent with data presented by other investigations where smoking status (never, current or previous) was unrelated to NHL overall [49-52, 54-58], although conflicting evidence has been presented [60, 63-65]. We did however fail to corroborate some studies that detected an association linking amount and/or duration of smoking tobacco and risk of NHL [56, 62, 65]. To some extent the inconsistent findings may be explained by differences in study design, study population, sample size and diversity in methods for exposure assessment and categorization or definition of for instance smoking status and smoking intensity (pack years vs. cigarettes per day). Similarly, conflicting results in terms of BMI may be partially assigned to discrepancies in classification of levels of BMI in accuracy of exposure ascertainment i.e. whether height and weight was self-reported or measured. Even though studies have been inconsistent there is emerging evidence of an association between excess weight and NHL, and there is an indication of a subtype specific relation to DLBCL.

The most important strengths of our investigation lie in the large size of the study cohort along with the extensive and in detailed prospectively collected information on tobacco use. In addition, the large study sample together with the comprehensive exposure information, gave us the opportunity to investigate oral moist snuff as an independent exposure. The long term follow up utilizing national registries provides high precision and high validity of outcome data. Nonetheless, in a recent evaluation of the completeness of the Swedish Cancer Register there was a higher frequency of underreporting for hematolymphoproliferative neoplasms than for most other site specific cancers [194]. This may have two main effects on the Construction workers study. Firstly, some misclassification could have been introduced if some subjects were falsely classified as non-cases. However, it is implausible that this would have a substantial effect on our results as it would not involve many individuals. Secondly, the power of the investigation is reduced if some incident cases in the cohort never became registered. An additional limitation of our study is the lack of data on histological subtypes which prevented us to investigate risk of specific NHL subentities independently. Also, results on intensity and duration of smoking should be interpreted cautiously considering that we do not know for how much longer a subject continued to utilize tobacco or if the daily amount stated remained consistent over time. Furthermore, due to constraints as to what would be computationally feasible, exposure information on smoking and BMI was restricted to data obtained at the first visit to the clinic. Hence, we were not able to adjust for changes in tobacco habits. The one point in time exposure measurement will only be an approximation and results will be biased towards the null (see methodological

considerations). However, taking into account that only a small fraction of never tobacco users started smoking, the reference group is fairly stable and we should have been able to discern a moderate positive association, if present. As discussed previously in the methodological considerations section of this thesis, a proportion of study subjects changed level of BMI during the follow-up period. If this would have an impact on our findings it might have concealed a true small positive association between level of BMI and NHL risk. BMI as a measure of overweight and obesity can be questioned since it is not very precise and does not take into account the body composition, i.e. percent body fat and muscle mass. BMI has been validated as a rather good predictor of percent body fat in persons of normal weight, but not at considerably high body weights [215].

There is evidence suggesting that smokers weigh less than nonsmokers and smoking cessation confers weight gain, more so among those who were heavy smokers [216]. Animal studies have reported a reduction in food intake and number of meals following administration of nicotine resulting in weight loss. After withdrawal of nicotine the exposed group ate more than double the amount of food than the control group and body weight normalized [217]. Nicotine activates the release of dopamine and serotonin in the hypothalamus. The sensitivity for satiety is augmented by serotonin resulting in a decrease in food intake and body weight [217]. Studies in humans indicate intake of carbohydrates to be an important factor that differentiates smokers and nonsmokers [216]. Another mechanism by which nicotine might have an effect on body weight is through changes of the metabolic rate. One study reported a 3% increase in metabolic rate for half an hour subsequent to consumption of one cigarette [218]. Physical activity does not seem to be a determinant for the described differences in body weight comparing smokers and nonsmokers or with the increase in weight after smoking cessation [216]. We adjusted for possible confounding by tobacco use and BMI on their respective association with NHL; nonetheless, some residual confounding cannot be fully excluded.

The putative biological mechanisms by which tobacco and BMI may cause lymphoma has been discussed in a previous section of this thesis (see page 9 and 12).

Although our study did not support a causal relation between tobacco use or level of BMI and lymphoma, the well established adverse effects including higher morbidity and mortality calls for stronger efforts in public health prevention globally. In particular, actions are warranted in some developing countries where tobacco products are heavily promoted by the industry, as well as nations with rapidly increasing prevalence of obesity.

The unavailability of information on specific disease subentities is a major limitation of our investigation. Taken together, the reports suggesting a relationship linking tobacco use to follicular lymphoma as well as the mixed cellularity subtype of HL gives incentive for additional studies in large study samples to confirm these associations.

In the light of reports of an elevated risk of several other forms of cancer such as oral cancer, esophageal cancer, pancreatic cancer and lung cancer associated with smokeless tobacco [75], the sparse knowledge about the role of smokeless tobacco and risk of lymphoma along with the indication in our study of a possible association between long term use of oral moist snuff and HL warrants further large scale investigations to elucidate the role of smokeless tobacco in lymphomagenesis.

9.2.2 Study II

In study II we observed current smokers to be at 50% greater risk of AML compared to never tobacco users. However, current smoking status was not associated with any of the other leukemia subtypes. We also found an indication of a relationship linking smoking intensity to ALL. We observed no association between oral moist snuff and risk of leukemia, nor did level of BMI influence the risk of any of the leukemia subtypes under study.

The number of studies investigating leukemia subtypes as distinct entities is limited; though two previous case-control studies have reported an association between current smoking and AML [177, 178] but smoking was unrelated to risk of ALL. Another case-control study [219] detected no significant association between smoking and AML overall but a twofold increased risk was observed for the French-American-British subtype M2 in addition to a significant dose response for number of cigarettes used per day and total years smoked (M2 subtype). In contrast, Björk et al [179] found no association between smoking and AML overall or the M2 subtype. However, an excess risk of AML was denoted at high cumulative doses of smoking.

Benzene is a known substance of tobacco smoke and may induce chromosomal aberrations which might be of significance in the causal pathway [220]. As discussed previously (see page 17) benzene is a well recognized risk factor for leukemia, notably AML. However, the exact mechanism explaining a subtype specific association between tobacco smoking and AML is ambiguous.

Additional studies are called for to verify a causal relationship between tobacco smoking and AML and to clarify the biological mechanisms that could explain a subtype specific causal relationship between tobacco and AML.

9.2.3 Study III

In study III we showed that immunosuppression with antithymocyte globulin (ATG) rather than the overall immunosuppressive load is an important determinant of NHL development subsequent to solid organ transplantation. Moreover, a high average daily dose of ATG conferred a more pronounced risk of post transplant NHL than a high total accumulated dose. This study is further consistent with preceding reports [110, 111] of an association between type of organ transplanted and NHL risk. Among heart and lung transplant recipients we observed a 30-fold and 20-fold elevated risk of NHL respectively, compared to kidney recipients. Patients receiving a liver graft were at a 17-fold albeit non-significantly increased risk of NHL.

This study further provides evidence of an indication of herpes virus group infections as a cofactor in the development of post-transplant NHL. This association appears to be driven largely by EBV. In our study we observed that EBV infection strictly arose in subjects later identified as cases. This, in addition to preexisting literature [116, 118, 119] suggest an imperative function of EBV in the etiology of NHL, notably early-onset NHL, following solid organ transplantation.

The strengths of study III include the population-based study design, utilization of the nationwide cancer register for case ascertainment as well as the extensive exposure information, particularly the detailed data collected on immnosuppressive medications. The major limitation however is lack of power due to a small sample size and in some instances missing data. Another weakness is the inability to look at drugs introduced in recent years, for instance Rapamycin inhibitors, some of the protein drugs, non-depleting antibodies and fusion proteins. Moreover, EBV status of tumor tissue was not at hand during the conduct of the study. Thus, the investigation is hampered by the incapacity of analyzing EBV positive and EBV negative lymphomas as separate entities. Nonetheless, taking the small sample size into account, the statistical power would probably be inadequate.

In conclusion, our results suggest that treatment with ATG is associated with subsequent risk of post transplant NHL among organ transplant recipients. More specifically, the average daily dose ATG administered may confer a greater risk than the cumulative dose. The interplay between EBV immortalizing B-lymphocytes and the level of impediment on the T-cell response by ATG is a putative model to explain the origination of post transplant NHL. However, if ATG is a risk factor for EBV-negative lymphomas as well, implied by our finding of an increased risk of both early- and late-onset NHL, other mechanisms may also be at play.

The field of organ transplantation has undergone an astonishing development in the past decades. With the growing population of organ transplant recipients the issue of post transplantation, malignancies becomes of matter of apprehension. Our findings advocate a more careful administration of ATG by physicians at the transplantations clinics, in particular avoiding high doses.

Heart transplant recipients typically tend to receive more ATG in comparison with kidney graft patients (G. Tufveson, personal communication) and come out as a high risk group in need of more close medical surveillance with the aim of achieving earlier detection of subclinical NHL.

As of today, very little is known about the etiological factors causing EBV-negative PTLD. Hence it would be of great interest to investigate this group independently. Moreover, in contrast to EBV positive PTLD, EBV-negative PTLD are generally less susceptible to reduction or withdrawal of immunosuppression and commonly presents with poor prognosis [221-223]. Therefore, better knowledge about this subentity is clinically relevant

9.2.4 Study IV

The knowledge about the role of genetic variation and susceptibility of lymphoma is scarce. A number of genes have been suggested to play a causal role (see genetic factors) but consistent findings to date have mainly been provided for *TNF* along with *IL-10* loci.

In the SCALE study (study IV) we found that genetic variability of the *TNF* gene confers a 50% excess risk of NHL overall which is in line with other investigations [128-132]. Moreover, a relation to T-cell lymphoma and Mantle cell lymphoma could be discerned. The study provided further evidence of an association between polymorphic variation in the *IL10* gene and risk of DLBCL and mantle cell lymphoma. Although, none of the observed estimates for *TNF* and *IL10* would have persisted adjustments for multiple testing, a high prior probability [224] fortifies these associations.

A causal pathway by which TNF might induce lymphomagenesis is the NF-κB pathway giving rise to cell proliferation and prevention of apoptosis [225]. With regard to IL10 a number of biological mechanisms have been postulated to explain a causal role in the origination of malignant lymphomas. IL-10 improves survival, proliferation and differentiation of B-cells [226]. Furthermore a reduction in IL-10 expression leads to diminished control of proinflammatory cytokines such as TNF [226]. The anti-inflammatory effects of IL-10 i.e. inhibition of cytokine production and individual cell types [226] have been suggested as potentially cancer-promoting [227].

In contrast, the results of our investigation did not indicate any clear causal relation connecting the translocation breakpoint genes *BCL2 and CCND1,* and *MYC* with risk of NHL development. Thus, we failed to confirm the report by Wang et al [228] presenting an association between the *CCND1* SNP rs603965 and NHL, that was noticeable also for DLBCL and marginal zone lymphomas. Nevertheless, among autoimmune patients in our investigation a suggestive relation between this SNP and DLBCL could be observed.

We did not detect any sign of effect measure modification in the gene-environment interaction models performed with tobacco smoking and autoimmune disorders respectively. However, the latter analyses may have been hampered by low power due to a low number of individuals with a positive history of autoimmune disease. Likewise, the study did not provide any evidence of a gene-gene interaction.

A weakness of study IV is the lack of power to distinguish small effects and any such true associations may thus remain undetected. Of more concern is however the possibility of selection bias that might have been introduced as a consequence of lower participation rates among controls for interviews as well as to provide blood samples. The absence of an association with autoimmunity in this study sample might indicate a selection of ill controls more prone to attend the outpatient clinics and hence leave a blood specimen. However, given the very small proportion of patients with the selected autoimmune disorders among our controls, the disappearance of the association of specific autoimmune disorders with NHL among blood donors may also have occurred at random.

To summarize, genetic variability in the immunoreulatory genes *TNF* and *IL10* may have a role in determining susceptibility to NHL. A more weak indication of a role in NHL development was found for *CCND1* while *BCL2* and *MYC* does not appear to be associated with NHL risk. However, we were not able to cover the investigated genes fully, for which reason the possibility of a relationship between genetic polymorphisms in these genes and susceptibility to NHL cannot be fully discarded.

Additional investigations are warranted to verify the putative association reported between *TNF* and *IL10* respectively, and NHL. In particular, studies with large sample sizes are needed to establish subtype specific associations reported in preceding studies as well as the novel findings of a possible relation between *TNF* and T-cell lymphoma as well as Mantle cell lymphoma presented in study IV. Additionally, if a true association lies between genetic variants in the immunoregulatory genes *TNF* and *IL10* and lymphoma development this might have clinical implications in terms of defining high-risk groups that possibly would be an object for closer medical surveillance.

Even though this study failed to provide support of a correlation between gene variants and the environmental exposures under investigation, future large scale studies with sufficient power to detect even modest association are called for to shed further light on the relative importance of genotype and environment on disease outcome. Thus, genome-wide association studies in pooled data in conjunction with functional studies would be the state of the art approach for upcoming studies within this field.

9.3 CONCLUSIONS

- Tobacco smoking does not appear to be associated with risk of non-Hodgkin lymphoma overall. Nevertheless, in the context of incumbent literature a causal link between smoking and follicular lymphoma cannot be excluded and warrants further investigation.

- Our finding indicating that long term use of oral moist snuff may be associated with an elevated risk of Hodgkin lymphoma, needs to be confirmed by other investigations.

- Level of body mass index does not seem to influence risk of non-Hodgkin or Hodgkin lymphoma.

- Current smoking may increase the risk of acute myeloid leukemia, and an association between smoking intensity and acute lymphatic leukemia cannot be excluded. However, tobacco smoking seems to be unrelated to chronic myeloid leukemia and multiple myeloma.

- Body mass index does not appear to be associated with risk of leukemia or multiple myeloma.

- Treatment with antithymocyte globulin (ATG) might be an important determinant of non-Hodgkin lymphoma in the setting of organ transplant recipients. The average daily dose appears to confer a higher risk of NHL than a high total accumulated dose. By impeding cytotoxic T-cells ATG could create a milieu where B-cells immortalized by Epstein Barr virus can elude tumor surveillance and progress into malignant lymphoma.

- Genetic polymorphisms in the *TNF* and *IL10* might play a role in the susceptibility of non-Hodgkin lymphoma and specific subtypes. In contrast, the chromosomal breakpoint genes *BCL1, CCND1* or *MYC* do not seem to be associated with non-Hodgkin lymphoma or specific disease entities.

9.4 SVENSK SAMMANFATTNING

Bakgrund
Lymfom är en mångfacetterad cancerform som uppkommer i immunförsvarets lymfocyter (en typ av vita blodkroppar). Lymfom kan indelas i två huvudgrupper; Hodgkin (HL) respektive non-Hodgkin lymfom (NHL) men dessa omfattar var för sig flera undergrupper. Leukemi har sitt ursprung i de vita blodkropparna i blodet och benmärgen. Multipelt Myelom härstammar från plasmaceller, en typ av högspecialiserade antikroppsproducerande lymfocyter.
NHL drabbar företrädelsevis äldre individer och ca 2000 personer insjuknar varje år i Sverige. Huvudsakliga kliniska symptom innefattar nattsvettningar, viktnedgång samt feber. Svullna oömma lymfkörtlar är också karakteristiskt. Diagnosen ställs baserat på klinisk undersökning och benmärgsprov. Expektans, cellgifts- och/eller strålbehandling är olika behandlingsalternativ som övervägs beroende på allvarlighetsgrad av sjukdomen. Prognosen varierar kraftigt och femårsöverlevnaden är mellan 30-70% beroende på subtyp av NHL.

Under 1970- till 1990-talet skedde en häpnadsväckande snabb ökning av NHL världen över, vilken kunde ses hos alla etniciteter, de flesta åldersgrupper och hos båda könen. En mindre del av ökningen kan tillskrivas förbättrade rutiner för diagnostisering, rapportering och klassificering av NHL. Varken detta eller någon av de kända eller misstänkta riskfaktorerna kan således enskilt eller sammantaget helt förklara den drastiska ökningen av NHL. Lika abrupt och oförklarligt har den stigande trenden avtagit de senaste åren.

Syfte
Det övergripande syftet med denna avhandling var att öka vår förståelse för bakomliggande orsaker till uppkomsten av lymfom och leukemier.
Våra specifika mål med studierna var:

- Att undersöka om tobaksrökning, snusanvändning eller övervikt/fetma är associerat med en ökad risk för att utveckla NHL, HL, leukemi eller multipelt myelom. (delarbete I-II)
- Att utreda om risken för lymfom, leukemier och multipelt myelom påverkas av intensitet och duration av tobaksbruk. (delarbete I-II)
- Att studera den relativa vikten av och samspelet mellan karaktäristika hos donator/mottagare, immunosuppressiva mediciner och infektionskomplikationer hos organtransplanterade individer. (delarbete III)
- Att undersöka om genetisk variation i kromosomala translokationsgener *(BCL2 och CCND1) MYC* och immunreglerande gener (*TNF,IL10*) är av betydelse för en ökad risk för NHL eller dess vanligaste undergrupper.

Material och metod
I Sverige utgör de nationella populationsbaserade registren tillsammans med individspecifika personnummer ett utmärkt grund för epidemiologisk forskning av hög kvalitet.
I avhandlingen ingående delarbeten har följande datakällor utnyttjats:

Slutenvårdsregistret: Startades 1964-1965 men har varit nationellt alltsedan 1987. Registret innefattar 50 miljoner diagnoskoder. För varje enskilt vårdtillfälle inom slutenvård fyller man i en blankett med information om in- och utskrivningsdatum, huvud- samt bidiagnoser, operationskoder och anestesiologiska procedurer etc.
Svenska cancerregistret: Initierades 1958 och får rapport om alla nydiagnostiserade maligniteter. Enligt svensk lag är samtliga läkare och patologer skyldiga att rapportera nya fall till ett av sex regionala register, vilket gör att registret håller mycket god kvalitet. Närmare 99% av alla nya cancerfall inrapporteras och uppemot 99% av dessa är morfologiskt verifierade.
Migrationsregistret: Innehåller information om antal individer som emigrerat och immigrerat samt datum för in- respektive utflyttning.

Dödsorsaksregistret: Data om alla dödsfall bland svenska medborgare registreras hos dödsorsaksregistret sedan 1958. Registret har blivit mer komplett och förbättrat sin kvalitet över tid. Vid varje enskilt dödsfall utfärdas ett dödsorsaksintyg av en läkare. Intyget ger, utöver datum för bortgång, även information om den underliggande orsaken till dödsfallet.
Danska cancerregistret: På samma sätt som det svenska cancerregistret innehåller dess danska motsvarighet statistik på alla nya cancerfall. Danskarna startade sitt register 1943 och har haft lagstadgad rapportering sedan 1978.
Danska nationella lymfomregisterorganisationen (LYFO): Samlar sedan 1983 in klinisk data på alla danska patienter med lymfomdiagnos. Registret innehåller bl.a. information om datum för diagnos, kliniskt stadium och vilka organ som påverkats av sjukdomen.
Danska patologiregistret: En nationell databank som grundades 1999. Registret fungerar som ett diagnostiskt verktyg för alla patologer i Danmark, men används även i forskningssammanhang då det har nästan 100% täckningsgrad.
Uppgifter insamlade från medicinska journaler

Delarbete I
”Bygghälsan” var ett samarbete mellan fackföreningar och arbetsgivareföreningen vilket pågick mellan 1969 och 1992. Projektet innebar att alla byggnadsarbetare erbjöds gratis hälsokontroller. I samband med dessa fick de fylla i ett frågeformulär med allmänna hälsouppgifter inklusive tobaksanvändning. Vi använde denna stora kohort av mer än 330,000 individer för att studera om olika typer av tobaksbruk (cigaretter, cigarrer, pipa samt snus) eller övervikt/fetma utifrån BMI (kroppsmasseindex = vikt i kg/längd i meter) påverkar risken för att utveckla lymfom. Vi fann inget samband mellan rökning och NHL eller HL. Att ha ett BMI klassificerat som överviktig eller fet medförde ingen ökad risk för NHL eller HL. Vi fann en indikation på att snusanvändning under mer än 30 års tid gav en nästan fyra gånger högre risk för HL men sambandet var baserat på få exponerade fall och bör därför tolkas med försiktighet.

Delarbete II
Ytterligare en studie på byggarbetarkohorten genomfördes, i vilken vi undersökte hur risken för leukemier och multipelt myelom influeras av tobak och övervikt/fetma. Vi använde i stort sett samma material och metod som i studie I. Våra data visade en 50% högre risk för undergruppen akut myeloisk leukemi (AML) hos nuvarande rökare. Dessutom såg vi en svag indikation på ett samband mellan intensitet av rökning(gram/dag) och akut lymfatisk leukemi (ALL).

Delarbete III
Baserat på en tidigare kohortstudie i vilken man följt upp samtliga patienter som genomgått organtransplantation i Sverige mellan 1970-1997 på cancerincidens genomförde vi en nested fall-kontroll studie med syftet att klargöra vilka faktorer som är av störst betydelse för den sedan tidigare kända överrisken för NHL hos denna patientgrupp. Tre kontroller per fall matchades för ålder vid transplantation (5-årsintervall) samt tidsperiod för transplantationen (5-årsintervall). Ett abstraktionsprotokoll utformades för att inhämta information från patienternas journaler med avseende på donator och recipient (ålder, kön, vitalstatus hos donator), typ av organ som transplanterats, infektionskomplikationer samt detaljrik data på de immunosuppressiva medicinerna (doser, datum för dosändring). Totalt 37 NHL-fall och 97 kontroller ingick i analyserna. Vi observerade ett samband mellan behandling med antithymocytglobulin (ATG) och NHL risk. En hög medeldos tycktes medföra en starkare riskökning än en hög total ackumulerad dos (8- respektive 5-faldigt högre risk). Genomgången herpesgruppvirusinfektion var associerat med en fyrfaldigt ökad risk för NHL, vilket sannolikt drevs av Epstein Barr virus.

Delarbete IV
Inom ramen för ett dansk-svenskt samarbete (SCALE) genomfördes en fall-kontrollstudie på 2410 NHL-fall och 1936 kontroller, matchade på ålder och kön inom respektive land. Studiepopulationen omfattade den danska och svenska befolkningen mellan 18-74 år under 1999-2002 (Sv) och 2000-2002 (Dk). Vi avsåg att studera om genetiska sekvensvariationer, sk. singel nukleotid polymorfismer (SNPs) i immunreglerande gener (*TNF, IL-10*), translokationsgener (*BCL2,CCND1*) samt *MYC* påverkar mottaglighet för NHL eller dess undergrupper. Samtliga deltagare i studien intervjuades per telefon om livsstilsfaktorer och sjukhistoria samt lämnade blodprov. Ur blodprovet extraherades DNA och genotypades. Individer med *TNF* rs1800629 AA-genotypen hade en 50% högre risk att drabbas av NHL och en 2-faldig riskökning observerades även för T-cellslymfom och mantelcellslymfom. *IL10* rs1800890 AA-genotypen var associerat med en ökad risk för diffust storcelligt B-cellslymfom samt mantelcellslymfom. Dock var det inget av dessa riskuppskattningar

som höll för s.k. multipla tester. Inget klart samband mellan de undersökta translokationsgenerna och NHL eller dess undergrupper kunde urskiljas.

Slutsatser

- Tobaksrökning verkar ej vara en betydande riskfaktor för NHL men utifrån tidigare studier kan ett samband med undergruppen follikulärt lymfom inte uteslutas och bör utredas vidare.

- Vårt fynd som indikerade att långvarig användning (>30år) av snus möjligtvis kan öka risken för HL måste verifieras av ytterligare studier.

- BMI tycks inte påverka risken att drabbas av lymfom.

- Rökare har möjligen en högre risk att drabbas av akut myeloisk leukemi, och ett samband mellan tobaksrökning och akut lymfatisk leukemi kan inte helt uteslutas.

- BMI verkar inte påverka risken för leukemier aller multipelt myelom.

- Hos organtransplanterade patienter kan ATG vara av betydelse för NHL-risk. En hög medeldos verkar vara av större betydelse än en hög ackumulerad dos. Herpesvirusinfektioner, framför allt Epstein Barr virus, är sannolikt en viktig delfaktor i detta sammanhang. ATG hämmar immunförvarets T-celler och skulle kunna skapa en miljö i vilken immortaliserade B-lymfocyter kan undgå att bli eliminerade och utvecklas till NHL.

- *TNF* respektive *IL-10* generna tycks öka mottagligheten för NHL och specifika undergupper. Dock verkar inte *BCL2, CCND1* eller *MYC* generna vara av betydelse för NHL risk.

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